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-

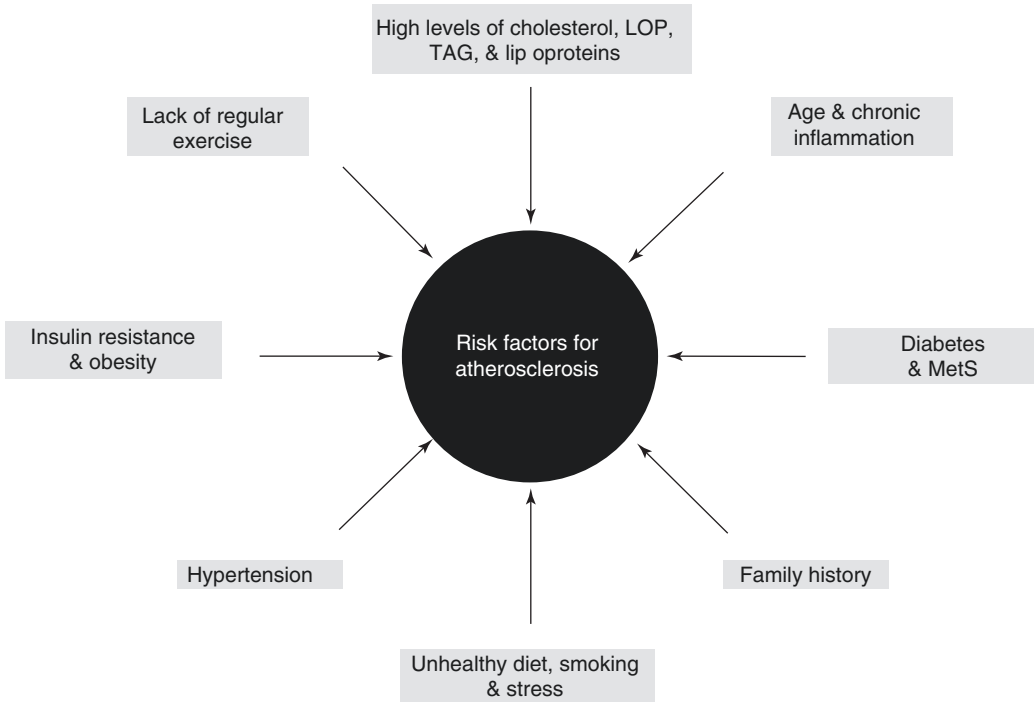

Fig. 1.1 Factors modulating atherosclerosis and cardiovascular diseases. *TAG* Triacylglycerol; *MetS* metabolic syndrome

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).

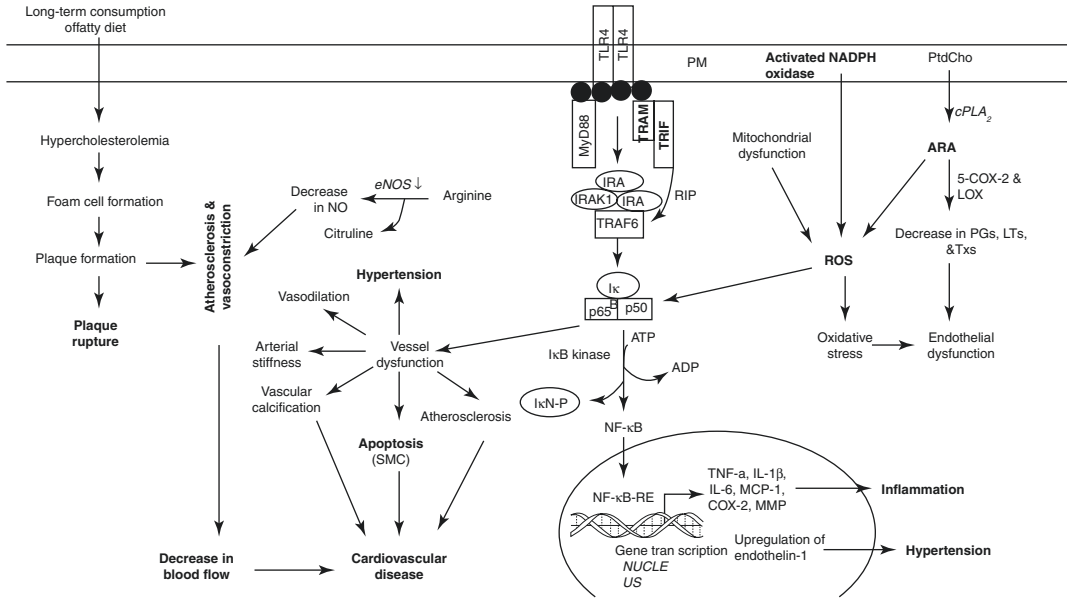

Fig. 1.2 Schematic diagram showing signal transduction mechanisms associated with the pathogenesis of cardiovascular disease. *PM* Plasma membrane; *PtdCho* phosphatidylcholine; *ARA* arachidonic acid; *lyso-PtdCho* lyso-phosphatidylcholine; *PAF* platelet activating factor; *cPLA*$_2$ cytosolic phospholipase 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

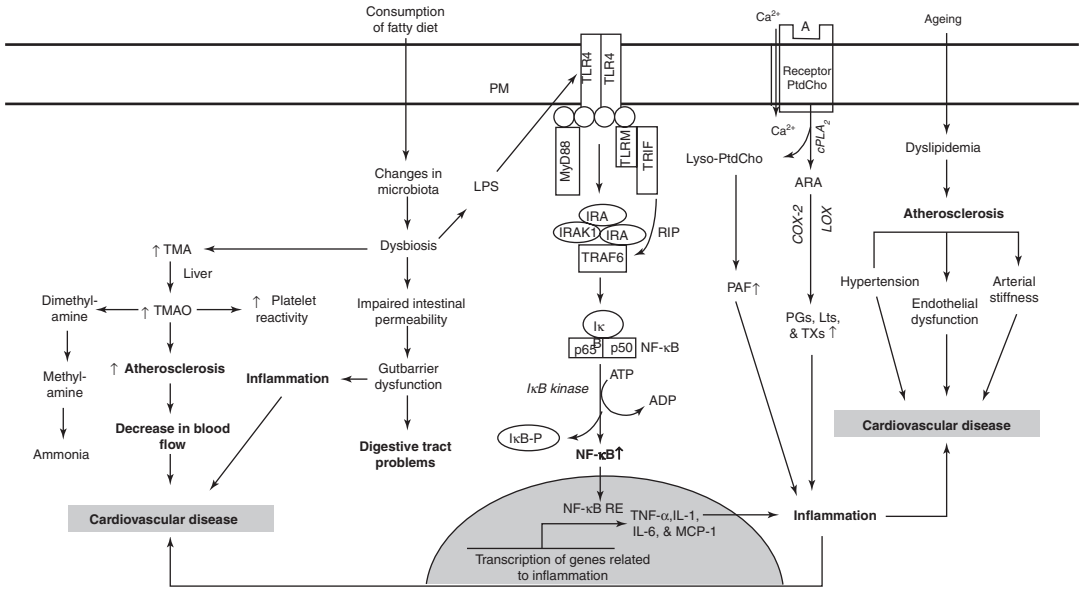

Fig. 1.3 Schematic diagram showing the contribution of microbiota in the pathogenesis of cardiovascular disease. *PM* Plasma membrane; *A* agonist; *R* receptor; *PtdCho* phosphatidylcholine; *ARA* arachidonic acid; *lyso-PtdCho* lyso-phosphatidylcholine; *PAF* platelet activating factor; *cPLA*$_2$ 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

of LPS in circulation is neutralized via binding with HDL resulting in its clearance through biliary excretion. Only a smaller part of LPS is associated with the activation of macrophages and the overproduction of potent inflammatory mediators. Both HDL and LPS are known to bind with the scavenger receptors class B type I (SR-BI). These receptors contribute to a cholesterol delivery system and are present in different types of cells, including adipocytes and type-two alveolar epithelial cells. LPS promotes inflammation through a cascade of inflammatory responses following the recognition of lipid A in LPS by immune cells. Lipid A is the toxic component of LPS and serves as the microbe-specific molecular signal that interacts with the TLR4 and myeloid differentiation factor 2 (MD2). At the molecular level, circulating LPS suppresses the expression of tight junction proteins, leading to an increase in intestinal permeability and subsequently the translocation of LPS from the gut into the blood from where LPS can enter any tissue and activate TLRs and their downstream targets [22, 23]. Elevated plasma LPS levels (over 50 pg/ml) are associated with a threefold increase in the risk of developing atherosclerosis, whereas the subpopulation of smokers or ex-smokers with the same LPS level exhibit a 13-fold increase indicating that there is a relationship between levels of plasma levels of LPS and atherosclerosis. Collective evidence suggests that cross-talk between gut microbiota and host intestinal tract not only involves multiple overlapping pathways (autonomic, neuroendocrine, and immune systems), but also metabolites-derived from gut microbiota such as trimethylamine N-oxide, short-chain fatty acids (acetate, propionate, butyrate), and secondary bile acids [20]. Consumption of dietary fibers results in lowering plasma cholesterol levels (with reductions in cholesterol level ranging from 0.5% to 2% per gram of fiber intake). Consumption of fiber reduces both total cholesterol and LDL cholesterol not only through an increase in bile acid excretion but also by downregulating the synthesis of hepatic cholesterol. Other potential mechanisms are related to the microbiota-dependent formation of acetate, propionate, and butyrate. These fatty acids are synthesized and used as a macronutrient source of energy. Alternatively, short-chain fatty acids can also act as hormone-like signals, entering the portal circulation to ultimately bind to G-protein-coupled receptors in numerous cells and inhibit the histone deacetylase, resulting in numerous epigenetic modifications in targeted cells. The cross-talk between gut microbiota and intestinal epithelial cells produces a variety of effects in the host. The gut microbiota has been reported to metabolize choline, phosphatidylcholine, and L-carnitine to produce trimethylamine (TMA), which is oxidized in the liver by flavin monooxygenase 3 into the pro-atherogenic metabolite, trimethylamine-*N*-oxide (TMAO). Increased TMAO levels are associated with macrophage foam cell formation not only by upregulating macrophage scavenger receptors, deregulating enterohepatic cholesterol and bile acid metabolism but also by impairing macrophage reverse cholesterol transport (RCT). All these processes are linked with the development of atherosclerotic plaques [24]. Collectively, these studies indicated that elevated TMAO plasma levels are linked with adverse cardiovascular events in humans. They can be normalized by antibiotic treatment. However, prolonged antibiosis for atherosclerosis prevention is potentially harmful as it poses an increased risk of antibiotic resistance and detrimental infections such as *Clostridium difficile*. Furthermore, flavin monooxygenase 3 may promote dyslipidemia by regulating multiple genes involved in hepatic lipogenesis and gluconeogenesis. In addition, flavin monooxygenase 3 is known to impair multiple aspects of cholesterol homeostasis, including transintestinal cholesterol export and macrophage-specific RCT [25]. Two processes contribute to the synthesis of TMAO. One involves ingestion of nutrients and the other via the synthesis of TMA by gut microbiota. As stated above, in the liver, TMA is oxidized by host hepatic flavin monooxygenase 3 leading to the production of TMAO. Inhibition of gut microbiota-dependent TMAO production has been shown as a promising strategy for the treatment of atherosclerosis [20, 24]. For example, inhibition of TMA-generating microbial enzymes

by dimethylbetane has been reported to reduce murine atherosclerosis. Furthermore, the consumption of western diet increases levels of TMAO. In contrast, consumption of a Mediterranean diet results in lower circulating levels of TMAO and this may account for the anti-inflammatory and health-promoting effects of Mediterranean diet. Recently developed, nontoxic potent inhibitors of gut microbial TMA lyase (halomethylcholines) are known to markedly inhibit platelet reactivity and thrombosis [20, 24].

1.1.4 Atherosclerotic Peripheral Artery Disease

PAD is a complex, multifactorial systemic disease characterized by reduced blood flow to the lower extremities most often caused by the development of atherosclerotic plaques, which lead to chronic vascular blood flow deficit caused by stenosis or occlusion of lower limb vessels. PAD affects 12% to 20% of Americans 60 years and older and more than 200 million people worldwide. The most significant risk factors for PAD are hyperlipidemia, diabetes mellitus, chronic kidney disease, smoking, and hypertension; the presence of three or more factors confers a tenfold increase in PAD risk. Intermittent claudication is the hallmark of atherosclerotic lower extremity PAD, but only about 10% of patients with PAD experience intermittent claudication [26]. Clinical manifestations of PAD include intermittent claudication, rest pain, and nonhealing ulcer.

The location of atherosclerotic manifestation in the arterial vessel tree differs according to the main risk factor profile. Patients with diabetes suffer more often from occlusion of the lower limb arteries while smokers develop mostly a stenotic disease of the iliac or femoral arteries [26]. These manifestations contribute to impaired quality of life in PAD subjects. PAD differs from coronary artery and cerebrovascular disease in its clinical presentation. In coronary artery and cerebrovascular diseases "plaque instability" results in either myocardial infarction or ischemic stroke. In contrast, in PAD such acute "events" are relatively uncommon and symptoms most often result from progressive arterial narrowing due to ongoing atherogenesis. It is therefore likely that risk factors (both genetic, environmental, and the intermediate biochemical pathways through which they act) contribute differently to PAD than to CVD or cerebrovascular disease [26, 27]. Two mechanisms are associated with the pathogenesis of PAD. They include abnormal skeletal muscle metabolism and histology (myopathy of PAD), and endothelial dysfunction (Fig. 1.4) [28]. Endothelial dysfunction in PAD involves a decrease in bioavailability of NO and impaired flow-mediated dilation (FMD) (Fig. 1.4) [29]. Those PAD patients, who show FMD closer to normal subjects can be improved with exercise [30], while low FMD has been shown to independently predict cardiovascular risk and risk of leg amputation [31]. In PAD, induction of ischemia-reperfusion (I/R) cycle increases the production of ROS and oxidative damage. This process contributes to the pathophysiology of PAD [32]. Collectively, these studies indicate that oxidative stress and inflammation play important roles in the pathogenesis of PAD. The higher production of ROS during oxidative stress and reduction in redox status are two crucial players in initiating and progressing PAD. Biomarkers for oxidative stress in PAD include beta-2 microglobulin, cystatin C, protein carbonylation or aldehyde/ketone adducts, nitration and sulfoxidation, DNA lesions such as 8-oxodG. These biomarkers interfere with physiological redox capability. Inflammatory biomarkers for PAD include acute-phase proteins, C reactive protein, fibrinogen, and pro-inflammatory cytokines. Most recently, other biochemical indices such as the chemochil ligand 2 have also been proposed to be useful in explaining the role of oxidative stress and inflammation in PAD pathophysiology and to diagnose more favorable biochemical pathways for PAD.

PAD is treated with lifestyle modifications such as cessation of smoking and introduction of

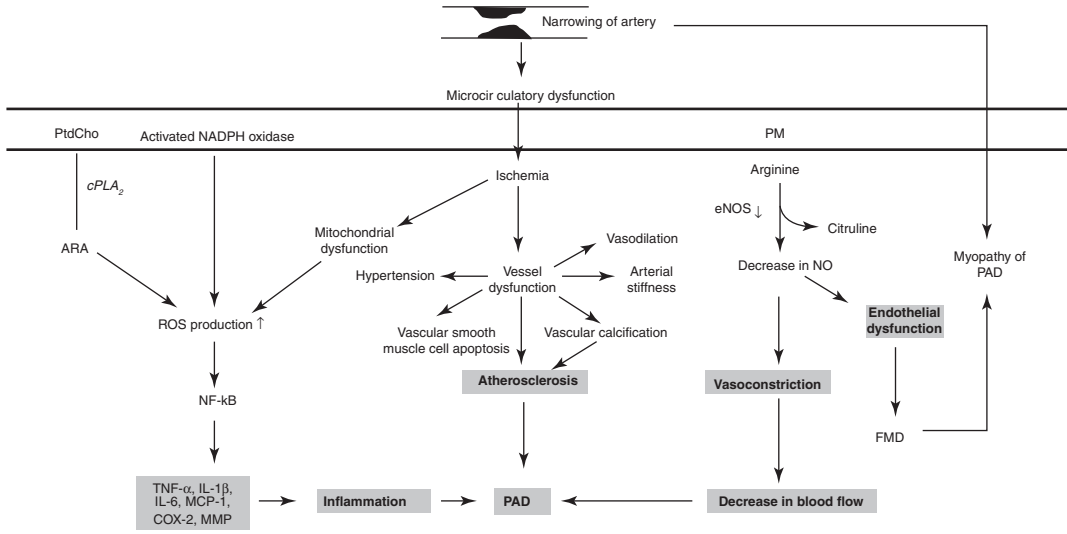

Fig. 1.4 Schematic diagram showing the pathogenesis of peripheral artery disease. *PM* Plasma membrane; *PtdCho* phosphatidylcholine; *ARA* arachidonic acid; *cPLA*$_2$ cytosolic phospholipase A$_2$; *ROS* reactive oxygen species; *NF-κB* nuclear factor-kappa B; *TNF-α* tumor necrosis factor-alpha; *IL-1β* interleukin-1beta; *IL-6* interleukin-6; *MCP1* monocyte chemotactic protein-1; *NO* nitric oxide; and *eNOS* endothelial nitric oxide synthase; *PAD* peripheral artery disease. Upward arrow indicates increase and downward arrow indicated decrease

limited physical activity (exercise). Several medications are used for the treatment of PAD including antiplatelet therapy, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, and statins. Surgical revascularization can also be used for the treatment of PAD patients.

1.2 Conclusion

Atherosclerosis is a multifactorial inflammatory disease of the arteries characterized by the accumulation of cholesterol, hydroxycholesterols, LOX, and triglycerides within the artery walls.

It is the leading cause of cardiovascular mortality and morbidity worldwide and is described as a complex disease involving several different cell types and their molecular products. The deposition of lipids is followed by foam cell formation with excessive production of connective tissue matrix components and, possibly, cellular proliferation and inflammatory reactions. 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. An increase in size of atherosclerotic plaques results in blocked blood flow to the heart causing not only CVD, angina, and carotid artery disease, but also PAD. Atherosclerosis also results in the induction of endothelial dysfunction, activation of the immune system, and the induction of vascular wall inflammation. Accumulating evidence also indicates the importance of gut microbiota in the development of atherosclerosis. Gut microbiota are not only considered as important regulators of immunity and metabolism but also to be possible antigenic sources for the development of atherosclerosis. However, the interplay between gut microbiota and host metabolism with regard to the modulation of atherosclerosis-associated immune responses remains poorly understood and more studies are needed to understanding the mechanisms by which the gut microbiota may influence atherogenesis, with particular focus on humoral immunity and B cells, especially the gut-immune-B2 cell axis.

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2 Effects of Dyslipidemia on the Cerebral Vessels

Chan-Hyuk Lee and Hyun Goo Kang

Abstract

Dyslipidemia indicates that the level of serum cholesterol measured after fasting exceeds the normal range. It has been reported that dyslipidemia is one of the modifiable risk factors for stroke and increases the risk of stroke by 2.19 times [1]. Cholesterol can be largely divided into total cholesterol, triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). When the endothelial cell of blood vessels is damaged by various cardiovascular risk factors such as hypertension and diabetes in the patients with hypercholesterolemia, LDL molecules of the bloodstream accumulate into the tunica intima of the artery. At the same time, macrophages in the blood enter the tunica intima to form free radicals. These free radicals oxidize LDLs and macrophages engulf them to generate a number of foam cells. If these processes are repeated, damaged foam cells, LDLs, smooth muscle cells, and their debris are cumulated under the endothelial cell lining. Then, the cumulated products form lipid cores, and these eventually form atheromatous plaques. High blood cholesterol, excluding HDL, eventually causes arteriosclerosis of blood vessels to narrow the diameter of the vessel. The fundamental molecular mechanism of arteriosclerosis induced by dyslipidemia is the same in all types of vessels. However, various clinical manifestations can be observed depending on where arteriosclerosis occurs in the human body.

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2.1 Effect of Dyslipidemia on Large Artery

The effect of atherosclerosis on blood vessels is particularly prominent in large vessels with a relatively large diameter such as the carotid artery compared to other vessels. Significant carotid artery stenosis accounts for almost 10% of the general population [2]. Rockman et al. reported that approximately 60% of patients with ischemic stroke developed carotid artery stenosis [3]. Statistically, approximately 20% of patients with ischemic stroke are owing to carotid arterial thrombosis. In other words, carotid artery stenosis is a relatively common disease and is directly associated with the occurrence of ischemic stroke. The main cause of carotid artery stenosis is atherosclerotic changes of the artery, and one of the main etiology of the deformity is dyslipidemia as described previously (Fig. 2.1).

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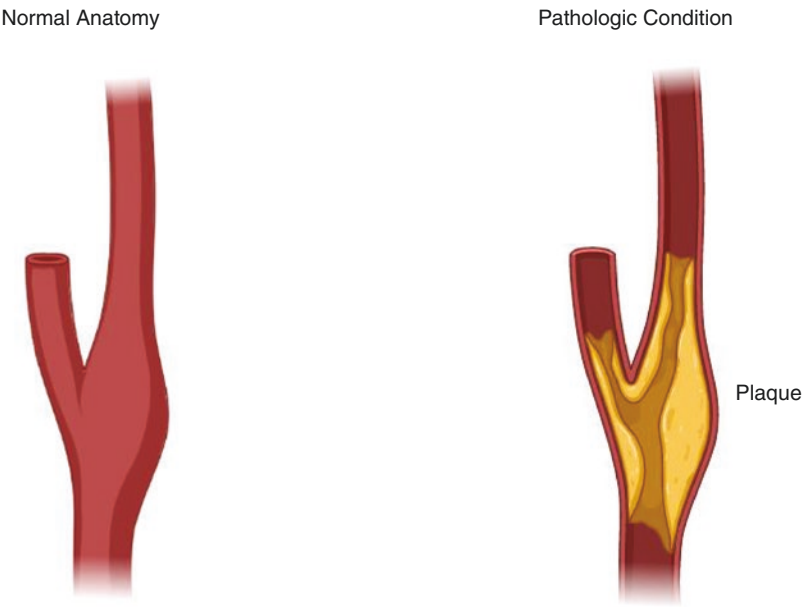

Fig. 2.1 Atherosclerosis of the carotid artery

The correlation between dyslipidemia and atherosclerotic stenosis of the carotid artery is relatively well known [4]. Dyslipidemia is directly related to the progression of carotid artery stenosis (high TC, LDL, and low HDL) [5]. It has been reported that TG, a component of lipids, may affect carotid artery stenosis [6]. Inflammatory reactions induced by dyslipidemia are associated with atherosclerotic carotid artery stenosis. It is known that LDL induces inflammatory reactions and promotes the increase of foam cells in blood vessels. Blackburn et al. proved this hypothesis: they reported a significantly ($p < 0.0001$) positive correlation between the degrees of carotid artery stenosis and CRP levels after evaluating 1051 patients diagnosed with dyslipidemia [7]. Intima-media thickness (IMT), which can be measured by carotid Doppler ultrasound, is known as a marker that indicates the risk of cardio-cerebrovascular disease. Previous studies revealed that children with hypercholesterolemia or patients with familial hypercholesterolemia had high carotid intima-media thickness (CIMT) [8, 9]. Moreover, it has been reported that blood LDL levels were related to CIMT [10, 11].

If the degree of the carotid artery stenosis is severe, the flow of the carotid artery can be improved by conducting carotid endarterectomy (CEA) or carotid artery stenting (CAS). Recently, these procedures are being considered more actively because the rate of perioperative complication has been lowered due to the development of devices and operator's techniques. However, restenosis of the carotid artery after the procedure is often reported as a complication. It has been reported that the restenosis rate after CEA is up to 22% and that after CAS is up to 33% [12, 13]. A meta-analysis of 17,000 patients who underwent CEA or CAS evaluated factors associated with arterial restenosis [14]. They reported that dyslipidemia, diabetes, gender (female), renal failure, hypertension, and smoking were risk factors related to restenosis. Atherosclerotic changes in the carotid artery mature according to stages. It was described in detail in the previous chapter. Among them, a vulnerable plaque refers to a thrombus that is likely to cause a rupture of a thrombus or a sudden change in a thrombus size, and it has a high possibility to induce an ischemic stroke than a non-vulnerable plaque. High lipid core, thin fibrous cap, and intraplaque hemorrhage (IPH) are the well-known characteristic findings of the vulnerable plaques. IPH is known to be observed in 40–49% of patients with plaques in the carotid artery and to increase the risk of ipsilateral ischemic events by 1.3–6 times [15].

As high-resolution vessel wall MR images have been advanced further in recent years, it has become easier to identify IPH owing to the development of sequences such as the magnetization-prepared rapid acquisition with gradient echo (MPRAGE), the simultaneous non-contrast angiography and IPH (SNAP) [16].

2.2 Effect of Dyslipidemia on Small Artery

Lacunar infarction accounts for approximately 25% of all ischemic strokes. Although it shows a better prognosis compared to ischemic stroke due to other causes, the recurrence rate of it relatively high. Therefore, it is necessary to cautiously treat patients with lacunar infarction. It has been reported that up to 60% of patients with lacunar infarction experienced aggravated neurological symptoms during hospitalization [17, 18]. It has been persistently suggested that lacunar infarction and dyslipidemia are associated. It was also reported that lacunar infarction patients with high TG had a higher risk of early neurological deterioration [19]. Other studies also indicated an association between TG and lacunar infarction [20]. TG is a major component of chylomicron and very low-density lipoprotein (VLDL). Although these complexes generally cannot pass through the endothelium of blood vessels due to their large molecular weights, complexes with relatively low molecular weights can be deposited in connective tissue by passing through the intima. TG complexes such as chylomicron and VLDL are accumulated in atherosclerotic thrombi. Monocytes that are moved inside of endothelial cells by oxidized LDL differentiate into macrophages. The macrophages engulf chylomicron and VLDL to differentiate into foam cells. In particular, high blood TG levels enhance coagulation factors (factors VII, X, XII, etc.) to promote blood coagulation. Thus, TG is an important factor in the occurrence of atherosclerotic changes and ischemic stroke at the molecular level.

If the level of LDL is high, the possibility of lacunar infarction occurrence increases at night time [21]. A study evaluated 127 lacunar stroke patients and reported that higher ox-LDL levels were associated with the neurological deterioration of lacunar infarction patients [22]. They speculated that oxidant stress might be associated with the progression of lacunar infarction. However, the relationship between LDL and lacunar infarction has not been proven. A large study with 2000 acute ischemic stroke patients concluded that LDL-C was related to large artery atherosclerosis (LAA) type stroke rather than lacunar infarction [23].

2.3 Effect of Dyslipidemia on Capillary Bed

Leukoaraiosis is a distinctive finding found in brain magnetic resonance imaging (MRI), and it refers to a high signal intensity frequently observed around the cerebral ventricle of the brain and corona radiata in fluid-attenuated inversion recovery. It looks like a small punctate in the early stage, but the number and area increase over time. In general, the number and range of it increase with age [24]. It is observed in more than 90% of healthy people over the age of 60. In many cases, it does not show any specific neurological symptoms, but some studies have reported that it is associated with stroke, dementia, gait impairment, and cognitive impairment [25, 26]. The grade of the leukoaraiosis is categorized by the Fazekas scale (0–3), and it is measured at the periventricular white matter and the deep white matter, respectively [27] (Table 2.1 and Fig. 2.2).

In China, leukoaraiosis was measured using 4683 patients who underwent MRI, and factors associated with it were analyzed. Guan et al.

Table 2.1 Fazekas scale in brain MRI

Fazekas scale	Periventricular white matter	Deep white matter
0	None	None
1	“Caps” or “pencil like”	Punctate
2	Smooth halo	Starting to confluence
3	Irregular and extending to deep white matter area	Large confluent

Adapted from Radiation Oncology, Copyright Springer Nature [27]

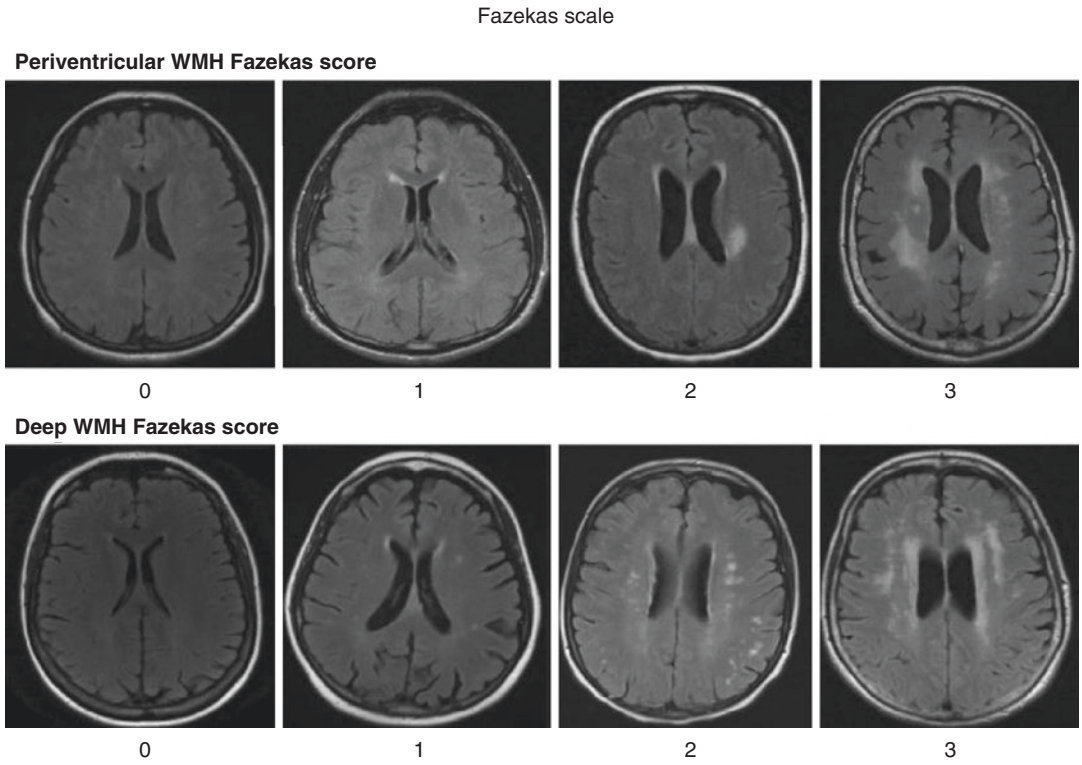

Fig. 2.2 Fazekas scale in brain MRI. Adapted with permission from Radiation Oncology, Copyright Springer Nature [27]

reported that the LDL level, along with age, gender, and hypertension, was related to the onset and progression of leukoaraiosis [28]. Schilling also examined the association between lipid profiles and leukoaraiosis using 2608 subjects [29]. They reported that there was a positive relationship between TG and leukoaraiosis, unlike other lipid factors (LDL and HDL). In other words, the high TG level increased the white matter hyperintensity volume. Additionally, it increased the frequency of lacunar infarction as well. The results implied that dyslipidemia could affect not only the large blood vessels but also the capillary blood vessels. Other studies also reported that TG and the grade of leukoaraiosis were associated [30]. However, other studies reported no association between them, and consensus between researchers has not yet been established [31]. How TG causes leukoaraiosis has not been clearly identified. Some suggested that it was related to inflammation. In other words, they argued that inflammation was related to leukoaraiosis and TG was a marker reflecting the state of inflammation. Additionally, some researchers hypothesized that it was caused by the weakened brain-blood barrier or the production of beta-amyloid with the subsequent promotion of the movement to the cerebral parenchyma. On the other hand, LDL is poorly associated with leukoaraiosis. One research team investigated the changes in small vessel disease according to the administration of pravastatin in 535 patients [32]. They followed up their brain MRI over the 3 years of administration. However, they did not find any difference in the degree of leukoaraiosis between the control group and the pravastatin-treated group. The results indirectly implied that even lowering the LDL level by using statin did not affect the course of leukoaraiosis. One study examined 1982 people in China, also reported that leukoaraiosis was associated with LAA-type stroke rather than lacunar infarction [23].

As such, dyslipidemia is closely related to the arteriosclerotic changes of cerebrovascular blood vessels. The relationship between large blood vessels (e.g., carotid artery) and dyslipidemia is already well known. Its association with lacunar infarction, related to the small artery, has also been revealed by many studies. However, such as leukoaraiosis, the association at the capillary level is still not clear. Further studies are needed to evaluate the different effects of dyslipidemia depending on the vessel diameter.

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Part II

Clinical Significance of Dyslipidemia in Stroke

3 Impact of Dyslipidemia on Ischemic Stroke

Yong-Jae Kim and Eung-Gyu Kim

Abstract

High cholesterol and lipids, especially low-density lipoprotein cholesterol (LDL-C) in the blood are associated with a higher risk of vascular events including stroke and myocardial infarction. In addition to therapeutic lifestyle changes, treatment with an HMG coenzyme-A reductase inhibitor (statin) medication is recommended for the primary prevention of ischemic stroke in patients estimated to have a high risk for cardiovascular events. Aggressive reduction of low-density lipoprotein cholesterol is likely to yield greater benefit than more modest reductions. Epidemiological evidence has suggested that high-density lipoprotein cholesterol (HDL-C) levels are inversely correlated with stroke risk. Nevertheless, direct evidence for the clinical benefit of elevating HDL-C is scarce, because the efficacy of lipid-modifying drugs that raise HDL-C levels had not been directly assessed in large-scale clinical trials in stroke patients.

Triglyceride (TG) level is high in many clinical situations and has influenced adverse cardiovascular diseases. Despite managing LDL-C adequately, residual risks still remain. Therefore, we must closely observe TGs for patients who are at high risk of atherosclerotic cardiovascular disease (ASCVD). Postprandial TGs could be a reasonable marker of average lipid concentration since people consume food on a daily basis and non-fasting hours are longer than fasting hours. A recent clinical trial proved that lowering TGs has positive effects on cardiovascular disease.

Lipoprotein(a) (Lp(a)) is an LDL-like particle and has an apolipoprotein(a) (apo (a)) bound to apolipoprotein B100 (apo 100). Increased levels of Lp(a) are associated with ASCVD and calcified aortic valve disease. Unfortunately, there is no approved effective Lp(a) lowering therapy as of today. Ongoing clinical trials for lowering Lp(a) are promising.

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3.1 Impact of LDL Cholesterol

While the risk of stroke is definitely associated with higher blood pressure, cigarette smoking, and diabetes, blood total cholesterol appears to be a weak predictor of stroke. Paradoxically, trials with statins in patients with hypertension,

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diabetes mellitus, coronary artery disease, previous stroke, or another high vascular risk, showed a decreased risk of stroke; a meta-analysis of over 90,000 patients included in these trials showed that stroke risk reduction was directly related to the extent to which total and low-density lipoprotein cholesterol (LDL-C) levels were lowered [1]. Moreover, in patients who had already had a stroke or a cerebral transient ischemic attack, the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial showed that lowering LDL-C reduced the risk of having another stroke or a revascularization procedure, including carotid endarterectomy.

A number of approaches for LDL-C lowering have been well studied. These include lifestyle interventions, pharmacologic treatment, intestinal bypass surgery, and lipid apheresis. For more than a decade, the main pharmacological option to prevent stroke and myocardial infarction through LDL-C lowering was the use of statins, i.e., HMG-CoA reductase inhibitors that inhibit the hepatic production of cholesterol. In recent years, two novel classes of drugs have proven their efficacy and safety to reduce LDL-C and prevent cardiovascular (CV) events in a number of large, well-conducted randomized controlled trials: ezetimibe and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Ezetimibe inhibits cholesterol absorption from the small intestine, and hence reduces the amount of cholesterol that becomes available for hepatocytes, driving these cells to increase cholesterol absorption from the circulation and thus reduce plasma cholesterol levels. PSCK9 inhibitors increase the number of available low-density lipoprotein (LDL) receptors on the surface of the hepatocyte which leads to higher cleavage of LDL-C from the circulation; this is mediated by the inhibition of the PSCK9 molecule which normally binds to the LDL receptor/LDL-cholesterol complex and prevents the LDL receptor to recycle back to the hepatocyte surface.

In this chapter, we review the current evidence regarding LDL-C lowering therapy, mainly the role of statins in stroke prevention and future directions in this field.

3.1.1 Primary Prevention Trials

First available evidence supporting the use of statin therapy in primary prevention of strokes derived mainly from studies designated to evaluate the efficacy of statins in primary and secondary prevention of coronary heart diseases (CHD). Earlier primary prevention trials with statins did not focus on stroke, but the unexpected finding of a reduced incidence of stroke in the first major statin trials conducted in patients with known CHD raised new expectative in stroke.

In the Scandinavian Simvastatin Survival Study (4S) trial of 4, 444 patients with CHD and high serum cholesterol levels, there was a significant reduction in strokes (30%) after 5 years in the simvastatin group as compared to the placebo group, although the number of deaths due to cerebrovascular disease was similar. The Cholesterol and Recurrent Events (CARE) and the Long-Term Intervention with pravastatin in Ischemic Disease (LIPID) studies confirmed the efficacy of statins in reducing the incidence of strokes in patients with CHD and cholesterol levels within the normal range or moderately elevated levels. In the CARE trial, the pravastatin group had a 31% lower incidence of all strokes ($p = 0.03$), although the incidence of fatal strokes, as in the 4S study, was about the same as in the control group. In the LIPID study, pravastatin significantly reduced the incidence of strokes by 19% ($p = 0.022$). Later, statins have shown a clear benefit in the primary prevention of stroke in other types of patients with vascular disease risk. In patients with high vascular risk: the HPS (Heart Protection Study), included 20, 536 UK adults (aged 40–80 years) with coronary disease, another occlusive arterial disease, or diabetes were randomly allocated to receive 40 mg simvastatin daily or matching placebo. Simvastatin treatment significantly reduced the relative risk of ischemic stroke by 28% ($p < 0.01$) [2].

3.1.2 Secondary Prevention Trials

Evidence for the benefit of statin therapy in secondary stroke prevention in patients with previ-

ous cerebrovascular disease was initially provided by the HPS. The HPS included 3280 randomly chosen stroke patients (none with transient ischemia attacks (TIAs)) and 1822 stroke patients without established CHD. In all stroke patients, there was a 19% relative risk reduction (RRR) of major vascular events, and in the stroke patients without established CHD, the reduction in the risk of major vascular events was 23%. However, the HPS did not find a reduction in stroke risk among patients with recurrent cerebrovascular disease (10.4% of patients in the statin group had a recurrent stroke as compared with 10.5% of patients in the placebo group). Therefore, in patients with a prior stroke, statins likely reduced the incidence of coronary events, but there was no proof that statins reduced the incidence of recurrent strokes. The SPARCL trial was specifically designed to investigate the effect of the reduction of cholesterol levels with a statin in secondary stroke prevention and was the first trial to show the benefits of statin therapy in preventing recurrent stroke. The SPARCL was a prospective, double-blind, placebo-controlled international trial in which 4731 patients with a history of non-disabling stroke or TIA in the preceding 1–6 months and with no CHD or hypercholesterolemia were randomized. Subjects were enrolled between September 1998 and March 2001. Patients received either atorvastatin 80 mg per day (n = 2365) or placebo (n = 2366) and were followed for an average of 4.9 years. The primary endpoint was the incidence of fatal or nonfatal stroke. A number of CV events were also measured as secondary outcomes. LDL-C levels were similar between the two groups at baseline and decreased by 53% in the atorvastatin group while remaining unchanged in the placebo group at 1 month after randomization. During the follow-up period, 265 patients (11.2%) receiving atorvastatin and 311 patients (13.1%) receiving placebo had a fatal or nonfatal stroke and represented a RRR of 16% (p = 0.03; 95% confidence interval (CI), 0.71–0.99). This effect was driven predominately by the reduced adjusted relative risk of fatal stroke which was decreased by 43% (p = 0.03), whereas atorvastatin had no significant effect on nonfatal stroke reduction (p = 0.11). A finding of note in SPARCL was the association of statin treatment with a higher incidence of hemorrhagic stroke (n = 55 (2.3%) for statin treatment versus n = 33 (1.4%) for placebo; hazard ratio (HR), 1.66; 95% CI, 1.08–2.55). A similar observation was seen in the subset of 3200 patients who had a stroke before randomization in the HPS, in which there was a 91% relative rise in the risk of hemorrhagic stroke in patients assigned to statin treatment. A later exploratory analysis of the SPARCL trial found that the factors significantly associated with intracerebral hemorrhage (ICH) in multivariable regression were: atorvastatin treatment, hemorrhage as the entry event (2% of the study population), male gender, increased age, and stage II hypertension (systolic blood pressure (SBP) > 160 mmHg or diastolic blood pressure (DBP) > 100 mmHg.). The largest risk was associated with having a previous hemorrhagic stroke. Importantly, there was no relationship of hemorrhage with the degree of LDL-C lowering, suggesting that it was not a dose-effect.

Based mainly on the SPARCL trial findings, the recent American Heart Association/American Stroke Association guidelines for the prevention of stroke in patients with ischemic stroke recommend high-intensity statin therapy for patients with TIA or ischemic stroke presumed to be of atherosclerotic origin to reduce the risk of stroke and CV events Several meta-analyses of randomized trials of statins in secondary prevention of stroke have been done. A meta-analysis that included eight studies involving approximately 10, 000 participants has shown that statin therapy in patients with a history of ischemic stroke or TIA significantly reduces subsequent major coronary events but only marginally reduces the risk of stroke recurrence (odds ratio (OR), 0.88; 95% CI, 0.77 to 1.00). However, in other meta-analyses intense reduction of LDL-C by statins significantly reduced the risk of recurrent stroke (RR, 0.84; 95% CI, 0.71–0.99; p = 0.03) and major CV events (RR, 0.80; 95% CI, 0.69–0.92; p = 0.002).

Recently, a trial on secondary stroke prevention, the Japan Statin Treatment Against Recurrent Stroke (J-STARS) trial has been

published [3]. This study examined whether treatment with low-dose pravastatin prevents stroke recurrence in ischemic stroke patients. A total of 1578 Japanese men and women aged 45 to 80 years with previous non-cardioembolic ischemic stroke and total cholesterol concentration between 180 mg/dl and 240 mg/dl were randomly assigned to open-label pravastatin 10 mg per day or to control, and followed for a mean of 4.9 years. Although, the authors found no difference in the primary endpoint of stroke or TIA (2.56% vs. 2.65% per year, adjusted HR, 0.97; 95% CI: 0.73 to 1.29). Allocation to pravastatin was however associated with a lower incidence of ischemic stroke due to atherothrombosis compared to control (0.21% vs. 0.64% per year, adjusted HR, 0.33; 95% CI, 0.15 to 0.74) without increasing intracranial hemorrhage (0.29% vs. 0.31% per year, adjusted HR, 1.00; 95% CI, 0.45 to 2.22). Nevertheless, several facts may explain the failure to prove the efficacy of pravastatin. The study only recruited about half ($n = 1578$) of the initial target sample size of 3000, and thus the study was underpowered to reliably identify clinically significant effects. Besides, the pravastatin dose used in this study is lower than that used in studies from previous studies (40 mg per day); however, this is the approved standard dose in Japan. Although the J-STARS study has certain limitations, it will also contribute to the establishment of guidelines for using statins to prevent strokes caused by larger artery atherosclerosis.

3.1.3 Future Therapeutic Directions

Statins inhibit the synthesis of HMG-CoA reductase, a key enzyme in cholesterol biosynthesis whose inhibition decreases hepatic cholesterol production, consequently decreasing hepatic LDL-C uptake and ultimately causes a 20–60% decrease in plasma LDL-C level depending on the type of and dose of statin. Although statins provide effective and substantial reductions in LDL-C, many patients do not achieve the recommended goals despite maximal therapy, and some patients cannot tolerate high-dose statin therapy thus remaining at unacceptably elevated risk. Moreover, although statins are highly effective, even those who have achieved significant LDL-C reductions with intensive statin therapy may still experience CV events, referred to as "residual risk." This risk is particularly high in certain patients such as those with diabetes and atherosclerosis affecting multiple vascular beds (e.g., cerebrovascular, peripheral vascular as well as coronary).

Recently, in the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), an additional reduction in LDL-C levels with the addition of ezetimibe, a cholesterol-absorption inhibitor, to a statin significantly reduced CV event rate, as compared with statin monotherapy [4]. These data have refocused attention on the potential cardiovascular benefit of greater LDL-C reduction through non-statin mechanisms. Ezetimibe targets the Niemann-Pick C1-like 1 (NPC1L1) protein, thereby reducing the absorption of cholesterol from the intestine. When added to statins, ezetimibe reduces LDL-C levels by an additional 23 to 24%, on average. The IMPROVE-IT evaluated the use of ezetimibe in addition to 40 mg of simvastatin daily in 18,144 post-acute coronary syndrome patients with LDL levels of 1.3 to 3.2 mmol/L. In this secondary prevention population, the addition of ezetimibe to statin therapy reduced the absolute risk of CV events—primarily nonfatal myocardial infarction or stroke—by 2% over the course of 7 years. The event rate for the primary endpoint was 32.7% in the simvastatin-ezetimibe group, as compared with 34.7% in the simvastatin monotherapy group (HR, 0.936; 95% CI, 0.89 to 0.99; $p = 0.016$), with no increased risk of adverse events. The risk of ischemic stroke was significantly lower with simvastatin-ezetimibe than with simvastatin monotherapy (difference, 0.7 percentage points; HR, 0.79; $p = 0.008$).

Moreover, in the last few years, other new agents for LDL-C lowering have also demonstrated their efficacy principally as add-on therapy to statins. The emerging therapeutic agents could be classified into two categories: those

interfering with lipoprotein synthesis such as apolipoprotein B (apoB) production or microsomal triglyceride transfer protein (MTP) inhibitors and those promoting lipoprotein catabolism such as PCSK9 inhibitors. Recent interest has focused on PCSK9 as a possible therapeutic target. In fact, for many, the PCSK9 inhibitors represent the pharmacotherapeutic innovation of the decade for the prevention of CV disease.

The first PCSK9 inhibitor, Alirocumab, was approved in July 2015 by the US Food and Drug Administration (FDA) as an adjunct to diet and maximally tolerated statin therapy for the treatment of adults with heterozygous familial hyperlipidemia or clinical ASCVD, who require additional lowering of LDL-C levels. Soon after, in August 2015, the FDA approved the second PCSK9 inhibitor, evolocumab.

The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) study is a randomized, placebo-controlled trial involving 27,500 high-risk patients with cardiovascular disease who are receiving background statin therapy; the primary endpoint is a composite of CV death, myocardial infarction, hospitalization for unstable angina, stroke, or coronary revascularization. Focusing on the ischemic stroke patient, it is reassuring to see that the beneficial cardiovascular effect of lowering LDL-cholesterol in the FOURIER trial was evident not only in the general population of patients with high cardiovascular risk but was also confirmed in the specific subgroup of ischemic stroke patients [5]. Although stroke is a heterogeneous syndrome of diverse etiologies with atherosclerosis being only one of the potential underlying causes, patient eligibility in the FOURIER trial was not restricted only to large artery atherosclerotic strokes but included any ischemic stroke patient regardless of the etiology. Although this could have potentially diluted the evolocumab effect in the stroke population, the FOURIER succeeded in detecting a significant effect. It could be hypothesized that a trial designed similar to FOURIER and focusing on the specific population of patients with ischemic stroke and significant carotid stenosis, specially recruited during the early post-stroke phase when the risk for stroke recurrence is the highest, would perhaps detect an even larger effect than the 30% RRR shown in the FOURIER stroke subgroup, and hence identify a well-defined patient population with very high cardiovascular risk for which the use of evolocumab could perhaps be more cost-effective than in the general FOURIER population. In addition, the detection of a significant treatment effect of evolocumab in the overall ischemic stroke population—and not only in large artery atherosclerotic strokes—underlines the importance of LDL-cholesterol lowering in all ischemic stroke patients regardless of the underlying etiology and, in line with previous observational studies, advocates in favor of intensive lipid-lowering treatment in all types of ischemic stroke. Of note, the stroke-alone subgroup in the FOURIER trial showed a large reduction in the rate of the primary endpoint (RRR of 30%) which was 2.5-fold higher than the effect identified in the myocardial-infarction-alone patients (RRR of 12%), with the corresponding numbers-needed-to-treat to prevent one primary endpoint being approximately 40 and 83 patients, respectively, for 2.2 years. This could imply that the cost-effectiveness of treatment with evolocumab is perhaps higher in the stroke population than in the myocardial infarction population, especially given that a large proportion of stroke patients have serious chronic sequelae which are associated with high direct and indirect costs. In addition, this may have implications in the research field, as the inclusion of a higher proportion of stroke patients in future trials of LDL-lowering could perhaps increase their power to detect a treatment effect, and hence allow for smaller target size populations and shorter follow-up duration.

Nevertheless, though preliminary data show that PCSK9 inhibitors added to statins safely decrease the number of strokes, we still do not know if those reductions are associated with clinical benefit in long-term use, or if those benefits are countered by harm (e.g., muscle-related events, hemorrhagic stroke, diabetes mellitus,

and neurocognitive defects) that might counterbalance the benefit. Only time will tell the real role of these new promising nonstatin lipid-modifying therapies on stroke prevention.

3.1.4 Conclusion

In the meantime, stroke physicians need to aim for low LDL-cholesterol levels by offering intensive statin treatment in all ischemic stroke patients regardless of the underlying etiology and consider PCSK9 inhibitors for those patients who are at the highest risk for recurrent stroke or another cardiovascular event and have unacceptable LDL-C levels despite aggressive statin treatment.

3.2 Impact of HDL Cholesterol

3.2.1 HDL: An Antiatherogenic Lipoprotein?

Abundant epidemiologic evidence establishes high-density lipoprotein (HDL) as an inverse risk factor for cardiovascular disease (CVD) including stroke. Recent evidence has furnished a mechanistic understanding of the method by which HDL likely mediates regress of cholesterol from lipid-laden foam cells. ABCA1 mediates the transfer of cholesterol to nascent HDL particles, whereas ABCG1 ferries cholesterol from cells to mature HDL particles. Scavenger receptor class B type I (SR-BI) appears to mediate uptake of cholesterol from HDL by steroidogenic organs and the liver. Thus, impaired reverse cholesterol transport could contribute to the cardiovascular risk associated with low levels of plasma HDL. Beyond the role of HDL in shuttling cholesterol, HDL may affect arterial biology as a carrier of anti-inflammatory and antioxidant proteins. Phospholipases associated with the HDL particle can catabolize some of the biologically active and proinflammatory oxidized phospholipids associated with modified LDL. Proteins such as platelet-activating factor acetyl-hydrolase and paraoxonase-1 exemplify such putative antioxidant proteins associated with HDL particles.

3.2.2 Low HDL Cholesterol

In the ACC/AHA guidelines, low HDL-C is defined as less than 40 mg/dL in men and less than 50 mg/dL in women for use in the Pooled Cohort Equation [6]. Low HDL-C may be caused by elevated TGs, diabetes, obesity, physical inactivity, a high-carbohydrate diet, smoking, and drugs such as β-blockers and anabolic steroids, as well as rare genetic disorders.

HDL-C may be increased with an increase in physical activity and with weight reduction. Niacin is the most efficacious HDL-C–raising drug; fibrates also increase HDL-C substantially, and statins provide modest increases in HDL-C. Although estrogen increases HDL-C, estrogen was not shown to reduce cardiovascular risk in the Women's Health Initiative and the Heart and Estrogen/Progestin Replacement Study, and therefore, it is not recommended for this purpose. Because of a lack of clinical trial data, the ACC/AHA guidelines make no recommendation for drug treatment specifically to increase HDL-C, and as such, they do not designate a target of treatment [7]. Although the inverse between HDL and cardiovascular disease remains undisputed, whether manipulation of HDL can benefit atherosclerosis still remains hypothetical. Finally, although HDL-C has been questioned as the appropriate inverse measure of CHD risk, it remains to be established whether newer assays that quantify HDL particle number or functionality will be clinically superior in CHD risk assessment.

3.2.3 Primary Prevention Trials

Some epidemiological studies have shown an inverse relationship between HDL-C and risk of stroke whereas others have not. The Emerging Risk Factors Collaboration performed a meta-analysis involving individual records on 302,430 people without vascular disease from 68 long-

term prospective studies [8]. Collectively, there were 2.79 million person-years of follow-up. The aggregated data set included 2534 ischemic strokes, 513 hemorrhagic strokes, and 2536 unclassified strokes. The analysis adjusted for risk factors other than lipid levels and corrected for regression dilution. The adjusted HRs were 0.93 (95% CI, 0.84–1.02) for ischemic stroke, 1.09 (95% CI, 0.92–1.29) for hemorrhagic stroke, and 0.87 (95% CI, 0.80–0.94) for unclassified stroke. There was modest heterogeneity among studies of ischemic stroke (I^2 = 27%). The absence of an association between HDL and ischemic stroke and between HDL and hemorrhagic stroke contrast with the clear inverse association between HDL-C and CHD observed in the same meta-analysis.

Niacin increases HDL-C and decreases plasma levels of lipoprotein(a) (Lp(a)). The Coronary Drug Project found that treatment with niacin reduced mortality in men with prior myocardial infarction. In the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High TG: Impact on Global Health Outcomes (AIM-HIGH) study of patients with established CVD, the addition of extended-release niacin to intensive simvastatin therapy did not reduce the risk of a composite of cardiovascular events, which included ischemic stroke [9]. In a meta-analysis of 11 studies comprising 9959 subjects, niacin use was associated with a significant reduction in cardiovascular events, including a composite of cardiac death, nonfatal myocardial infarction, hospitalization for acute coronary syndrome, stroke, or revascularization procedure (OR, 0.66; 95% CI, 0.49–0.89). There was an association between niacin therapy and coronary heart disease event (OR, 0.75; 95% CI, 0.59–0.96) but not with the incidence of stroke (OR, 0.88; 95% CI, 0.5–1.54).

However, there are serious safety concerns about niacin therapy. The Heart Protection Study 2—Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2- THRIVE) trial involving 25,693 patients at high risk for vascular disease showed that extended-release niacin with laropiprant (a prostaglandin D2 signal blocker) caused a significant fourfold increase in the risk of myopathy in patients taking simvastatin [10]. Fibric acid derivatives such as gemfibrozil, fenofibrate, and bezafibrate lower TG levels and increase HDL-C. The Bezafibrate Infarction Prevention study, which included patients with prior myocardial infarction or stable angina and HDL-Cl ≤45 mg/dL, found that bezafibrate did not significantly decrease either the risk of myocardial infarction or sudden death (primary endpoint) or stroke (secondary endpoint). In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, fenofibrate neither decreased the composite primary endpoint of coronary heart disease death or nonfatal myocardial infarction nor decreased the risk of stroke [11]. In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) study of patients with type 2 diabetes mellitus, adding fenofibrate to simvastatin did not reduce fatal cardiovascular events, nonfatal myocardial infarction, or nonfatal stroke compared with simvastatin alone [12]. Based on these results, niacin may be considered for patients with low HDL-C or elevated Lp(a), but its efficacy in preventing ischemic stroke in patients with these conditions is not established. Caution should be used with niacin because it increases the risk of myopathy. Fibric acid derivatives may be considered for patients with hypertriglyceridemia, but their efficacy in preventing ischemic stroke is not established.

3.2.4 Secondary Prevention Trials

Recently, the role of niacin among patients with established CVD and low HDL-C levels receiving intensive statin therapy was addressed in the AIM-HIGH trial [13]. AIM-HIGH evaluated whether extended-release niacin added to intensive statin therapy versus statin therapy alone would reduce the risk of cardiovascular events in 3414 patients with known atherosclerotic disease and atherogenic dyslipidemia (low levels of HDL-C, elevated TG levels, and small, dense particles of LDL-C). Patients in the niacin group received niacin at a dose of 1500 to 2000 mg/D. In both groups, the dose of the statin was adjusted to achieve and maintain the LDL-C level in the

range of 40–80 mg/dL. The trial was stopped after an average follow-up period of 3 years because of a lack of efficacy. By 2 years of follow-up, add-on niacin therapy had boosted the median HDL-C level from 35 to 42 mg/dL, reduced the TG level from 164 to 122 mg/dL, and lowered the LDL-C level from 74 to 62 mg/dL. The primary endpoint occurred in 282 patients (16.4%) in the niacin group versus 274 (16.2%) in the placebo group (HR, 1.02; 95% CI, 0.87–1.21; $P = 0.79$). Of note, there was an unexpected imbalance in the rate of ischemic stroke as the first event between patients assigned to niacin versus placebo (27 [1.6%] versus 15 patients [0.9%]). Even when all the patients with ischemic strokes were considered (versus just those in whom stroke was the first study event), the pattern persisted (albeit non-significant: 29 [1.7%] versus 18 patients [1.1%]; HR, 1.61; 95% CI, 0.89–2.90; $P = 0.11$). It is not clear whether this observation seen in AIM-HIGH reflects a causal relationship or the play of chance.

Inhibition of cholesteryl ester transfer protein (CETP) increases HDL-C levels, and the hypothesis that cholesteryl ester transfer protein inhibitors will enhance cardiovascular outcomes has been tested in 2 clinical trials. The Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial evaluated whether torcetrapib lowered the risk of clinical cardiovascular events in 15,067 patients with a history of CVD. Although there was a rise in HDL-C level of 72% and a drop of 25% in LDL-C level at 12 months among those who received torcetrapib, there was also an increase of 5.4 mm Hg in SBP, electrolyte derangements, and a higher rate of cardiovascular events [14]. The HR estimate for stroke was 1.08 (95% CI, 0.70–1.66; $p = 0.74$). The dal-OUTCOMES study randomly assigned 15,871 patients who had a recent acute coronary syndrome to receive dalcetrapib 600 mg daily versus placebo. HDL-C levels rose from baseline by 31% to 40% in the dalcetrapib group. Dalcetrapib had a minimal effect on LDL-C levels. The trial was terminated for futility; compared with placebo, dalcetrapib did not significantly affect the risk of the primary endpoint nor any component of the primary endpoint, including stroke of presumed atherothrombotic cause (HR, 1.25; 95% CI, 0.92–1.70; $P = 0.16$).

3.2.5 Conclusion

Despite the impressive observational data during the past five decades that have identified HDL-C as an independent predictor of CHD risk, considerable doubt has suddenly been cast on the "HDL hypothesis." This reflects, in part, the disappointing results of recent clinical trials that have failed to demonstrate favorable outcomes with raising HDL-C. However, although nicotinic acid–based therapies and the CETP inhibitor dalcetrapib did not exhibit CHD and stroke risk, newer and more potent CETP inhibitors are in phase 3 clinical testing.

3.3 Association of Hypertriglyceridemia with Ischemic Stroke

TGs are considered a risk factor for coronary artery disease as well as increased mortality for decades. Patients with coronary artery disease whose cholesterol level is not high, are associated with mildly elevated LDL-C, low HDL-C, and high TG level. Therefore, this pattern of lipid profile is strongly related to atherosclerosis and is referred to as atherogenic dyslipidemia.

TGs could be high in various clinical situations such as hypothyroidism, diabetes mellitus, chronic kidney disease, systemic inflammation (including rheumatoid arthritis, systemic lupus erythematosus), high intake of alcohol or sugar, glucocorticoids, thiazide and loop diuretics, non-selective beta-blockers, and atypical antipsychotics (clozapine, olanzapine, risperidone). Therefore, we must take these situations into in patients with hypertriglyceridemia. Correlation between TGs and clinical outcomes is shown in Fig. 3.1.

In spite of managing LDL-C adequately to a normal or low range, residual risk persists. Taking this into consideration, other factors must be

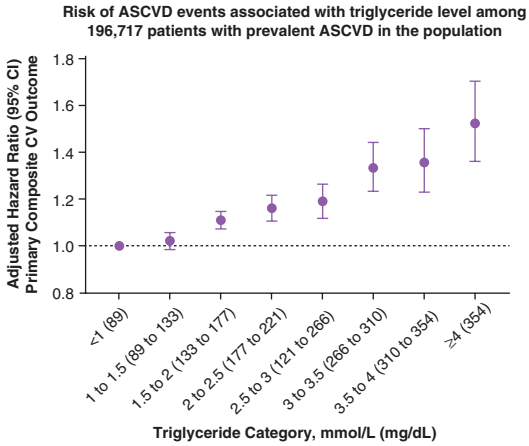

Fig. 3.1 Triglyceride level and clinical outcomes. Adapted with permission from Postgradeuate Medicine, Copyright Taylor & Francis Group [15]. *ASCVD* atherosclerotic cardiovascular disease

managed outside of LDL-C management. Indeed, approximately 25% of patients with ASCVD whose LDL-C were well-treated within the range of 41–100 mg/dL, showed elevated TG levels (135-149 mg/dL). In this patient group, the CV events increase without managing TG levels. Regardless of LDL-C level, TGs increase coronary artery disease mortality.

Nine clinical trials involving 4957 patients with coronary artery disease underwent intravascular ultrasonography to see atheroma volume changes and to evaluate the role of non-HDL-C and TG levels. These studies showed that coronary atheroma volume increase was closely related to non-HDL-C levels than LDL-C level and associated with high TG levels only above 200 mg/dL [16]. The ACCORD-Lipid trial randomized 5519 patients to either fenofibrate plus simvastatin or simvastatin only. There was no difference between the combination and non-combination groups in primary endpoints (fatal cardiovascular events, nonfatal myocardial infarction, or nonfatal stroke). However, primary endpoints were reduced in patients who initially had the highest level of TGs (more than 204 mg/dL) and lowest HDL-C (below 34 mg/dL) taking fenofibrate and simvastatin in comparison to same patient group taking simvastatin only. Therefore, the ACCORD trial delineated the role of fenofibrate to reduce cardiovascular disease especially in the patient population with high TGs and low HDL-C.

The efficacy of fenofibrate was proven in Korean metabolic syndrome populations. In the Korean National Health Insurance Service-Health Screening Cohort (NHIS-HEALS), 29,771 adults with metabolic syndrome (≥40-year-old) receiving statin treatment were enrolled. Based on propensity score matching, 2156 patients with statin and fenofibrate in comparison to 8549 patients with statin only were analyzed. The composite CV endpoints were significantly reduced in the combination group by 26%. There were no big safety issues in combination groups. Compared to the previous fenofibrate trial (ACCORD-Lipid and FIELD trial), this Korean population had a higher level of TGs.

The adverse effects of TGs on the coronary artery are more pronounced in diabetic patients. Diabetic patients have a higher risk of death due to increased coronary artery disease than the normal population, especially for higher tertile of TG level. This trend is more prominent in women than men. Therefore, diabetic patients with high levels of TGs should be closely monitored.

3.3.1 Fasting Versus Non-fasting TGs

Generally, measuring TG is done after overnight fasting, and guidelines have defined a normal level of fasting serum TG to be less than 150 mg/dL, borderline as 150–199 mg/dL, high defined as 200–499 mg/dL, and very high defined as ≥500 mg/dL. The risk of acute pancreatitis is increased in very high level of TGs.

Abnormal fasting triglycerides levels (more than 150 mg/dL) are related to cardiovascular disease. On the other hand, the Women's Health study which enrolled 20,509 healthy US women highlighted the importance of postprandial TG. Postprandial TG levels are associated with incident CV events, apart from the effect of traditional cardiovascular factors and level of other lipids. When TG tertile is at its highest, the risk of cardiovascular events doubles. An increase in

intermediate-density lipoprotein and remnants can cause adverse cardiovascular events.

We eat meals regularly every day and non-fasting condition is more common than fasting. Ideally, non-fasting TGs levels could be a reasonable marker of average lipid concentration. An abnormal cut-off value of non-fasting TGs is more than 175 mg/dL which can predict the cardiovascular risk [17]. Non-fasting TGs level is higher (about 27 mg/dL) than fasting, but increased TG's clinical significance is not too significant in comparison and non-fasting screening is more comfortable for patients. Therefore, a number of guidelines recommend non-fasting TG screening.

Postprandial increased TGs adversely influence on blood vessels. First, postprandial TGs induce obesity through deposition in the adipose tissue. Second, postprandial TGs accelerate atherosclerosis via increased formation of small dense LDL- and TG-rich remnant lipoprotein. Epidemiologic studies reported the significant role of non-fasting TGs on atherosclerotic CVD. A detailed mechanism of TGs increasing the risk of CVD is illustrated in Fig. 3.2.

A report of the American College of Cardiology/American Heart Association Taskforce on Clinical practice guidelines adopted the term of ASCVD risk enhancers. Persistently elevated TG (≥175 mg/dL, ≥2.0 mmol/L) is one of the ASCVD risk enhancers. In patients with borderline ASCVD risk (10-year ASCVD risk score between 5% and 7.5%) for primary prevention, risk discussion is necessary. If risk enhancers are present, then discussion regarding moderate-intensity statin therapy is required. Therefore, persistent hypertriglyceridemia itself could be eligible to favor statin therapy for the primary prevention goal.

3.3.2 Clinical Evidence for Lowering of TGs on CVD Outcomes

Eicosapentaenoic acid (EPA) is a long-chain polyunsaturated fatty acid (PUFA). Omega-3 PUFA include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA). (Fig. 3.3).

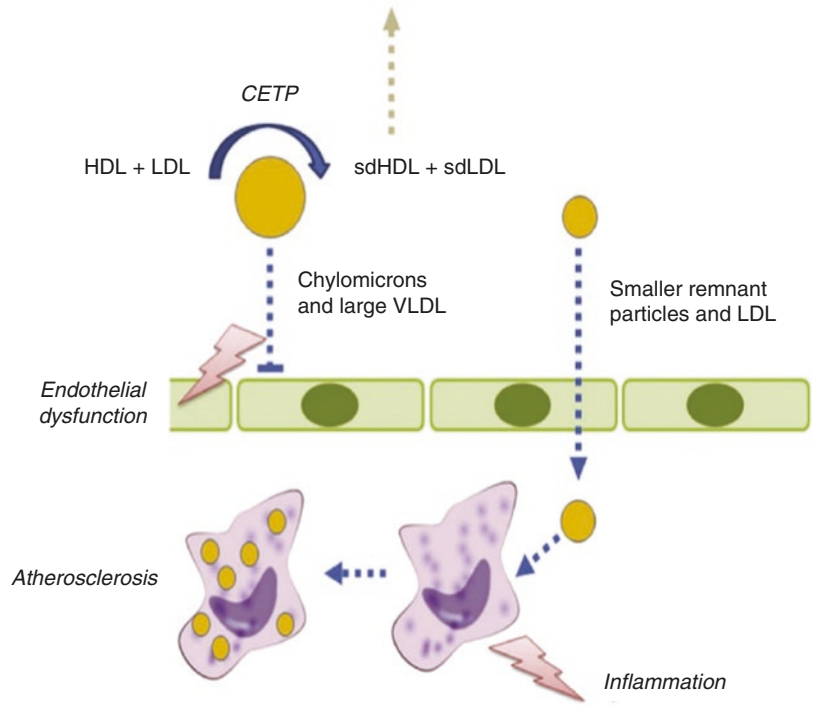

Fig. 3.2 Mechanisms for how triglyceride-rich lipoprotein induce cardiovascular disease. Adapted with permission from Clinica Chimica Acta, Copyright Elsevier [18]. Large vert low-density lipoprotein (VLDL) and chylomicrone (CM) are too large to enter the arterial wall, but smaller remnants and low-density lipoprotein (LDL) penetrate the arterial intima and bind to artery wall proteoglycans. *CETP* cholesteryl ester transfer protein

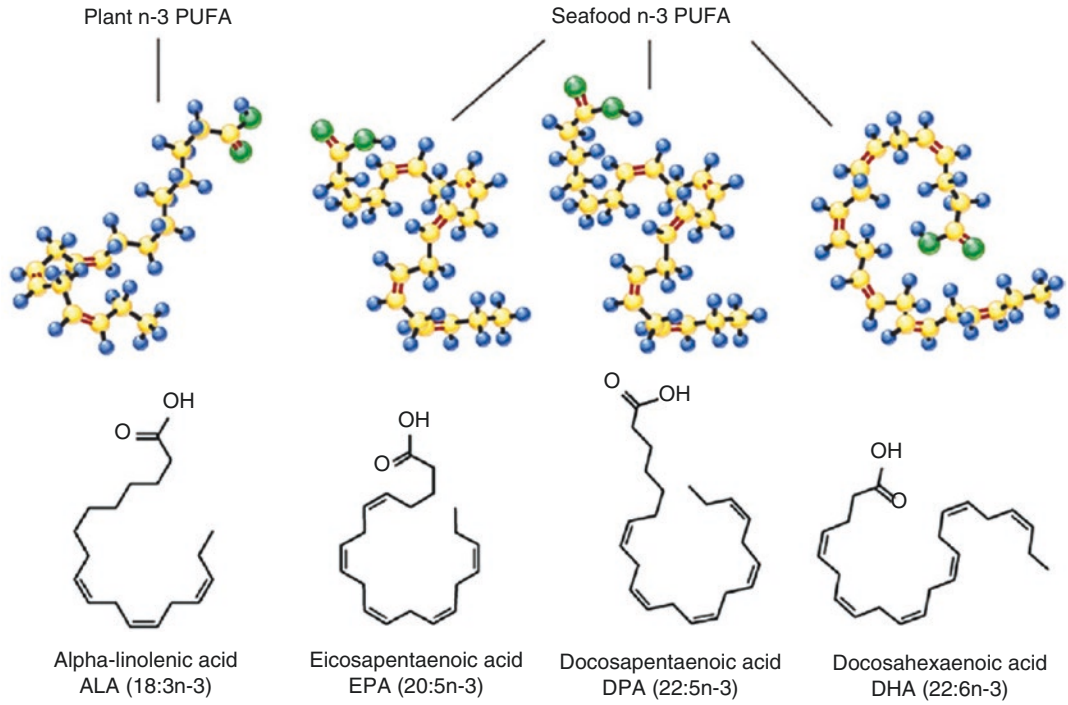

Fig. 3.3 Structure of n-3 PUFA. Adapted with permission from Journal of the American College of Cardiology, Copyright Elsevier [19]. *PUFA* polyunsaturated fatty acid

SPARCL trial originally randomized 4731 patients to either atorvastatin 80 mg/d or placebo. These patients recently had a stroke or TIA with no known coronary heart disease. The SPARCL trial determined that an elevation of baselineT G level was associated with major cardiovascular events (MACE). Furthermore, a suitable decrease of TG (less than 150 mg/dL) lowered the risk of stroke by 38%. Therefore, it is crucial for effective secondary stroke prevention in stroke patients to decrease the TG level along with lowering LDL-C.

Japan EPA Lipid Intervention Study (JELIS) trial is to evaluate the effectiveness of long-term use of EPA for prevention of major coronary events in hypercholesterolemic patients in Japan patients given statins [20]. At a mean follow-up of 4.6 years, any major coronary event, including sudden cardiac death, fatal and nonfatal myocardial infarction, and other nonfatal events including unstable angina pectoris, angioplasty, stenting, or coronary artery bypass grafting were reduced in the active treated group compared to placebo by 19%. Serum LDL-C was not a significant factor in a reduction of risk for major coronary events. But in patients without a history of coronary artery disease, a non-significant 18% reduction was shown in major coronary artery events. But the event rate of ischemic and hemorrhagic strokes did not show any significant difference between the two groups, and neither did all-cause mortality.

The Vitamin D and Omega-3 trial (VITAL) is a primary prevention trial on the patient group not taking statin with normal TG to evaluate the efficacy of supplementation with vitamin D3 (at a dose of 2000 IU per day) and n-3 fatty acids (1 g per day as a fish oil capsule containing 460 mg of EPA and 380 mg of DHA, which is a lower dosage and different compositions compared to Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT)). Major cardiovascular events (a composite of myocardial infarction, stroke, or death from cardiovascular causes) were not significantly different between active and placebo

groups [21]. Contrary to the REDUCE-IT trial, perhaps the reason for negative results of the VITAL trial is that population was too low risk to show positive effects of omega-3 polyunsaturated fatty acid (PUFA). Furthermore, the trial dose of n-3 fatty acids may have been too low and different compositions.

REDUCE-IT was a double-blinded, placebo-controlled trial in patients with established cardiovascular disease or diabetes mellitus and at least one additional cardiovascular risk factors with fasting TG level of 150 to 499 mg/dL (1.69 to 5.63 mmol/L), LDL level of 41 to 100 mg/dL (1.06 to 2.59 mmol/L) and had been receiving a statin. The patients were randomly assigned to either 2 g of icosapent ethyl (IPE, highly purified stable EPA) twice daily or placebo. Majority of REDUCE-IT population is secondary cohort (70.7%). It showed that the primary endpoint (composite of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina) was reduced by 25% with the number needed to treat (NNT) of 21. Total CV events (composite of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke) were also reduced by 26% over a median follow-up of 4.9 years. Hospitalization for atrial fibrillation or flutter was significantly higher in the IPE group (3.1% vs 2.1%, $p = 0.004$). Serious bleeding events were higher in the IPE group (2.7% vs 2.1%, $p = 0.006$) but there were no increased rates of hemorrhagic stroke, serious central nervous system bleeding, and gastrointestinal bleeding.

The beneficial effect of EPA was observed across a broad set of patient subgroups based on baseline characteristics, including TG and LDL-C levels, primary and secondary cohorts, and baseline intensity of statin use. The exact mechanisms for the beneficial effect of IPE in the REDUCE-IT trial are not known but might be explained by pleiotropic effect other than lowering TG levels. First, primary endpoint was not changed by baseline TG levels (no difference in the subgroup in patients whether the cutoffs were 150 or 200 mg/dL). Second, the beneficial effect of IPE was not influenced by whether patients achieved triglyceride levels (<150 mg/dL vs ≥150 mg/dL) at 1-year mark. Another plausible mechanism of beneficial action of IPE is improving endothelial function, reducing anti-inflammatory and anti-oxidative properties of HDL particles in patients with dyslipidemia.

Effect of icosapent ethyl on the progression of coronary atherosclerosis in patients with elevated triglycerides on statin therapy (EVAPORATE trial) may explain possible mechanisms of IPE. The trial evaluated the efficacy of IPE in patients on a statin and elevated TG with coronary atherosclerosis documented by multidetector computed tomography (MDCT). Plaque volume regressed in the IPE group compared to placebo, including low attenuation plaque, total plaque, fibrous, and fibrofatty plaque. Interestingly, low attenuation volume (109%) and fibrofatty plaque (32%) progressed in the placebo group [22].

EPA treatment for ischemic stroke patients with hypertriglyceridemia and stable coronary artery disease who were already taking statin also retard the progression of atherosclerosis through decreased small dense LDL particles and increased larger HDL particles [23].

Recently published meta-analysis and systematic review which included 17 studies ($n = 83{,}617$) showed that low dose of omega-3 supplement as ≤1 capsule/day did not have positive effects on CV outcomes, but 2 capsules/day exhibited significant reduction of cardiac death, and more than 3 capsules/day reduced cardiac death, sudden death, and stroke.

Meta-analysis using marine omega-3 supplementation, including 13 trials of 127,477 participants, showed a significantly lower risk of myocardial infarction, cardiac death, and total cardiovascular disease. Omega 3 dose and clinical outcomes were shown in Fig. 3.4. These beneficial effects are related to marine omega-3 dose linearly [25]. Therefore, composition and dose of omega-3 PUFAs are important for cardiovascular events protection. Ongoing trial, Statin-Residual Risk Reduction With Epanova in High Cardiovascular Risk Patients With Hypertriglyceridemia (STRENGTH, NCT02104817) will help us understand the beneficial effects of omega-3 PUFAs for cardiovascular events in patients with high cardiovascular risk already taking statin [26].

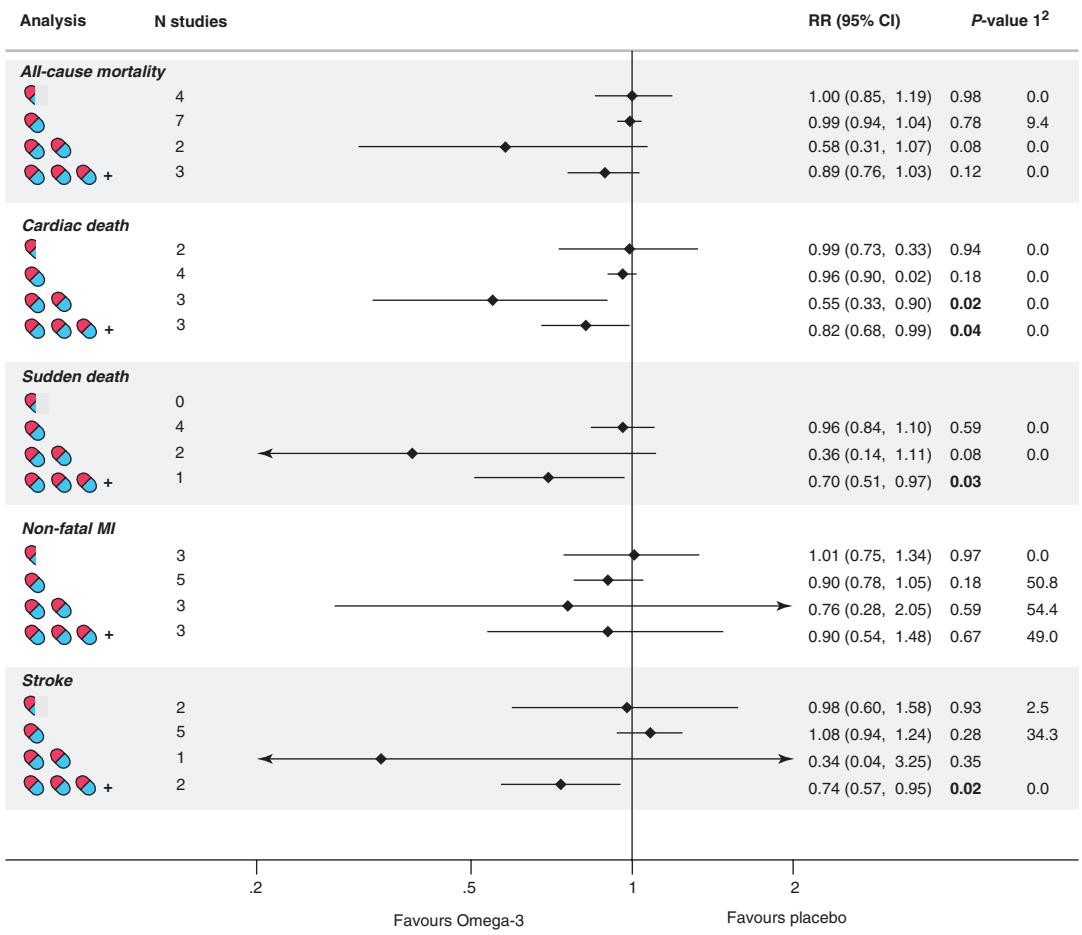

Fig. 3.4 Omega-3 dose and clinical outcomes. Adapted with permission from Heart, Copyright BMC Publishing Group Ltd. [24]

3.3.3 Management of Hypertriglyderidemia

Hypertriglyceridemia itself is not easy to control through lifestyle modification which included weight control, exercise, restriction of alcohol intake, and diet control. Therefore, medical management is needed. Detailed guidelines and management for lipid control will be mentioned in Chap. 13.

I would like to briefly describe American and European Society of Cardiology guidelines here. The American Heart Association Science Advisory Committee comments on the potential cardiovascular disease benefit for EPA with DHA or EPA only at a dose of 4 g/day and European guidelines describe that IPE 4 g/day in addition to statin can be considered for high risk or very high-risk patients with TG level 135–499 mg/dL [27, 28].

3.4 Association of Other Lipids with Ischemic Stroke

2018 AHA/ACC lipid guidelines adopted a risk enhancer which is helpful to use statin therapy. Lipoprotein(a), also known as Lp(a), is also one of the risk enhancers. This chapter will describe Lp(a) and its association with ischemic stroke.

Lp(a) is an LDL-like particle (Table 3.1) and has an apolipoprotein(a) (apo (a)) bound to apoli-

Table 3.1 Lp(a) and LDL-C composition

	Lp(a)	LDL
Physico-chemical properties		
Molecular mass (Da)	3.8–4.0 × 10^6	2.9 × 10^6
Diameter (nm)	28.3 ± 0.5	25.9 ± 0.1
Density (g/ml)	1.006–1.125	1.019–1.063
Electrophoretic mobility	Pre-ß1	ß
Composition (%)		
Protein	17–29	26–31
Carbohydrates	8	2
Free cholesterol	6–9	9
Esterified cholesterol	35–46	40–43
Triglycerides	4–8	4–6
Total phospholipids	17–24	22–22
Phospholipid composition (% of total phospholipids)		
Phosphatidylcholine	64.8 ± 0.2	66.0 ± 0.2
Choline plasmalogen	<<1	<<1
Phosphatidylethanolamine	1.9 ± 0.2	1.5 ± 0.2
Ethanolamine plasmalogen	4.7 ± 0.4	3.3 ± 0.2
Sphingomyelin	28.6 ± 0.5	29.2 ± 0.6

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poprotein B100 (apo B 100), which is synthesized in the liver. Thanks to the small diameter of Lp(a),70 nm, it easily fluxes across the endothelial cells. It is well known that apo B 100 is closely related to atherosclerosis through cholesterol deposition. Therefore, a high Lp(a) level is related to ASCVD and calcified aortic valve disease. Apo(a) is similar to plasminogen and competes with plasminogen on endothelial cells and decreases plasmin formation. Finally, apo(a) is related to thrombus formation through delayed clot lysis and decreases the risk of hemorrhagic stroke.

Lp(a) also independently predicts carotid stenosis and occlusion. The plausible pathophysiology of Lp(a) on atherosclerosis and thrombosis is shown in Table 3.2.

Lp(a) is a heterogenous lipoprotein and composed of apo(a) (46%) and apo B 100 (42%). Besides apo(a) and apoB100, more than 35 proteins are expressed in Lp(a) (Table 3.3).

Table 3.2 Lp(a) pathophysiolgical mechanisms

Proatherogenic molecular mechanisms
↑endothelial cell adhesion molecule expression
↑expression of pro-inflammatory cytokines
Carrier of oxidezed phospholipids
↑vascular permeability by activating various inflammatory signals
↑smooth muscle cell proliferation, migration, and binding to elastin
↑monocyte chemoattractant activity
↑foam cell formation
Prothrombotic molecular mechanisms
↓plasminogen binding to fibrin
↓plasmin formation
↓tissue factor pathway inhibitor (TFPI) activity
↑activity and expression of endothelial cells plasminogen activator inhibitor-1 (PAI-1)
↑platelet responsiveness

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3.4.1 Human Genetics of Lp(a)

Apo(a) gene is linked to chromosome 6 and serum level of Lp(a) is mostly determined by variation in the LPA, which encodes apo(a). Genetic variation in the Lp(a) locus and other genes determine the Lp(a) levels.

Apo(a) has plasminogen-like kringle (triple loop stabilized by 3 disulfide bonds) 4 (KIV) and 5 (KV). There are 10 subtypes of KIV. (Fig. 3.5) The isoform size of apo(a) is determined by krin-

Table 3.3 Main proteins associated to Lp(a)

Apoprotein	Function
ApoB100	Interaction with membrane receptors
Apo(a)	Homologs to plasminogen
ApoA1	Efflux of lipids from cells; cofactor for lecithin cholesterol-acyltransferase (LCAT), anticlotting effect
ApoC1	Modulatory effect of enzymes involved in lipoprotein metabolism (LCAT, LPL, HL)
	Modulatory effect of interactions between lipoproteins and cell receptors
ApoC3	Modulatory effect of enzymes involved in lipoprotein metabolism (LCAT, LPL, HL)
	Modulatory effect of interactions between lipoproteins and cell receptors
ApoF	Cholesterol transport and/or esterification
Complement components (C3, C4A and C5)	Regulatory role of various steps of inflammatory response
Histidine-rich glycoprotein (HRG)	Regulation of processes such as coagulation, fibrinolysis, and complement activation
ß2-integrin Mac-1	Plasmin inhibitor
α2-macroglobulin	Inhibitor of fibrinolysis
Serine proteinase inhibitor A1	tPA inhibitor
ß2-glycoprotein-1	Anticoagulant properties
Enzyme	
Lp-PLA2	Hydrolysis of oxidized fatty acids at the sn-2 position in oxPLs
Autotaxin (ATX)	Conversion of lysophosphatidylcholine into lysophosphatidic acid

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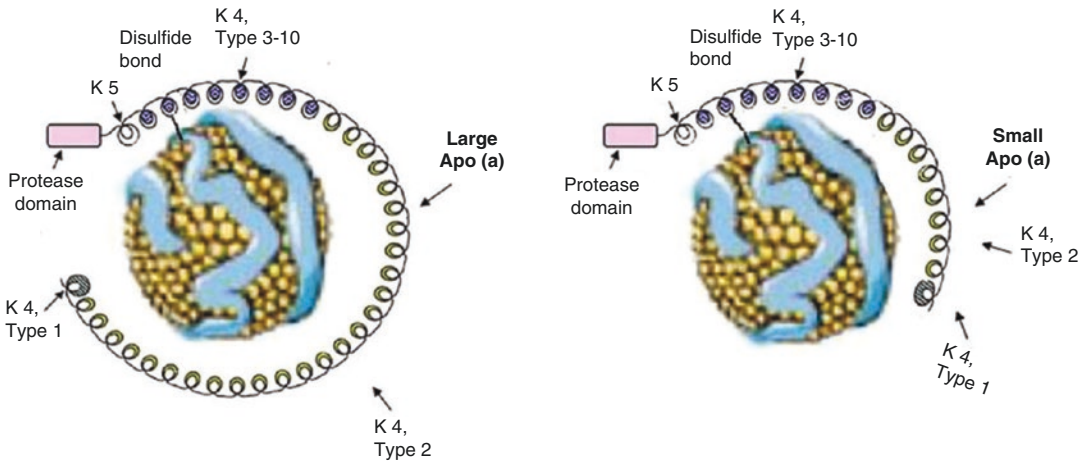

Fig. 3.5 Structures of lipoprotein (a) particles. Adapted with permission from Journal of Cellular Physiology, Copyright John Wiley and Sons [29]

gle 4-II (KIV2) which has expanded repeat numbers (from 1 to more than 40). Molecular weight of apo(a) is widely varied (200–800 Kda) due to the varying number of KIV2. Besides many subtypes of KIV, there are multiple repetitions in the KIV2 domain 3, which causes Lp(a) gene complexity.

Other genetics related to Lp(a), such as determinants of the number of kringle domains, include single-nucleotide polymorphism (SNP).

Rs10455872 and rs3798220 could account for 36% of Lp(a) plasm levels variation and rs41272114 and rs143431368 are loss-of-function variants related to lower Lp(a) levels.

3.4.2 Clinical Evidence Related to Lp(a)

Lp(a) measurement is useful in the following population: (1) patients with young stroke, (2) family history of premature cardiovascular disease, (3) very high inherited Lp(a) levels (above 180 mg/dL) whose lifetime risk of ASCVD equivalent to the risk associated with heterozygous familial hypercholesterolemia, (4) borderline or intermediate lifetime ASCVD risk patients whether to use statin or not, (5) not explained by traditional risk factors in ASCVD patients.

Plasma levels of Lp(a) are genetically determined by variation in the Lp(a) gene. Up to 20% of general population has a high level of Lp(a). Lp(a) levels vary by race. African-American have a higher mean Lp(a) level compared to white, Chinese, and Hispanics. Therefore, the significance of a high level of Lp(a) is underpowered. But high level of Lp(a) increased the risk of early-onset ischemic heart and blood vessel disease by two-2 to fourfold compared to people without genetic inherited conditions. It is reasonable to check Lp(a) level in patients with family history of early-onset cardiovascular disease and high LDL-C levels despite statin and non-statin treatment. Blood level of Lp(a) is genetically determined by apo(a) gene polymorphism. With decreasing apo(a) size, plasma Lp(a) level is increasing in general.

The correlation between high level of Lp(a) and risk of ischemic and hemorrhagic stroke and ischemic heart disease has been reported, but results are not consistent. Meta-analysis of Lp(a) as a risk factor for ischemic stroke evaluated 90,904 subjects and showed that high level of Lp(a) is an independent risk factor for ischemic stroke and more in young stroke population [30].

U.K. Biobank data including 112,338 used 4 single nucleotide polymorphism (SNPs) related to Lp(a) levels (re10455878, rs3798220, rs41272118, and rs143431368) to evaluate cardiometabolic disease and other disorders. This trial showed that loss of function variants decrease Lp(a) levels and reduces CHD risk. Because of dose-dependent relationship between Lp(a) and CVD risk, lowering Lp(a) levels may be beneficial effects on CVD risk reduction [31].

Copenhagen General population study (49,699 individuals) and Copenhagen City Heart Study (10,813 individuals) measured Lp(a) levels, KIV2 number of repeats, rs10455872, and ischemic stroke events in individuals with Lp(a) levels <10 mg/dL. Ischemic stroke (HR,1.20), KIV2 number of repeats (HR,1.20), and rs10455872 (HR,1.27) in patients with more than 50 mg/dL of Lp(a) levels were observed. An increase in ischemic stroke incidence was observed with increasing Lp(a) levels [32].

Other trials regarding primary and secondary prevention of Lp(a) are shown in Table 3.4.

3.4.3 Management of High Levels of Lp(a)

There is no approved effective Lp(a) lowering therapy until now and no randomized control trial supports Lp(a) level as a treatment goal (Table 3.5). But correlation between Lp(a) level and high ASCVD risk is present, recent guidelines emphasis the evaluation of Lp(a) as Class IIa in both American and European guidelines.Lipid aphesis remarkably reduced Lp(a) and decreased MI and composite CV outcomes but have not been reported beneficial effects further.

Table 3.4 Primary and secondary prevention trial of Lp(a)

Primary prevention trials of Lp(a)						
Author, year	Country	Size	Age (years)	Gender (Male)	Follow-up (years)	Main results
Kamstrup, 2009	Denmark	40,486	58	48%	16	Graded risk for MI with elevated Lp(a)
Kouvari, 2019	Greece	3042	44	48%	10	Lp(a) > 50 mg/dL with high risk of MACE
Saleheen, 2017	Pakistan	17,644	54	80%		Lp(a) concentration associated with MI risk
Agrawala, 2017	US	14,154	55	45%	23.4	Lp(a) levels were associated with incident MI related heart failure
Arosnis, 2017	US	9908	63	43%	15	High Lp(a) was associated with a 42% relative increase in stroke risk
Cock, 2018	US	8158	54	0%	10	Direct linear relationship of Lp(a) with MACE
Verbeek, 2018	Denmark & UK	26,102	59	45%	Minimum 5	Lp(a) ≥80th percentile was at increased CVD risk
Secondary prevention trials of Lp(a)						
Albers, 2013	US & Canada	3144	64	85%	2	Lp(a) was predictive of MACE
Nestel, 2013	Australia & new Zealand	7863	62	83%	6	Baseline Lp(a) was associated with CV death
Lincoff, 2017	Multinational	12,092	65	77%	2.3	Lp(a) in the highest quartile had higher events
O'Donoghue, 2019	Multinational	27,564	63	75%	1	Lp(a) lowering with PCSK9i had reduced MACE
Gaudet, 2017	Multinational	4915	62	60%	1.5	Alirocumab resulted in 23–29% reductions in Lp(a)
Willeit, 2018	Multinational	29,069	62	72%	3	Increase in MACE with high Lp(a) level on statin therapy

Adapted with permission from The American Journal of Cardiology, Copyright Elsevier. *MACE* Major adverse cardiovascular event; *HF* heart failure; *MI* myocardial infarction [33]

Lp(a) lowering effect of PCSK9 in FOURIER trial confirmed that evolocumab lowered Lp(a) level significantly, independent of LDL-C level, and patients with higher baseline Lp(a) levels more pronounced reduction of Lp(a) and greater benefit for coronary heart disease death, myocardial infarction, or urgent revascularization [35]. But another agent, inclisiran, did not decrease Lp(a) level.Mipomersen is an antisense oligonucleotides (ASO) and is targeting apo B. Mipomersen is injected subcutaneously. It binds the mRNA of Apo B 100 and preventing the translation of Apo B 100 that causes lowering the LDL and Lp(a) levels. A major adverse event is hepatic toxicity. CV outcome benefits are yet to be demonstrated.

Recent promising trials using ASO were published. IONIS-APO(a)RX trial decreased Lp(a) level about 62.7%–67.7%. But higher study dose was needed to enter the hepatocyte that makes apoA. IONIS-APO(a)RX-Lrx, covalently attaching triantennary N-acetyl-galatosamin (GALNAc), was modified first trial IONIS-APO(a)RX. This trial showed a more potent Lp(a) level reduction of 92.49% without adverse reactions.

Table 3.5 Summary of therapeutic agents to lower Lp(a)

Agent	Class	% Lp(a)↓	Mechanism of action
Antibodies			
Evolocumab	mAb	30	Inhibition of PCSK9; ↑LDLR; ↓apo(a) secretion
Alirocumab	mAb	30	Inhibition of PCSK9; ↑LDLR; ↓apo(a) secretion
Tocilizumab	mAb	30	IL-6 receptor blockade; ↓transcription of apo(a) mRNA
Nucleic acid based			
Mipomersen	ASO	21–39	↓*APOB* mRNA
IONIS-APO(a)rx	ASO	70	↓*LPA* mRNA
AKCEA-APO(a)-Lrx	ASO	90	↓*LPA* mRNA
Small molecule			
Niacin	Small molecule	25	Unknown (transcription of *LPA*?)
Lomitapide	Small molecule	15–20	MTP inhibitor; ↓secretion of apoB-containing lipoproteins
Anacetrapib	Small molecule	36	Unknown (secretion of apoB?)
Other			
Lipid apheresis		70(acute) 25–40(sustained)	apoB or apo(a) affinity-based removal of lipoproteins from plasma

Adapted with permission from Trends in Pharmacological Sciences, Copyright Elsevier [34]. *Lp(a)* Lipoprotein (a); *mAb* monoclonal antibody; *PCSK9* proprotein convertase subtilisin/kexin type 9; *LDLR* low-density lipoprotein receptor; *apo(a)* apolipoprotein (a); *ASO* antisense oligonucleotides; *MTP* microsomal triglyceride transfer protein; *apoB* apolipoprotein B

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Dyslipidemia and Hemorrhagic Stroke

4

Sung-Il Sohn

Abstract

Dyslipidemia is not as strong a risk factor as hypertension for hemorrhagic stroke. Moreover, studies have reported opposite or neutral results regarding the association between dyslipidemia and intracerebral hemorrhage (ICH) according to patient age, race, and research years. A meta-analysis of well-designed studies concluded that the risk of hemorrhagic stroke is inversely related to serum levels of total cholesterol and low-density lipoprotein cholesterol. However, the risk of hemorrhagic stroke was not associated with the serum triglyceride and high-density lipoprotein cholesterol levels. Statin or lipid-lowering therapy has routinely been used the past two decades to avoid cardiovascular events. However, concern is increasing about the risk of ICH following lipid-lowering therapy. In meta-analyses, there was no association between statin or lipid-lowering therapy and the risk of ICH in primary stroke prevention. In secondary stroke prevention, the risk of ICH showed a nonsignificant trend for statin therapy and was significantly associated with lipid-lowering therapy. The risk of ICH from statin or lipid-lowering therapy is offset by the prevention of ischemic stroke and substantial and significant improvement in mortality and functional outcomes. Therefore, clinicians should not stop statin or lipid-lowering therapies to prevent cardiovascular events.

4.1 Impact of Total Cholesterol

In the early 1990s, etiologic studies of intracerebral hemorrhage (ICH) that were mainly conducted of Japanese people in Japan and Hawaii showed an inverse relationship between serum cholesterol levels and the incidence of ICH [1, 2]. The large observational Multiple Risk Factor Intervention Trial (MRFIT) showed that the 6-year risk of death of hemorrhagic stroke is three times higher in men with total cholesterol (TC) levels <160 mg/dL (<4.14 mmol/L) than in men with higher cholesterol levels [3]. This inverse association between cholesterol level and the risk of fatal ICH was observed only in men with a diastolic blood pressure > 90 mmHg [3]. Subsequently, cohort studies on related topics have been published in various countries.

In 2013, Wang et al. published a meta-analysis of cholesterol levels and risk of hemorrhagic stroke, including both ICH and subarachnoid hemorrhage (SAH) [4]. Twenty-three studies

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were included, consisting of 19 prospective cohort studies and four nested case–control studies involving 1,43,141 participants with 7650 (5.6%) hemorrhagic stroke events [1, 5–21]. In the high and low meta-analyses, an inverse association was observed between the risk of hemorrhagic stroke and TC level (relative risk [RR], 0.69; 95% confidence interval [CI], 0.59–0.81; $p < 0.01$). In the dose-response meta-analysis, this study showed that an increment of 1 mmol/L (38.7 mg/dL) in TC level was associated with a 15% decreased risk of hemorrhagic stroke (RR, 0.85; 95% CI, 0.80–0.91; $p < 0.01$).

Jin et al. recently published a new meta-analysis based on cohort studies performed between 1993 and 2019 for cholesterol levels and risk of hemorrhagic stroke [22]. Thirty-one studies with 2,291,643 participants and 12,147 hemorrhagic stroke cases were included [1, 5, 6, 8–16, 18, 19, 23–31]. In the high and low meta-analyses, an inverse association was observed between the risk of hemorrhagic stroke and TC (RR, 0.72; 95% CI, 0.64–0.82; p heterogeneity = 0.002). (Fig. 4.1) In a dose-response meta-analysis, there was a nonlinear dose-response trend between the risk of hemorrhagic stroke and TC. The risk of hemorrhagic stroke was reduced by approximately 55% when the level of TC level was approximately 232 mg/dL (6 mmol/L) ($p = 0.04$) [22]. (Fig. 4.2a) The association between TC and the risk of hemorrhagic stroke was consistent with ethnicity, number of participants, number of events, and follow-up time. However, no statistically significant difference was observed in SAH, adjusted lipid-lowering drug use, or unadjusted blood pressure.

In a meta-analysis of risk factors by ICH location, hypercholesterolemia was a protective factor for lobar and non-lobar ICH, which did not differ between the two locations [32].

The relationship between lipid status and SAH remains controversial, with studies reporting both increased and decreased associations. Lindhohm et al. reported a meta-analysis of 21 studies of the association between lipid profiles and the risk of SAH [33]. However, only two among them had a low risk of bias [19, 24]. The two studies suggest that elevated TC levels

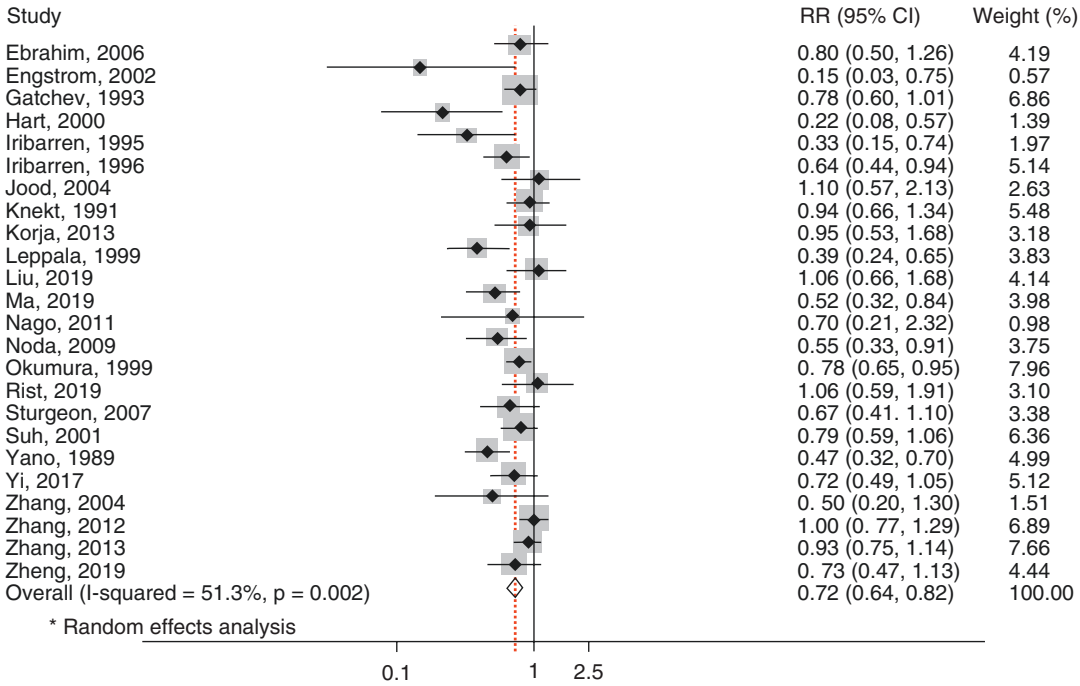

Fig. 4.1 Forest plot of total cholesterol and risk of hemorrhagic stroke. Adapted with permission from Nutrition, Metabolism & Cardiovascular disease, Copyright Elsevier [22]

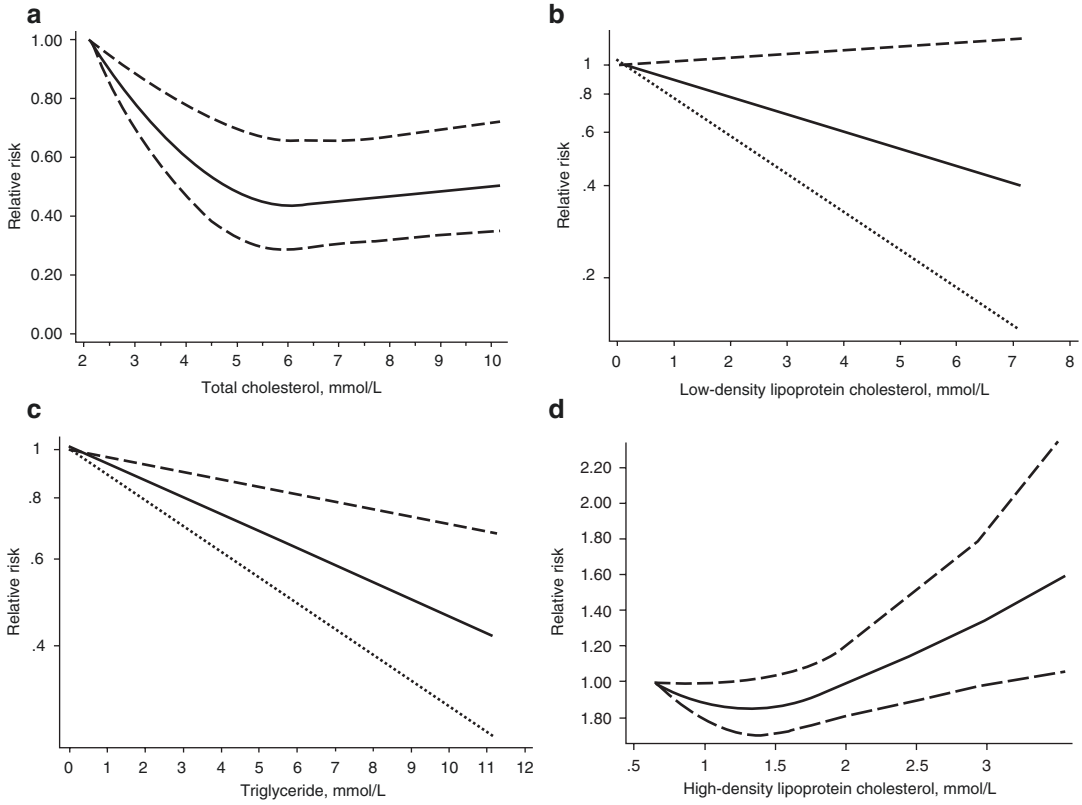

Fig. 4.2 Dose-response between total cholesterol (**a**), lower-density lipoprotein cholesterol (**b**), triglyceride (**c**), high-density lipoprotein cholesterol (**d**) and the risk of hemorrhagic stroke. Reproduced by permission of Nutrition, Metabolism & Cardiovascular disease, Copyright Elsevier [22]

increase the risk of SAH in men. In the largest case–control study, elevated high-density lipoprotein (HDL) cholesterol levels and the use of lipid-lowering agents were associated with a significantly lower risk of intracranial aneurysm rupture [34].

4.2 Impact of Low-Density Lipoprotein Cholesterol

Low-density lipoprotein (LDL) cholesterol is a well-established risk factor for the development of atherosclerotic cardiovascular diseases. The risk of hemorrhagic stroke is inversely related to LDL cholesterol and TC levels. A meta-analysis of LDL cholesterol and hemorrhagic stroke that included 12 prospective studies with 476,173 participants and 7587 hemorrhagic stroke cases showed that a 10 mg/dL increase in LDL cholesterol was associated with a 3% lower risk of hemorrhagic stroke (pooled RR, 0.97; 95% CI, 0.95–0.98) [16, 17, 26, 27, 35–43]. The association appeared to be more pronounced in Asians (pooled RR, 0.95; 95% CI, 0.92–0.98) than in Caucasians (pooled RR, 0.98; 95% CI, 0.97–1.00; p heterogeneity = 0.05).

Jin et al. also analyzed LDL cholesterol level and the risk of hemorrhagic stroke in10 studies with 2129 hemorrhagic stroke cases [17, 22, 25–27, 30, 38, 40, 42, 43]. They reported an inverse relationship between LDL cholesterol level and the risk of hemorrhagic stroke (RR, 0.69; 95% CI, 0.53–0.89; p heterogeneity = 0.04). (Fig. 4.3) No evidence of a nonlinear association between LDL cholesterol and the risk of hemorrhagic stroke was detected. No statistically significant differences were noted on a linear dose-response

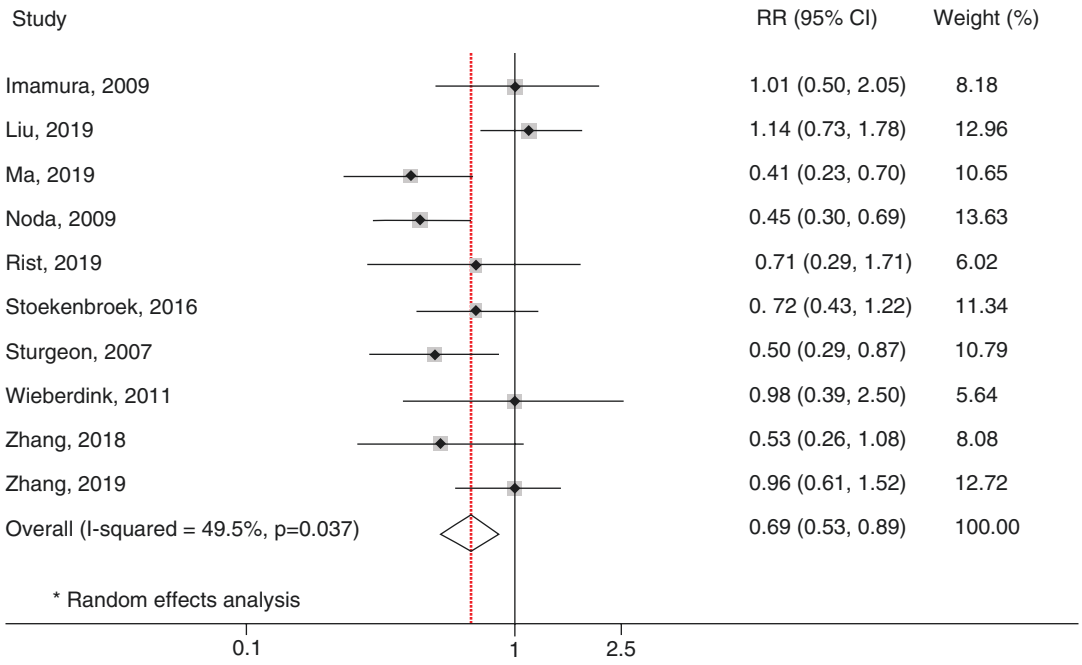

Fig. 4.3 Forest plot of low-density lipoprotein cholesterol and risk of hemorrhagic stroke Adapted by permission of Nutrition, Metabolism & Cardiovascular disease, Copyright Elsevier [22]

analysis (RR, 0.88; 95% CI, 0.75–1.02; $p = 0.1$) [22]. (Fig 4.2b).

A higher LDL cholesterol level at hospital admission can be an independent predictor of a lower likelihood of hematoma expansion and decreased in-hospital mortality in patients with acute spontaneous ICH [44].

4.3 Impact of Triglyceride

Limited data have been published regarding the risk of hemorrhagic stroke and triglyceride (TG) levels. A meta-analysis of eight studies with 1715 hemorrhagic stroke events [16, 17, 22, 25–27, 30, 42, 45] reported no association between TG level and the risk of hemorrhagic stroke (RR, 0.72; 95% CI, 0.52–1.01; p heterogeneity < 0.01) [16, 17, 22, 25–27, 30, 42, 45]. (Fig. 4.4) In a dose-response meta-analysis of six studies, the nonlinear association between TG level and hemorrhagic stroke was nonsignificant. The linear trend suggested that for every 1 mmol/L (88.6 mg/dL) increase in TG level, the risk of hemorrhagic stroke decreased by 7% (RR, 0.93; 95% CI, 0.89–0.97; $p = 0.000$) [22]. (Fig 4.2c) A subgroup analysis of this study showed an inverse relationship between TG level and hemorrhagic stroke in the subgroup of America and Europe participants, fewer events, higher quality, SAH, and unadjusted blood pressure.

4.4 Impact of HDL Cholesterol

There are few studies on the risk of hemorrhagic stroke and HDL cholesterol levels. The results of high and low meta-analyses of 11 studies with 3499 hemorrhagic stroke cases showed no association between HDL cholesterol level and the risk of hemorrhagic stroke (RR, 0.94; 95% CI, 0.83–1.08; p heterogeneity = 0.6) [8, 16, 19, 22, 25–27, 30, 42, 46, 47]. (Fig. 4.5) A dose-response meta-analysis of eight studies revealed a nonlinear dose-response trend. The risk of hemorrhagic stroke was lowest when the HDL cholesterol level was about 1.3 mmol/L (50.3 mg/dL) [22]. (Fig 4.2d) A recent observational cohort study of

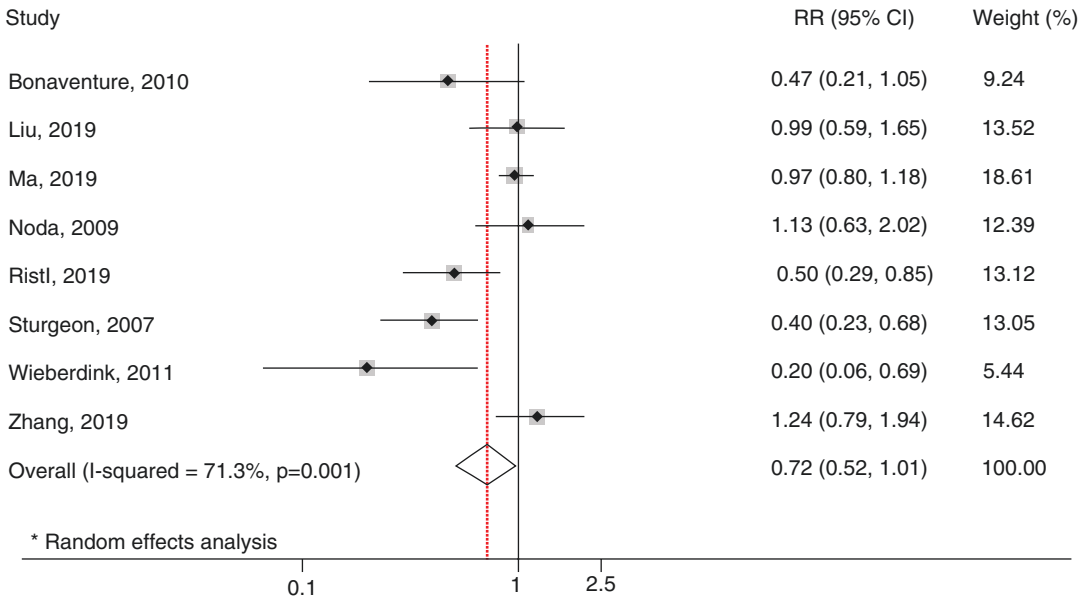

Fig. 4.4 Forest plot of triglyceride and risk of hemorrhagic stroke. Adapted by permission of Nutrition, Metabolism & Cardiovascular disease, Copyright Elsevier [22]

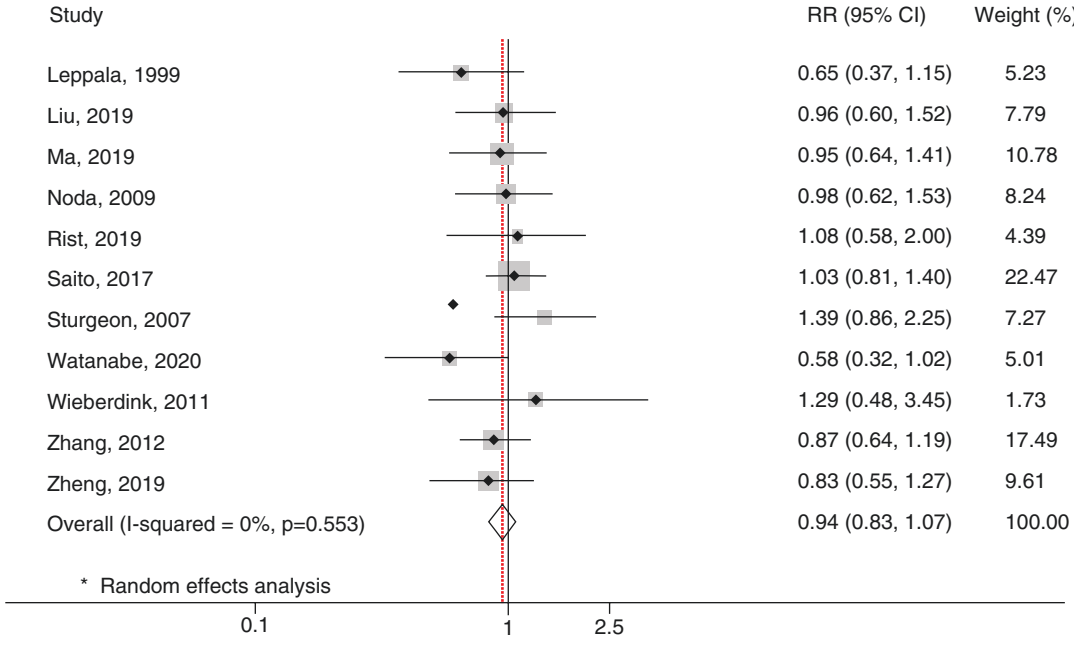

Fig. 4.5 Forest plot of high-density lipoprotein cholesterol and risk of hemorrhagic stroke. Adapted by permission of Nutrition, Metabolism & Cardiovascular disease, Copyright Elsevier [22]

11,027 Japanese individuals without a history of stroke showed that a high HDL cholesterol level was associated with a decreased incidence of ICH in women (hazard ratio [HR], 0.23; 95% CI, 0.06–0.89) but not in men (HR, 0.73; 95% CI, 0.27–1.97) [47].

4.5 Postulated Pathophysiology of Hemorrhagic Stroke with Low Cholesterol Level

The exact pathophysiology by which dyslipidemia causes hemorrhagic stroke is unknown, but we can infer several mechanisms based on limited animal and clinical data. Cholesterol is the main component of the cell membrane. Lipid composition affects the physical properties of membranes [48]. Polyunsaturated fatty acids in glycerophospholipids reduce membrane rigidity and affect processes that accompany membrane deformation. Low levels of cholesterol within membranes can increase membrane fragility [49]. Decreased endothelial fragility may lead to angionecrosis and microaneurysm formation, which may then result in hemorrhagic stroke [50].

Another postulated mechanism is that lipid components may affect platelet activity and the coagulation cascade. Elevated serum TC, elevated LDL cholesterol, and low HDL cholesterol are well-established risk expressions and functions of procoagulant, fibrinolytic, and rheological factors [51]. TG-enriched lipoproteins seem to increase factor VII clotting activity and blood viscosity [52]. LDL cholesterol promotes platelet activation and tissue factor expression. HDL cholesterol may inhibit platelet aggravation, reduce viscosity, and suppress tissue factors [53]. These possible biological effects suggest that increased TC levels promote thrombosis, whereas decreased TC levels increase changes in bleeding. Therefore, lipid components can influence hemostasis and potential tissue damage resulting from the vascular injury.

4.6 Lipid-Lowering Treatment and Hemorrhagic Stroke

4.6.1 Lipid-Lowering Treatment and Risk of ICH

Statin, ezetimibe, and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have been proven to reduce the risk of stroke and cardiovascular events in patients at cardiovascular risk. Each 1 mmol/L reduction in LDL cholesterol with lipid-lowering treatment reduces the risk of major cardiovascular events by at least 19% [54]. This reduction effect was reportedly similar regardless of the initial cholesterol level, age, or degree of cardiovascular risk.

Cohort studies and meta-analyses showed an inverse association between the risk of hemorrhagic stroke and TC and LDL cholesterol levels, especially in secondary stroke prevention. The Stroke Prevention with Aggressive Reductions in Cholesterol Levels (SPARCL) trial of treatment with high-dose atorvastatin (80 mg/day) demonstrated a 16% reduction in the combined risk of fatal and nonfatal stroke in patients with a recent stroke or transient ischemic attack and no known coronary heart disease (11.2% vs 13.1% over 4.9 years; HR, 0.84; 95% CI, 0.71–0.99; $p = 0.03$) [55]. A post hoc analysis of the SPARCL trial found that treatment with atorvastatin was independently associated with an increased risk of hemorrhagic stroke (HR, 1.68; 95% CI, 1.09–2.59) [56]. In the Heart Protection Study (HPS) for patients with a previous history of cerebrovascular events, statin treatment showed a trend of an increased risk of ICH, but the difference was not significant [57]. A combined analysis of the HPS and the SPARCL showed a significant increase in the odds of hemorrhagic stroke with statin therapy (odds ratio, 1.72; 95% CI, 1.20–2.49) in secondary stroke prevention [58]. Therefore, there is concern about the potential risk of ICH with statins in patients with a history of previous stroke, especially intracerebral bleeding.

The 2012 meta-analysis of the Cholesterol Treatment Trialists' (CTT) Collaboration showed no impact of statins on the incidence of hemorrhagic stroke (RR, 1.15; 95% CI, 0.97–1.38), a neutral effect that did not differ according to the baseline vascular risk [59]. This analysis suggested that for every 1.0 mmol/L reduction in LDL cholesterol, the annual risk of ICH was 0.5 per 1000 patients who were treated with statins for at least 5 years. This increase in the risk of hemorrhagic stroke was offset by the significant reduction in the risk of ischemic stroke and the risk of major vascular events [59]. A recent meta-analysis included 43 studies with a combined total

of 317,291 patient-years of follow-up for patients with previous ischemic or hemorrhagic stroke [55, 57, 60–64]. In patients with previous ischemic stroke, although statin use was associated with a nonsignificant trend of ICH (RR, 1.36; 95% CI, 0.96–1.91), there was a significant beneficial effect on recurrent ischemic stroke (RR, 0.74; 95% CI, 0.66–0.83), mortality (RR, 0.68; 95% CI, 0.50–0.92), and functional outcome (RR, 0.83; 95% CI, 0.76–091) [60]. (Fig. 4.6) In patients with previous ICH, statin therapy resulted in significantly reduced mortality (RR, 0.49; 95%

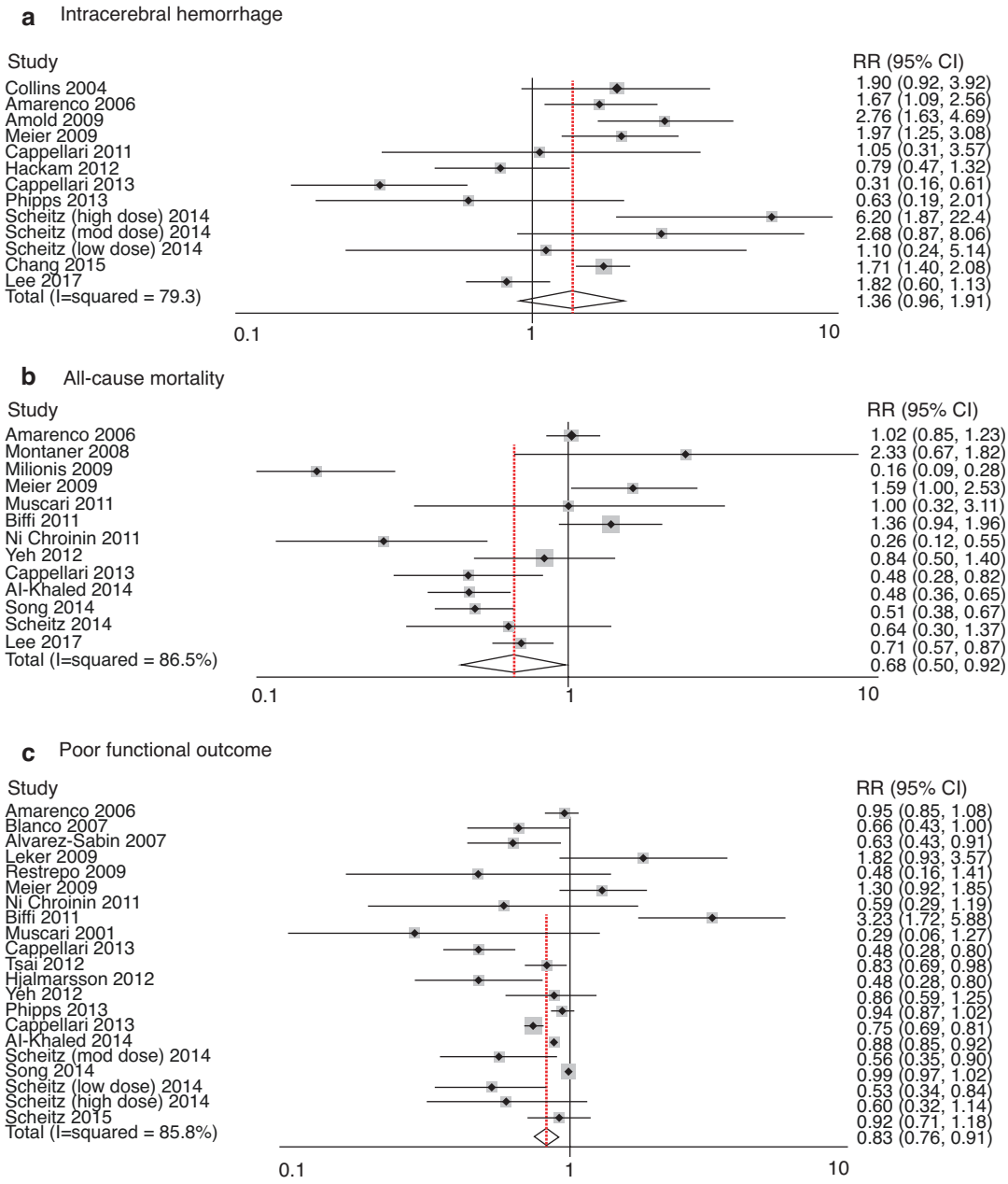

Fig. 4.6 Forest plot of statin and clinical outcome in patients with previous ischemic stroke. A. intracerebral hemorrhage, B. all-cause mortality, C. poor functional outcome. Reproduced by permission of *Journal of Neurology Neurosurgery and Psychiatry, Copyright BMJ Publishing Group Ltd* [60]

CI, 0.36–0.67) and poor functional outcome (RR, 0.71; 95% CI, 0.67–0.75) without any significant change in the rates of recurrent ICH (RR, 1.04; 95% CI, 0.86–1.25) [60].

A recent large population-based propensity score-matched cohort study including a total of 55,692 unique individuals initiating statin treatment after a first-time stroke diagnosis (4.9% ICH, 95.1% ischemic stroke) showed that the risk of ICH was similar for statin users and nonusers when evaluated among those with prior ICH (HR, 0.90; 95% CI, 0.72–1.12) and that the risk was halved among those with prior ischemic stroke (HR, 0.53; 95% CI, 0.45–0.62) [65]. The Target Stroke to Target (TST) trial including 2860 patients with recent ischemic stroke or transient ischemic attack and mean baseline LDL cholesterol level > 135 mg/dL (3.5 mmol/L) showed that the group with a target LDL cholesterol <70 mg/dL (1.8 mmol/L) was associated with a lower risk of cardiovascular events than the group with a target LDL cholesterol level of 90–110 mg/dL (2.3–2.8 mmol/L) after a median of 3.5 years of follow-up (8.5% vs 10.9%, adjusted HR 0.78; 95% CI 0.61–0.98) [66]. There was no significant intergroup difference in the occurrence of ICH (1.3% vs 0.9%; HR, 1.38; 95% CI, 0.68–2.82).

Recent meta-analyses or large observational studies have shown conflicting findings with past studies. Consequently, the current clinical guidelines state that there is a need for further exploration of the risk of hemorrhagic stroke in particular patients [67]. The SPARCL study and observational studies showed that hemorrhagic stroke episodes with statin medication use occurred more frequently in patients with a hemorrhagic stroke as an entry event, particularly in patients who were male, of advanced age, had poorly controlled hypertension during statin therapy, had lobar ICH associated with cerebral amyloid angiopathy, and had multiple microbleeds [56, 65, 68, 69]. However, the overall benefit of statins on ischemic and hemorrhagic stroke greatly outweighs this small and uncertain hazard.

4.6.2 Statin Treatment in Patients with ICH

With regard to hemorrhagic stroke followed by statin therapy, many physicians are very careful to administer statins to patients with ICH and often stop statin medication immediately after the onset of ICH. Statins have been identified as potential neuroprotective agents in addition to lipid-lowering agents [70, 71]. Animal studies have shown the neuroprotective and recovery enhancement effects of statins, including reduced cerebral edema, increased angiogenesis and neurogenesis, and accelerated hematoma clearance [72–76]. Retrospective observational clinical studies reported that pre-ICH statin use reduced perihematomal edema, decreased mortality rates, and improved functional outcomes [63, 77–79]. On the other hand, the sudden withdrawal of statin treatment may lead to rebound effects that may impair vascular function and induce adverse clinical effects in patients with ICH [61, 80–83]. Statin therapy after ICH might be of great benefit for decreasing mortality, especially in younger patients with fewer pre-existing comorbidities [63, 78, 79]. A recent observational study from Sweden of 6082 patients with a first ICH showed that statin use was associated with a reduced risk of death (adjusted HR, 0.71; 95% CI, 0.60–0.84) but not with the risk of recurrent ICH (adjusted HR, 0.82; 95% CI, 0.55–1.22) during a mean follow-up period of 3.1 years [84].

These clinical results suggest that statin therapy should not be discontinued in the acute phase of ICH. The decision should be made to continue or discontinue statin therapy after the acute phase of ICH. It is recommended that patients continue therapy if they have high cardiovascular risk factors but discontinue therapy if they have lobar hemorrhage, severe small vessel disease such as multiple microbleeds, or poorly controlled hypertension [82]. There is insufficient evidence to recommend starting statin therapy in patients with statin-naive ICH [71, 82]; rather, the results of randomized trials are needed first.

4.7 Conclusion

The current study showed that the risk of ICH was inversely related to TC and LDL cholesterol levels but not TG and HDL cholesterol levels. The inverse relationship was a nonlinear trend in the dose-response analysis in TC. This finding suggests that maintaining lipid levels may reduce the risk of ICH. The relationship with SAH remains controversial and requires confirmation in large cohort or randomized trials. Lipid-lowering therapy was associated with a nonsignificant or marginally significant increased risk of ICH in secondary stroke prevention, but not in primary prevention or in patients with ICH [22, 60, 85]. However, this concern regarding the risk of ICH was counterbalanced by a significant reduction in mortality and the risk of major vascular events, including ischemic stroke. In addition, sudden withdrawal of statins may have adverse clinical effects in patients with ICH.

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Clinical Biomarkers of Dyslipidemia

5

Hee-Kwon Park

Abstract

There are controversies about the utility of nontraditional lipid biomarkers like apolipoproteins in addition to the evaluation of traditional risk factors like total cholesterol LDL cholesterol and HDL cholesterol. However, apolipoproteins like Apo B100 sometimes can be better predictor for assessing the risk of the cardiovascular disease and stroke. The stroke pathomechanism is heterogeneous and multifactorial. Oxidative stress involves the injury to cellular membrane and apoptosis of neuron. Assays for lipid peroxidation like isoprostanes and malondialdehyde are commonly used for assessing the oxidative stress or damage, although the role of oxidative stress in cerebrovascular disease is complex. Comprehensive approaches of metabolomics and lipidomics give the dynamic bioprofile and elucidate these complex pathogenesis and disease progress, which lead to discover new biomarkers for early prediction and drug development. Here, we reviewed the use of nontraditional lipid biomarkers including apolipoproteins and lipid peroxidation and discussed the role of metabolomics and lipidomics in improving stroke risk evaluation and monitoring the drug effects.

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5.1 Apolipoproteins

Apolipoproteins and lipids form lipoproteins like low-density lipoprotein (LDL) and high-density lipoprotein (HDL) to transport lipids throughout lymphatic and circulatory systems. Eight classes of apolipoproteins (apo) with many subclasses are known**,** which have a unique function and are associated with neurodegenerative and cardiovascular diseases, cancer autoimmunity, and others (Table 5.1). Cholesterol levels like serum level of LDL are still used to predict stroke risk and there is some controversy about the utility of nontraditional lipid biomarkers like apo B for stroke risk prediction. However, apolipoproteins also are relevant biomarkers and sometimes are better at assessing the risk and starting the medication.

Apo B is the major apolipoprotein embedded in very low-density lipoprotein (VLDL), intermediate-density lipoprotein (ILD), chylomicrons, and LDL particles. Measurement of the total apo B-100 concentration in the circulation can quantify the number of lipoprotein particles because there is 1 apo B-100 molecule per hepatic-derived lipoprotein (Fig. 5.1). Apo A-1

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Table 5.1 Overview of apolipoproteins

Analyte	Main Function	Gene ID	Related diseases
Apo A1	Protein component of HDL	335	Atherosclerosis, diabetes, autoimmune, sepsis
Apo A2	Protein component of HDL	336	Atherosclerosis, cancer
Apo B	Main component of LDL, VLDL, and intermediate-density lipoprotein (IDL).	338	Atherosclerosis, diabetes, cancer, autoimmune
Apo C1	Differentiation of monocytes into macrophages	341	Atherosclerosis, autoimmune, diabetes, neurodegenerative, sepsis
Apo C3	Component of VLDL	345	Atherosclerosis, diabetes, cancer, autoimmune
Apo D	Component of HDL, associated with lipoprotein metabolism	347	Neurodegenerative disease, cancer
Apo E	Primarily found in IDL essential for the normal catabolism of triglyceride-rich lipoprotein cholesterol carrier in human brain	348	Alzheimer's disease, neurodegenerative disease, atherosclerosis, diabetes,
Apo H	Bind cardiolipin for involvement in phospholipid binding. Agglutination of platelets. Anti-coagulation activity	350	Lupus
Apo J	Molecular chaperone, apoptosis	1191	Bone healing, cancer, neurodegenerative disease

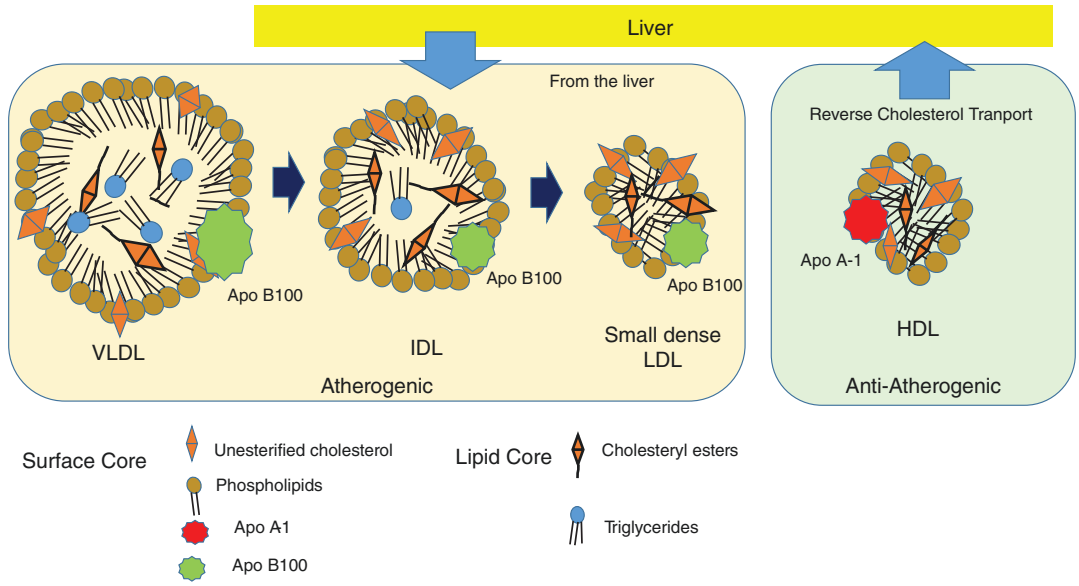

Fig. 5.1 Structure and component of various lipoprotein particles

accounts for 70% of all HDL-associated proteins and mediates the anti-atherogenic effect of HDL [1]. Although serum level of HDL is correlated with risk protection for stroke and cardiovascular disease, HDL particles have heterogeneous composition and size. So, Apo A-1 is a candidate for more accurate biomarker than HDL. ApoA1 also has anti-inflammatory and antioxidant effects.

High serum levels of Apo B and Apo A1 is associated with atherosclerosis like stroke and coronary disease across all ethnic groups and sexes. A large-scale review showed that apo B was the stronger marker of cardiovascular risk (relative risk reduction [RRR], 1.43; 95% confidence interval (CI), 1.35–1.51), and LDL-C was the least potent marker (RRR, 1.25; 95% CI,

1.18–1.33) and is a superior indicator of atherosclerosis in people aged 40 years or younger, than older ones, which is helpful for decision of initiating lipid-lowering therapy in young patients and may have advantages in patients with hypertriglyceridemia [2–4]. The Apo B/A1 ratio is a better indicator than the LDL/HDL ratio for estimating the risk of the proatherogenic to antiatherogenic cholesterol and is associated with asymptomatic deep subcortical ischemic burden in patients with intracranial stenosis [5, 6]. The Apo B/A1 ratio also can identify the familial hyperlipidemia in children [7].

On four clinical trials, meta-analysis recommended measurement of apo B and apo B/apo A ratio in an addition to the traditional risk factors for the high risk persons, who are defined as men >35 years, women >45 years, and adults ≥20 years old with multiple cardiovascular risk factors [8].

However, apo B measurement has also some limitation like that it is costly, and may not be reliable in some laboratories [9]. The ratio of Apo B/Apo A1, Apo C3, and APOE ε2 also are associated with diabetes mellitus and are candidate for new biomarker to predict the diabetes mellitus [10–12]. On the other hand, low serum level of Apo C3 is related to decreased risk of cardiovascular diseases [13].

5.2 Lipid Peroxidation

Imbalance between the production and elimination of reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been called "oxidative stress," which leads to the formation of atherosclerotic plaques and increases the risk of type 2 diabetes mellitus. Lipid peroxidation causes impairment of cellular membrane and cell death. Because ROS and RNS are short lived and do not accumulate, measurement of them is very difficult and failed in showing a consistent correlation between clinical disease severity and oxidative stress level. The different parameters for the oxidative stress do not always come to the same results. The lack of correlation between different parameters might be caused by the different production and elimination of each biomarkers (Fig. 5.2). Assays for lipid peroxidation are commonly used for assessing the oxidative stress or damage, because lipids in biological membranes and lipoproteins are one of major peroxidation targets.

The hydroxyl radical(-OH), the most reactive radical, plays an important role in the lipid peroxidation. The hydroxyl radical has very short half-life in vivo and oxidizes lipids like polyunsaturated fatty acids (PUFAs). Lipid oxidation generates hydroperoxides, which subsequently

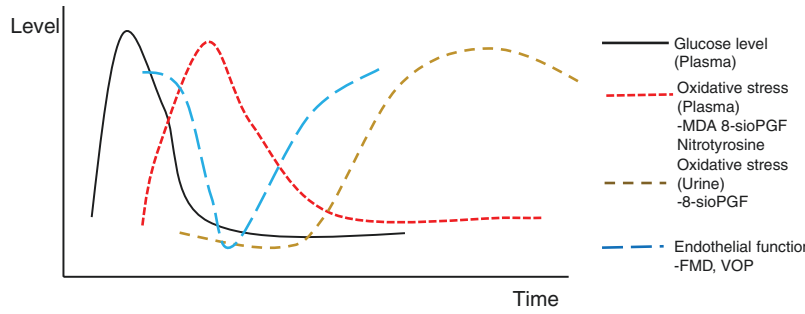

Fig. 5.2 Time course of changes in plasma glucose, plasma/urine oxidative stress, and endothelial function. Modified from antioxidants [14]. The causal relationship between glucose level and oxidative stress explored by plasma/urine samples taken at different times. First, plasma glucose levels increase after eating and then return to the baseline. Oxidative stress assessed by plasma malondialdehyde (MDA), 8-iso-prostaglandin F (PGF), and nitrotyrosine increases after peak of glucose level and return to baseline by gradual excretion through urine. Endothelial function assessed by venous occlusion plethysmography (VOP) and flow-mediated dilatation (FMD) is impaired concomitantly with the increase in plasma levels of glucose and oxidative stress

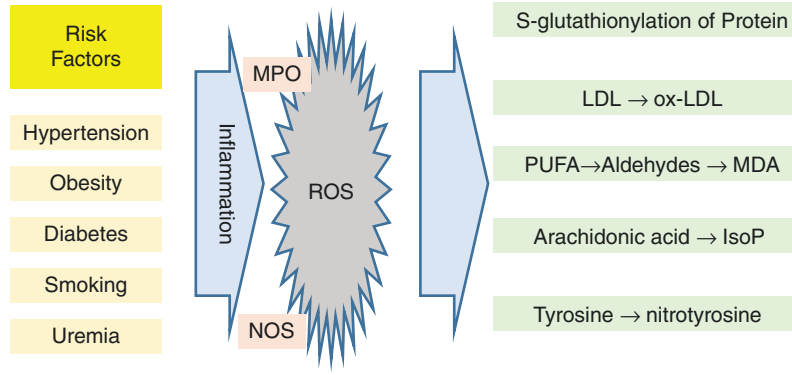

Fig. 5.3 This scheme shows the formation of ROS, leading to various products of lipid peroxidation. MDA, malondialdehyde; PUFAs poly unsaturated fatty acids

fragment into various reactive intermediates, like various bioactive aldehydes, such as 4-hydroxynonenal (4-HNE), acrolein, prostaglandin F2-IsoPs, and malondialdehyde (MDA). These aldehydes have toxic effects by the alternation of cellular proteins, which is related to atherosclerosis, diabetes, neurodegenerative disease, and liver disease (Fig. 5.3). For measuring lipid peroxidation, there are two assay methods; (i) measurements of lipid peroxides concentrations; and (ii) assessing the end products of lipid peroxidation, like isoprostanes (8-isoPGF2 α), MDA, and dihydropyridine-lysine.

The isoprostanes are bioactive prostaglandin-like substances and are significantly higher in HDL compared with LDL or VLDL. Isoprostanes are primarily formed in a free radical-dependent and nonenzymatic fashion, but can also be generated by activation of enzymes, like cyclooxygenase-2. Although the relative contribution of enzymatic pathways, isoprostane is a good biomarker for the oxidative stress.

MDA is a reactive carbonyl compound and a chemically stable end product of enzyme- and free radical-catalyzed peroxidation of a variety of PUFAs (Fig. 5.3). The amount of MDA is much more than that of isoprostane at the molecular level. So, it is easier to be measured and is perhaps the most commonly used biomarkers for lipid peroxidation. The plasma levels of MDA correlate closely with plasma 8-isoPGF2 α levels. Because MDA concentrations increased during storage after sampling, the plasma MDA levels should be measured as soon as possible after sampling.

The diacron reactive oxygen metabolites (d-ROMs) test is a simple commercial available assay, and can quantify the early peroxidation species, rather than their end products. In actuality, the d-ROMs test measures not only preexisting lipid peroxides but also those generated by a radical chain reaction. This test is based on the principle that transition metal ions released from serum proteins under acidic conditions stimulate the conversion of hydroperoxides to alkoxyl or peroxyl radicals, leading to the generation of colored materials that can be measured spectrophotometrically. Although reaction require plasma iron ions and the individual difference in iron levels might affect the accuracy of the d-ROMs test, the d-ROMs test has been applied in many clinical trials, because it is a simple, rapid, and cost-effective test. The Fe-ROMs test is a d-ROMs-modified method. In this test, iron ions are added to the sample exogenously and serum transferrin-derived Fe3+ is not required. The Fe-ROMs test can measure the oxidized HDL levels in addition to the oxidative stress status. Because of oxidization of HDL weakens the atheroprotective effect of HDL like removing oxidized lipids from oxidized LDL, the Fe-ROMs test can be a good test for measuring the protection function of HDL in daily clinical trial or practice. Moreover, antioxidant biomarkers can be assessed for evaluation of the total antioxidant capacity (TAC), enzymatic antioxidants, such as catalase, glutathione peroxidase, superoxide dismutase; or nonenzymatic markers like vitamins, glutathione, and uric acid.

Hyperglycemia, especially glycemic variability is associated with massive production of ROS and is strongly associated with oxidative stress [15]. Monnier et al. studied that the oxidative stress was more associated with glycemic variability during the postprandial period than chronic sustained hyperglycemia [16]. This result suggested large glucose burden during postprandial periods cause the excess ROS formation over antioxidant capacity. The d-ROMs test also correlated significantly with HbA1c ($p = 0.007$), mean amplitude of glycemic excursions (MAGE) ($p < 0.001$), and mean of daily differences (MODD) ($p < 0.001$) [17]. Increased oxidative stress is one of the risk factors for insulin resistance. ROS has also been reported to inhibit insulin signal transduction by activation of PKC and nuclear factor kappa B (NF-κB). Moreover, antioxidant biomarkers can also be evaluated assessing the total antioxidant capacity (TAC) or nonenzymatic markers like vitamins, and uric acid.

The exact role of oxidative stress in coronary and cerebrovascular damage is complex. Vascular function of endothelial cells is controlled by several vasodilators like Endothelium-derived NO (eNO). The eNO is the major chemical that regulates the vasodilation, platelet activation, leukocyte adhesion, and vessel wall permeability. Hyperglycemia and hyperlipidemia induce endothelial dysfunction through ROS production and the reduction of eNO. Total-ox/HDL values ratio, measured by the Fe-ROMs test, is associated with endothelial dysfunction and arterial stiffness and is a reliable biomarker for measuring oxidative stress, obesity, and chronic inflammation [15].

Steinberg and his colleagues suggested that oxidative modification of LDL is a critical initial event in the atherosclerosis generation [18]. Oxidized LDL has also the pro-inflammatory effects. OxLDL levels were higher in subjects with cardiovascular disease, and high level of OxLDL was associated with the severity of coronary disease [19]. OxLDL levels also is a good predictor for future coronary disease in healthy persons [20]. Although achieving significant increases in HDL levels, niacin failed to reduce the vascular events in the Heart protection Study2. It might be explained by a dysfunction of HDL [21].

The free radicals are produced excessively in ischemic and hemorrhagic stroke, and oxidative stress is one of main pathomechanisms of brain damage. Peters revealed an increase in superoxide radical anions in the penumbral region not only during the initial ischemic phase but also after reperfusion [22]. In acute ischemic stroke, MDA levels, thiobarbituric acid-reactive substances (TBARs), isoprostanes, and Cholesteryl ester hydroperoxides (CEOOH) were of higher levels in stroke patients than in controls at hospital admission and its levels did not change in the following 7 days. Furthermore, the levels of MDA and CEOOH also correlated with infarct size, clinical severity, and outcome [23, 24]. The level of oxLDL of patients with acute cortical infarction reached the highest peak on the third day and then rapidly disappeared [25]. Elevated levels of isoprostane and Oxygen Radical Absorbance Capacity were associated with PWI-DWI mismatch in patients with hyperacute ischemic stroke [26]. Increased activities of antioxidant enzymes after acute ischemic stroke remained elevated even at 3 months [27]. The increased intracranial pressure after hemorrhagic stroke and the release of vasoactive and toxic substances from hematoma can impair cerebral perfusion and can cause brain damages. Several studies in animal model showed an increase in free radicals but the related clinical data for hemorrhagic stroke is scanty.

Although the lipid peroxidation substances are non-specific and there is no commercial available biomarker for the clinical practice, these biomarkers might assess oxidative stress, disease severity, and effectiveness of antioxidants. Considering chemical stability in human fluids, lipid hydroxides might be more suitable than hydroperoxides. The level of some lipid peroxidation biomarkers may not increase in accordance with the disease severity or progress. So, the repeated measurement of multiple biomarkers at different time points could be recommended to overcome this limitation (Fig. 5.2).

5.3 Metabolomics

The metabolome means the total set of low-molecular-weight metabolites under a specific environmental conditions and can be the min to min profile of metabolite change to the environmental stimuli such as adaptive metabolic shift [28]. So, metabolomics is one of new technologies for the measurements of multiple metabolites in biological specimens to find new biomarker. The metabolite profiling in human is a comparatively small number of human metabolites (7000) compared to the numbers of genes (25,000), transcripts (100,000), and proteins (1,000,000). Compared to the genome, the metabolome is more dynamic and closer to the disease manifestation. The fact suggest that the metabolomics could be helpful in understanding of the molecular mechanism responsible for the disease progression. However, new high throughput metabolite profiling techniques has led to rapid progress to metabolome-wide approach in cerebrovascular disease. Metabolome profiling is performed commonly by two core methods. First tool is nuclear magnetic resonance (NMR), which separates metabolites by their magnetic resonance shift. The other one is mass-spectroscopy (MS), which is separated by mass/charge ratio. NMR needs small amount of sample and has low sensitivity. But it is not destructive to the sample even in vivo, with easy preparation and the results are quantitative, and reproducible. But NMR has low sensitivity and cannot evaluate the small amount of metabolite. MS is highly sensitive, but requires sample ionization by radiation or electron beams, which cause irreversible damage to sample. MS needs also time consuming preparation, and is not easy to quantify. Microdialysis is also performed for continuous measurement of molecules, including neurotransmitters, metabolites, and hormone in the extracellular fluid. Metabolome profiling can be "closed targeted" or "open non-targeted." The non-targeted method can measure as many metabolites as possible and require identification before data analysis. Targeted approach can measure the predefined metabolite profiles and can also quantify the substance [29]. NMR can identify the metabolite by comparison to spectral database of known reference compound and MS can confirm the metabolite by comparison to authentic standards substances. Matching m/z ratio and isotope abundance also help the identification of the molecule.

Metabolomics in cardiovascular studies were applied to coronary artery disease, cardiogenic shock, heart failure, risk of atherosclerosis dyslipidemia and diabetes mellitus, atrial fibrillation, and stroke. Although none of metabolomics has made it to diagnostic clinical practice, the metabolome discovered new metabolite-stroke associations related to excitotoxicity or neurotoxicity, oxidative stress, and inflammation [29, 30] (Table 5.2).

Excitotoxicity means the rapid release and inhibited reuptake of excitatory amino acid and neurotransmitter like glutamate. It is the first molecular mechanism of ischemic brain tissue injury as a result of energy failure. Oxidative stress is a downstream consequence of excitotoxicity. The free radicals increase lipid peroxidation, mitochondrial and DNA damage, protein nitration and oxidation. Matrix metalloproteinase-9 may be an important mediator of microvascular blood–brain barrier (BBB) injury and hemorrhagic transformation. Metabolites involved in one-carbon metabolism pathways such as homocysteine sulfinic acid and S-adenosyl homocysteine were associated with ischemic stroke. The metabolites associated with a perturbation of normal amino acid metabolism also appear like alanine, aspartate, and glutamate. Kelly et al. found that elevated plasma levels of F2IP, the marker of lipid peroxidation were correlated with plasma levels of MMP-9 in the acute stroke patients who received IV thrombolysis [31]. This results supports that oxidative stress may be followed by MMP activation, BBB injury, and hemorrhagic transformation.

Energy deficits after cerebral ischemia also evoked the upregulations of lactate and pyruvate and decrease the level of citrate and citric acid. The inflammatory response after ischemic stroke affects phospholipid metabolism like phosphatidylethanolamine (PE), phosphatidylcholine (PC), and lysophosphatidylcholine (LysoPC).

Table 5.2 Summary of metabolites and lipidome associated with acute ischemic stroke. Adapted from Journal of Stroke, Copyright Korean Stroke Society [29]

Metabolite	Excitotoxicity	Oxidative stress	Inflammation	Other
Increased	Glutamate Glutamine Phenylalanine Tyrosine Homocysteine Methionine Tryptophan Aspartate Alanine	Hypoxanthine, Lactate Pyruvate*, uric acid F2-isoprostanes matrix Metalloproteinase(MMP) Sphingosine 1-phosphate Homocysteine, Formate Glycolate, tetrahydrofolate Cysteine, glycine S-adenosylhomocystein Oxidized glutathione Dimethylarginine Short- and medium-chain acylcarnitines	Kynurenine Lysophosphatidylethanolamine (LysoPE) Phosphatidylserine Phosphatidylethanolamine (PE) Phosphatidylcholine (PC) Lysophosphatidylcholines Ceramides (16:0, 22:0, 24:0, and 24:1)	Carnitine N-acetylneuraminic acid Creatinine Hydroxyeicosatetraenoic acid, Hydroxyoctadecadienoic acid, Short- and medium-chain acylcarnitines Homocysteine sulfinic acid
Decreased	Glutamine Proline Pyroglutamate	Valine, isoleucine Citric acid, Dimethylamine Glycine, Hippurate Methanol Serine, stearic acid	Tryptophan, 3-indole propionic acid	Long chain fatty acids Hydroxyoctadecadienoic Hydroxyeicosatetraenoic acids Adenosine Phosphatidylcholine, Phosphoethanolamine, Sphingomyelin Free fatty acid

These are important signaling molecules with diverse biological function and related to backbone of neural membrane. One study found that low concentrations of specific LysoPC was associated with stroke recurrence and low level of some LysoPC was a potential biomarker for large artery atherosclerosis [32]. In ischemic stroke, three branched chain amino acids (isoleucine, leucine, and valine) are decreased but in other prospective study, the high level of these amino acids was associated with risk of ischemic stroke [33, 34]. In 2019, Ke et al. reviewed the previous papers and suggested that isoleucine, leucine, valine, glycine, lysine, glutamate, LysoPC(16:0), LysoPC(18:2), serine, uric acid, citrate, and palmitic acid may be potential biomarkers of ischemic stroke [35]. Interestingly, Sun et al. showed that a direct-infusion MS method, which requires only a few minutes, is helpful for early differential diagnosis of acute ischemic stroke from patients with vertigo [36]. For early differentiation of ischemic stroke from hemorrhagic stroke, Hu et al. identified several molecules like asparagine, citrulline, and leucine, which were increased in ischemic stroke, compared to intracranial hemorrhage [37]. Zhang et al. found 20-OH-LTB4 was a potential biomarker for intracranial hemorrhage (ICH) and can discriminate ICH patients from healthy people and the patients with acute ischemic stroke [38].

There are the contradictory findings of glutamate levels in cerebral ischemia, which may be explained, in part, by the fact that glutamate cannot cross the BBB. Unfortunately, there have been confounding results for alanine, glutamine, proline, carnitine, and creatinine in ischemic stroke. Metabolomics tools are not yet mature but metabolomics combined with genomic and proteomic would have great potential for clinical application in the near future.

5.4 Lipidomics

Lipids can be divided into eight categories including fatty acids, glycerolipids, glycerophospholipids, sterol lipids, sphingolipids, saccharolipids, prenol lipids, and polyketides. Lipidome means the total molecules of lipid in cells. Lipidomics is an emerging subfield of metabolomics and included the identification, quantification of the lipidome. The strategies of lipidomics are divided into targeted, untargeted, and shotgun ones. Like metabolomics, lipids can also be evaluated by MS imaging, shotgun, and liquid chromatography (LC)-based technologies. In lipidomics, liquid chromatography is applicable to a broad range of lipid species and the principal separation technique. LC separates and concentrates lipids simultaneously, based on their physico-chemical properties like carbon-chain length and the number of double bonds. The LC–MS can measure thousands of lipids with high sensitivity, in spite of low cost and very small sample volume. However, LC–MS cannot detect structural and positional isomers of lipid. LC-MS also have the low detection capability and measurement accuracy due to ion suppression. Shotgun technique directly infuses lipids into an electrospray ionization mass spectrometer (ESI/MS). Shotgun lipidomics separates and quantifies hundreds of lipids by electrospray ionization with relative simple operation and short run times. However, the method has lower sensitivity than LC-based methods. The application of NMR spectroscopy to lipidomics is limited because of low sensitivity.

Many lipid molecules have been known to be related to cardiovascular disease outcomes, which include ceramides (d18:1/16:0, d18:1/18:0, and d18:1/24:1), phosphatidylcholines with saturated(SFA) and mono-unsaturated (MUFA) fatty acyl chains, and Lysophosphatidylcholines [39]. Some lipid species increase the risk of ischemic stroke, which include ceramides, docosatrienoic acid, diacylglycerol, hydroxyeicosatetraenoic acid, lysophosphatidylcholines (LPC), hydroxyoctadecadienoic acid, LPC (20:4 and 20:5), and triacylglycerols (Table 5.2). The ischemic stroke patients showed the decreased level of linoleic acid, oleic acid, palmitic acid, stearic acid, free fatty acid, and LPC (16:0) [40]. The LPC (16:0 and 20:4), phosphatidylcholines (16:0/20:4 and 16:0/18:1), phosphatidyl ethanol amine s(18:1/18:0), and arachidonic acid can be a good predictor for

stroke recurrence [32, 40]. Purroy et al. also found that 11 molecules including lyso phosphatidic acid and cholesterol-related molecules, creatinine, threoninyl-threonine, and N-acetyl-glucosamine increased in the patients with acute ischemic lesions on Diffusion weighted images. The ischemic lesion volume was associated with ten molecules like lysophosphatidylcholine, hypoxanthine, and leucines. Lysophospholipids and creatinine clearly increased in the subjects with the subcortical DWI lesions [41].

Ding et al. found that in the patients with post-stroke depression, palmitic acid, oleic acid, and linoleic acid increased, although oxalate decreased, compared to post-stroke nondepression [42]. Zhang et al. also indicated the high level of azelaic acid, glyceric acid, and tyrosine in urine of the patients with post-stroke depression [43]. Glutamine, kynurenine, and LPC (18:2) are a good candidate of biomarkers for early diagnosis of post-stroke cognitive impairment [44]. For the discrimination between symptomatic and asymptomatic carotid plaque, metabolite related to the eicosanoid pathway, acylcarnitine species, and β-oxidation was identified for good biomarkers [45].

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Part III

Treatment of Dyslipidemia in Stroke Patients

Therapeutic Lifestyle Modification

6

Gyeongsil Lee, Yun Hwan Oh, Seulggie Choi, and Sang Min Park

Abstract

(*Dietary modification*) A healthy dietary pattern is associated with decreased TC, LDL-C, and TG levels. A dietary pattern with emphasis on vegetables, fruit, legumes, nuts, whole grains, and fish intake is recommended. Avoid trans fats and reduce saturated fatty acid intake as much as possible. Reduce dietary simple carbohydrates and replace them with monounsaturated fats or polyunsaturated fats. Avoid added sugar and sugar-sweetened beverages. Limit alcohol use.

(*Physical activity*) Physical activity significantly increases HDL-C levels and decreases TG levels. At least 150 minutes per week of moderate-intensity, or 75 minutes per week of vigorous-intensity aerobic physical activity. At least, avoid complete physical inactivity.

(*Smoking cessation*) No exposure to tobacco in any form. Cigarette smoking leads to lipid profile deterioration, including increased TC and TG levels as well as reduced HDL-C levels. Smoking cessation helps improve HDL-C levels to non-smoker levels as long as smoking cessation is maintained. Passive smoke exposure may lead to higher TC and TG levels. Smoking cessation is recommended for all smokers for improvement of their lipid profiles.

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6.1 Dietary Modification in Dyslipidemia

This section is primarily based on the 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease Lifestyle Factors Affecting Cardiovascular Risk and 2019 ESC/EAS Guidelines for the Management of Dyslipidaemias: Lipid Modification to Reduce Cardiovascular Risk [1, 2].

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6.1.1 Energy

Given that obesity, including abdominal obesity, contributes to dyslipidemia, total caloric intake should be reduced. Body weight reduction, even to modest (5–10% of basal body weight) degrees, among individuals with obesity improves lipid abnormalities [3]. Generally, a low-calorie diet with 500 kcal less than the usual intake is reasonable to adhere to as it has no particular harm to health [4].

6.1.2 Fats and Carbohydrates

In general, trans fats and saturated fats are regarded as detrimental factors and unsaturated fats are regarded as helpful factors on total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels with robust evidence [1, 2, 4]. The detrimental effect on cholesterol of dietary trans fats and saturated fats are similar. However, saturated fats could increase high-density lipoprotein cholesterol (HDL-C) levels, while trans fats could decrease them [5]. Given these effects on the lipid profile, trans fats intake should be avoided, and saturated fatty acid intake should be reduced as much as possible.

However, physicians should take into consideration upon nutrition education to patients "the nutritional balloon effect" in which excessive fat intake restriction may bring side effects of increased carbohydrates intake, which induce not only increasing triglyceride (TG) levels but also blood glucose levels [6]. For the general population, sugar-sweetened beverages should be used in moderation and should be limited drastically in individuals with visceral adiposity or elevated TG values [7–9]. An observational study from the Prospective Urban Rural Epidemiological (PURE) trial data demonstrated that saturated and unsaturated fat intake were associated with reduced stroke and mortality when used instead of refined carbohydrates [10].

6.1.3 Dietary Pattern

As foods are mixtures of diverse nutrients, attributing the health effects of food to only one of its components is not appropriate. For this reason, the studies on the effect of specific nutrients, including carbohydrates, protein, and fats on lipid profiles and atherosclerotic cardiovascular disease (ASCVD) outcomes, lack in concordance. To overcome this, nutrition research has recently focused on both the relationship between ASCVD and foods and dietary patterns, as opposed to single nutrients.

The Prevención con Dieta Mediterránea (PREDIMED) trial demonstrated that an extra-virgin olive oil or nuts-supplemented Mediterranean diet was associated with stroke reduction [5]. In the REGARDS (REasons for Geographic and Racial Differences in Stroke) trial, a dietary pattern characteristic of southern United States was identified as substantially increasing (30%) stroke risk [11]. This pattern consisted heavily of added fats, fried food, egg dishes, organ meats, processed meats, and sugar-sweetened beverages. A diet composed of juice and sweetened beverages, refined grains, potatoes/fries, and sweets may result in a higher coronary event risk compared to the increase seen with animal product consumption [12]. Given the additional risk these various food products are associated with, clinicians should warn individuals about their associated harm and recommend avoiding these foods when possible.

6.1.4 Alcohol

Given excessive alcohol consumption [>10 g/day (1 unit)] significantly increases TG, limit alcohol use [13].

6.1.5 Other Foods Patients Are Curious about

Dietary Fiber Largely present in wholegrain cereals (e.g., oats and barley), legumes, vegeta-

bles and fruits, dietary fiber (in particular the soluble type) has a hypocholesterolemic effect. Dietary fiber may be useful in maximizing the effects of the diet on LDL-C levels and minimizing the effects of a high-carbohydrate diet on other lipoproteins upon substitution with saturated fats [14, 15]. As both higher and lower proportions of carbohydrate diet is associated with increased risk of mortality, 45-55% of total energy intake should be composed of carbohydrate intake [10, 16].

Phytosterols While there may be a certain degree of heterogeneity among individuals, reduction of TC and LDL-C levels by 7-10% is possible upon daily consumption of 2 g of phytosterols [17]. Phytosterols modulate TC levels via direct competition for intestinal absorption with cholesterol. The principal phytosterols are sitosterol, campesterol, and stigmasterol. Phytosterols occur naturally in vegetable oils as well as in nuts, grains, legumes, and fresh fruits and vegetables to a smaller extent.

Coffee Previous studies on the association between coffee consumption and ASCVD has yielded mixed results, including detrimental and protective associations [18–20]. Recently, one cohort reported that no coffee consumption was associated with higher mortality risk compared to filtered brew, while unfiltered brew was also associated with higher mortality than filtered brew [21]. Compared to that of filtered coffee, the concentration of the lipid-raising cafestol and diterpenes kahweol is about 30 times higher [22]. Cafestol amount in filtered coffee is determined by the particle size of the ground roasted coffee as well as the porosity of the filter [23].

6.2 Physical Activity in Dyslipidemia

In general, aerobic exercise decreases TG levels while increasing HDL-C levels, with little changes to LDL-C concentration [24–27].

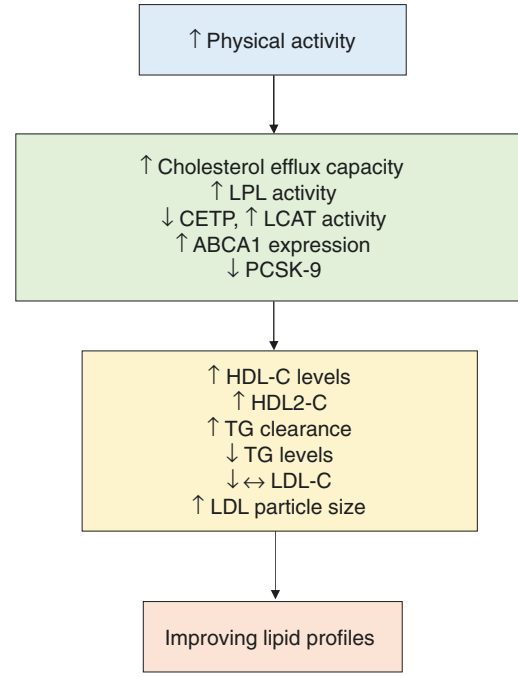

Fig. 6.1 Relationship of physical activity to lipid profiles. *LPL* lipoprotein lipase; *CETP* cholesterol ester transfer protein; *LCAT* lecithin cholesterol acyltransferase; *ABCA1* ATP-binding cassette transporter A-1; *PSCK-9* Proprotein convertase subtilisin/Kexin type 9; *HDL* high-density lipoprotein; *TG* triglyceride; *LDL* low-density lipoprotein

Possible relationship between physical activity and lipid profiles is described briefly in Fig. 6.1.

6.2.1 Effects of Physical Activity on HDL-C

Physical activity has the most significant effect on HDL-C levels as compared with any other lipoprotein [24]. Significantly higher HDL-C levels are observed in physically active men and women compared to those of physically inactive participants [28, 29]. Exercise intervention increased HDL-C by approximately 4.3 mg/dL [27]. The Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) Study reported individuals with normal serum lipid levels who participated in 20 weeks of exercise training showed

an increase of about 3.6% of HDL-C level across the entire group compared with baseline [30].

6.2.2 Effects of Physical Activity on TG

There is an inverse correlation between physical activity and serum TG level [24]. The physical activity volumes that elicit energy expenditures ≥1200 kcal during the week are most frequently associated with reduced serum TG levels [24]. In the case of men, the effect of exercise on TG is relatively greater than that of women [30]. One trial reported that exercise intervention decreases TG level by about 10–26% conversely, control group without exercise increase TG increases by about 18% [27].

6.2.3 Effects of Physical Activity on LDL-C

After several months of exercise intervention in dysplipidemic patients, there was no significant difference in LDL-C levels, but the small LDL particles are known as pattern B LDL decreased, and the overall size of the LDL particles increased [31]. A few months of aerobic exercise decreased the concentration of apolipoprotein B (apoB) in participants with hyperlipidemia [32]. But more studies about the relationship between exercise and apoB are needed because of other controversial studies [30].

6.2.4 Mechanism behind the Effects of Physical Activity on Lipid Profile

Exercise appears to reduce plasma lipid levels by improving the ability of the skeletal muscle to utilize lipids as opposed to glycogen [33]. This mechanism is thought to be involved in the increase of lipoprotein lipase (LPL) activity. Significantly increased plasma LPL activity and subsequently higher levels of LPL-mediated TG hydrolysis were observed upon prolonged aerobic exercise [34]. "Reverse cholesterol transport" describes the process of cholesterol removal. Upon exercise, increases in lecithin cholesterol acetyltransferase (LCAT) and reductions in cholesterol ester transfer protein (CEPT) results in the removal of cholesterol from circulation for disposal [35]. The ability of the muscle to oxidize fatty acids originating from plasma, including very low-density cholesterol (VLDL-C) or TG, is enhanced by the increased LCAT and CETP enzymatic activities.

Increased expression of ATP-binding cassette transporter A-1 (ABCA1) in macrophages is another important mechanism for explaining exercise effect on lipid metabolism. Increased ABCA1 has a strong effect on reverse cholesterol transport (RCT), and increasing in HDL-C level [36]. A previous study found that exercise significantly increased ABCA1 mRNA expression after exercise, and also regardless of exercise intensity [37].

6.2.5 Physical Activity Suggestion for Stroke Prevention

The 2019 AHA guideline provides more detailed guidelines on improving physical activity [38]. First of all, the level of physical activity must be regularly assessed and promoted by health care providers. This is because physical inactivity is still pandemic despite various studies showing that physical activity reduces CVD risk [39]. A strong dose-responsive relationship between the amount of physical activity and the risk of CVD exists [40]. The general principles related to the promotion of physical activity are as follows. At least 150 minutes per week of accumulated moderate-intensity aerobic or 75 minutes per week of vigorous-intensity aerobic physical activity (or an equivalent combination of moderate and vigorous activity) is recommended for all adults to lower cardiovascular disease (CVD) risk. About 10 minutes of short bout exercise is also effective if the accumulative amount of exercise per week is enough [41]. If someone cannot achieve the minimum recommended amount, at least a little amount of moderate-to-vigorous

physical activity is still beneficial to reduce CVD risk [42]. The proverb is still applied that less is better than none.

Prolonged sedentary behavior such as sitting and reclining has become our new concern. According to several reports, the prolonged sedentary time has deleterious effects on CVD [40]. Although there is not enough evidence to recommend specific maximum thresholds for sedentary behavior, everyone, irrespective of their age or abilities, should try to limit their daily sedentary time and replace it with physical activity of any intensity [43]. Before participating in exercise, the American College of Sports Medicine (ACSM) recommends preparticipation screening tests and medical clearance for individuals with multiple risk factors or established CVD [44].

6.3 Smoking Cessation in Dyslipidemia

6.3.1 Cigarette Smoking and Lipid Profiles

Extensive literature demonstrates that cigarette smoking is associated with deteriorating lipid profiles [45, 46]. It has been shown that cigarette smoking may lead to increases in TC, TG, and VLDL-C levels, as well as decreases in HDL-C and apolipoprotein A1 levels (summary of possible effects of cigarette smoking on lipid profile regulation in Fig. 6.2 [45]). Smoking may reduce the activity of LPL at the skeletal muscle, an enzyme that contributes to the metabolism of TG [47, 48]. Upon decreased levels of skeletal muscle LPL, slower metabolism of TG-rich lipoproteins such as chylomicrons and VLDL-C may lead to impaired TG clearance, ultimately resulting in higher TG levels among smokers [48].

Interestingly, LDL-C levels are not as affected by smoking compared to other lipoproteins [45].

Despite this, smoking may nonetheless have negative impacts on LDL via contributing to the reduction of LDL size to smaller, denser particles, which are more prone to being lodged in arterial walls [49]. Moreover, the tendency of LDL particles to induce immune response after it has been lodged in the arterial intima may be facilitated by smoking-related free radicals that cause lipid peroxidation [46]. Ultimately, smaller LDL particles due to smoking may lead to lipid peroxidation-mediated immune response and subsequently initiate atherosclerotic plaque development in the arterial wall.

Smoking also reduces LCAT activity, a key enzyme in esterifying free cholesterol and moving esterified cholesterol into the HDL core [50]. The reduction in esterified cholesterol movement into the HDL core due to smoking ultimately results in lower excretion and catabolism rates of cholesterol by the liver. Furthermore, smoking can also disrupt activities of hepatic lipase (HL) or CETP levels as well [51–53]. Since HL, CETP, and LCAT are key enzymes involved in the metabolism of HDL-C, the existing evidence

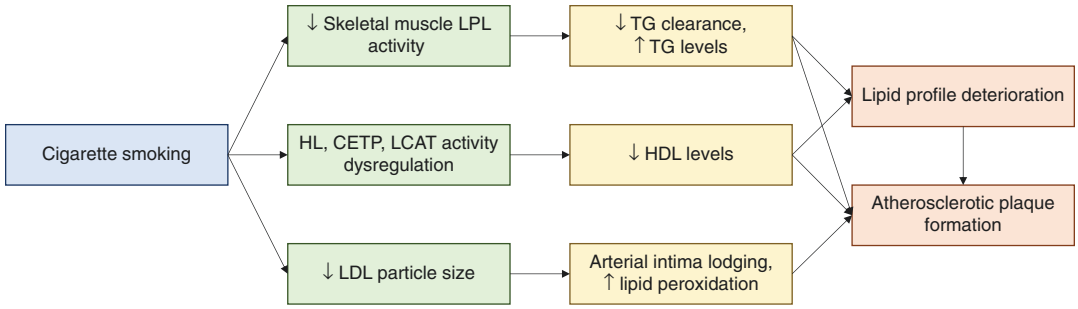

Fig. 6.2 Schematic diagram of possible effects of cigarette smoking on lipid profile regulation. Acronyms: *LPL* lipoprotein lipase; *TG* triglyceride; *HL* hepatic lipase; *CETP* cholesterol ester transfer protein; *LCAT* lecithin cholesterol acyl-transferase; *HDL* high-density lipoprotein; *LDL* low-density lipoprotein

suggests that alterations in HDL-related enzyme due to smoking activity contributes to reduced HDL-C levels among smokers.

6.3.2 Changes in Lipid Profiles upon Smoking Cessation

As cigarette smoking may lead to the disruption of lipid profiles, smoking cessation is recommended for the improvement of lipid metabolism [54]. A meta-analysis of 27 studies has shown that smoking cessation may lead to increased HDL-C levels [55]. Interestingly, other lipoproteins, including LDL-C and TG levels, do not appear to be altered upon smoking cessation [55, 56]. Upon smoking cessation, improvements in HDL-C levels can be expected in as early as 17 days, and HDL-C levels will continue to improve towards non-smoking levels upon maintained smoking cessation [57–59].

Nicotine replacement therapy (NRT) such as nicotine patches are often used in order to aid the smoking cessation process. In contrast to other previous studies that do not demonstrate a correlation between NRT and lipid profiles, one study suggests that NRT may hinder the HDL-C normalization process upon smoking cessation [60]. This impedance of HDL-C level normalization due to NRT persists only when the nicotine patch is used, and HDL-C levels increase upon removal of the patch as long as smoking cessation is maintained [60]. Therefore, while NRT may hinder HDL-C recovery, smoking cessation is recommended even with NRT use as a means of successful smoking cessation under the assumption that NRT is temporary.

6.3.3 Other Forms of Cigarette Smoke Exposure: Passive Smoking and Electronic Cigarettes

Passive smoking has been shown to be associated with higher levels of TC and TG [61]. Moreover, passive smoke exposure was associated with higher TC:HDL ratios and non-HDL-cholesterol levels [61]. With the increasing popularity of electronic cigarettes, interest grows to what extent electronic cigarette smoking may lead to lipid profile alterations. Preliminary data from the Cardiovascular Injury due to Tobacco Use (CITU) study demonstrate that electronic cigarette use was associated with higher TC, TG, and LDL-C levels compared to non-smokers [62]. In contrast, another study failed to depict any changes in lipid profiles between non-smokers and electronic cigarette smokers [63]. Taken together, while some evidence supports electronic cigarette-mediated lipid profile alterations, further studies are needed.

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Statins

7

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Abstract

Statins, also known as β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, are a class of drugs frequently prescribed for lowering cholesterols. The statins have been used for more than 30 years for the prevention of stroke and coronary artery disease. Their primary mechanism of action is via inhibition of the mevalonate pathway, resulting in a decrease of cholesterol and isoprenoid synthesis. Reduction of cholesterol synthesis enhances the uptake of extracellular low-density lipoprotein cholesterol (LDL-C) via upregulation of LDL-C receptors. The inhibition of isoprenoid synthesis results in the so-called pleiotropic effects of statins, including anti-inflammatory action, antioxidant effect, improvement of endothelial function, prevention of platelet aggregation, plaque stabilization, and regression of atherosclerosis. There are now seven commercially available statins, including Rosuvastatin, Atorvastatin, Simvastatin, Fluvastatin, Pravastatin, Lovastatin, and Pitavastatin. The key pharmacological properties of each statin are slightly different according to their solubility and chemical features. As for their implications on stroke trials, the association between pre- and post-stroke statins on stroke-related outcomes is not always consistent. Nevertheless, statins reduced the risk of stroke by 24.5–48%.

Lipid-lowering agents include several classes of medications such as β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase inhibitor known as statin, cholesterol absorption inhibitor, fibric acid derivative, bile acid sequestrant, and nicotinic acid. These drugs differ in mechanism of action and common side effects and the type and degree of lipid reduction. In this chapter, we reviewed the history of statin development, mechanism of action, and drug characteristics of each statin. We also described the pleiotropic effects of statin other than lowering lipid levels on cardiovascular outcomes. Lastly, we summarized the implication of statin administration on several stroke-related outcomes.

7.1 History of Statin

Since the Seven Country Studies and the Framingham Heart Study in the 1950s, the causal relationship between high cholesterol levels and coronary artery diseases (CAD) had been

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elucidated [1, 2]. Further researches regarding this "lipid hypothesis" revealed that the CAD was mainly attributed to low-density lipoprotein cholesterol (LDL-C), while high-density lipoprotein cholesterol (HDL-C) showed an inverse correlation with severity and mortality of CHD [3]. Since then, multiple groups have endeavored to develop medications aiming to reduce the level of LDL-C and possibly elevate the level of HDL-C.

The first two HMG-CoA reductase inhibitors were discovered in 1971 and 1973 by a Japanese biochemist, Dr. Akira Endo. His hypothesis was driven by the fact that the fungi use ergosterol, not cholesterol, for the synthesis of the cell wall, unlike bacteria. He proposed that fungi survive by producing a chemical that inhibits HMG-CoA reductase hence damaging the cell wall of nearby bacteria. He tested more than six thousand fungi and eventually discovered citrinin and compactin, latter from the fungus *Penicillium citrinum* [4, 5]. Though Citrinin strongly inhibited HMG-CoA reductase and lowered serum cholesterol levels in rats, further development was suspended due to its renal toxicity. Compactin has demonstrated lipid-lowering effect in both animal and human trials, but commercialization was discontinued because compactin caused lymphoma in dogs that received very high doses [6–8]. Later in 1978, Alfred Albert and other researchers from Merck discovered a statin called lovastatin (a.k.a Mevinolin) in a fermentation broth of *Aspergillus terreus* [9]. After several animal and clinical trials, lovastatin demonstrated significant activity in lowering plasma LDL cholesterol without serious adverse reactions [10]. Eventually, lovastatin became the first statin to have gained U.S. FDA approval in 1987.

After the first commercialized statin came out to the market, six statins including 4 synthetic and 2 fungal-derived statins have been introduced [11]. The next statin after lovastatin was its fungal derivative, simvastatin, in 1988 by Merck followed by pravastatin (fungal-derived) by Sankyo in 1989, fluvastatin (synthetic) in 1994, atorvastatin (synthetic) in 1997, rosuvastatin (synthetic) and pitavastatin (synthetic) in 2003 [8]. Recently, the most commonly prescribed statins worldwide are simvastatin and atorvastatin, followed by pravastatin and rosuvastatin [12]. The molecular structures of the different statins are demonstrated in Fig. 7.1.

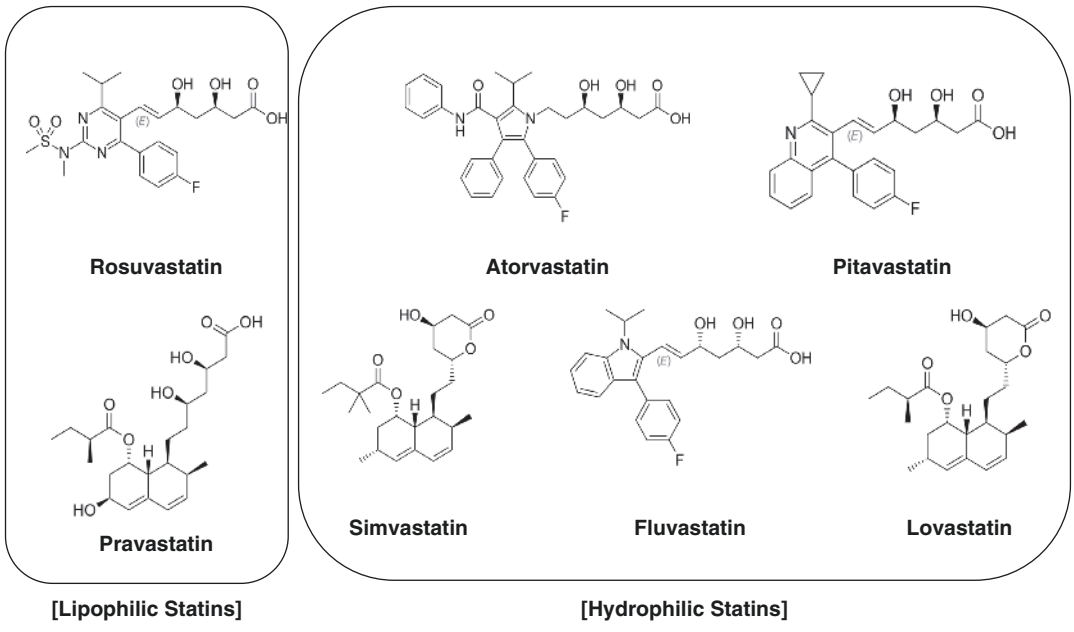

Fig. 7.1 Chemical structures of commercially available statins according to the solubility

7.2 Mechanism of Action

The mevalonate (MVA) pathway, also referred to as the cholesterol-isoprenoid bio-synthetic pathway, is a crucial metabolic pathway expressed in all mammalian cells (Fig. 7.2). The MVA pathway mainly produces cholesterols, as well as other important end-products that are potentially related to the pleiotropic effects of statins, which will be elaborated later in this chapter.

Among the whole pathway represented in Fig. 7.2, HMG-CoA reductase is the key and rate-limiting enzyme and is responsible for the transformation from HMG-CoA into MVA. As statins are structurally similar to HMG-CoA, they competitively and reversibly inhibit the HMG-CoA reductase by occupying an active site with their side chains and lactone ring. This competition reduces the rate of conversion from HMG-CoA into mevalonate, and eventually decreases the synthesis of cholesterol [13]. Reduction of cholesterol synthesis causes the hepatic cells to enhance the uptake of extracellular LDL cholesterol via activating a protease that cleaves sterol regulatory element-binding proteins (SREBPs) from the endoplasmic reticulum (ER). SREBPs are transcription factors that are embedded in the ER in inactive forms. The SREBPs translocate to the nucleus via interaction with the SREBP cleavage activating protein (SCAP) when intra-cellular sterol content is low. Then, SCAP escorts SREBPs to the Golgi apparatus and are proteolytically cleaved. The cleaved fragments migrate into the nucleus and upregulate the expression of the LDL receptor gene. As LDL receptor expression is elevated, endocytosis of LDL and very low-density lipoprotein (VLDL) is enhanced and eventually lowers serum LDL and triglyceride levels [14].

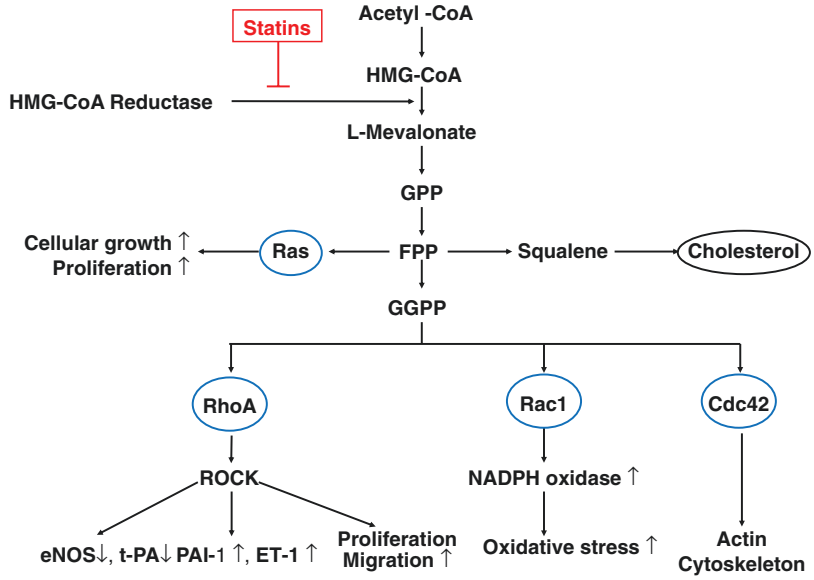

Fig. 7.2 The Mevalonate Pathway that shows the mechanism of statins on cholesterol and isoprenoid synthesis. Inhibition of the farnesylation of Ras and geranylgeranylation of Rho and Rac family leads to modulation of various pathways resulting in the pleiotropic effects of the statins. *CoA* coenzyme A, *HMG-CoA* β-hydroxy β-methylglutaryl Coenzyme A, *GPP* geranyl pyrophosphate, *FPP* farnesyl pyrophosphate, *GGPP* geranylgeranyl diphosphate, *ROCK* Rho-kinases, *NADPH* Nicotinamide adenine dinucleotide phosphate, *eNOS* endothelial nitric oxide synthase, *t-PA* tissue plasminogen activator, *PAI-1* plasminogen activator inhibitor-1, *ET-1* endothelin 1

7.3 Types of each Statin

7.3.1 Pharmacological Properties of Statin

Statins are classified into lipophilic and hydrophilic groups according to the tissue selectivity. Hydrophilic statins are specific to hepatic cells, while lipophilic statins are broadly distributed in different tissues [15]. Because hydrophilic statins use carrier-mediated mechanisms for uptake, they are known to have reduced non-lipid effects on extrahepatic tissues, meaning less pleiotropic properties [15]. Rosuvastatin and pravastatin are relatively hydrophilic due to its methane sulfonamide group, while atorvastatin, simvastatin, lovastatin, fluvastatin, and pitavastatin are rather lipophilic due to polar hydroxyl group [16, 17].

The key pharmacological properties of each statin are summarized in Table 7.1. All statins are absorbed rapidly, reaching peak serum concentration within 1–5 h. Since the rate of cholesterol synthesis is highest in the evening, statins with relatively shorter half-lives (simvastatin, pravastatin, fluvastatin, and lovastatin) are best administered in the evening or bedtime. Meanwhile, statins with long half-lives (atorvastatin, rosuvastatin, and pitavastatin) can be administered at any time of the day. Because of long half-lives, atorvastatin (14 h), rosuvastatin (19 h), and pitavastatin (12 h) have higher efficacy for lowering LDL cholesterol compared to other statins [15].

Drug intake during a meal has variable effects on the absorption of statin. While lovastatin absorption is increased with food intake, the bioavailability of pravastatin and pitavastatin is decreased. However, the overall lipid-lowering effect of the currently available statins does not appear to be affected with respect to food intake [18]. Most of the statins have generally low bioavailability, meaning extensive intestinal endothelial absorption and first-pass metabolism. Given that the first-pass metabolism of statin occurs in the liver and the liver itself is the target organ, initial uptake may be more important than high bioavailability for the lipid-lowering effect of statin [15]. Except for pravastatin, all statins are substantially bound to serum proteins in the blood circulation, leading to less systemic exposure in their active state [19]. Though unbound pravastatin level is relatively higher than other statins, its hydrophilic nature prevents its extensive tissue distribution [20].

Statins are primarily metabolized by the Cytochrome P450 (CYP), composed of more than 30 enzymes [21]. Simvastatin, lovastatin, and to a lesser extent, atorvastatin are extensively metabolized by the CYP3A4 isoenzyme. Thus, the risks of serious side effects including muscle injury are elevated with concurrent use of drugs that interfere with CYP3A4 via elevation of the plasma level of aforementioned statins. Rosuvastatin and fluvastatin are chiefly metabolized by the CYP2C9 isoenzyme, and pitavastatin is minimally metabolized by the CYP2C9. Thus, pravastatin may be preferred for patients using multiple medications [22]. The majority of statins are predominantly eliminated via the bile by the liver [23]. Thus, the administration of statins in patients with hepatic impairment may elevate the risk of statin-induced myopathy. Rosuvastatin, however, is known to maintain its pharmacological properties in patients with mild to moderate hepatic failure [24].

7.3.2 Efficacy of each Statin on Lipid Profile

Statins are primarily prescribed in the treatment of hypercholesterolemia and for the prevention of cardiovascular diseases. Statins are the most efficient drugs for lowering the level of LDL-C, with maximal reductions up to 63 percent [25]. The intensity of statin therapy can be divided into three groups: high-intensity, moderate-intensity, and low-intensity (Table 7.2). The high-intensity groups consist of 40–80 mg of atorvastatin and 20–40 mg of rosuvastatin. Although 80 mg of simvastatin poses high intensity, it is not recommended by the FDA due to the increased risk of myopathy and rhabdomyolysis. Rosuvastatin is more potent than atorvastatin in general, and both statins are significantly more potent than simvastatin, pravastatin, lovastatin, and fluvastatin [26]. The moderate-intensity groups include 10–20 mg

Table 7.1 Pharmacological properties of the statins

	Atorvastatin	Simvastatin	Rosuvastatin	Pravastatin	Pitavastatin	Fluvastatin	Lovastatin
LDL-C reduction	38–54%	28–41%	52–63%	19–40%	31–41%	17–33%	29–48%
Optimal time of dosing	Any time of day	Evening	Any time of day	Evening	Any time of day	Evening	Evening
Effect of food	No effect	No effect	No effect	Bioavailability decreased	Bioavailability decreased	No effect	Increased absorption
Bioavailability (%)	14	5	20	17	43–51	24	5
Tmax(h)	1–2	4	3–5	1–1.5	1	<1	2–4
Solubility	Lipophilic	Lipophilic	Hydrophilic	Hydrophilic	Lipophilic	Lipophilic	Lipophilic
Protein binding (%)	>98	95	88	50	99	98	>95
CYP hepatic enzyme	3A4	3A4	2C9	None	2C9(2C8 minor)	2C9(2C8, 3A4 minor)	3A4
Pro-drug	No	Yes	No	No	No	No	Yes
Active metabolites	Yes	Yes	Minimal	No	No	No	Yes
Renal excretion (%)	<2	13	10	20	15	5	10
t1/2(h)	14	2	19	1.8	12	3	2–3

CYP Cytochrome P450, *Tmax* Time until maximum serum concentration achieved, *t1/2* drug half life

Table 7.2 Statin classifications according to the intensity of LDL-C reduction

	High-Intensity	Moderate-Intensity	Low-Intensity
LDL-C reduction	≥50%	30–49%	<30%
Statins	**Atorvastatin 40–80 mg**	**Atorvastatin 10**-20 mg	
	Rosuvastatin 20–40 mg	**Rosuvastatin** 5–**10 mg**	
		Simvastatin 20–40 mg	Simvastatin 10 mg
		Pravastatin 40–80 mg	**Pravastatin 10–20 mg**
		Lovastatin 40–80 mg	**Lovastatin 20 mg**
		Fluvastatin XL 80 mg	Fluvastatin 20–40 mg
		Fluvastatin 40 mg BID	
		Pitavastatin 1–4 mg	

LDL-C Low-density lipoprotein cholesterol
Specific statins and doses with bold font indicate that they were evaluated in randomized clinical trials

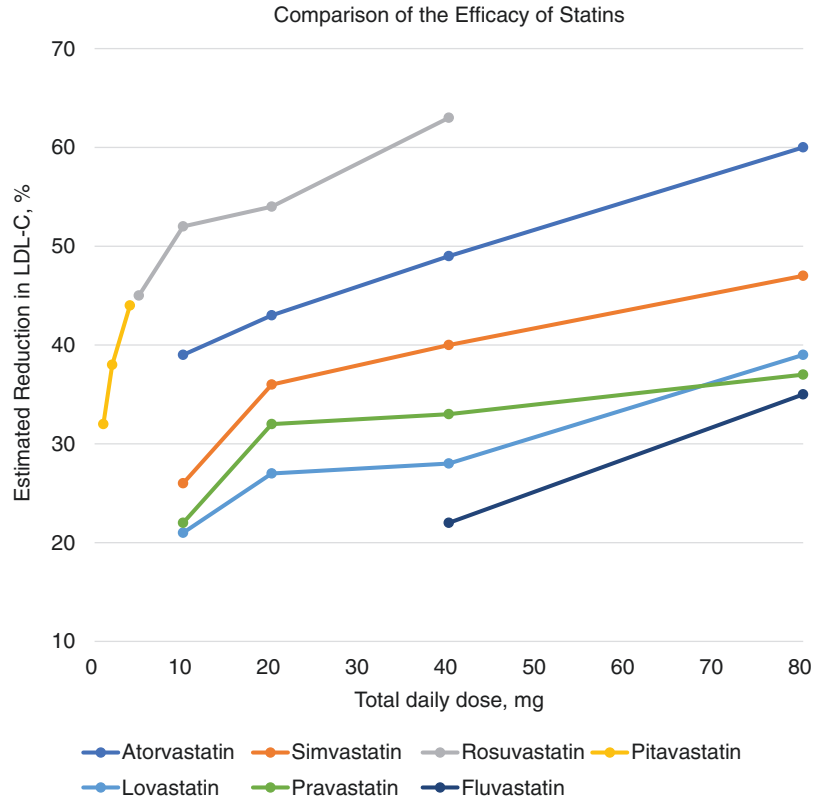

Fig. 7.3 Comparison of the percent reduction in serum LDL-C with each statin at its variable doses. *LDL-C* Low-density lipoprotein cholesterol

of atorvastatin, 5-10 mg of rosuvastatin, 20–40 mg of simvastatin, and maximal doses of pravastatin, lovastatin, and fluvastatin. They are known to reduce the LDL-C level up to 30–49%. The low-intensity group reduces less than 30% of LDL-C and includes 10 mg of simvastatin, 10–20 mg of pravastatin, 20 mg of lovastatin, and 20–40 mg of fluvastatin. The intensity of statin therapy is determined according to the age, degree of atherosclerotic cardiovascular disease (ASCVD) risks, and the level of serum LDL-C [27]. The percent reduction in serum LDL-C with each statin at its variable doses is described in Fig. 7.3.

Statins are also effective in reducing the level of TG in a dose-dependent manner, though the observed magnitude of percent reduction is smaller than that of LDL-C. Of them, rosuvastatin and atorvastatin are more effective than other statins [25]. Statins also alter HDL-C levels to varying degrees, typically by raising them. Unlike its effects on LDL-C and TG, statins do not always increase HDL levels in a dose-response relationship. For example, rosuvastatin and simvastatin raise HDL-C in a dose-dependent manner, but a higher dose of atorvastatin attenuates its effect on HDL-C [28]. In some patients, HDL-C may even decline with statin therapy. The observed variable effects of statin may be attributed to their effects on hepatic microRNA33 induction via decreasing ATP-binding cassette transporter A1 expression [29].

7.3.3 Side Effects of Statins

Adverse effects occur less frequently with statins compared to other classes of lipid-lowering agents. Most concerning adverse events include myotoxicity ranging from myopathy, myositis to rhabdomyolysis, hepatic dysfunctions, and possible new-onset diabetes [30–32]. A detailed and insightful description of side effects and drug interaction of statins will be discussed in Chap. 17.

7.4 Pleiotropic Effects of Statin on Cardiovascular Outcomes

7.4.1 Mechanism of Action of Pleiotropic Effects

Aside from LDL-C lowering properties, statins also exert pleiotropic effects via LDL-C independent manners [33]. As illustrated in Fig. 7.2, inhibition of mevalonate synthesis by statins not only reduces the synthesis of cholesterol but also prevents the production of isoprenoid intermediates [34]. Inhibition of isoprenoid production may exert pleiotropic effects including anti-inflammatory action, antioxidant effect, improvement of endothelial function, prevention of platelet aggregation, plaque stabilization, and regression of atherosclerosis.

Isoprenoid intermediates including farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) act as lipid attachments for the modification of heterotrimeric G proteins such as Ras and Rho family. Ras and Rho are small signaling GTP-binding proteins, involved in the regulation of cell proliferation, differentiation, migration, and cytoskeleton, and are found in nearly all eukaryotes [35]. Ras and Rho translocation is dependent on farnesylation and geranylgeranylation, respectively. Rho family is composed of RhoA, Rac1, and Cdc42 which exerts different cellular functions.

GGPP decrement by statin prevents geranylgeranylation of Rho and eventually reduces its activation of Rho kinase (ROCK). ROCKs are serine/threonine kinases that mediate the downstream event of Rho GTPases [36]. ROCK activity is a marker of cardiovascular disease, affecting endothelial cells, fibroblasts, inflammatory cells, vascular smooth muscle cells (SMC), and cardiomyocytes that induce atherosclerosis and cardiac remodeling. In vivo study showed that ROCK inhibitor (fasudil and Y27632) had cardiovascular effects similar to statins, by reducing cardiac fibrosis, hypertrophy, and pathological remodeling [37]. Statin administration has also demonstrated reduced ROCK activity in humans with coronary artery disease independent of LDL-c reduction [38]. Among the Rho GTPase subfamily, Rac1 modulates critical functions on tight junction and adherence junction integrity [39]. Rac1 increases the production of reactive oxygen species (ROS) by activation of nicotine amide adenine dinucleotide phosphate (NADPH) oxidase. ROS in turn leads to cardiac remodeling and left ventricular hypertrophy. ROS also oxidizes LDL-C, which mediates foam cell formation and eventually causes atherosclerosis [40]. Multiple in vivo studies showed that statins reduce the activity of Rac1 and NADPH oxidase, and the formation of ROS, which may explain a part of the pleiotropic effects of statin [41, 42].

Aside from the isoprenoid synthesis pathway, statins also have been shown to activate peroxisome proliferator-activated receptors (PPARs) [43]. Statins reduced lipopolysaccharide-related inflammation in wild-type mice while not in PPARα-Knock out mice [44]. Statins increase PPAR-γ activity and prevent lipopolysaccharide-induced tumor necrosis factor-α and chemotactic protein-1 activity of monocytes, leading to anti-inflammation [45]. Atorvastatin has been shown to reduce fibroblast proliferation and cardiac fibrosis which was reversed with PPAR-γ antagonist [46]. By augmenting the mRNA expression of the PPAR-γ, statins also reduced ROS production [47].

7.4.2 Pleiotropic Effects of Statins on Atherosclerosis

Atherosclerosis is a chronic inflammatory process in the intima of the vascular wall. The process is initiated by an excessive level of LDL-C and mediated by endothelial dysfunction, activated macrophages, T and B lymphocytes, and SMCs [48]. Statins exert an anti-inflammatory process by reducing inflammatory cytokines such as interleukin-6, interleukin-9, and monocyte chemotactic protein-1. Both the innate and adaptive immune responses are modulated by statins [49]. In the adaptive immune system, T cell differentiation is reduced by statin in a geranylgeranylation-dependent manner which may improve plaque stability [50].

Statins also alleviate endothelial dysfunction resulting from atherosclerosis by upregulation of endothelial nitric oxide synthase (eNOS) expression. Endothelial nitric oxide (NO) is crucial for vasodilation, platelet aggregation, endothelial–leukocyte interactions, and vascular smooth muscle proliferation [51]. Statins increase endothelial NO production via inhibition of geranylgeranylation of Rho and Rock signaling. Statins decrease the interaction between leukocytes and endothelial cells that occurs in the atherogenic process. Statins decrease monocyte adhesion to endothelial cells by inhibiting the clustering of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1. Vascular SMCs proliferation is also important in atherogenesis [52]. Statin administration reduces platelet-derived growth factor-induced DNA synthesis in SMCs by inhibition of the Rho pathway. In a study with simvastatin, statin reduced SMC proliferation, leukocyte accumulation, and eventually decreased intimal thickening [53].

7.4.3 Pleiotropic Effects on Cardiovascular Outcomes

We demonstrated that most of the pleiotropic effects of statins are derived from the inhibition of the mevalonate pathway and the subsequent reduction of downstream events of Ras and Rho GTPase activation. However, in clinical trials, it is difficult to separate the lipid-lowering effects from potential pleiotropic effects due to a strong association between elevated LDL-C and cardiovascular outcomes [54]. Furthermore, as inhibition of cholesterol synthesis and isoprenoid synthesis are combined effects of statins, independent assessment of pleiotropic effects aside from lipid-lowering effects is difficult. Besides, since clinical trials require standard therapy in the control arm, mostly statin, quantification of independent pleiotropic effects is often not allowed.

Despite these limitations, several studies have tested the concept of anti-inflammatory function of statins. Rosuvastatin to Prevent Vascular Events in Men and Women With Elevated C-Reactive Protein (CRP.JUPITOR) compared rosuvastatin and placebo for patients with an LDL-C less than 130 mg/dL and CRP more than 2.0 mg/L. Rosuvastatin administration decreased LDL-C by 50%, CRP by 37%, and vascular event rate by 44% [55]. The reduction of vascular event was greater than expected benefit based on LDL-C reduction from the regression line of Cholesterol Treatment Trialists' (CTT) Collaboration, implicating an anti-inflammatory property of statin [56]. The Myocardial Ischemia Reduction With Aggressive Cholesterol Lowering (MIRACL) trial also showed anti-inflammatory effects of atorvastatin by reducing CRP by 83% [57].

Studies comparing statin and non-statin lipid-lowering agents with clamped cholesterol designs also revealed some pleiotropic effects. Ezetimibe, an inhibitor of intestinal cholesterol absorption, has been used for this purpose. For example, a heart failure study comparing simvastatin 10 mg and ezetimibe 10 mg found approximately 15% reduction in LDL-c for both groups but only simvastatin showed improvement of flow-dependent vasodilation, increased superoxide dismutase, and increased endothelial progenitor cells [58]. Several studies comparing high dose statins and combination of low dose statins and ezetimibe revealed greater improvement in vascular inflammation and endothelial function, despite the similar reduction of LDL-C in both groups [59, 60]. These findings represent potential pleiotropic effects of statin on ischemic stroke.

Proprotein convertase subtilisin-kexin type9 (PCSK9) inhibitor, which has a lipid-lowering mechanism similar to that of statin, does not inhibit the mevalonate pathway. Thus, PCSK9 inhibitors do not reduce the level of CRP, interleukins, or tumor necrosis factor-α which are markers of inflammation [61]. As PCSK9 inhibitors exert high efficacy in LDL-C reduction, further studies comparing high dose statin and PCSK9 inhibitors may provide us with evidence regarding the pleiotropic effects of statins at their high doses.

7.5 Implication of Statin on Stroke Trials

Although elevated LDL-C is a well-defined risk factor for ischemic stroke in many clinical trials and observational studies, the relationship is not always consistent compared to coronary artery disease [62]. Nevertheless, statins reduced the risk of stroke by 24.5% in the Heart Protection Study and 48% in the JUPITER trial [63, 64]. Atorvastatin showed its effectiveness for secondary prevention of stroke in the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial [65]. In this section, we reviewed the effects of statin on multiple stroke-related outcomes. Major randomized clinical trials including SPARCL, 4S, and LIPID will be discussed separately in Chap. 15.

7.5.1 Effects of Prestroke Statins

Several studies have revealed that statin administration before stroke was significantly associated with milder initial stroke severity, better functional outcome at 3 months, and lower short-term mortality. A meta-analysis pooled 7 studies involving 6806 patients and showed that prestroke use of statins was associated with mild stroke severity at stroke onset (odds ratio (OR), 1.24; 95% confidence interval (CI), 1.05–1.48). The neuroprotective effects of statins on stroke may be explained by animal studies and imaging clinical trials which demonstrated less infarct volume and better leptomeningeal collaterals with statin administration [66, 67].

Regarding functional outcomes, a pooled meta-analysis with 30,942 patients showed that prestroke statin was associated with good functional outcome (OR, 1.50; 95% CI, 1.29–1.75) [68]. Another meta-analysis with ischemic stroke demonstrated that prestroke statin was significantly associated with good functional outcomes in small vessel occlusion and large artery atherosclerosis, but not in cardioembolic stroke [69]. Prestroke statin was also associated with lower 30-day and 90-day mortality. A meta-analysis with 4508 patients showed that prestroke statin was significantly associated with lower mortality (OR, 0.42; 95% CI, 0.21–0.82) [68].

7.5.2 Effects of Post-Stroke Statins

Statin administration after hospitalization due to stroke was also associated with better functional outcomes and lower short-term mortality. A meta-analysis with 37,153 patients revealed that in-hospital statin administration was significantly associated with better functional outcome (OR, 1.31; 95% CI, 1.12–1.53) [68]. Regarding short-term mortality, a meta-analysis of observational studies with 20,681 patients showed that post-stroke statin was significantly associated with

less mortality (OR, 0.41; 95% CI, 0.29–0.58) [68]. Beside less initial stroke severity and better functional outcome, prevention of stroke recurrence by statins may account for the benefit on stroke-related mortality. However, in a meta-analysis including 7 randomized clinical trials with 431 patients with acute ischemic stroke or TIA, all-cause mortality was not different between the statin and placebo groups (OR, 1.51; 95% CI, 0.60–3.81) [70].

7.5.3 Effects of Statins in Patients with Thrombolysis

The association between prestroke statin and the outcome of thrombolytic therapies after stroke has been inconsistent throughout the studies. Three earlier meta-analyses showed that statin was not associated with good functional outcome after thrombolysis. However, recent studies including one meta-analysis with 10,876 patients showed that prestroke statin was significantly associated with good functional outcomes after thrombolysis (OR, 1.44; 95% CI, 1.10–1.89) [68, 71–73]. As for mortality, the results have been even more controversial. A meta-analysis showed that statin was associated with a higher incidence of mortality, while other studies have shown non-significant association. As of now, only THRaST (Thrombolysis and STatins) study with 2072 patients showed that statin use was significantly associated with lower mortality (OR, 0.48; 95% CI, 0.28–0.82) [73, 74].

These inconsistent observations in patients with thrombolysis may be attributed to a higher incidence of symptomatic hemorrhagic transformation with statin treatment. A meta-analysis showed that prestroke statin was associated with an increased risk of symptomatic hemorrhagic transformation (OR, 1.63; 95% CI, 1.04–2.56) which may have been mediated by anti-fibrinolytic and anti-thrombotic effects of statins [68]. However, the functional outcome is better with prestroke statin despite the increased risk of hemorrhage, prestroke statin is not a contraindication for thrombolytic therapy.

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8 Ezetimibe

Min Kyoung Kang

Abstract

Despite the current vast repertoire of therapeutic cholesterol-lowering agents, a considerable proportion of the hypercholesterolemic population is not able to maintain optimal serum cholesterol levels. Further, safety issues associated with high-dose statins may hinder maintenance treatments that are required to reduce cardiovascular disease risks. Combination therapies based on statins and non-statin cholesterol-lowering agents, including ezetimibe, have been investigated in the above-mentioned clinical settings for the purpose of reducing side effects of statin and maintenance of optimal serum cholesterol level. Ezetimibe inhibits cholesterol absorption in the intestine and the biliary lymphatic system and may reduce low-density lipoprotein cholesterol levels when used as monotherapy or in combination with statins. Regardless of the acceptable safety profile and the known cholesterol-lowering effects of ezetimibe, many clinicians are still not convinced of the clinical benefits of ezetimibe. This chapter aims to elucidate the developmental history, pharmacokinetic profiles, mechanisms of action, therapeutic adjustment for practical use, and clinical trial data that may support the usage of ezetimibe in ischemic stroke patients.

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8.1 History of Ezetimibe

Statins, the most widely used lipid-lowering agents, inhibit hepatic cholesterol biosynthesis. Drugs that inhibit intestinal cholesterol absorption may be presumed to exert additive effects when used in combination with statins. In a large-scale project to identify the novel inhibitors of acyl-coenzyme A: cholesterol acyltransferase (ACAT), which could block the absorption of intestinal cholesterol, Burnett et al. investigated conformationally restricted compounds [1, 2]. The original compound assay showed that an azetidinone nucleus was essential for in vivo activity [3]. The crucial elements for inhibiting cholesterol absorption were confirmed to be an N-1-aryl group, a 4S-alkoxyaryl substituent, and C-3 arylalkyl substituent, and that led to the synthesis of compound Scheme 48461 [4]. (Fig 8.1a) Further studies of its metabolites led to the modification of Scheme 48461 by introducing fluorine to block nonproductive metabolism, and by stereospecific benzylic hydroxylation to pre-activate productive metabolism. Subsequently, series of assays were used to develop Scheme 58235 (termed ezetimibe) that showed higher potency and retention in the intestinal wall [5, 6] (Fig 8.1b).

The concept of "rational drug design" was introduced in the 1980s and focused on objective-

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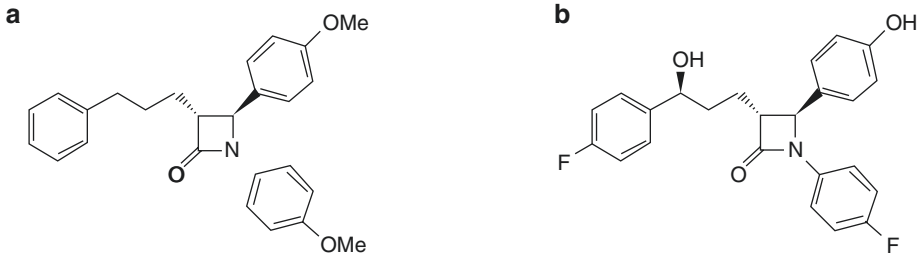

SCH 48461

Ezetimibe (SCH 58235)

1-(4-fluorophenyl)-(3*R*)-[3-(4-fluorophenyl)-(3*S*)-hydroxypropyl]-(4*S*)-(4-hydroxyphenyl)-2-azetidinone
$C_{24}H_{21}F_2NO_3$ CAS registry number: 163222-33-1

Fig. 8.1 Structure of Scheme 48461 and Scheme 58235 (ezetimibe). Adapted with permission from Nature Reviews Drug Discovery, Copyright Springer Nature [2]

Table 8.1 Commercially available products containing Ezetimibe

Ezetimibe	10 mg
Ezetimibe/ atorvastatin	10 mg/10 mg, 10 mg/20 mg, 10 mg/40 mg, 10 mg/80 mg
Ezetimibe/ rosuvastatin	10 mg/5 mg, 10 mg/10 mg, 10 mg/20 mg
Ezetimibe/ simvastatin	10 mg/20 mg, 10 mg/40 mg
Ezetimibe/ pitavastatin	10 mg/2 mg, 10 mg/4 mg

driven methods of drug discovery; however, the developmental history of ezetimibe as a novel cholesterol-lowering agent stood out from these trends. The biological activity profile of this drug was improved through serial assays of its conformationally restricted analogs; however, the developers did not understand its pivotal mechanism of intestinal absorption of cholesterol at the initial developmental stages [2].

Ezetimibe was approved by the FDA in 2002, and it is indicated for reducing total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), apolipoprotein B (apoB), and non-high-density lipoprotein (non-HDL) levels in patients with primary hyperlipidemia, mixed hyperlipidemia, and familial hypercholesterolemia (HoFH). Ezetimibe is the only therapeutic drug for treating homozygous sitosterolemia (phytosterolemia), and it can be a monotherapy or combination therapy with fenofibrate or statins. The first combination compound of ezetimibe and simvastatin has been available since 2002, indicated for primary or mixed lipidaemia and HoFH. Subsequently, combinations of ezetimibe at various doses, including atorvastatin, rosuvastatin, and pitavastatin have been available commercially (Table 8.1).

8.2 Mechanisms of Action

The plasma levels of cholesterol depend on two crucial factors: hepatic cholesterol biosynthesis and intestinal absorption of dietary and biliary cholesterol [7] (Fig. 8.2). One of the steps of cholesterol biosynthesis is the conversion of acetyl-CoA to mevalonate by 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase (as described in the Chap. 7). In this chapter, we focus on the mechanisms of action of ezetimibe.

Absorption of dietary and biliary cholesterol, which contributes to serum cholesterol levels, occurs predominantly in the duodenum and proximal jejunum [8]. Dietary intake accounts for approximately 25% of the cholesterol absorbed through the intestinal lumen. The remaining 75% of absorbed cholesterol originates from biliary cholesterol excreted from the liver. In the intestine, free cholesterol is incorporated into mixed micelles followed by delipidation, and these micelles are imported by the intestinal enterocyte membrane sterol influx transporters [9]. In 2004, Altmann et al. reported the discovery of the Niemann–Pick C1-like 1 protein (NPC1L1) as a human sterol transport protein expressed at the enterocyte apical and hepatobiliary canalicular

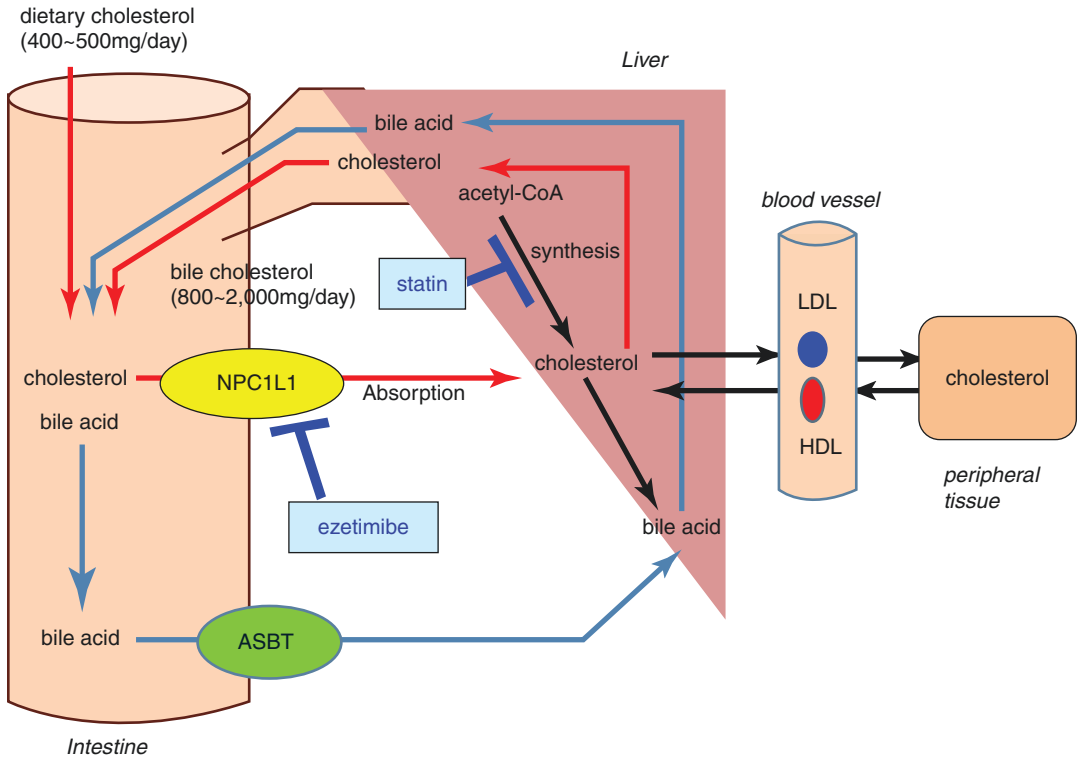

Fig. 8.2 Schematic presentation of blood cholesterol regulation and inhibitory medicines. Adapted from International Journal of Molecular Science, Copyright MDPI [7]. *NPC1L1* Niemann–Pick C1-like 1, *ASBT* apical sodium-dependent bile acid transporter, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein

interface [10]. NPC1L1 is composed of 180 amino acids, constituting 13 membrane-spanning helices. Five of these helices comprise the sterol-sensing domain that can bind to dietary or biliary cholesterol. After cholesterol binds to the sterol-sensing domain, the NPC1L1/cholesterol complex is internalized via a vesicle complex composed of Aps-clathrin, as shown in Fig. 8.3 [11]. At high concentrations of intercellular cholesterol, the vesicle complex is translocated to an endosomal storage site referred to as the endocytic recycling compartment. In case of low intracellular cholesterol levels, NPC1L1 is released from the endocytic recycling compartment and translocated to the cell membrane.

Such interactive homeostasis of serum cholesterol regulation was also found to occur between hepatic cholesterol biosynthesis and intestinal cholesterol absorption [12]. Statins inhibit hepatic cholesterol biosynthesis by inhibiting HMG-CoA reductase [7] (Fig. 8.2). In response to reduced hepatic cholesterol biosynthesis, hepatic LDL receptors are upregulated, resulting in the decrease of circulating LDL in the bloodstream. Further, statins enhance intestinal cholesterol absorption by upregulating gene expression of apoB or ACAT [13].

Ezetimibe is a known blocker of NPC1L1 protein which is essential for the uptake of cholesterol micelles into enterocytes in the jejunal brush border of the intestinal epithelium or hepatocytes [6]. The exact mechanism is so far not entirely clear; however, Ge et al. suggested that ezetimibe can hinder the interaction of NPC1L1/sterol complex with adapt protein 2 in clathrin-coated vesicles [14]. By reducing cholesterol absorption, chylomicron formation, and efflux of cholesterol into the bile, ezetimibe depletes the hepatic cholesterol pool and upregulates the expression of LDL receptors on hepatocytes, leading to an increase in LDL-C removal from the blood [15]. In summary, an effective reduction

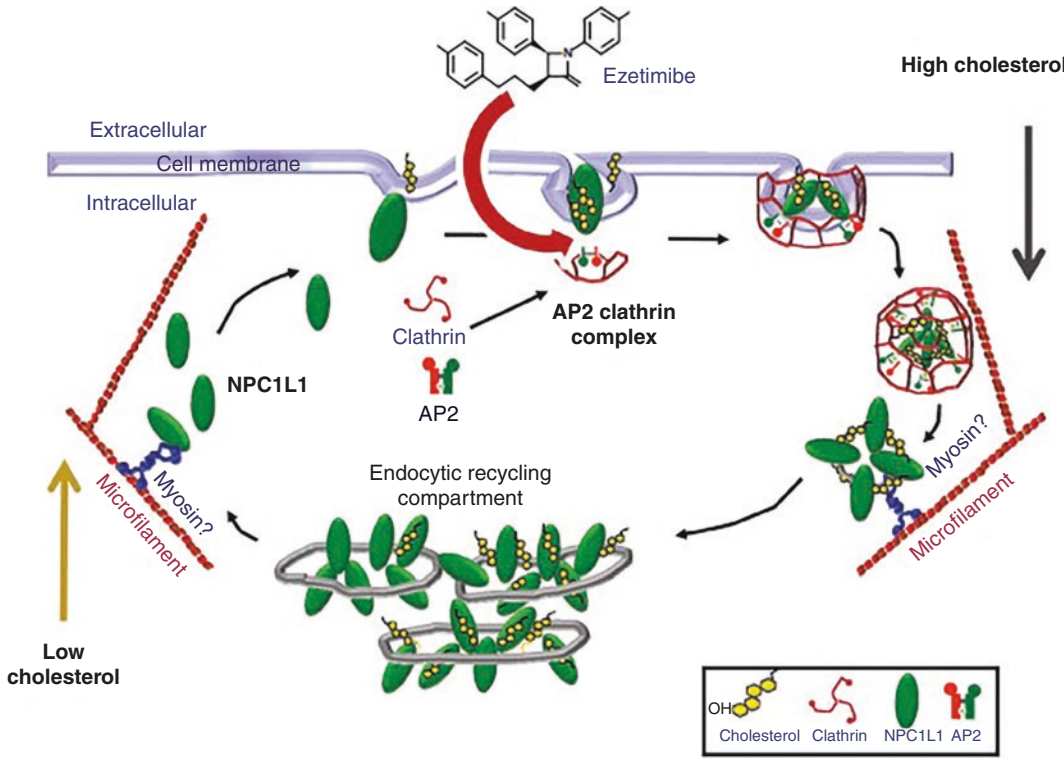

Fig. 8.3 Effect of ezetimibe on NPC1L1-mediated internalization of cholesterol. Adapted from Vascular Health and Risk Management, Copyright Dove Medical Press Ltd. [11]. *NPC1L1* Niemann–Pick C1-like 1, *AP2* adaptor protein 2

of serum cholesterol levels by ezetimibe can be attained via dual active sites, enterocyte and hepatocyte [16].

Other hypotheses have been proposed. Ezetimibe has been reported to bind to aminopeptidase N, which blocks endocytosis of cholesterol micelles or effectively disrupts the annexin2-caveolin1 complex, thereby regulating cellular cholesterol accumulation [17].

While statin therapy still consists predominantly of dyslipidemia treatment, statin-induced downregulation of hepatic cholesterol biosynthesis results in a compensatory increase of the hepatic cholesterol pool due to upregulation of hepatic LDL receptors and intestinal cholesterol absorption. Considering these homeostatic effects, an adjunctive strategy for hypercholesterolemia using cholesterol absorption inhibitors, such as ezetimibe, in combination with statins is expected to exert an additive effect on the reduction of serum cholesterol levels.

8.3 Information for the Practical Use of Ezetimibe

Possible dosages for monotherapy, drug combinations, and ezetimibe are shown in Table 8.1.

8.3.1 Brief Review of Pharmacokinetics

Ezetimibe is rapidly absorbed within 30 min of administration, reaching maximum serum concentration (Cmax) in 1.3 h; it is extensively metabolized by glucuronidation of the 4-hydroxy phenyl group, and more than 90% of the total absorbed dose was converted to the pharmacologically active ezetimibe-glucuronide metabolite [18]. Ezetimibe is predominantly excreted in feces (78% of the administered dosage). The half-life of ezetimibe was estimated at 22–24 h; therefore, one dose per day is recommended. Ezetimibe reaches steady-state after approximately 10 repeated doses. Generally, concomi-

tant food intake did not affect ezetimibe's pharmacokinetic characteristics. However, high-fat meals were found to increase the risk of elevated ezetimibe Cmax values [19].

8.3.2 Special Subgroups

Based on its phase II clinical trial data, ezetimibe is generally not recommended in cases of moderate or severe hepatic insufficiency (Pugh score > 7) and for children under 10 years of age [20]. Except for those cases, no dosage adjustment is necessary based on sex, race, age, or diseased condition.

8.3.3 Drug–Drug Interactions

Ezetimibe is generally accepted to produce safe interaction profiles, because ezetimibe does not induce or inhibit the hepatic enzyme system including cytochrome P450 system. No additional adjustment is required for co-administration with statins, glucose-lowering medication, antacids, digoxin, warfarin, or oral contraceptives [21].

8.3.4 Safety

Ezetimibe is generally considered safe. Minor side effects may include muscle pain, nasal congestion, sore throat, and diarrhea, which typically do not require medical attention. In randomized control trials, the incidence of adverse events was not statistically different between the ezetimibe group and placebo group [22].

8.4 Implications in Stroke Patients

In a study, of approximately 5000 patients suffering from or at high risk for coronary heart disease, only 38% of the patients achieved the National Cholesterol Education Program-designated LDL-C target levels [23]. A meta-analysis of data from 170,000 subjects showed that reductions in LDL-C levels below the accepted target levels led to additional reductions in cardiovascular (CVD) events [24]. This suggests that aggressive lowering of LDL-C concentrations below current target values will benefit patients at high risk for CVD. Additionally, epidemiological studies support the hypothesis that lowering LDL-C concentrations may be expected to reduce CVD events, regardless of the chosen hypolipidemic treatment.

Ezetimibe, a promising agent for therapy beyond statin monotherapy, was proven to lower serum cholesterol levels during monotherapy and in combination with statins among various population segments, including those with familial hypercholesterolemia, sitosterolemia, and insulin resistance.

8.4.1 Monotherapy

In hypercholesterolemic patients, ezetimibe reduces levels of LDL-C, TC, ApoB, and triglycerides and increases the levels of HDL-C, where each component is associated with a risk of atherosclerosis. In two studies, monotherapy with ezetimibe showed an 18% reduction in LDL-C compared with the 1% increase in the placebo group during the 3-month study period [2]. Moreover, a meta-analysis of data from 2700 subjects showed that monotherapy with ezetimibe for more than 12 weeks reduced serum LDL-C levels (18.5%), triglyceride levels (8%), and increased HDL-C levels (3%) compared with those in placebo treatments [25].

8.4.2 Combination Therapy

Ezetimibe may be used in combination with statins. Table 8.2 shows pivotal trial results of ezetimibe combination therapy [26–31]. The results of the trial were summarized as decreased LDL and cardiovascular output. Addition of ezetimibe to statin therapy produced consistently improved lipid profiles. However, the clinical outcome of therapy remains controversial. In

Table 8.2 The pivotal phase III clinical trial of ezetimibe-combination therapy

Trial	Year	Number of patients	Study population	Study design	Results
SANDS	2008	499	American-Indian men and women aged 40 years or older with type 2 diabetes and no prior CVD events	Aggressive group (LDL-C < 70, SBP < 115 mmHg) (n = 252) vs. standard group (LDL-C < 100, SBP < 130 mmHg) (n = 247) Secondary analysis : ezetimibe (n = 69) vs. nonezetimibe (n = 154) subgroup within the aggressive group	After 36 months, CIMT regressed in the ezetimibe group significantly differed from the nonezetimibe group (−0.025 mm vs. 0.012 mm; P = 0.01); There was no significant difference in mean change of plaque score between the ezetimibe and nonezetimibe groups (0.42 vs. 0.61; P = 0.50)
ENHANCE	2008	720	Patients of heterozygous FH	Simvastatin 80 mg (n = 363) vs. simvastatin 80 mg plus ezetimibe 10 mg (n = 357)	Ezetimibe plus simvastatin did not produce a significant reduction in CIMT despite the further reduction in LDL-C and hs-CRP achieved (change in mean CIMT after 2 year, 0.0111 mm vs 0.0058 mm; P = 0.29)
ARBITER 6	2010	315	Patients of CAD/CAD equivalent with LDL-C < 100 mg/dl and HDL-C < 50 mg/dl for men or 55 mg/dl for women (on statin treatment)	Ezetimibe (10 mg/day) (n = 161) vs. extended-release niacin (target dose, 2000 mg/day) (n = 154)	Patients on niacin had significant regression in both mean CIMT (−0.0102 ± 0.0026 mm; P < 0.001) and maximal CIMT (−0.0124 ± 0.0036 mm; P = 0.001). Ezetimibe did not reduce mean CIMT (−0.0016 ± 0.0024 mm; P = 0.88) or maximal CIMT (−0.0005 ± 0.0029 mm; P = 0.88) compared with baseline
VYCTOR	2009	90	Mexican patients 40–72 years of age with 10-year absolute risk of CAD > 20%	Pravastatin 40 mg (n = 30) vs. simvastatin 40 mg (n = 30) vs. simvastatin 20 mg with ezetimibe 10 mg (n = 30)	After 1 year of therapy, a significant reduction in LDL-C to a mean level of 45–48 mg/dL was seen with a significant reduction in all three groups and CIMT values were 1.33 ± 0.32 to 0.93 ± 0.13 mm, 1.30 ± 0.11 to 0.90 ± 0.11 mm, and 1.23 ± 0.28 to 0.92 ± 0.01 mm for groups 1, 2, and 3, respectively (P < 0.01), without difference between treatment groups

Table 8.2 (continued)

Trial	Year	Number of patients	Study population	Study design	Results
SHARP	2011	9,270	Patients with CKD	Simvastatin/ezetimibe 20 mg/10 mg (n = 4,650) vs. placebo (n = 4,620)	LDL-C lowering with combination therapy reduced major atherosclerotic events (coronary death, myocardial infarction, non-hemorrhagic stroke, or any revascularization) (risk ratio 0.83; 95% CI, 0.74–0.94; log-rank P = 0.0022)
SEAS	2008	1,873	Patients with asymptomatic AS	Simvastatin/ezetimibe 40 mg/10 mg (n = 944) vs. simvastatin 40 mg (n = 929)	Ezetimibe plus simvastatin did not produce significant effect on primary composite outcome and AS progression-related events. There were fewer patients with the secondary composite outcome of ischemic cardiovascular events in the combination therapy (hazard ratio 0.78; 95% CI, 0.63–0.97; P = 0.02)
IMPROVE-IT	2010	18,144	High-risk post-ACS patients	Simvastatin/ezetimibe 40 mg/10 mg (n = 9,067) vs. simvastatin 40 mg (n = 9,077)	Simvastatin/ezetimibe combination was superior to simvastatin monotherapy in reducing cardiovascular death, myocardial infarction, documented unstable angina requiring rehospitalization, coronary revascularization, or stroke events (hazard ratio 0.94; 95% CI, 0.89–0.98, 32.7 vs. 34.7%, P = 0.016)

Adapted from Cardiology Research and Practice, Copyright Hindawi [26]. *SANDS* Stop Atherosclerosis in Native Diabetics Study, *ENHANCE* Efficacy and Safety Study of Prolonged-Release Fampridine in Participants With Multiple Sclerosis, *ARBITER 6* Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol-6, *VYCTOR* Vytorin on and Overall Arterial Rigidity study, *SHARP* Study of Heart and Renal Protection, *SEAS*, Simvastatin and Ezetimibe in Aortic Stenosis, *IMPROVE-IT* IMProved Reduction of Outcomes: Vytorin Efficacy International Trial, *CVD* cardiovascular disease, *FH* familial hypercholesterolemia, *CAD* coronary artery disease, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *CIMT* carotid intima-media thickness, *CRP* C-reactive protein, *CKD* chronic kidney disease, *CI* confidence interval, *AS* aortic stenosis, *SBP* systolic blood pressure

summary, the efficacy of ezetimibe was demonstrated by atherosclerosis regression in carotid intima-media thickness (CIMT) in the Stop Atherosclerosis in Native Diabetics Study (SANDS) and Vytorin on and Overall Arterial Rigidity study (VYCTOR), and cardiovascular benefits were observed in Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) and the Study of Heart and Renal Protection (SHARP) studies via combination therapy with statins [28, 29, 32, 33]. However, the failure to show a practical effect on CIMT and femoral intima-media thickness in Ezetimibe and simvastatin in the Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) trial and the adverse effects of ezetimibe and simvastatin on cardiovascular outcomes observed during the Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol-6 (ARBITER 6) study generated tremendous controversy and dampened the expectations for ezetimibe as a treatment of hypercholesterolemia [34]. This provoked comments such as: "Until outcome trials provide additional insights into the effects of ezetimibe on cardiovascular events, this drug should only be considered an expen-

sive tool to provide a cosmetic effect on blood examinations" [35].

In 2015, however, the situation changed. The IMProved Reduction of Outcomes: Vytorin Efficacy International Trial showed that ezetimibe in combination with simvastatin reduced LDL-C levels, in contrast to simvastatin monotherapy that was unable to control LDL-C levels [27]. Addition of ezetimibe to simvastatin (40 mg) further reduced LDL-C levels by 16.9 mg/dL. The combination therapy reduced the risk of composite cardiovascular adverse effects by 6% (hazard ratio 0.94, 32.7% versus 34.7%; $P = 0.016$) at the end of 7 years of follow-up. This degree of reduction and the time taken to prove their effect may seem somewhat disappointing; however, we emphasize the low costs, safety profiles, and substantially beneficial risk for the prevention of ischemic stroke of ezetimibe in combination with statins.

In a meta-analysis of data from 31,048 patients, ezetimibe combination therapy significantly reduced the risk of myocardial infarction (13.5%) and of any stroke (16.0%) compared with that in statin monotherapy, over a median follow-up period of 3 years [36] (Fig. 8.4). Taken together, we conclude that ezetimibe can significantly reduce the risk of myocardial infarction and stroke without any effect on general or cardiovascular mortality.

8.4.3 Guidelines

According to the 2018 American College of Cardiology/American Heart Association (ACC/AHA) lipid guidelines, patients at high risk for clinical atherosclerotic cardiovascular disease (ASCVD), LDL-C levels <70 mg/dL or LDL-C level reduction from baseline values >50% is rec-

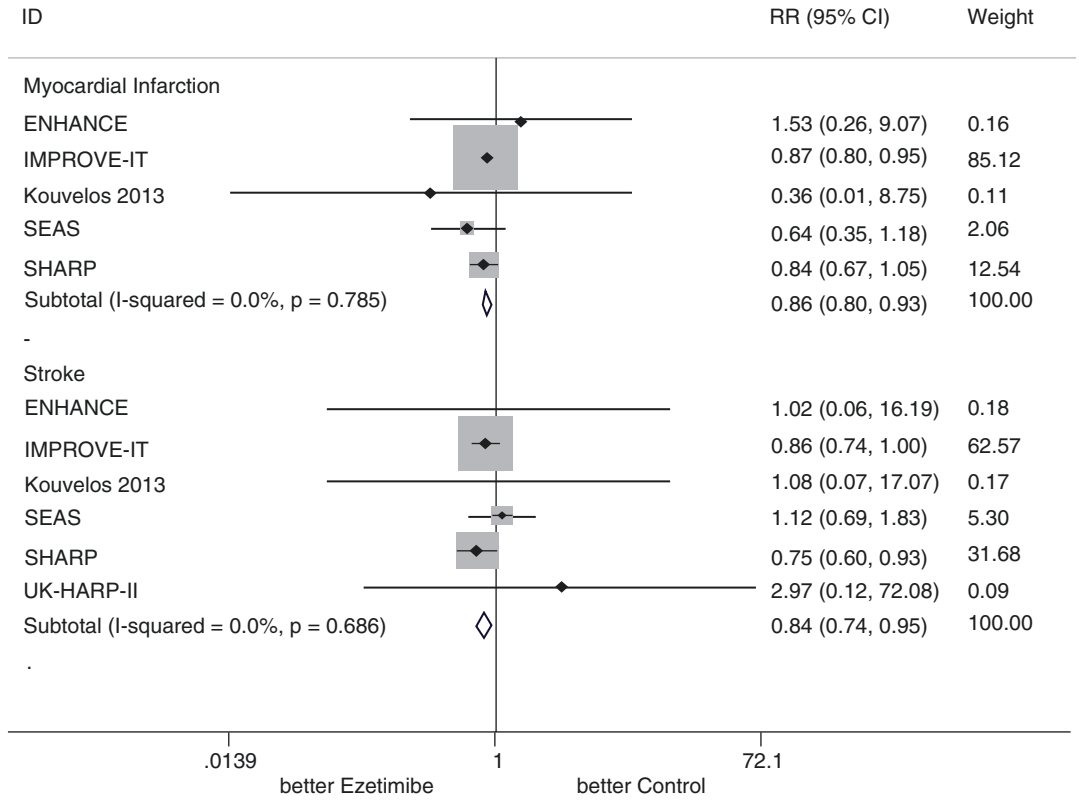

Fig. 8.4 Forest plots comparing risk ratios for myocardial infarction and stroke according to ezetimibe treatment. Adapted with permission from International Journal of Cardiology, Copyright Elsevier [36]. *RR* risk ratio, *CI* confidence intervals

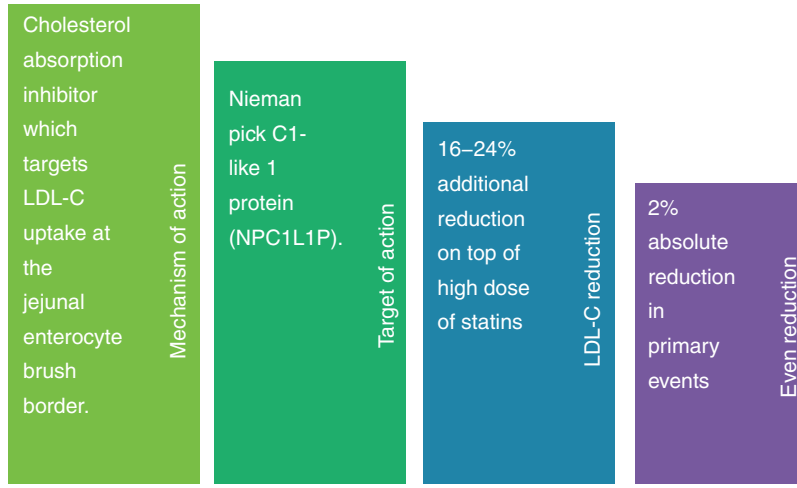

Fig. 8.5 Salient features of ezetimibe. Adapted from Cardiology Research and Practice, Copyright Hindawi [26]

ommended [37]. However, the 2019 European Society of Cardiology (ESC) guidelines for the management of dyslipidemia recommend LDL-C level reduction of >50% from baseline values or LDL-C level < 55 mg/dL for secondary prevention [38]. When these target levels cannot be achieved even by the maximum tolerated dosage of statins, the addition of ezetimibe is a class IIa recommendation according to the ACC/AHA guidelines, whereas the ESC guidelines suggest that the addition of ezetimibe is a class I, level B recommendation. In Korea, ezetimibe-statin combination therapy is considered an effective treatment option for dyslipidemia control and shows rising popularity, particularly regarding the combination of rosuvastatin and ezetimibe.

8.5 Conclusion

Statins are the most effective lipid-lowering treatment option as they may reduce LDL-C levels by 36–60%; however, it is unclear whether this reduction is sufficient or whether lower LDL-C levels are even truly beneficial. Based on the "6% rule" (which suggests that each doubling of the statin dose produces an additional 6% reduction in LDL-C), statins are evidently limited regarding their potency. Considering the safety, cost-effectiveness, and superiority of statin therapy with respect to reducing LDL-C levels, a combination of ezetimibe with statins is well worth as a method of choice of lipid-lowering treatment, despite the prevalence of biological drugs (Fig. 8.5). It should be noted that there is still potential for improvement regarding the clinical outcome in patients with dyslipidemia and ASCVD to facilitate achieving clinical benefits beyond statin therapy.

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Fibrate and Niacin

9

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Abstract

Fibrates and niacin are conventional medications that have long been used for the treatment of dyslipidemia. Clinical studies have shown that fibrates and niacin have lipid-modifying effects. Specifically, fibrates, which are peroxisome proliferator-activated receptor-alpha agonists, affect lipid metabolism. That is, it decreases triglyceride and low-density lipoprotein cholesterol (LDL-C) and increases high-density lipoprotein cholesterol (HDL-C) levels. Moreover, niacin, or vitamin B3, is a water-soluble vitamin that increases HDL-C and decreases LDL-C levels. In the era of statins for stroke and cardiovascular prevention, the role of fibrates and niacin is limited in clinical use. Fibrates were considered beneficial as they prevent cardiovascular events, particularly in individuals with hypertriglyceridemia. However, the effect of these medications in preventing stroke remains unclear based on clinical trials. Also, recent studies have shown neutral results in that the application of combined niacin and statins can prevent stroke and cardiovascular diseases. Hence, in the future, the effect of fibrates and niacin in decreasing the residual risk of stroke and cardiovascular diseases should be assessed.

Dyslipidemia, which is characterized by high low-density lipoprotein cholesterol (LDL-C) and/or low high-density lipoprotein cholesterol (HDL-C) level, is a major risk factor for atherothrombosis. Large clinical trials have commonly assessed the effect of statin (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor) in decreasing LDL-C levels. Results showed that this drug reduces the risk of stroke and cardiovascular diseases [1, 2]. In the era of statins, which are utilized for the prevention of stroke and cardiovascular diseases, the role and efficacy of fibrates and niacin, which were used for treating dyslipidemia for more than 50 years in the market, were questioned. Therefore, based on major treatment guidelines, the use of fibrates and/or niacin is limited in a subset of patients with hypertriglyceridemia and/or dyslipidemia. However, their effect in preventing stroke and cardiovascular diseases has not yet been strongly established [3–5]. In this chapter, the history, mechanism, type, and implication for stroke prevention of fibrates and niacin were assessed and discussed.

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9.1 History of Fibrate and Niacin

Fibrates are lipid-modifying drugs that mainly decrease triglyceride (TG) and increase HDL-C levels. These drugs were initially synthesized and were found to have hypolipidemic properties in the mid-1950s [6]. In 1962, clofibrate was discovered by Thorp and Waring and was the first compound clinically approved for the treatment of hypercholesterolemia [7]. Although the mechanism of action of clofibrate was unclear at that time, its hypocholesterolemic effect was assessed in clinical studies. Results showed that clofibrate modifies lipid profiles in hypercholesterolemic patients, primarily by decreasing very-low-density lipoprotein (VLDL) levels and, less likely, LDL fraction [8, 9]. Therefore, clofibrate was approved for clinical use in the USA in 1967. However, there were still some concerns regarding hepatoxicity caused by the long-term use of clofibrate [10]. With intensive studies on improving the pharmacokinetic and pharmacological activities of clofibrate, fenofibrate, also known as procetofen, was synthesized and introduced in clinical practice in France in 1974. Then, it was introduced in the USA in 1998 [6]. Moreover, novel fibrates, including gemfibrozil, were introduced in the late 1970s and early 1980s in the USA and bezafibrate and ciprofibrate in Europe.

Niacin, or vitamin B3, is a water-soluble vitamin that has two major pharmacological actions: a vitamin that is potent in milligram doses and a lipid-modifying drug that is potent in gram doses. Niacin affects the concentrations of all major classes of plasma lipoproteins, which specifically decrease TG and LDL-C and increase HDL-C levels based on the niacin dose used. In the mid-twentieth century, based on the study of Altschul, niacin was found to decrease plasma cholesterol levels in rabbits [11]. In 1955, Altschul et al. first reported that nicotinic acid could decrease plasma cholesterol levels in normal and hypercholesterolemic participants. Interestingly, they also revealed that nicotinamide, which is a nutritional equivalent of nicotinic acid, also known as vitamin B3, did not affect plasma cholesterol levels [12]. Succeeding studies were then conducted to assess the role of niacin on lipid metabolism. Results showed that the cholesterol- and triglyceride-lowering effects of this drug were associated with decreased beta (β)-lipoprotein (i.e., LDL-C) levels and β-to-alpha (α)1-cholesterol (i.e., LDL-C to HDL-C) levels, as observed on lipoprotein electrophoresis, and the immediate but transitory lowering of free fatty acid concentration in the plasma in participants who were subjected to fasting [13–15].

9.2 Mechanism of Action

As mentioned previously in this chapter, fibrates are generally effective in lowering TG and cholesterol levels, and such changes are based on the patient's pretreatment lipoprotein status, and the relative potency of the fibrate used [16]. In particular, along with decreased LDL-C and increased HDL-C levels, the most prominent effect of fibrates was decreasing plasma TG levels. Moreover, a small paradoxical increase in LDL-C levels may be observed with high TG levels. However, the mechanisms of action underlying lipoprotein modulation caused by fibrates were not clearly elucidated until the early 1990s. Based on data obtained from animal and human studies, the major mechanisms of fibrates were as follows [17]: (1) triglyceride-rich lipoprotein (TRL) lipolysis with changes in intrinsic lipoprotein lipase (LPL) activity or increased accessibility of TRLs for lipolysis by LPL; (2) hepatic fatty acid (FA) uptake associated with FA transporter protein and acyl-CoA synthetase activity, which increase FA update and conversion to acyl-CoA in the liver, and decreased hepatic triglyceride production caused by the β-oxidation pathway with a concomitant decrease in FA synthesis; (3) increased removal of LDL particles by the formation of LDL with a higher affinity to the LDL receptor and subsequent high rapid catabolism; (4) reduced neutral lipid (cholesteryl ester and triglyceride) exchange between VLDL and HDL, which may be caused by decreased TRL levels in the plasma; and (5) increased HDL production and stimulation of reverse cholesterol transport via greater production of apolipoprotein A-I and A-II in the liver. These lipid-modifying activities

of fibrates are mediated mainly via the peroxisome proliferator-activator receptor (PPAR) activation, particularly PPARα [18].

After the initial discovery of the lipid-modifying effects of niacin in the 1950s, a significant progress has been made in elucidating the mechanism of action on plasma lipids. That is, they were found to increase HDL-C and TG and decrease LDL-C levels. Based on recent studies, the mechanisms of action of niacin on lipid metabolism were as follows [19]: (1) inhibition of hepatic diacylglycerol acyltransferase 2 production, which reduces hepatic TG synthesis and increases intrahepatic apolipoprotein B degradation (decreased VLDL and LDL levels); (2) suppression of the surface expression of hepatocyte β chain ATP synthase, a putative HDL receptor, which decreases the uptake of HDL-apolipoprotein A-I (increase in HDL by slowing the removal of HDL particles from the circulation); and (3) promotion of the membrane protein ATP-binding cassette transporter A1 activity at the cell surface, which increases the production of nascent HDL particles [20–22].

9.3 Types of Agents

After the introduction of clofibrate in the 1960s, fibrates were considered the most effective drug that can lower TG levels. This drug was well tolerated with an excellent safety profile. Currently, the available fibrates include gemfibrozil, bezafibrate, and fenofibrate. (Table 9.1) The incidence of fibrate-associated toxicity in each organ system is low [23]. The most common side effects are gastrointestinal complaints (e.g., nausea, abdominal pain), which affect approximately 5% of patients and are less common in those taking fenofibrate [24]. The risk of developing cancer caused by fibrate use was observed in rodent models, and the mechanism may be correlated with peroxisome proliferation. However, this finding was not definite [25]. In humans, the long-term administration of various fibrates did not cause peroxisome proliferation or any other morphological changes in the liver [26, 27]. However, the extrapolation of this evidence on carcinogenesis remains uncertain. In terms of clinically relevant interactions, fibrates cause rhabdomyolysis and decreased bioavailability when used along with statins and some bile acid sequestrants, respectively [23]. Fibrates are excreted by the kidney and, therefore, accumulate in the serum of patients with renal failure [28]. In addition, the anticoagulant effect of warfarin may cause bleeding, which requires up to 30% decrease in the use of this drug [29].

Niacin has been used to treat dyslipidemia for more than 50 years. (Table 9.1) Although it has favorable characteristics such as its lipid-modifying effect, general prescription and widespread acceptance have been limited by the need to take it four times a day and by the high incidence of flushing, which is a side effect. The extended-release formulation of niacin is easier to take at bedtime (1000–2000 mg daily) and has a lower incidence of side effects such as flushing [30]. Currently, niacin is available in immediate-, sustained-, and extended-release (ER) formulations. Apart from flushing, other side effects, including glucose intolerance, increased uric acid levels, cystoid macular edema, and liver-function abnormalities, were reported. Compared with other formulations, ER niacin at doses of up to 2000 mg/day is associated with a lower incidence of hepatic toxicities. In addition, caution is generally required when using niacin at high doses in diabetic patients because it can increase glucose levels. However, the Arterial Disease Multiple Intervention Trial showed that niacin could be safely used in patients with diabetes mellitus (DM) [31].

9.4 Implication of Dyslipidemia Medication on Stroke Trials

In clinical trials, fibrates, which are potent lipid-modifying drugs, are used mainly for the prevention of cardiovascular diseases. Unfortunately, studies that focus on the effect of fibrates in preventing stroke have not been conducted to date. The Helsinki Heart Study was conducted in 1987 and was the first clinical trial on fibrates. It included 4081 asymptomatic middle-aged men

Table 9.1 Available fibrates and niacin and their characteristics

Drug	Dosage	Clinical trials	Effect on lipid profiles	Side effects	Drug interactions
Gemfibrozil	1200 mg/day	HHS, VA-HIT	10% ↓ in TC levels 10% ↓ in LCL-C levels 41% ↓ in TG levels 10% ↑ in HDL-C levels	Rash; gastrointestinal irritation; abdominal pain (gemfibrozil); cholesterol-saturated bile; increased incidence of gallstone formation (1%–2%), erectile dysfunction (clofibrate), and myositis with impaired renal function; and high serum aminotransferase levels	Increased anticoagulation, decreased absorption of fibrates when utilized with bile acid resins, higher incidence rates of myopathy and, rarely, rhabdomyolysis when used in combination with statins, enhanced blood glucose-lowering effects when taken along with oral hypoglycemics (gemfibrozil), nephrotoxicity with cyclosporin (fenofibrate)
Bezafibrate	400 mg/day	BIP	4.5% ↓ in TC levels 6.5% ↓ in LDL-C levels 21% ↓ in TG levels 18% ↑ in HDL-C levels		
Fenofibrate	200 mg/day	FIELD	11% ↓ in TC levels 12% ↓ in LDL-C levels 29% ↓ in TG levels 5% ↑ in HDL-C levels		
Niacin (immediate-release, sustained-release, extended-release)	2000 mg/day Up to 4500 mg/day to reduce LDL-C levels	HATS AFREGS AIM-HIGH HPS2-THRIVE	23% ↓ in LDL-C levels 33% ↑ in HDL-C levels 35% ↓ in lipoprotein (a) levels at a higher niacin dosage	Flushing (most common), dry skin, pruritus, ichthyosis, acanthosis nigricans, conjunctivitis, cystoid macular edema, retinal detachment, nasal stuffiness, supraventricular arrhythmia, heartburn, loose bowel movement, mild increase in serum aminotransferase levels, hepatitis with nausea and fatigue, myositis, hyperglycemia, and increased serum uric acid levels	Could enhance adverse effects caused by alcohol intake, diminish the therapeutic effect of antidiabetic agents, and cause myopathic conditions (rhabdomyolysis) when used in combination with statins The absorption of niacin may decrease when utilized along with bile acid sequestrants The use of niacin extended-release formulations and laropiprant along with simvastatin increases the risk of myopathy

HHS Helsinki Heart Study, *VA-HIT* Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial, *BIP* Bezafibrate Infarction Prevention, *FIELD* Fenofibrate Intervention and Event Lowering in Diabetes, *HATS* High-density Lipoprotein Atherosclerosis Treatment Study, *AFREGS* Armed Forces Regression Study, *AIM-HIGH* Atherothrombosis Intervention in Metabolic Syndrome with Low High-density lipoprotein/High Triglycerides: Impact on Global Health Outcomes, *HPS2-THRIVE* Heart Protection Study 2–Treatment of High-Density Lipoprotein to Reduce the Incidence of Vascular Events, *TC* total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TG* triglyceride

with primary dyslipidemia but without the confirmed cardiovascular disease [32]. The primary endpoint was fatal and nonfatal myocardial infarction (MI) or cardiac-related mortality. Results showed that treatment with gemfibrozil significantly reduced the primary endpoints, with a relative risk reduction of 34%, and increased HDL-C and decreased total cholesterol, LDL-C, and non-LDL-C levels. In conclusion, treatment with gemfibrozil could reduce the incidence of coronary heart disease in men with dyslipidemia.

In terms of secondary prevention trials, the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial included 2531 men with coronary heart disease and low HDL-C (≤40 mg/dL) and low LDL-C (≤140 mg/dL) levels [33]. The primary outcome was nonfatal MI or coronary heart disease-related mortality. Results showed that the use of gemfibrozil (1200 mg/day) significantly reduced the incidence of the primary outcome (17.3% vs. 21.7%; $p = 0.006$), with a relative risk reduction of 22%. Moreover, the incidence rate of ischemic stroke and the combined mortality rate of coronary heart disease, nonfatal MI, and stroke decreased with gemfibrozil treatment. The Bezafibrate Infarction Prevention (BIP) included 3090 patients with a previous history of MI or stable angina with the following baseline lipid profiles: total cholesterol (TC) level, 180–250 mg/dL; HDL-C level, ≤45 mg/dL; TG level ≤ 300 mg/dL; and LDL-C level, ≤180 mg/dL [34]. The primary endpoint was fatal or nonfatal MI or sudden death. The results of this study, unlike the VA-HIT study, were neutral in terms of primary outcome or secondary outcome (stroke), with a mean follow-up of 6.2 years. In a post hoc analysis of a subgroup with a high baseline TG level (≥200 mg/dL), the cumulative probability of the primary outcome after bezafibrate treatment decreased to 39.5% ($p = 0.02$). Hence, bezafibrate had cardiovascular preventive effects in patients with high baseline TG levels.

In 2005, the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial was the first clinical trial on fenofibrate and fibrates for type 2 DM. Moreover, it included 9795 statin-naive patients with type 2 DM at baseline [35]. Results showed that fenofibrate neither decreased the composite primary outcome of coronary heart disease-related mortality or nonfatal MI nor the risk of stroke. The presumed negative results of the FIELD trial might be attributed to the increased use of lipid-lowering agents other than fenofibrate in the placebo compared with the treatment group (36% vs. 19%). Subsequently, the Action to Control Cardiovascular Risk in Diabetes trial aimed to show the additional benefits of fenofibrate when used in combination with statins in patients with type 2 DM. This study included 5518 patients with type 2DM who were receiving simvastatin treatment [36]. The primary outcome was the first occurrence of nonfatal MI, nonfatal stroke, or cardiac-related mortality. Results showed that the use of fenofibrate combined with simvastatin, compared with simvastatin alone, did not reduce the incidence of the primary endpoint, including nonfatal stroke. However, as in the BIP study, there was a substantial risk reduction of 28.6% in a subgroup of patients with low HDL-C (≤34 mg/dL) and high TG (≥204 mg/dL) levels. A meta-analysis of 18 trials on fibrates showed that fibrate therapy was associated with a relative risk reduction of 10% for major cardiovascular events, but not stroke [37]. Another meta-analysis of ten trials revealed the effects of fibrates against stroke [38]. Overall, fibrate therapy was not associated with a significant reduction in the risk of stroke (relative risk [RR], 1.02; 95% confidence interval [CI], 0.90–1.16; $p = 0.79$). However, a subgroup analysis emphasized two points: (1) gemfibrozil therapy had a beneficial effect against stroke (RR, 0.72; 95% CI, 0.53–0.98; $p = 0.04$), and (2) fibrate therapy had an effect on a fetal stroke when only high-quality clinical trials were included.

To date, the effect of niacin in preventing stroke has not been evaluated. However, it has been commonly assessed in clinical trials on cardiovascular diseases, including stroke. The Coronary Drug Project was conducted in 1975, and it first evaluated the effect of niacin. In this research, 3908 participants (excluding women) with a previous history of MI on electrocardiogram were randomized to either the niacin or placebo group [39]. In a 5-year trial, niacin was associated with a 27% reduction in the incidence

of MI. Nevertheless, results initially showed that it did not have an effect on mortality. After a mean follow-up of 15 years, the overall mortality rate of the niacin group decreased by 11% [40].

The use of niacin combined with statins or fibrates has been evaluated in multiple studies. In earlier trials, including the Stockholm Ischemic Heart Disease Secondary Prevention Study, High-density Lipoprotein Atherosclerosis Treatment Study, and Armed Forces Regression Study, the use of niacin combined with fibrates or statins was associated with a lower risk of coronary heart disease, mortality, and coronary stenosis on angiography accompanied by changes in plasma lipid concentrations (increased HDL-C and decreased TG and LDL-C levels) [41–43]. Due to the small sample size and few outcome events of stroke, significant conclusions regarding the effect of niacin in preventing stroke are challenging to obtain.

The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) trial was conducted in 2011. This study included 3414 patients with confirmed cardiovascular disease and dyslipidemia. However, the sample size was limited due to the availability of funds [44]. The primary efficacy endpoint was the first event of the composite of death from coronary heart disease, nonfatal MI, ischemic stroke, hospitalization due to the acute coronary syndrome, or symptom-driven coronary or cerebral revascularization. The trial, which was event-driven, was discontinued early, based on futility, after only 3 years and after about 70% of the planned events (total: 800) have occurred. Niacin therapy had significantly improved lipid profiles by increasing HDL-C and decreasing TG and LDL-C levels after 2 years. However, the use of combined ER niacin (1500–2000 mg/day) and intensive simvastatin therapy, compared with simvastatin therapy alone, did not reduce the risk of the composite of cardiovascular events, including ischemic stroke (16.4% vs. 16.2%; hazard ratio, 1.02; 95% CI, 0.87–1.21; $p = 0.79$). Moreover, there was a concern about the unexpected increase in the incidence of ischemic stroke with niacin therapy. However, the causal association or statistical "play of chance" was not determined in this study.

In early 2013, a meta-analysis on 11 trials, including the AIM-HIGH trial, comprising 9959 participants, aimed to assess the efficacy of niacin in reducing the incidence of cardiovascular events [45]. This drug was associated with a significant reduction in the composite end points of any cardiovascular disease event (odds ratio (OR) 0.66; 95% CI, 0.49–0.89; $p = 0.007$) and major coronary heart disease event (OR: 0.75; 95% CI, 0.59–0.96; $p = 0.02$). However, there was no significant association between niacin therapy and stroke (OR, 0.88; 95% CI, 0.5–1.54; $p = 0.65$). In addition, the difference in on-treatment HDL-C levels was not associated with the effect of niacin on outcomes between treatment arms.

However, there were serious safety concerns about niacin therapy. The Heart Protection Study 2–Treatment of High-Density Lipoprotein to Reduce the Incidence of Vascular Events trial, which was performed in 2013, included 25,673 patients at high risk of vascular events [46, 47]. This was a randomized, placebo-controlled trial of ER niacin 2000 mg and laropiprant 40 mg (a prostaglandin D2 signal blocker) combined with simvastatin 40 mg daily. The primary outcome was nonfatal MI, fatal coronary heart disease, fatal or nonfatal stroke, or coronary or other revascularization. The results were negative in two significant perspectives. First, ER niacin with laropiprant did not reduce major vascular events [48]. Second, ER niacin with laropiprant caused a significant increase in the risk of myopathy in patients taking simvastatin. This phenomenon led to the withdrawal of this formulation from the market [46, 48].

9.5 Conclusion

In conclusion, fibrates and niacin have long been used for the treatment of dyslipidemia. Moreover, they are widely available and have good safety profiles even before the era of statin. Major fibrates were found to be beneficial in the prevention of cardiovascular diseases, particularly in patients with hypertriglyceridemia. However, the role of

fibrates in preventing stroke remains unclear. The cardiovascular and stroke preventive effects of niacin when concomitantly used with statins were not clearly elucidated. In the era of statins, which are used to prevent stroke and cardiovascular diseases, fibrates and niacin could be used as an additional regimen to further decrease the risk of these diseases. Hence, clinical trials should be conducted in the future to validate this notion.

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Fish Oils

10

Jae-Myung Kim and Man-Seok Park

Abstract

Fish oil is a significant source of long-chain omega-3 polyunsaturated fatty acids (PUFAs). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the essential PUFAs found in fish oil. Early epidemiologic researches have shown a reverse relationship between fish consumption and the risk of coronary heart disease. Since then, several experimental investigations and clinical studies have demonstrated the advantages of fish oil supplementation in decreasing the development and progression of atherosclerosis, myocardial infarction, arrhythmias, heart failure, and ischemic stroke. Possible mechanisms have been investigated, such as lowering triglycerides, altering membrane fluidity, modulation of cardiac ion channels, anti-inflammatory, antithrombotic, and antiarrhythmic effects. This chapter will review the mechanism of action of omega-3 PUFAs and their clinical implications in cardiovascular disease and stroke.

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10.1 History of Fish Oils

In the 1970s, Danish physicians Jorn Dyerberg and Hans Olaf Bang first discovered evidence that fish oils might have a positive effect on cardiovascular disease [1, 2]. They found that the total amount of fat intake of Americans, Danes, and Greenland Inuits was almost the same. However, coronary atherosclerotic disease mortality was significantly lower among the Greenland Eskimos than the Danes and Americans. They predicted that these results might come from the difference in the diet of the three cohort groups and found that Greenland Eskimo's diet was much different from that of the Americans and Danes. Greenland Eskimos mainly consumed fat from whales, seals, and fish, which were very rich sources of omega-3 fatty acids. The reason Eskimo was free from heart disease even after consuming the same amount of fat as Americans and Danish was speculated to be due to omega-3-rich fish oil. Since then, fish oil has attracted people's attention, several epidemiologic studies have been conducted, and similar results were produced [3–5]. Further researches have shown that the beneficial effects of fish oil are principally mediated by omega-3 polyunsaturated fatty acids (n-3 PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

There have been several clinical studies conducted proving the protective effects of omega-3 fatty acids on cardiovascular disease. In 1989,

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Burr et al. reported the first clinical trial, wherein the group who ingested oily fish twice a week significantly decreased the fatal arrhythmia than the control group [6]. Numerous randomized controlled trials (RCTs) are going on so far for fish oils and cardiovascular diseases. Ecological data suggested beneficial effects of fish consumption on the risk of stroke and mortality [7, 8]. Several but not all prospective cohort studies demonstrated an inverse relationship between fish intake and the risk of stroke after potential confounding factors were adjusted [9, 10]. Recently, several prospective RCTs have been conducted regarding fish oil and stroke risk.

10.2 Mechanism of Action

After absorption from the intestine as chylomicrons, polyunsaturated fatty acids (PUFAs) are transported to the liver and other tissues. PUFAs are subsequently incorporated into the lipid bilayer of plasma membranes and maintain membrane fluidity and signaling. The beneficial effects of fish oil (n-3 PUFA) on cardiovascular and metabolic systems have various mechanisms, such as lowering of blood pressure, reducing serum triglyceride levels, stabilizing heart rate, improving cardiac function, improving endothelial function, inhibiting platelet aggregation, and potentiating anti-inflammatory effect [11–17]. Physiological effects of n-3 PUFA on atherosclerosis and cardiovascular disease are well described in Fig. 10.1.

10.2.1 Triglyceride

n-3 PUFAs affect lipid metabolism positively. Genetic and epidemiologic studies revealed that high fasting triglyceride (TG) levels are associated with the risk of developing cardiovascular disease [19, 20]. Intake of marine-derived, long-chain omega-3 fatty acids has been shown to reduce TG markedly. DHA and EPA reduce serum TG levels by 30%, which can reduce the risk for cardiovascular morbidity and mortality [21]. Previous studies proposed that, at effective doses, n-3 PUFAs lower serum TG levels by reducing TG synthesis, inhibiting the incorporation of TG into VLDL (very-low-density lipoprotein), decreasing TG secretion, and accelerating TG clearance from VLDL particles [22]. n-3 PUFA exerts these TG-lowering effects through several mechanisms: (1) n-3 PUFA reduced hepatic lipogenesis, (2) increased fatty acid

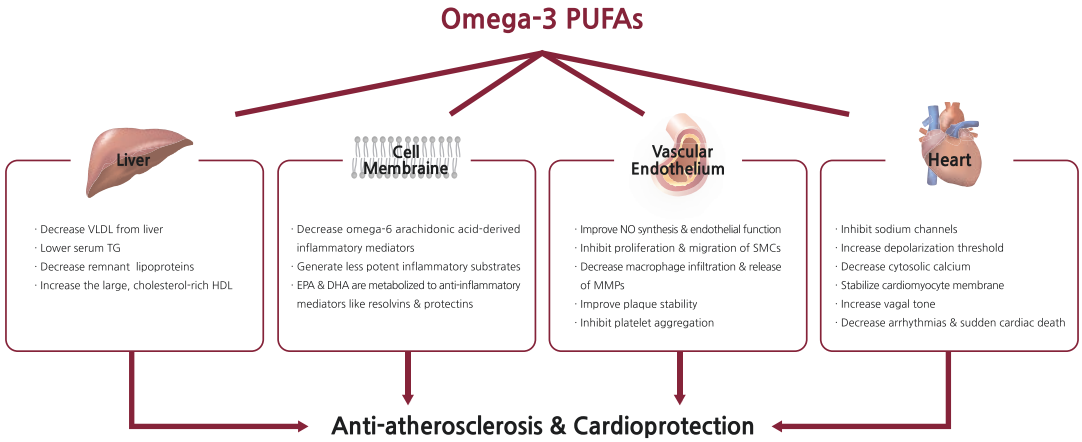

Fig. 10.1 Pleiotropic cardioprotective effects of n-3 PUFAs. Adapted from International Journal of Molecular Sciences, Copyright MDPI [18]. *PUFA* polyunsaturated fatty acids, *VLDL* very-low-density lipoprotein, *TG* triglycerides, *HDL* high-density lipoprotein, *EPA* including eicosapentaenoic acid, *DHA* docosahexaenoic acid, *NO* nitric oxide, *SMC* smooth muscle cell, *MMP* matrix metalloproteinase

β-oxidation and reduced delivery of non-esterized fatty acids required for the synthesis of TG and VLDL, (3) inhibiting a critical hepatic enzyme involved in TG synthesis, (4) enhancing the expression of lipoprotein lipase (LPL), and (5) increasing TG removal from circulating VLDL and chylomicron particles [23–27]. (Fig. 10.2) Based on previous RCTs, TG lowering is linearly dose-dependent across a wide range of consumption but with variable individual responses. TG-lowering effect was much more significant in trials of individuals with higher baseline TG levels [28].

10.2.2 Heart Rate and Blood Pressure

Fish oil consumption appears to reduce heart rate (HR) and systolic and diastolic blood pressure [15, 29]. In a meta-analysis of 30 RCTs, fish oil supplementation (median dose of 3.5 g/day, median duration of 8 weeks) reduced resting HR by 1.6 beats per minute (bpm) [15]. Experimental animal studies propose that HR lowering could result from direct effects on cardiac electrophysiological pathways, and n-3 PUFA might also lower HR through more indirect effects in humans by improving the left ventricular diastolic filling or augmenting vagal tone [30]. Experimental and observational studies in humans indicated that the BP-lowering effect of fish oil results from a reduced systemic vascular resistance, with unchanged cardiac output [31, 32]. In vitro studies revealed that n-3 PUFA consumption induces nitric oxide production, modulates vasodilatory responses, and improves arterial compliance [33, 34]. These effects could lead to reduced systemic vascular resistance and blood pressure.

10.2.3 Platelet Function

n-3 PUFAs are essential components of the platelet phospholipid membrane and modulate the role of the cell receptors in these mem-

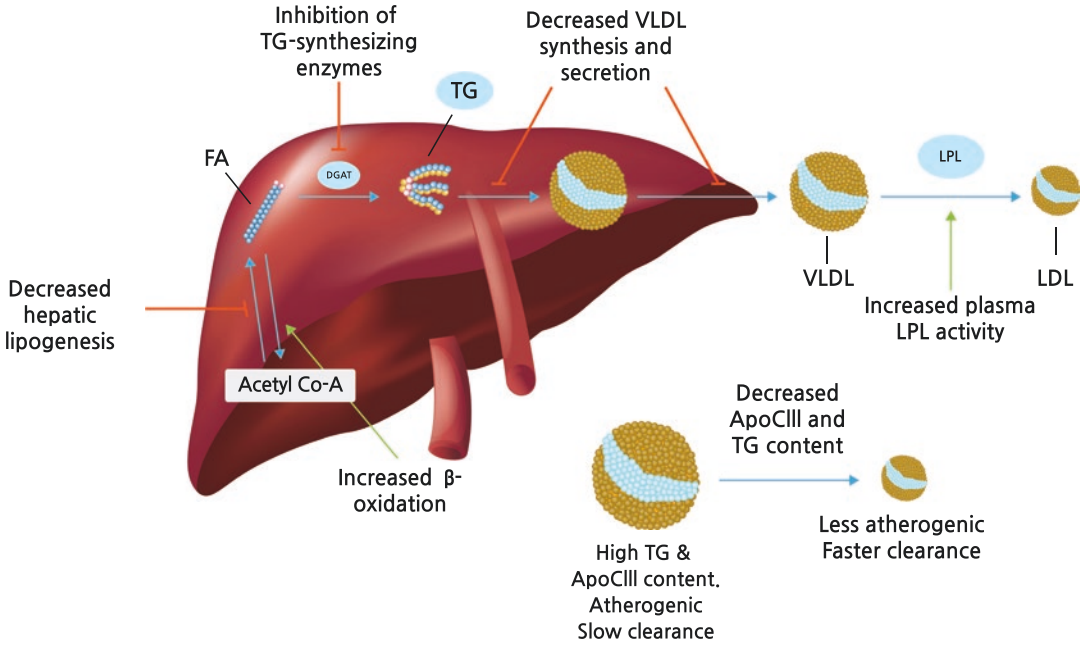

Fig. 10.2 Triglyceride-lowering mechanisms of action of long-chain omega-3 fatty acids. Adapted from Lipids in Health and Disease, Copyright Springer Nature [21]. *ApoCIII* apolipoprotein CIII, *Acetyl Co-A* acetyl coenzyme A, *DGAT* diglyceride acyltransferase, *FA* fatty acid, *LPL* lipoprotein lipase, *TG* triglyceride, *VLDL* very-low-density lipoprotein

branes. n-3 PUFAs decrease the platelet tendency toward adhesion and aggregation by inhibiting the synthesis of prothrombotic compounds, including thromboxane A2 and platelet-activating factor, increasing prostacyclin level and tissue plasminogen activity, and decreasing fibrinogen concentration [35, 36]. n-3 PUFAs inhibit platelet-activating factors in vitro experimental studies, but in human trials, significant effects of PUFAs on platelet aggregation are not firmly evident [37, 38]. Omega-3 fatty acids can also stabilize the atheromatous plaque, decreasing the restenosis incidence after coronary angioplasty [39].

10.2.4 Inflammation

n-3 PUFAs can serve as an essential regulator of inflammation. These fatty acids can partly inhibit several aspects of inflammation, including leucocyte chemotaxis, adhesion molecule expression, and leucocyte–endothelial adhesive interactions. Anti-inflammatory mechanisms of the EPA and DHA contain altering cell membrane phospholipid fatty acid composition, disrupting lipid rafts, inhibiting the activation of the pro-inflammatory transcription factor nuclear factor κB. Besides, EPA and DHA give rise to anti-inflammatory and inflammation-resolving mediators, including protectins, resolvins, and maresins. They specifically regulate the resolution phase of inflammation, which is now known as a highly regulated, active, and complicated program that terminates the inflammatory response [40].

10.2.5 Vascular Endothelium

The underlying mechanisms of improving endothelial function by n-3 PUFAs in human subjects have not been completely elucidated. n-3 PUFAs can improve endothelial function by increasing NO levels [41]. DHA and EPA activate endothelial nitric oxide synthase (eNOS) in human endothelial culture cells, while dietary n-3 PUFA supplementation enhances eNOS activation in the mouse aorta [42–44]. Another probable mechanism is reducing reactive oxygen species (ROS). Vascular ROS can decrease NO bioavailability and increase endothelial-derived vasoconstrictive factors, thus leading to impaired endothelial-dependent vasodilation. In an animal experiment, dietary intake of n-3 PUFAs considerably reduces the expression of two oxidative stress markers, 8-isoprostane and heme oxygenase-1 mRNA [45]. Endothelial activation causing endothelial dysfunction is related to increases in the surface expression of adhesion molecules. Both DHA and EPA can reduce the expression of adhesion molecule and leukocyte adhesion to endothelial cells [17, 46].

Several, but not all, clinical trials in humans have demonstrated that fish oil intake reduces the circulating markers of endothelial dysfunction, including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin [47, 48].

10.3 Types of Fish Oils

Fish oil is a major source of long-chain omega-3 PUFAs. Fatty acids are long-chain hydrocarbons that can be classified according to the number of double bonds in their side chains: PUFAs (two or more double bonds), MUFAs (one double bond), and saturated fatty acids (no double bond). PUFAs can be categorized further by the location of the first double bond from the methyl terminal into n-6 or n-3 PUFAs. For example, the first double bond of linoleic acid (LA) is at the sixth position from the methyl terminal; thus, it is an n-6 PUFA. Similarly, alpha-linolenic acid (ALA) has three double bonds, with the first double bond at the third position from the methyl terminal; thus, it is an n-3 PUFA. Both LA and ALA are regarded as essential fatty acids because they cannot be synthesized by humans and must be obtained through their diet. n-6 and n-3 PUFAs have opposite effects on the body. Diets rich in n-6 PUFAs are linked to inflammation, platelet aggregation, and vasoconstriction. Acute inflammation might be an essential and protective response to infection and injury [49]. However,

excessive and inappropriate inflammation has been associated with atherosclerosis and cancer. Conversely, n-3 PUFAs are precursors of anti-inflammatory molecules and deliver benefits against chronic inflammatory conditions, such as diabetes, cancer, and atherosclerotic vascular disease [50].

LA and ALA, the essential fatty acids, are metabolized into other fatty acids through desaturase and elongase enzymes. LA (n-6 PUFAs) is metabolized into arachidonic acid. Similarly, ALA (n-3 PUFAs) is converted into EPA, docosapentaenoic acid (DPA), and DHA. Thus, EPA, DPA, and DHA are traditionally considered non-essential since, technically speaking, they can be synthesized from ALA. However, this pathway is slow and inefficient [51]. Usually, only 0.5%–5% of ALA is converted into DHA and 1%–10% into EPA. Moreover, the conversion rate depends on the levels of other nutrients, including magnesium, iron, copper, zinc, calcium, and vitamins B6 and B7. The modern diet, especially vegetarianism, lacks some of these nutrients. Therefore, the dietary intake of EPA, DPA, and DHA is essential and crucial to obtain health benefits. Figure 10.3 shows the structure and metabolism of major PUFAs. Evidence supporting that certain effects may be specific and unique to individual omega-3 PUFAs is now growing. For instance, the beneficial effects of EPA on mood and affective disorders have more consistently been reported in clinical trials, whereas high DHA consumption has been linked to a lower risk of neurodegenerative conditions, including Alzheimer's disease [52, 53].

Omega-3 fatty acids are available by prescription or as nutritional supplements. Supplement products usually contain the labeled amount of EPA and DHA. Preparations available by prescription are (1) omega-3-acid ethyl esters (Lovaza◯R, Omtryg◯R) in which each 1 g capsule contains about 465 mg of EPA and 375 mg of DHA, (2) icosapent ethyl (Vascepa◯R) containing about 878 mg of highly purified EPA, (3) mega-3-carboxylic acids (Epanova◯R) containing about 550 mg of EPA and 200 mg of DHA, and (4) omega-3 phospholipid (CaPre◯R containing phospholipids and free fatty acids collected from krill oils, including about 310 mg of EPA and DHA.

Fatty predatory fish such as sharks, swordfish, tilefish, and albacore tuna may be high in omega-3 fatty acids, which they obtained from the plankton, microalgae, or prey fish in their diets. Considering their top position at the food chain, toxic substances may also be accumulated

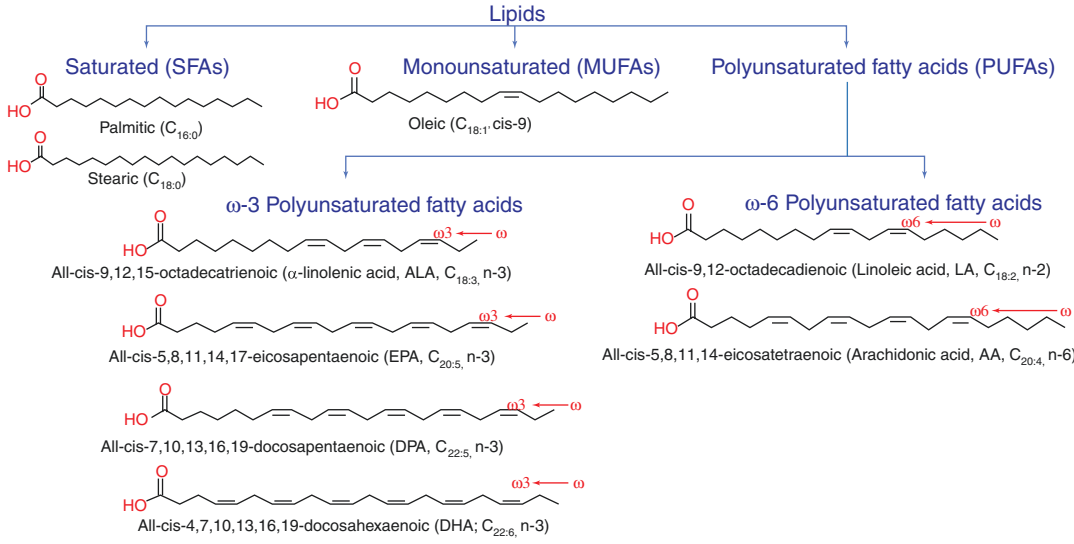

Fig. 10.3 Structures f major fatty acids found in plant and animal-based diet. Adapted with permission from Life Sciences, Copyright Elsevier [50]

through biomagnification in these species. For this reason, women of childbearing age need to be restricted from eating certain predatory fish species due to high levels of the toxic substances that they contain [54].

10.4 Implication of Fish Oils on Stroke Trials

Since the initial reports showing the association of n-3 PUFA-rich diet of the Greenland Inuit people with low mortality from ischemic heart disease, n-3 PUFAs have been given the great interest in the prevention of various vascular diseases, including cardiovascular disease (CVD) and stroke [1, 55–58]. In previous studies, a majority had focused on the association between n-3 PUFAs and CVD [59]. Epidemiological studies have supported the relationship between high n-3 PUFA intake and lower CVD rates [48, 56, 60]. However, recent major RCTs regarding this issue have shown inconsistent results [56, 61–64]. First, A Study of Cardiovascular Events in Diabetes (ASCEND), 1 g of n-3 PUFAs supplementation failed to show significant risk reduction of cardiovascular events compared to placebo [61]. Second, VITamin D and OmegA-3 Trial (VITAL), which aimed to identify the benefits of vitamin D3 and marine n-3 PUFAs (1 g/day) in primary prevention of CVD and cancer among healthy, middle-aged people, also did not find any significant statistical difference in risk reduction of cardiovascular events [63]. Conversely, Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT), a trial that studied the effect of icosapent ethyl (EPA 4 g/day) on patients with elevated triglyceride levels who were taking statins, indicated a significant risk reduction of major endpoints including cardiovascular death, despite the use of statins [62]. Recent Cochrane review concluded that (1) n-3 PUFAs have little or no effect on cardiovascular events or mortality, (2) EPA and DHA reduce triglyceride levels, and (3) n-3 PUFA may slightly reduce the risk of morbidity and mortality of the coronary heart disease [56].

10.4.1 Fish Oils on Stroke Trials

Recently, most studies have mentioned the association between n-3 PUFAs and stroke as a part of subgroup analysis or one of the secondary endpoints [59]. In the early 2000s, the initial observational studies showed the beneficial effect of higher consumption of fish and n-3 PUFAs in reducing the risk of stroke, especially ischemic subtype [65–68]. Another prospective cohort study showed different risk reduction effects in ischemic stroke according to its subtypes [69]. That study indicated that EPA was associated with lower risks of most types of ischemic stroke, apart from cardioembolism, which suggested different effects based on its pathomechanisms [69].

To identify the effect of marine n-3 PUFAs in stroke or TIA, results of various clinical trials were analyzed, including a comprehensive Cochrane review of RCTs [59, 70–80]. (Table 10.1) Unfortunately, most of the outcome measurements, including efficacy, adverse events, and functional outcome, were indifferent regardless of marine n-3 PUFA intervention. Fatal and nonfatal combined recurrent events were assessed in three trials, and two of them showed significant statistical differences [74, 76, 78]. However, the effect varied among studies. One study revealed an increased risk of recurrent events with n-3 PUFA treatment (GISSI HF: relative risk (RR), 2.42; 95% confidence interval (CI), 1.02–5.74) [74], whereas another study suggested risk reduction (JELIS: RR, 0.65; 95% CI, 0.42–0.99) [78]. The other study found no significant effect (Risk and Prevention Study: RR, 1.07; 95% CI, 0.60–1.88) [76]. Thus, the quality of the evidence was deficient due to inconsistency and imprecision [59]. A trial that studied the effect of n-3 PUFAs on mood showed favorable outcomes in the control group (mean difference (MD), 1.41; 95% CI, 0.07–2.75) [59, 72]. However, the evidence was thought to be weak since the scoring tools were unclear and not fully verified [59]. Moreover, the other trial with longer follow-up (more than 3 months) revealed no effect (MD, 1.00; 95% CI, −2.07–4.07; $p = 0.61$) [70].

Table 10.1 Summary of stroke-related randomized controlled trials

Trial	Study population	Stroke type	Number of participants (study)	Regimen of intervention	Length of intervention	Primary outcome	Stroke-related outcome	Relative effect
CVD + stroke								
ALPHA OMEGA [77]	Patients with history of MI up to 10 years	Not mentioned	345 (with history of stroke)	Average 20 g/day of margarine with 400 mg of EPA–DHA or 400 mg EPA–DHA + 2 g of ALA	40 months	Major cardiovascular events	Vascular-related death Fatal recurrent events	**Vascular-related death:** RR 1.09 (0.67–1.80) **Fatal recurrent events:** RR 1.09 (0.34–3.50)
FOILS [72]	Patients with a history of ischemic stroke (> 3 months)	Ischemic	102	3 g/day of fish oil (1.2 g n-3 PUFAs)	12 weeks	Change in serum triglycerides	[a]Mood [b]QOL Vascular-related death (d/t MI)	**Mood:** MD 1.41 (0.07–2.75, $p = 0.04$) **SF-36 physical scale:** MD -2.31 (−4.81–0.19) **SF-36 mental scale:** MD -2.16 (−5.91–1.59) **Vascular-related death:** RR 0.33 (0.01–8.00)
Foroughinia 2018 [71]	Patients with a history of ischemic stroke and CAS	Ischemic	18	3 g loading dose of n-3 PUFA (990 mg of EPA, 660 mg of DHA)	Single dose, 12 h before CAS	Composite outcome of any stroke, MI, and/or mortality	Adverse events (extracranial hemorrhage) Incidence (hemorrhagic) Recurrent events (ischemic)	**Adverse events:** RR 0.25 (0.04–1.73) **Incidence:** RR 6.11 (0.33–111.71) **Recurrent events:** RR 0.41 (0.02–8.84)
GISSI HF [74]	Patients with clinical evidence of heart failure of any cause	Not mentioned	346 (with stroke of history)	1 g of n-3 PUFA (850 to 882 mg of EPA–DHA (average ratio of 1:1.2)	Median (IQR) 3.9 (3.0 to 4.5) years	Composite time to death and time of admission to hospital for cardiovascular reasons	Recurrent events Vascular-related death	**Recurrent events:** RR 2.42 (1.02–5.74) **Vascular-related death:** RR 1.19 (0.86–1.64)

(continued)

Table 10.1 (continued)

Trial	Study population	Stroke type	Number of participants (study)	Regimen of intervention	Length of intervention	Primary outcome	Stroke-related outcome	Relative effect
JELIS [78]	Patients with a total cholesterol of 250 mg/dL or more	Both	942 (with stroke of history)	EPA 1800 mg/day with statin or statin only	Up to 5 years	Major coronary events	Recurrent events	**Recurrent events:** RR 0.65 (0.42–0.99)
NUTRISTROKE [75]	First-ever ischemic stroke survivors admitted to rehabilitation unit	Ischemic	72	500 mg of n-3 PUFA (250 mg DHA, 250 mg EPA) with/ without antioxidants	12 months	Neurological impairment [c]Functional disability	Vascular-related death Efficacy (BI, RMI)	**BI**: MD 7.09 higher (−5.16–19.34) **RMI**: MD 1.30 (−1.31–3.91) **Vascular-related death:** RR 0.10 (0.01–1.79)
OPAL [70]	Cognitively normal adults aged 70–79 years	Not mentioned	19 (with history of stroke)	700 mg/day of n-3 PUFA (200 mg EPA + 500 mg DHA)	24 months	[d]Cognitive function	Vascular-related death Recurrent events [a]Mood	**Vascular-related death:** RR 0.30 (0.01–6.62) **Recurrent events:** RR 0.30 (0.01–6.62) **Mood:** MD 1.00 (2.07–4.07)
Risk and Prevention Study [76]	Patients with atherosclerotic CVD + multiple risk factors	Not mentioned	1455 (with history of stroke)	1 g of n-3 PUFA (850 to 882 mg of EPA–DHA (average ratio of 1:1.2)	Median (IQR) 5 (4.0 to 5.5) years	Time to death from cardiovascular causes or hospital admission for cardiovascular causes	Vascular-related death Recurrent events Adverse events	**Vascular-related death:** RR 0.85 (0.55–1.33) **Recurrent events:** RR 1.07 (0.60–1.88) **Adverse events:** RR 0.94 (0.56–1.58)
Stroke only								
Rist 2020 [73] (subanalysis of VITAL)	Patients with a history of stroke during follow-up period	Both	197	1 g/day of fish oil capsule (840 mg of n-3 PUFAs: EPA 460 mg DHA 380 mg)	Median of 1.4 years after stroke diagnosis	[e]Functional limitation [f]Physical disability	Same as primary outcome	**Functional limitation**: OR 0.55 (0.28–1.09) **Physical disability: RB scale**: OR 0.56 (0.31–1.02) **Katz ADL scale**: OR 0.32 (0.08–1.24)

Saito 2017 [80]	Within 72 h after SAH	Hemorrhagic (SAH)	40	Intravenous (IV): 100 mL/day of fish oil-based lipid emulsion, FOLE Oral: fish oil capsule 4 g/day, EPA 1840 mg, DHA 1520 mg	60 days (5 days of IV treatment followed by 55 days of oral treatment)	[g]Functional outcome Adverse events	Same as primary outcome	**Poor clinical outcome:** RR 0.78 (0.36–1.68) **Vascular-related death:** RR 0.33 (0.01–7.72) **Bleeding complication:** RR 0.32 (0.01–7.35) **Cerebral infarction by DCI:** RR 0.63 (0.25–1.58)
Tanaka 2008 [79] (subanalysis of JELIS)	Patients with a total cholesterol of 250 mg/dL or more	Both	Primary: 17,703 Secondary: 942 (with stroke history)	EPA 1800 mg/day with statin or statin only	Up to 5 years	Primary and secondary prevention of stroke	Same as primary outcome	**Primary prevention:** HR 1.08 (0.95–1.22) **Secondary prevention:** HR 0.80 (0.63–0.997)

Abbreviations: *ALA* α-linolenic acid, *CAS* carotid artery stenting, *CVD* cardiovascular disease, *DCI* delayed cerebral ischemia, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *HR* hazard ratio, *IQR* interquartile range, *MD* mean difference, *MI* myocardial infarction, *n-3 PUFA* omega-3 polyunsaturated fatty acid, *OR* odds ratio, *RR* risk ratio, *SAH* subarachnoid hemorrhage

[a]Assessed by GHQ-28 (general health questionnaire)

[b]Assessed by physical scale and mental scale of SF-36 (36-item short-form survey)

[c]Assessed by Barthel Index (BI) and Rivermead Mobility Index (RMI)

[d]Assessed by California Verbal Learning Test (CVLT)

[e]Assessed by Nagi scale

[f]Assessed by Rosow–Breslau (RB) scale, Katz ADL scale

[g]Assessed by GOSE (Glasgow outcome scale-extended) score at 3 months

Another Cochrane review, more focused on preventive effect, was recently published [56]. That review included the trials assessing the effects of higher omega-3 intake versus lower omega-3 intake (i.e., placebo, no supplementation, usual diet, or lower dose of omega-3) for at least a year on the heart and circulatory disease. It concluded that n-3 PUFA intake had little effect on stroke risk (RR, 1.02; 95% CI, 0.94–1.12; 31 trials reported 2850 strokes) with moderate-certainty evidence. In detail, the risk of hemorrhagic stroke was slightly increased (RR, 1.23; 95% CI, 0.93–1.64), while ischemic stroke was unchanged (RR, 0.98; 95% CI, 0.79–1.20). Subgroup analysis did not show any significant differences in stroke outcome regardless of intervention type, replacement, statin use, trial duration, baseline triglycerides, or diabetic status. Furthermore, metaregression to assess the effect of n-3 PUFA dose did not find any clear dose-response relationship on the risk of stroke ($p = 0.12$) [56].

On the other hand, even with the preventive effect of n-3 PUFAs in stroke, considerable preclinical studies have revealed that n-3 PUFA supplementation might benefit the long-term outcome of ischemic stroke patients. It was mainly due to anti-inflammatory effects or enhanced brain repair processes, including angiogenesis, neurogenesis, and white matter restoration [81–85]. However, compared to the emerging shreds of evidence of the neuroprotective effect of n-3 PUFAs in stroke, human studies to verify the theories are still lacking. Accordingly, the long-term functional outcome and stroke recovery directly in the acute phase need to be more addressed, particularly for further studies [59]. Indeed, several ongoing trials awaiting assessment are focused on the neuroprotective effect of n-3 PUFAs (especially cognitive function) [56, 59].

10.4.2 Conclusion

To summarize, evidence to adopt marine-derived n-3 PUFAs as a therapy for stroke at this point is insufficient [59]. In other words, n-3 PUFAs have little or no effect on stroke in primary or secondary prevention [56]. Despite disappointingly negative results of prior trials, clinicians are still focusing on the protective effect of n-3 PUFAs for stroke and CVD, probably since given the prevalence of CVD, stroke, and their disease burden, lifestyle modifications including dietary adjustments (e.g., increased fish intake) could be easy instead of finding effective ways to prevent those diseases [64]. Also, studies regarding various outcome measurements other than primary and secondary prevention of stroke (especially functional outcome) are limited, which might be more critical in stroke than other CVDs. Therefore, considering numerous conditions affecting the research results, including delivery way or dose, combinations of various n-3 PUFAs, and ethnicity of participants, well-designed RCTs in various clinical settings are expected to elucidate the pleiotropic effects of n-3 PUFAs in stroke.

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PCSK9 Inhibiting Monoclonal Antibodies

11

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Abstract

Low-density lipoprotein cholesterol (LDL-C), a component of dyslipidemia, is a modifiable risk factor for cerebrovascular disease including ischemic stroke and TIAs. The current guidelines recommend the use of lipid-lowering medications to reduce the risk of stroke among patients with ischemic stroke or TIA presumed to be of atherosclerotic origin. Statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, in addition to healthy lifestyle modifications are a mainstay in the treatment of hyperlipidemia and stroke risk reduction. However, there are limitations to statins use. Some patients, despite being on maximum doses of high-intensity statins in conjunction with other lipid-lowering medications like ezetimibe, continue to have persistently elevated LDL-C levels, while a subset of patients is unable to tolerate statins due to their adverse effects, namely statin-induced myopathy. A relatively new class of monoclonal antibodies have been developed that inhibit proprotein convertase subtilisin/kexin type 9 (PCSK9), a protein involved in lipid metabolism, and can address therapeutic limitations in this subset of patients. A second class of drugs using small interfering RNA that lower PCSK9 are also currently under active investigation. This chapter will focus on PCSK9 inhibitors by discussing pharmacology, clinical trials with a focus on stroke outcomes, and clinical use in practice.

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11.1 Biology of PCSK9

Autosomal dominant familial hypercholesterolemia (also known as familial hypercholesterolemia) is a genetic disorder characterized by significantly elevated levels of low-density lipoprotein cholesterol (LDL-C) leading to premature atherosclerotic cardiovascular disease (ASCVD). Familial hypercholesterolemia affects 1 in 250 people with a prevalence of 30 million

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people worldwide [1]. Mutations in *LDLR* (encoding the low-density lipoprotein receptor) account for 90% of cases, and *APOB* (encoding apolipoprotein B) account for 5%. In the early 2000s, investigators identified a French family with familial hypercholesterolemia who did not have mutations in either *LDLR* or *APOB* genes [2]. Researchers identified a novel region on the short arm of chromosome 1 with a gain of function mutations that was linked to this phenotype. In 2003, proprotein convertase subtilisin/kexin type 9 (*PCSK9)* was identified as the third gene to be implicated in the pathogenesis of familial hypercholesterolemia. In 2005, researchers identified loss-of-function mutations in PCSK9, leading to lowering of LDL-C levels and thereby implicating PCSK9 in the LDL pathway. Thus, this became a potential biological target for drug development.

PCSK9 cDNA has 3619 base pairs that encode 692 amino acid called NARC-1, a novel serine protease that is primarily expressed in the liver, intestine, and kidney [3, 4]. The protein consists of four main regions: an N-terminal signal sequence, a prodomain (amino acids 31–152), a catalytic domain (amino acids 153–451) that is responsible for the protein's protease function, and a C-terminal domain (amino acids 452–692) [5]. The protein is initially translated as an inactive zymogen and is posttranslationally modified within the cell's endoplasmic reticulum via glycosylation, phosphorylation, and sulfation [6]. The prodomain of the protein is cleaved within the endoplasmic reticulum, but remains tightly associated with the mature protein and serves as a chaperone to allow for secretion of PCSK9 outside of the cell and into the bloodstream [4]. PCSK9 normally binds low-density lipoprotein receptors (LDLR) on hepatocytes and promotes their degradation leading to elevated LDL-C levels (Fig. 11.1).

11.2 Pharmacology of Monoclonal Antibodies Against PCSK9

Within a decade of discovering *PCSK9* and its role in lipid metabolism, several antibody-based therapeutics were developed targeting PCSK9 protein. There are currently two PCSK9 inhibitors that are approved by the U.S. Food and Drug Administration (FDA): (1) **evolocumab** (Repatha) and (2) **alirocumab** (Praluent). A third candidate, **bococizumab**, was discontinued due to the development of immunogenicity leading to antidrug antibodies that reduced its long-term efficacy. This section will focus on the pharmacology of evolocumab and alirocumab.

11.2.1 Alirocumab

11.2.1.1 Indications

Alirocumab (tradename Praulent™, co-developed by Regeneron Pharmaceuticals and

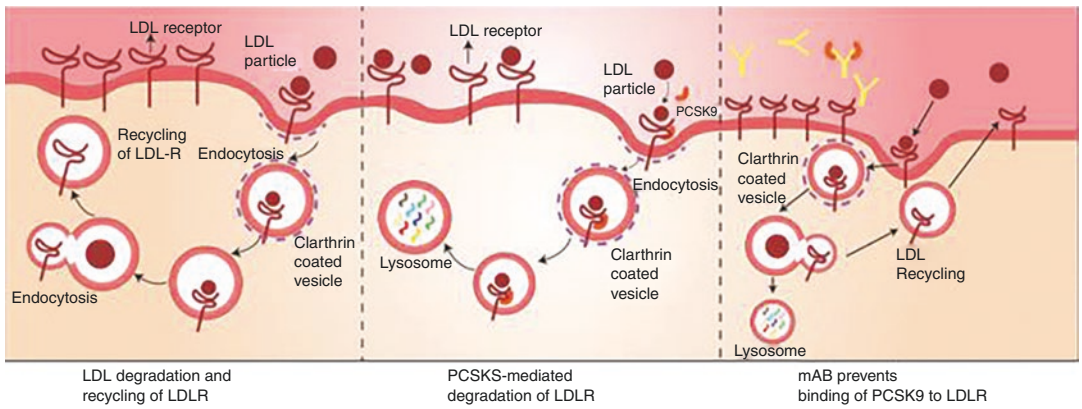

Fig. 11.1 PCSK9-mediated degradation of LDLR

Sanofi) was approved by the FDA in July 2015 for adult patients:

- As adjunctive treatment to diet and maximally tolerated lipid lowering medications, such as statins and/or ezetimibe, in patients with primary hyperlipidemia or those with heterozygous familial hypercholesterolemia who require additional lowering of LDL-C.

11.2.1.2 Mechanism

Alirocumab is a human monoclonal IgG1 antibody (146 kD) that binds to and inhibits PCSK9 [1]. Since LDLR normally clears circulating LDL-C and PCSK9 promotes the degradation of LDLR, alirocumab prevents degradation of LDLR resulting in an increase in LDLR, thereby lowering circulating LDL-C levels [2–4].

11.2.1.3 Dose/Administration, Absorption, Distribution, Metabolism, and Clearance, Interactions

The recommended starting dose is 75 mg by subcutaneous injection (available as a prefilled pen or syringe) every 2 weeks and maybe titrated up to 150 mg if LDL-C lowering effects are not adequate after 4–8 weeks [1, 5]. (Table 11.1) One injection of alirocumab results in maximum suppression of free PCSK9 in 4–8 h in a dose-dependent fashion [6]. A maximum serum concentration is achieved between 3–7 days, and

Table 11.1 Pharmacology of Alirocumab and Evolocumab

	Alirocumab (Praluent)	Evolocumab (Repatha)
Mechanism of action	Humanized monoclonal IgG1 (146 kDa) antibody directed against PCSK9.	Humanized monoclonal IgG2 (144 kDa) antibody directed against PCSK9.
Indications [7]	HeFH, ASCVD	HeFH, HoFH, PH, ASCVD
Method of administration	SC injection every 2 weeks	SC injection every 2 weeks OR Once monthly injection in abdomen, thigh or upper arm.
Pertinent drug interactions	None	None
Adverse reactions	• Nasopharyngitis • Injection site reactions • Influenza • Urinary tract infections • Diarrhea	• Nasopharyngitis • Injection site reactions • Upper respiratory tract infections • Arthralgia • Nausea
Effects on lipids	For patients with prior ACS event: −58% decrease in LDL-C, −36% on total cholesterol, −50% on non-HDL-cholesterol, and − 51% on ApoB at 24 weeks compared to baseline (50 mg alirocumab every 2 weeks)	For HoFH: −31% decrease LDL-C levels at week 12 compared to baseline For PH: −71% decrease in LDL-C, −59% decrease in non-HDL-C, −55% in Apo B, −40% in total cholesterol compared to placebo for 2 week doses (140 mg given every 2 weeks)
Half life	17–20 days	11–17 days
Bioavailability	85%	72%
Volume of distribution	0.04–0.05 L/kg (primarily distributed in circulatory system)	3.3 L
Time to maximum serum concentration	3–7 days	3–4 days
Time to maximum suppression of PCSK9	4–8 h	4 h
Mechanism of elimination	• High concentrations: Serum proteolysis. • Low concentrations: Binding to PCSK9 and resultant intracellular degradation.	• High concentrations: Serum proteolysis. • Low concentrations: Binding to PCSK9 and resultant intracellular degradation.

bioavailability is around 85%. Doubling the dose of alirocumab results in between 2.1–2.7 times the increase in total alirocumab concentrations, and steady state is reached after 2–3 doses. The volume of distribution is likely primarily circulatory (0.04–0.05 L/kg). There are no known drug interactions with alirocumab. No dose adjustments are necessary for mild or moderate hepatic or renal impairment, and safety and efficacy have not been established in children [1].

Because alirocumab is a protein and thus expected to degrade into amino acids, specific metabolism studies were not conducted. At low concentrations, alirocumab was primarily eliminated through PCSK9 binding, and at high concentrations, it was eliminated through proteolytic pathways. The median apparent half-life is 17–20 days [1].

11.2.1.4 Efficacy

In the ODESSEY OUTCOMES, a multicenter, double-blind, placebo-controlled trial of 18,924 patients, researchers found that a composite endpoint of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization was significantly lower in the alirocumab-treated group than placebo (9.5% vs. 11.0%) [8]. (Table 11.2) In terms of stroke, alirocumab reduced the risk of any stroke (hazard ratio (HR), 0.72; 95% confidence interval (CI), 0.57–0.91) and ischemic stroke (HR, 0.73; 95% CI, 0.57–0.93), with no difference in effect in patients who had previously had cerebrovascular disease [17]. Another study of 216 patients found that the difference at week 12 in LDL-C was −46% in alirocumab compared with placebo [18]. Other studies found similar results, with one finding that if patients started at 75 mg with little effect at 12 weeks but then was uptitrated to 150 mg, patients on alirocumab would experience −54% change in LDL-C over placebo, which was sustained to week 52 [1, 19, 20]. A meta-analysis found that the reduction in LDL-C was seen in placebo and ezetimibe-controlled trials, and patients on alirocumab experienced an increase of 6.15% in HDL, 40% reduction in ApoB, 40% reduction in non-HDL-C, 33% reduction in total cholesterol, and 11% reduction in triglycerides [4]. The overall effects of alirocumab on cerebrovascular morbidity and mortality have not yet been determined [4].

11.2.1.5 Adverse Reactions

In an analysis of 9 randomized control trials of alirocumab of 2476 patients, the most common adverse reactions that occurred more frequently than placebo were nasopharyngitis (11.3%), injection site reactions (7.2%), influenza (5.7%), urinary tract infection (4.8%), and diarrhea (4.7%) [1]. Out of the 5.3% of patients who discontinued it due to adverse effects, the most common reasons were for allergic reactions (0.6%) and elevated liver enzymes (0.3%). The overall allergic reaction rate was 8.6% and elevated liver enzymes rate was 2.5%. A greater number of patients had local injection site reactions on alirocumab over placebo (7.2% vs. 5.1%), but this led to few treatment discontinuations. Furthermore, more neurocognitive events were reported with alirocumab than placebo (0.8% vs. 0.7%). Some patients experienced two consecutive calculated LDL-C values <25 mg/dL, but changes to statins or alirocumab were not made, and no serious side effects were experienced [1].

11.2.1.6 Cost

A 2020 study found that in the United States, the annual treatment cost of alirocumab is $5850, which makes the incremental cost-effectiveness ratio $92,200. This was broken down into $41,800 per QALY for patients with baseline LDL-C ≥ 100 mg/dL and $299,300 for patients with LDL-C < 100 mg/dL, thus rendering it cost-effective only for patients with LDL-C ≥ 100 mg/dL [21].

11.2.2 Evolocumab

11.2.2.1 Indications

Evolucumab (tradename Repatha®, developed by Amgen) was approved by the FDA in August 2015 for adult patients:

Table 11.2 Phase 3 randomized clinical trials involving Alirocumab and Evolocumab

Name	Drug Regime	Sample size	Inclusion Criteria	Control Group	Time (Weeks)	Primary Outcome	Baseline LDL-C	% Reduction in LDL-C[a]	Neurologic End Points (HR)	P-value
MENDEL-2 (2014) [9]	Evolocumab	614	LDL: 100-190 mg/dL & Framhingham risk <10%	Placebo (1) Ezetimibe (2)	12	% change in LDL-C at week 12 compared to baseline	143 mg/dL	55–57% (1) 38–40% (2)	N/A	$p < 0.001$ (1) $p < 0.001$ (2)
ODYSSEY MONO (2014) [10]	Alirocumab	103	LDL: 100-190 mg/dL and ASCVD: 1–5%	Ezetimibe	24	% change in LDL-C at week 24 compared to baseline	141.1 mg/dL	47.2%[b] (Ali. Group) 15.6%[b] (Eze. Group)	N/A	$p < 0.0001$
LAPLACE-2 [11] (2014)	Evolocumab + statin	1899	Primary hypercholesterolemia and mixed dyslipidemia	Placebo	12	% change in LDL-C at week 12 compared to baseline	109.7 mg/dL	63–75%	N/A	$p < 0.05$
DESCARTES (2014) [12]	Evolocumab + statin	901	Hyperlipidemia	Placebo	52	% change in LDL-C at week 52 compared to baseline	104 mg/dL	55–59%	N/A	$p < 0.001$
ODYSSEY COMBO I (2015) [13]	Alirocumab + statin	316	CAD and hypercholesterolemia	Placebo	24	% change in LDL-C at week 24 compared to baseline	97 mg/dL	44–52%	N/A	$p < 0.001$
ODYSSEY COMBO II (2015) [14]	Alirocumab + statin	720	High cardiovascular risk and high LDL-C, statin	Ezetimibe + statin	104	% change in LDL-C at week 24 compared to baseline	107 mg/dL	49–52%[b] (Ali. Group) 18–23%[b] (Eze. Group)	N/A	$p < 0.0001$
ODYSSEY OUTCOMES (2019) [15]	Alirocumab	18,924	Recent CAS, and dyslipidemia, despite statin	Placebo	104 (median F/U)	CVD, NFMI, ischemic/hemorrhagic stroke, or UA requiring hospitalization	<80 mg/dL (7164) 80–100 mg/dL (6128) >100 mg/dL (5629)	N/A	Any stroke: 0.72 (Ali. Group) (1) Ischemic stroke: 0.73 (Ali. Group) (2)	$p < 0.05$ (1) $p < 0.05$ (2)
FOURIER (2020) [16]	Evolocumab	27,564	Atherosclerosis, or prior MI or prior stroke and LDL >80 mg/dL, despite statin	Placebo	115 (median F/U)	CVD, NFMI, ischemic/hemorrhagic stroke, or UA requiring hospitalization	92.8 mg/dL (median)	N/A	Any stroke: 0.79 (Evol. Group) (1) Ischemic stroke: 0.75 (Evol. Group) (2)	$p < 0.05$ (1) $p < 0.05$ (2)

Abbreviations: *CAS* Coronary Artery Syndrome, *CVD* Cardiovascular Disease, *HR* Hazard Ratio, *NFHI* Nonfatal Myocardial Infarction, *UA* Unstable Angina

[a]Reductions generally in reference to the control arm of the trial

[b]Absolute reductions

- To reduce the risk of myocardial infarction, stroke, and coronary revascularization in patients with known cardiovascular disease.
- As adjunctive treatment to diet and maximally tolerated lipid-lowering medications, such as statins and/or ezetimibe, in patients with primary hyperlipidemia or those with heterozygous familial hypercholesterolemia who require additional lowering of LDL-C.

11.2.2.2 Mechanism

Evolocumab is a human monoclonal IgG2 antibody (144 kD) that inhibits PCSK9 with a mechanism of action that is similar to that of alirocumab [2].

11.2.2.3 Dose/Administration, Absorption, Distribution, Metabolism and Clearance, Interactions

For adults with established cardiovascular disease or primary hyperlipidemia, evolocumab is administered subcutaneously 140 mg every 2 weeks or 420 mg once monthly in the abdomen, thigh, or upper arm (Table 11.1). For patients with HoFH, it is administered 420 mg once monthly. No dose adjustments are needed in patients with renal impairment or mild to moderate hepatic impairment [22].

One injection of evolocumab results in maximum suppression of free PCSK9 in 4 h. One 120 mg dose results in a maximum concentration of 18.6 μg/mL, while a 420 mg dose results in 59.0 μg/mL with clearance of 12 (2) mL/h. Median peak serum concentrations are attained in 3–4 days with estimated absolute bioavailability of 72%, and steady-state volume of distribution is 3.3 (0.5) L. Similar to alirocumab, evolocumab is eliminated through PCSK9 binding at lower concentrations and proteolysis at higher concentrations and has an estimated half-life of 11–17 days [22].

11.2.2.4 Efficacy

Efficacy for prevention of cerebrovascular and cardiovascular events was evaluated in the FOURIER trial of 27,564 patients with known ASCVD including either a history of MI, nonhemorrhagic stroke, who were already on high or moderate-intensity statins with persistently elevated LDL-C ≥ 70 mg/dL or non-HDL-CC ≥100 mg/dL [23]. Endpoints of the trial included cardiovascular disease, nonfatal myocardial infarction, ischemic/hemorrhagic stroke, or unstable angina requiring hospitalization. Evolocumab significantly reduced primary composite endpoint events (time to first cardiovascular death, MI, stroke, hospitalization for unstable angina, coronary revascularization) as well as secondary endpoint (time to first cardiovascular death, MI, or stroke). At week 72, patients on evolocumab had a mean −57% change in LDL-C from baseline, and nearly half of patients had LDL-C < 25 mg/dL at 48 weeks.

A subgroup analysis was subsequently performed to assess the efficacy of evolocumab to reduce overall stroke and stroke subtypes. Median age of the patient population was 63 years old with 19% with prior nonhemorrhagic stroke. There was a median follow-up of 2.2 years. Of the 27,564 patients, 469 (1.7%) patients suffered from 503 strokes, with 421 (84%) being ischemic in nature. The results demonstrated that the use of evolocumab reduced the risk of all stroke by 21% when compared to placebo (HR, 0.79; 95% CI, 0.66–0.95; $P = 0.01$) with a 25% decrease in risk of ischemic stroke (HR 0.75; 95% CI, 0.62–0.92; $P = 0.005$), with no difference in hemorrhagic stroke (HR 1.16; 95% CIl 0.68–1.98; $P = 0.59$). The study also assessed cardiovascular endpoints by subgroups based on history of a stroke. This trial demonstrated that PCSK9 with evolocumab when added to statin therapy in patients with known ACVSD reduced ischemic stroke in the total population and in patients with prior ischemic strokes.

Several studies have also looked at evolocumab's efficacy in primary hyperlipidemia [22]. The LAPLACE-2 study showed that evolocumab resulted in −71% decrease in LDL-C for 140 mg every 2 weeks and − 63% for 420 mg monthly doses compared to placebo [24]. (Table 11.2) Furthermore, it resulted in a − 59% decrease in Non-HDL-C, −55% in Apo B, and − 40% in total

cholesterol compared to placebo for 2 week doses. Similar lipid-lowering effects were seen in the DESCARTES trial, MENDEL-2, and RUTHERFORD-2 trials [7, 9, 25]. One meta-analysis showed that evolocumab may be more effective than alirocumab. 140 mg of evolocumab every 2 weeks at 10 and 12 weeks resulted in −20% and − 13% bigger reductions in LDL-C levels than alirocumab [10].

The TESLA study, which evaluated patients with homozygous familial hypercholesterolemia (HoFH), showed that at week 12, evolocumab users experienced a − 31% decrease LDL-C levels from baseline, but patients with known two LDL-receptor negative alleles did not respond [11].

11.2.2.5 Adverse Reactions

Overall, the adverse event rates were similar between evolocumab and control in trials [12]. For patients receiving evolocumab for primary hyperlipidemia, in a 52-week, placebo-controlled trial of 901 patients receiving 402 mg monthly, the most common adverse effects seen significantly higher in patients receiving evolocumab over placebo were nasopharyngitis (10.5%), upper respiratory tract infection (9.3%), influenza (7.5%), back pain (6.2%), and injection site reactions (5.7%) [7, 25]. Of the 2.2% of patients who discontinued therapy, the most common reason was for myalgia (0.3%). In seven pooled, 12-week trials of 3276 patients, the most common adverse reactions were nasopharyngitis (4.0%), back pain (2.3%), upper respiratory tract infection (2.1%), arthralgia (1.8%), and nausea (1.8%). Local site injection reactions occurred in 3.2% of patients and led to treatment discontinuation in 0.1% of patients, while allergic reactions occurred in 5.1% of patients [22].

For patients receiving evolocumab to improve cardiovascular outcomes, one trial of 27,525 patients found that serious adverse events occurred in 24.8% of treated patients, with adverse events leading to discontinuation in 4.4% of patients (vs 4.2% in placebo) [23]. The most common adverse effects seen significantly higher in patients receiving evolocumab over placebo were diabetes mellitus (8.8%), nasopharyngitis (7.8%), and upper respiratory tract infection (5.1%). Similar results were seen in a trial of patients with HoFH [11].

11.2.2.6 Cognitive Function

There was initial concern for a potential association between statin use and impaired neurocognitive function, including memory loss, concentration difficulties, and confusion, leading to the FDA to issue a warning in 2012 [8, 17]. Further studies demonstrated inconclusive evidence with the Statin Cognitive Safety Task Force ultimately concluding that there was no association between the use of statins and memory dysfunction [17]. Similarly, there was some initial evidence that showed a potential association between the use of PCSK9 inhibitors and adverse cognitive events reported by patients, with a meta-analysis demonstrating an increased incidence of neurocognitive adverse events (odds ratio (OR), 2.34; 95% CI, 1.11–4.93; $P = 0.02$) in patients taking PCSK9 inhibitors as compared to placebo [18]. These findings prompted the Evaluating PCSK9 Binding Antibody Influence on Cognitive Health in High Cardiovascular Risk Subjects (EBBINGHAUS) study to use a validated test to assess cognitive function in a subgroup of 1204 patients from the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial, which assessed efficacy and safety of evolocumab [19–21]. Researchers assessed mean change over time in score for spatial working memory as an index of executive function as a primary endpoint which demonstrated −0.21 ± 2.62 change in raw score for evolocumab group versus −0.29 ± 2.81 for the placebo group ($P < 0.001$ for noninferiority; $P = 0.85$ for superiority), suggesting no significant differences in cognitive function between patients who received evolocumab and placebo over a median study period of 19 months. Similarly, there were no changes in raw score for secondary endpoints of working memory, episodic memory, or psychomotor speed between patients who received evolocumab vs. placebo.

11.2.2.7 Cost

In 2018, evolocumab was available in the United States for a reduced annual list price of $5850 per year. One analysis found that when added to standard background therapy (maximally tolerated statins with or without ezetimibe), evolocumab's incremental cost-effectiveness ratios ranged from $56,655 to $7667 per quality-adjusted life-year increase, depending on the level of risk in the population [13]. Thus, it has an acceptable level of the cost-effectiveness ratio. Evolocumab requires prior authorization.

11.3 Current Guidelines on PCSK-9 in Patients with ASCVD

The 2018 guidelines on the management of blood cholesterol from the American Heart Association and American College of Cardiology include the use of PCSK-19 inhibitors, a new addition from the prior 2013 guidelines [25]. Per guidelines, it is a Class IIa indication to add PCSK9 inhibitor as secondary prevention to high-risk patients who have known ASCVD who are already on maximally tolerated statins and ezetimibe who continue to have LDL-C levels of 70 mg/dL or higher (≥1.8 mmol/L) or a non-HDL-C level of 100 or higher (≥ 2.6 mmol/L). The guidelines emphasized that this should be shared decision-making between clinician and patient after considering benefit, safety, and costs of PCSK9 inhibitors. It is a Class I recommendation to continue maximally tolerated statins and ezetimibe in these patients who are being started on PCSK9 inhibitors.

11.4 Conclusion

Monoclonal antibodies that target PCSK9, including alirocumab and evolocumab, are novel non-statin, lipid-lowering medications that when used as monotherapy and/or in conjunction with statins and ezetimibe have shown to lower LDL-C levels in patients with established ASCVD. Small interfering RNA that inhibits hepatic synthesis of PCSK9 remain under active investigation in the United States but have recently received approval for use in Europe. Future clinical studies are required to assess long-term safety and cerebrovascular outcomes of PCSK9 inhibitors in patients with elevated LDL-C levels.

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PCSK9 Inhibiting siRNA

12

R. Scott Wright

Abstract

Currently available therapies reduce low-density lipoprotein cholesterol (LDL-C) through a variety of mechanisms, with statins being the most widely utilized of all therapies. However, patients who do not respond to statin therapy or other oral agents, or who develop side effects from taking the medications often need novel treatments. Inclisiran is a new and novel small interfering RNA compound which inhibits the translation of mRNA for Proprotein convertase subtilisin-kexin 9 (PCSK9) and thus reduces plasma levels of LDL-C. This chapter will focus on small interfering RNA compound PCSK9 inhibitors, which is currently under active investigation, by discussing pharmacology, clinical trials with a focus on stroke outcomes, and clinical use in practice.

Managing dyslipidemia can be simple yet challenging. Currently available therapies reduce low-density lipoprotein cholesterol (LDL-C) through a variety of mechanisms, with statins being the most widely utilized of all therapies [1]. Patients who do not respond to statin therapy or other oral agents, or who develop side effects from taking the medications often need novel treatments. Additionally, there are patients with cardiovascular disease who need more aggressive LDL-C reductions than can be achieved alone with oral therapy [1]. And it is worth mentioning that at least 30% of patients fail to take their oral medications daily, prompting the need for new pharmacotherapies which ensure adherence [2].

Inclisiran is a new and novel small interfering RNA compound which inhibits the translation of mRNA for Proprotein convertase subtilisin-kexin 9 (PCSK9) and thus reduces circulating levels of PCSK9 [3]. Inclisiran reduces plasma levels of LDL-C, because of its actions on PCSK9. How does inclisiran work? It interrupts the translation of the mRNA for PCSK9 by interacting with the body's natural RNA silencing complex (RISC) in hepatocytes [4, 5]. This mechanism of protein synthesis regulation is a relatively novel discovery and has now become the target of emerging pharmacotherapeutics [6, 7].

Inclisiran is a double stranded small RNA molecule with synthetic copies of the sense and anti-sense strands of the mRNA for PCSK9. Inclisiran is synthetically modified as 44 nucleotides, with modifications designed to reduce immune-mediated alteration and destruction. The nucleotides have one 2′-deoxy, eleven 2′-fluoro and thirty-two 2′-*O*-methyl modifications and the terminals of the nucleotides have attached

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phosphorothioates, all of which reduce immunogenicity [4, 5]. Inclisiran is designed to have high affinity for hepatocytes to minimize any off-target actions. Its affinity for hepatocytes is due to the attachment of a triantennary *n*-acetyl galactosamine moiety (GalNec) at one end of the double stranded RNA compound. The GALNec moiety has a high affinity for the asialoglycoprotein receptor (ASGPR) on hepatocytes, which facilitates the uptake of inclisiran through clathrin coated vesicles into the cells of hepatocytes via endocytosis (Fig. 12.1). The GALNec-

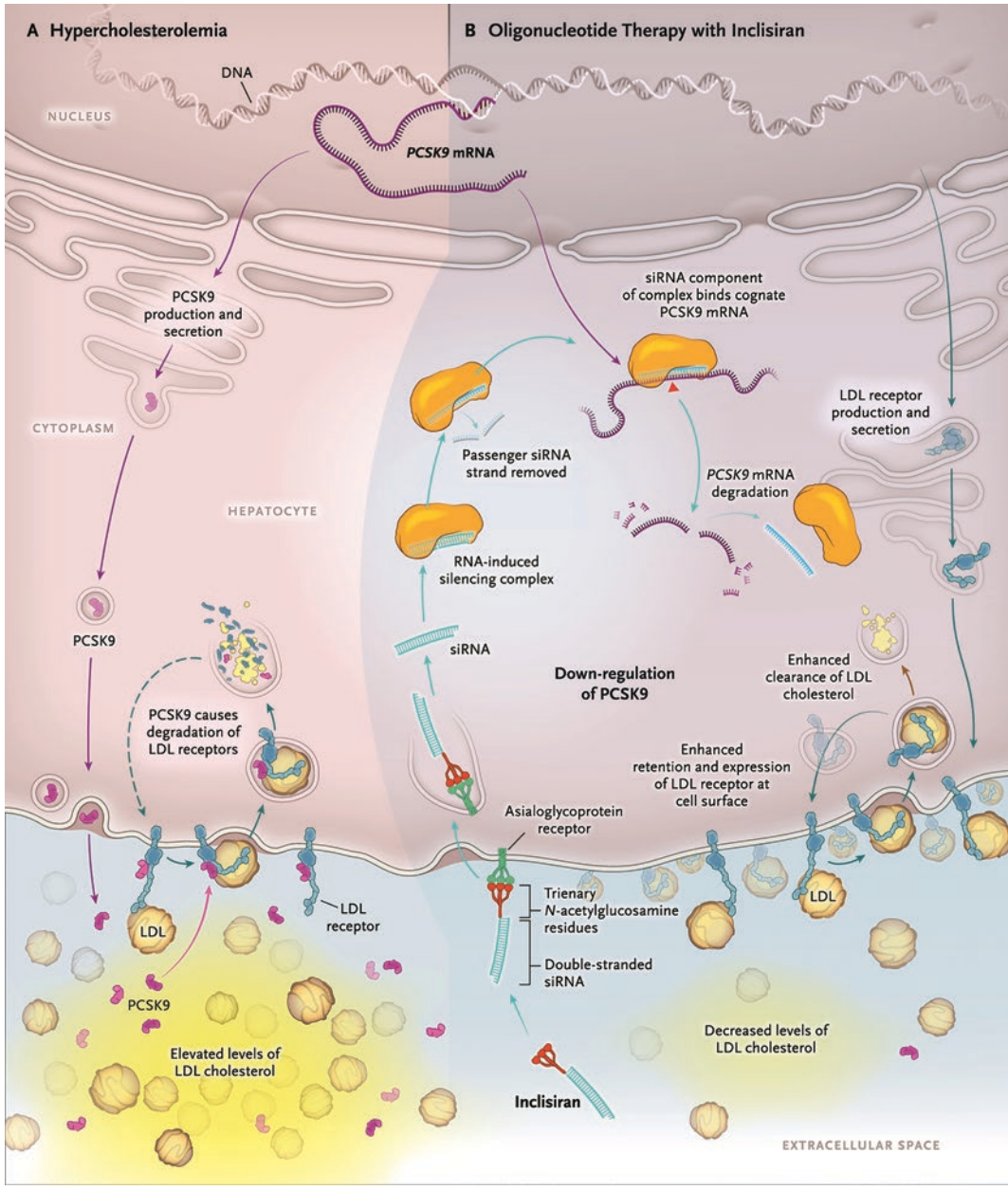

Fig. 12.1 This figure depicts the uptake of inclisiran by the liver and its RISC-Mediated cleavage of PCSK9 mRNA. Adapted with permission from the New England Journal of Medicine, Copyright Massachusetts Medical Society [5]

ASGPR affinity allows inclisiran to be exclusively delivered to the hepatocyte after subcutaneous administration After uptake into the liver cell, inclisiran's double stranded RNA separates into the sense and anti-sense strands. The anti-sense strand binds within RISC and potently inhibits the translation of PCSK9 mRNA into PCSK9 [5]. The inhibition of the translation of mRNA into PCSK9 results in substantial reductions of circulating levels of PCSK 9. The reductions in plasma PCSK9 functionally interrupt the degradation and recycling of the LDL-C receptor as it enters the hepatocyte through endocytosis after binding LDL-C. These actions prolong the lifespan of the LDL-C receptor on the surface of hepatocytes. The lack of recycling of the LDL-C receptor facilitates additional removal of LDL-C from plasma, lowering plasma LDL-C levels while raising intracellular LDL-C concentrations in the hepatocytes. The rise in LDL-C concentrations in the cytoplasm of the hepatocyte results in down regulation of de novo cholesterol biosynthesis, as initially postulated by Brown and Goldstein [8, 9]. Together, these actions lower plasma LDL-C, which makes it useful as a treatment for dyslipidemia. Inclisiran's mechanism of action as a small, interfering RNA that inhibits PCSK9 Synthesis sets it apart from the monoclonal antibodies which bind circulating PCSK9 and lower LDL-C through this extrinsic binding rather than inhibition of PCSK9 biosynthesis [4].

12.1 Phase 1 and 2 Clinical Studies

An early phase 1 clinical trial evaluated a precursor of inclisiran in a small number (n = 32) of patients [10]. The precursor of inclisiran was injected intravenously in a series of doses: 0.015 mg/kg, 0.045 mg/kg, 0.090 mg/kg, 0.150 mg/kg, 0.250 mg/kg, and 0.4 mg/kg and compared to placebo with regard to efficacy of PCSK9 and LDL-C lowering, safety, and tolerability. The formulation was an intravenous compound that had been suspended in lipid microparticles. The participants who received the 0.4 mg/kg dosing (n = 6) experienced a 70% lowering in circulating PCSK9 and approximately a 40% reduction in LDL-C compared with placebo. Its duration of action was short but the observed efficacy was judged as a proof of concept that a small, interfering RNA therapy might have clinical efficacy. The rates of treatment emergent adverse events were similar between the placebo and treated groups, supporting satisfactory tolerability and safety.

A second phase I trial was conducted by Alnylam Pharmaceuticals which randomized 24 healthy volunteers in a 3:1 fashion to placebo or multiple doses of a newly formulated inclisiran: 25 mg, 100 mg, 300 mg, 500 mg, and 800 mg doses [11]. Participants had to be between ages 18 and 60 with an LDL-C ≥ 100 mg/dl and if taking statin therapy, the dose had to be considered a stable dose. Participants with triglycerides ≥400 mg/dl were excluded from consideration. The newly formatted inclisiran was administered subcutaneously instead of intravenously as in the first phase I study. The dosing of inclisiran was complex as the participants were divided into six cohorts. One group received inclisiran 125 mg weekly for 4 weeks while a second group received 250 mg every 2 weeks for 4 weeks. Two groups received monthly inclisiran at 300 mg/month for 2 months; two additional groups received 500 mg/month for 2 months. The results demonstrated only grade 1 or grade 2 adverse events, and no treatment discontinuations occurred. The most common reported adverse events were nasopharyngitis, cough, musculoskeletal pain, headache, back pain, and diarrhea. The lipid data demonstrated placebo-adjusted reductions at day 84 in LDL-C ranging from 31% to 49% again confirming the potential efficacy of inclisiran as a therapy for LDL-C. The placebo-adjusted reductions at day 84 in PCSK9 range from 62% to 100%, while the placebo-adjusted reductions in total cholesterol ranged from 17% to 33%. Placebo-adjusted changes at day 84 in non-HDL C ranged from 25% to 46%, changes in apo B ranged from 24% to 40%, and changes in Lipoprotein (a) ranged from 13% to 37%.

The compound was acquired by The Medicines Company after completion of the Phase 1 trials.

The sponsor designed a series of phase 2 and phase 3 studies (The ORION Program) to further evaluate the safety and efficacy of inclisiran as depicted in Fig. 12.2 [4]. Its initial Phase 2 trial (ORION 1) was a phase 2 placebo-controlled, multicenter randomized trial which evaluated multiple doses of inclisiran against placebo on LDL-C lowering at 180 days [12]. The participants included patients with established cardiovascular disease and an LDL-C >70 mg/dl or patients without a history of disease but who had an elevated LDL-C >100 mg/dl. Both groups had to be on a stable dose of maximally tolerated statin therapy for at least 30 days. Inclisiran was administered in several doses (200 mg, 300 mg, or 500 mg) as a single injection or as two injections given on days 1 and 90, or 90 days apart against placebo administered in the same fashion. The investigators observed reductions in LDL-C from 28% to 42% at day 180 when inclisiran was administered as a single dose, and reductions in LDL-C from 35% to 53% when inclisiran was administered in two doses, 90 days apart across the ranges of doses given. Total cholesterol was reduced 18–27% when inclisiran was administered as a single dose, and reduced 22% to 33% when administered as two doses, 90 days apart. Non-HDL cholesterol was reduced 25–37% with single doses of inclisiran and by 32–46% with two doses of inclisiran administered on days 1 and 90. Apo B was also significantly reduced across the dose ranges of inclisiran: 23–33% with single dose injections, and 28–41% across dose ranges of 200–500 mg when inclisiran was injected on days 1 and 90. The injections of inclisiran significantly reduced plasma PCSK9 by 48–59% when inclisiran was administered as a single dose of 200 mg, 300 mg, or 500 mg on day 1 and by 53%, 66%, and 69% when administered across the dose ranges with injections given on days 1 and 90. All of these reductions were statistically significant compared to placebo associated changes in the same measured biomarkers. The investigators observed no significant changes in AST, ALT, alkaline phosphatase, and bilirubin levels across any dose of inclisiran evaluated compared to placebo. One patient had a creatine kinase level that went to eight times the upper limit of the normal range (ULN) in the single dose group and one patient had a creatine kinase level that went to four times the ULN in the double dose group. The data from the phase 2 study confirmed the potential clinical efficacy and utility of inclisiran and suggested that 300 mg administered on days 1 and 90, then every 6 months thereafter would be the most appropriate

Study	Description	Estimated number of subjects	Duration of follow-up†	Status as on second-half of 2018
ORION-1	Phase II dose finding	501	Up to 360 days	Completed
ORION-2	Homozygous FH pilot study	4	Up to 300 days	Ongoing
ORION-3	Open-label extension study to ORION-1	374	Up to 4 years	Ongoing
ORION-4	Cardiovascular outcomes trial	15,000	Event-driven/median duration ≥5years	Start-up
ORION-5	Homozygous FH Phase III	45	Up to 2 years	Start-up
ORION-6	Hepatic impairment	24	Up to 180 days	Start-up
ORION-7	Renal Impairment	31	Up to 180 days	Completed
ORION-8	Phase III extension study ORION-9, -10, -11)	Up to 3600	Up to 3 years	Will start after completion of ORION-9, ORION-10 and ORION-11
ORION-9	Phase III heterozygous FH	482	Up to 540 days	Ongoing
ORION-10	LDL-C lowering in ASCVD (USA)	1561	Up to 540 days	Ongoing
ORION-11	LDL-C lowering in ASCVD and ASCVD risk equivalents (EU)	1617	Up to 540 days	Ongoing
ORION-12	TQTc	Appr.48	Up to 180 days	Start-up

Fig. 12.2 This figure represents the clinical study program evaluating the safety and efficacy of Inclisiran across a range of disease states and clinical scenarios. Adapted with permission from Future Cardiology, Copyright Future Medicine Ltd. [4]. †The primary trial end point may be before the maximum duration of follow-up; for example, in ORION-1, the primary end point was at day 180, whereas follow-up continued until return to 80% of baseline LDL-C with a maximum of 360 days. *ASCVD* Atherosclerotic cardiovascular disease, *FH* Familial hypercholesterolemia, *LDL-C* Low-density lipoprotein cholesterol

strategy to test in larger, phase 3 placebo-controlled trials.

12.2 Phase III Studies

The ORION Program continued with several large Phase 3 trials to further evaluate the clinical efficacy and safety of inclisiran (Fig. 12.2). These trials used a 300 mg dose of inclisiran sodium (also called Inclisiran 284 mg) based upon the findings of ORION 1. ORION 9 was a placebo-controlled, randomized clinical trial evaluating the efficacy and safety of inclisiran in 482 participants with heterozygous familial hypercholesterolemia [13]. ORION 10 was a placebo-controlled, randomized clinical trial evaluating the efficacy and safety of inclisiran in 1561 participants with atherosclerotic cardiovascular disease (ASCVD) who were in the United States, while ORION 11 was a placebo-controlled, randomized clinical trial evaluating the efficacy and safety of inclisiran in 1617 participants with ASCVD or ASCVD risk equivalents who lived in Europe or South Africa [14]. Each of these trials administered inclisiran or placebo on day 1 and again at day 90, followed by every 6 months thereafter until the end of the study. The primary end-points in each trial were two: placebo-adjusted change in LDL-C at day 510 and time-averaged, placebo-adjusted change in LDL-C (Inclisiran minus placebo) from day 90 through day 540. All patients had to be on maximally tolerated statin or other oral lipid lowering therapy and have an entry LDL-C of ≥70 mg/dl if enrolled with ASCVD or Heterozygous FH or ≥100 mg/dl if enrolled with ASCVD risk equivalents. These trials were designed to allow eventual pooling of the data for an integrated analysis [15].

Plasma LDL-C was reduced in a placebo-adjusted manner by 48% in ORION 9 at day 150 by randomization to inclisiran and the time-averaged LDL-C between days 90 and 540 was reduced by 44% among those randomized to inclisiran. Inclisiran reduced PCSK9 values by 78% at day 510, total cholesterol by 33% at day 510, Non-HDL Cholesterol by 44% at day 510, and ApoB by 37% at day 510, all with *p*-values that were significant. There were no observed differences in treatment emergent adverse and serious adverse events, except for a slightly higher rate of injection site reactions among those randomized to inclisiran (17.0 vs. 1.7%).

ORION 10 and 11 were published jointly in a side by side analysis with similar findings given the overlap of their studied populations [14]. Plasma LDL-C was reduced by 52% in ORION 10 and by 50% in ORION 11, at Day 510. The time-averaged reduction in LDL-C between days 90 and 540 was 54% in ORION 10 and 49% in ORION 11. PCSK9 plasma concentrations were reduced by 83% in ORION 10 and by 79% in ORION 11 at day 510. Inclisiran reduced total cholesterol at day 510 by 33% in ORION 10 and by 30% in ORION 11. Inclisiran reduced non-HDL C at day 510 by 47% in ORION 10 and by 43% in ORION 11. Inclisiran reduced ApoB by 43% in ORION 10 and by 39% in ORION 11 at day 510, all $p < 0.01$.

A subsequent patient-level pooled analysis integrating the data of ORION 9, 10, and 11 demonstrated that inclisiran reduced LDL-C at day 510 by 51% and a time-averaged LDL-C reduction over days 90 through 540 of 45% [15]. The same analysis demonstrated that inclisiran reduced PCSK9 by 81% at day 510, total cholesterol by 32% at day 510, non-HDL C by 46% at day 510, and Apo B by 42% at day 510. Inclisiran also reduced triglycerides by 13% at day 510, raised HDL-C by 8%, and reduced Lp (a) by 19.5%, all $p < 0.01$ [15]. The rate of treatment emergent adverse events and serious adverse events were not different between inclisiran and placebo participants, except for a slightly higher rate of injection site reactions in those receiving inclisiran (5.0 vs. 0.7%) and self-reported bronchitis (4.3 vs. 2.7%).

ORION 7 examined the tolerability and efficacy of inclisiran in participants with renal impairment ($n = 31$). Its data were combined with prior studied patients from ORION 1 to evaluate the impact of mild and moderate renal dysfunction on lipid lowering efficacy and safety, and the small number of subjects from ORION 7 was also analyzed for the pharmacodynamic profile

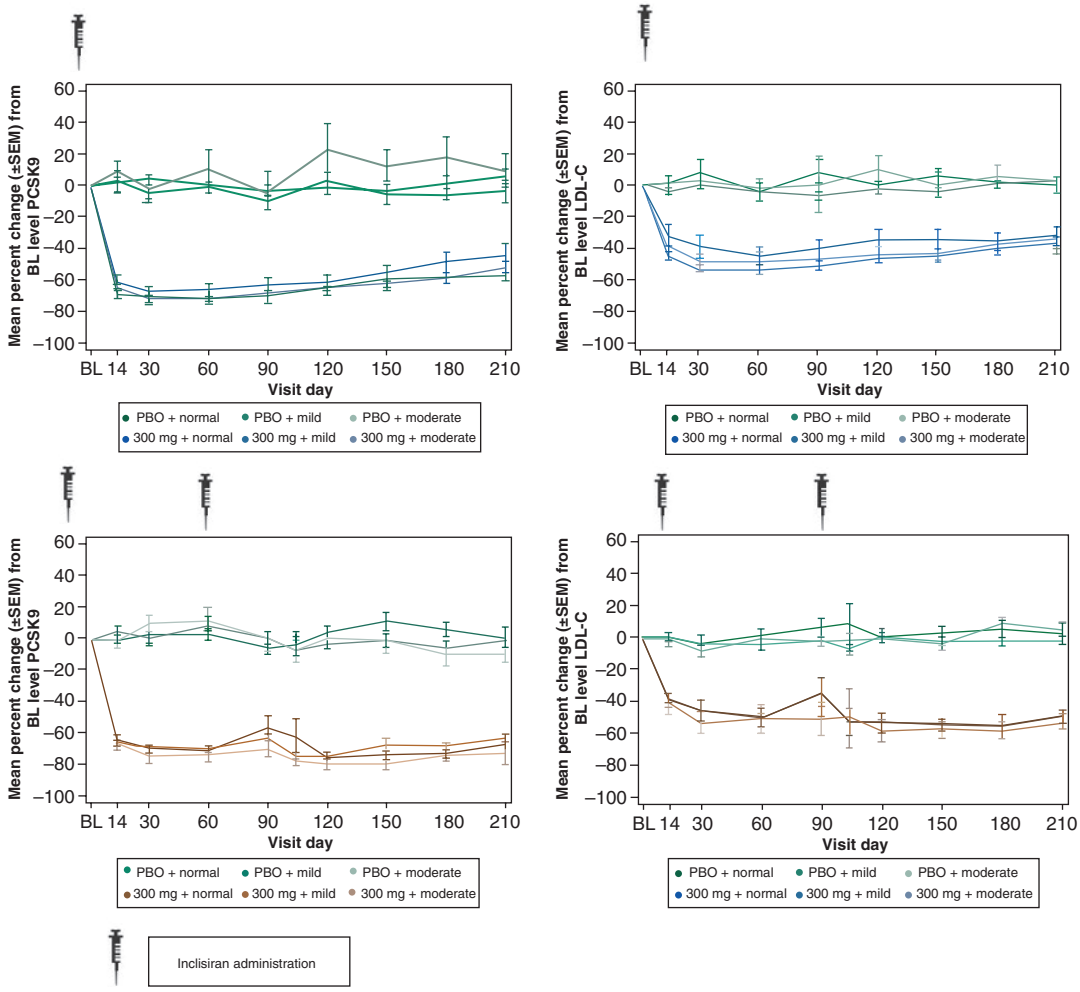

Fig. 12.3 This figure demonstrates the impact of renal impairment on the efficacy of inclisiran with regard to lowering plasma LDL and PCSK9. Adapted with Permission from Mayo Clinic Proceedings, Copyright Elsevier [16]. *BL* baseline, *LDL-C* low-density lipoprotein cholesterol, *Pbo* placebo, *PCSK9* proprotein convertase subtilisin-kexin type 9

of inclisiran in those with renal impairment [16] (Fig. 12.3). Inclisiran potently reduced both LDL-C and PCSK9 in all subgroups of renal impairment suggesting its potential utility as a lipid lowering agent in this population. Figure 12.4 illustrates the clearance of inclisiran across the varying ranges of renal function from normal to severe impairment, demonstrating that renal impairment was associated with higher peak plasma levels after administration but with no attenuation or prolongation of plasma clearance of the drug.

12.3 What Side Effects or Other Symptoms Should Clinicians Watch for?

The pooled phase 3 data suggest that injection site reactions—erythema, itchiness, redness, or a slightly raised area—are the most common adverse reaction in those randomized to inclisiran. Nearly all of these reactions were mild or moderate and transient. Few episodes occurred in the same patient on more than one occasion. The use of inclisiran is infrequent—typically twice

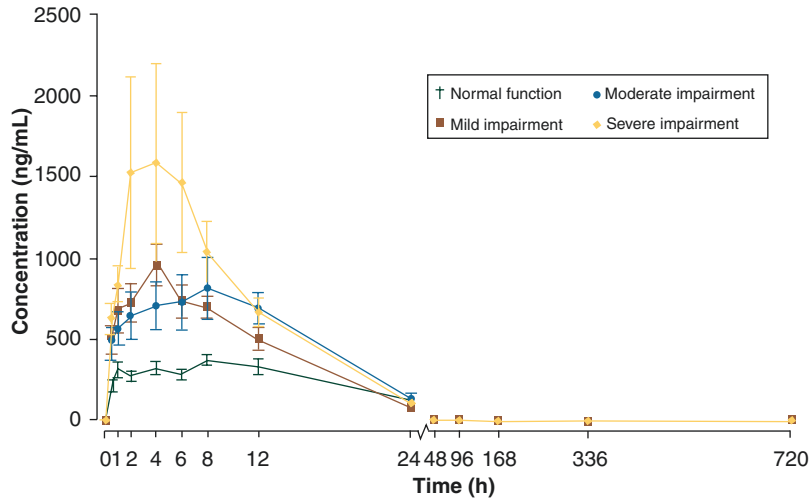

Fig. 12.4 This figure is a graph of the inclisiran plasma concentrations over time in the ORION 7 trial. Adapted with Permission from Mayo Clinic Proceedings, Copyright Elsevier [16]

yearly—so the reactions would be expected to be infrequent. Post-market approval surveillance will provide further insight into the frequency and severity of injection site reactions. The pooled phase 3 data also observed a slightly higher rate of self-reported bronchitis among those randomized to inclisiran (4.3%) versus placebo (2.7%), while rates of upper respiratory infections were not different (5.7 vs. 5.7%). The significance of these observations are unclear and post-market approval surveillance will provide further insight into whether bronchitis occurs more frequently.

12.4 Will Inclisiran Enhance LDL-C Goal/Threshold Attainment?

It is expected that inclisiran will be added to established oral therapies for managing dyslipidemia in the great majority of patients. The pooled patient-level analysis of Inclisiran in the Phase 3 program suggested that inclisiran use can result in a 50% or greater additional reduction in LDL-C on top of established oral therapies compared to 2.2% on placebo (15). These data also indicate that Inclisiran will achieve a LDL-C of <70 mg/dl in 87% of patients compared to 31% in the placebo groups, and a LDL-C < 50 mg/dl in 52% of patients compared to 2% of placebo patients. These data underscore the potential utility of inclisiran as an adjunct treatment for additional LDL-C reduction and achievement of important LDL-C threshold levels in higher risk patient subgroups.

12.5 What About Outcome Data?

The Phase 3 data reported to date have not been powered to adjudicate whether use of inclisiran significantly alters prospective cardiovascular events such as stroke, myocardial infarction, unstable angina, and non-ST acute coronary syndromes. There is an ad hoc reported pooled summary by non-ORION investigators utilizing publicly reported data at the late breaking sessions of the preliminary phase III data. Their report is not patient-specific and should be not considered as adjudicated, especially as the ORION 9, 10, and 11 studies were not powered to report on prospective major adverse cardiovascular events [17]. The ORION 4 trial is a properly powered prospective outcomes trial designed to determine if use of inclisiran to lower LDL-C reduces major adverse cardiovascular events [18].

12.6 Comparison to Other PCSK 9 Therapies

There are at least two monoclonal antibodies against PCSK9 available for treatment in patients with dyslipidemia. Both reduce LDL-C, non-

	Evolucomab	Alirocumab	Inclisiran
LDL-C reduction	59%	55-63%	50-55%
Apo B reduction	46%	58%	42%
Total Cholesterol reduction	35.5%	40%	35%
Triglyceride reduction	16.2%	20%	12%
Lp(a) reduction	27%	24-29%	20%
Dosing/administration	140 mg SQ q 2 weeks or 420 mg SQ monthly	75/150 mg SQ q 2 weeks or 300 mg SQ monthly	284 mg SQ q 6 months (after a starter dose at days 1, 90)
Secondary prevention benefit	Yes[1]	Yes[2]	Not Yet [3]

Fig. 12.5 This figure is a comparison of the two commercially available PCSK9 monoclonal antibodies and Inclisiran. 1, Fourier Study; 2, Odyssey Outcomes Study; 3, ORION 4 Study (in progress)

HDL-C cholesterol, apo (b), and Lipoprotein a significantly compared to placebo (Fig. 12.5). There are no head-to head studies comparing monoclonal antibody therapy to inclisiran so direct comparisons are difficult. All of the available therapies which lower LDL-C by reducing PCSK9 are important tools for clinicians who are managing patients with dyslipidemia in whom statin and/or other oral therapies are insufficient to achieve specific LDL-C goals or thresholds. The presence of multiple therapeutic options offers important choice and options to patients who may not tolerate one of the available therapies as well as likely exert pressure on the costs of these therapies due to market competition. One potential advantage to inclisiran will be the infrequent need for dosing, typically twice yearly, compared to 26 times per year for monoclonal antibody therapies.

12.7 Summary

Inclisiran is a new and exciting adjunctive therapy for treating patients with dyslipidemia who do not achieve satisfactory LDL-C reduction from oral therapies alone. It is a small, interfering RNA therapy which inhibits the translation of mRNA of PCSK9 into the PCSK9 protein. The phase 3 data clearly have established the efficacy and safety of inclisiran to date, and demonstrate that use of inclisiran as an adjunct to maximally tolerated statin therapy will lower LDL-C by at least an additional 50%. Furthermore, most patients on inclisiran will achieve an on-treatment LDL-C of <70 mg/dl in most patients and <50 mg/dl in about half of patients. Inclisiran is administered by subcutaneous injection with a total volume of approximately 1.5 cc. It needs to be administered on days 1 and 90 when starting it, then every 6 months thereafter. It has the potential to assist clinicians and patients reach aggressive LDL-C goals/thresholds more completely and to improve LDL-C control regardless of oral therapy adherence. It is a new therapy, currently approved in Europe but not in the United States of America at the time of this writing. Further experience after clinical use may further inform our understanding of benefits and risks.

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13 Understanding of Clinical Practice Guidelines for Dyslipidemia

Eun-Jung Rhee

Abstract

The establishment of clinical practice guidelines for dyslipidemia has a briefer history than might be expected. Since the release of the National Cholesterol Education Program-Adult Treatment Panel (NCEP-ATP) I in 1988, guidelines from many associations have evolved. ATP I through III and an updated version of ATP III have been released between 1988 and 2004. These guidelines established the core standards of dyslipidemia treatment and have identified low-density lipoprotein cholesterol (LDL-C) as the main target for prevention of cardiovascular diseases, the need for cardiovascular risk group stratification, and the establishment of risk calculators for different ethnic groups. In 2013, the American College of Cardiology and American Heart Association cholesterol guidelines omitted the LDL-C target levels and suggested the use of statins with appropriate intensity according to the four statin benefit groups. European Society of Cardiology/European Atherosclerosis Society joint dyslipidemia guidelines were serially released and have evolved to maintain LDL-C levels as the main targets and define cardiovascular risk group categories. In this chapter, the recent dyslipidemia guidelines have been reviewed.

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13.1 History of the Clinical Practice Guidelines

The history of establishing clinical practice guidelines for dyslipidemia closely follows that of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in the National Cholesterol Education Program-Adult Treatment Panel (NCEP-ATP). ATP I, published in 1988, first emphasized that the prevention of cardiovascular disease should focus on lowering low-density lipoprotein cholesterol (LDL-C) levels, and ATP II, released in 1993, recommended different treatments for cardiovascular (CV) risk groups stratified based on risk factors and risk severity [1, 2]. ATP III, published in 2001, emphasized that LDL-C concentration in the high-risk group should be <100 mg/dL, and diabetes was included in the coronary artery disease equivalent group to be classified as a high-risk group inclusive condition [3]. In the updated ATP III guidelines, a lower LDL-C target of <70 mg/dL was introduced for the very high-risk group [4].

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In 2013, the American College of Cardiology and American Heart Association (ACC/AHA) guidelines were published [5]. In these guidelines, LDL-C target levels were eliminated, and those who fulfilled "four statin benefit groups" were recommended for statins with at least moderate intensity by classifying statins themselves into three groups of high, moderate, and low intensity. However, these guidelines were not readily endorsed by many associations because they did not consider ethnic differences, in that most cited studies were conducted in Caucasian populations. Further, the guidelines eliminated LDL-C target levels, and no mention of recommendations for non-statin treatments was included.

The first joint European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) guideline was published in 2011 [6]. In this guideline, the Systemic Coronary Risk Evaluation (SCORE) estimator, based on European data, was used to stratify patients into very high, high, moderate, or low CV risk groups as bases for treatment decisions. They recommended clinicians to aim for LDL-C levels below <3.0 mmol/L (115 mg/dL) in moderate-risk patients, <2.5 mmol/L (100 mg/dL) in high-risk patients and <1.8 mmol/L (70 mg/dL) and at least 50% reduction in levels or both if this target cannot be reached in very high-risk patients. After this guideline was published, two more revised versions were published in 2016 and 2019 [7, 8]. LDL-C level remained the main treatment target for the prevention of cardiovascular disease (CVD) across the guidelines.

In 2018, the ACC/AHA task force on clinical practice guidelines updated its 2013 cholesterol guideline [9]. These guidelines narrow the use of the atherosclerotic cardiovascular disease (ASCVD) risk calculator, provide more guidance on the use of risk-enhancing factors in making statin therapy decisions, and recommends therapy options for achieving LDL-C targets. The biggest change from the 2013 guidelines was that LDL-C targets reappeared and were stratified based on risk groups (Fig. 13.1). The updated guidelines also include recommendations for non-statin lipid-lowering agents that can be used as statin add-ons to meet the recommended LDL-C thresholds.

13.2 NCEP-ATP III Guidelines

Following ATP I and ATP II in 1988 and 1993, ATP III was published in 2001 [3]. This guideline differed from the existing guidelines, which set treatment targets by dividing between primary and secondary prevention while focusing on assessing risk levels and improving risk factors. The main focus was on identifying subjects with multiple risk factors for coronary heart disease (CHD), and it was recommended that subjects belonging to higher risk groups undergo stronger cholesterol-lowering treatments. The optimal level of high-density lipoprotein cholesterol (HDL-C) concentration was increased from 35 mg/dL to 40 mg/dL, and the treatment ranges of triglyceride (TG) were further subdivided. It also recommended new dietary regimens and lifestyle changes to mitigate other risk factors and lower LDL-C. Diabetes was considered equally high-risk as established CVD; therefore, similarly, strict treatment targets for dyslipidemia were applied. The 10-year CVD risk was calculated based on age, sex, total cholesterol and HDL-C, smoking status, and systolic blood pressure using Framingham scoring. The treatment target was reinforced in the same manner as those of CHD patients in cases above 20%. In addition, the criteria for metabolic syndrome (MS) were included and were also considered as equally high risk as those with extant CHD. Substantial therapeutic lifestyle changes were emphasized.

In 2004, updated ATP III guidelines were released, and this guideline recommended a new risk group, the "very high risk" group, which included patients with CVD with multiple risk factors, especially diabetes, MS, or acute coronary syndrome (ACS) [4]. The guidelines recommended lowering LDL-C to <70 mg/dL in patients in this group.

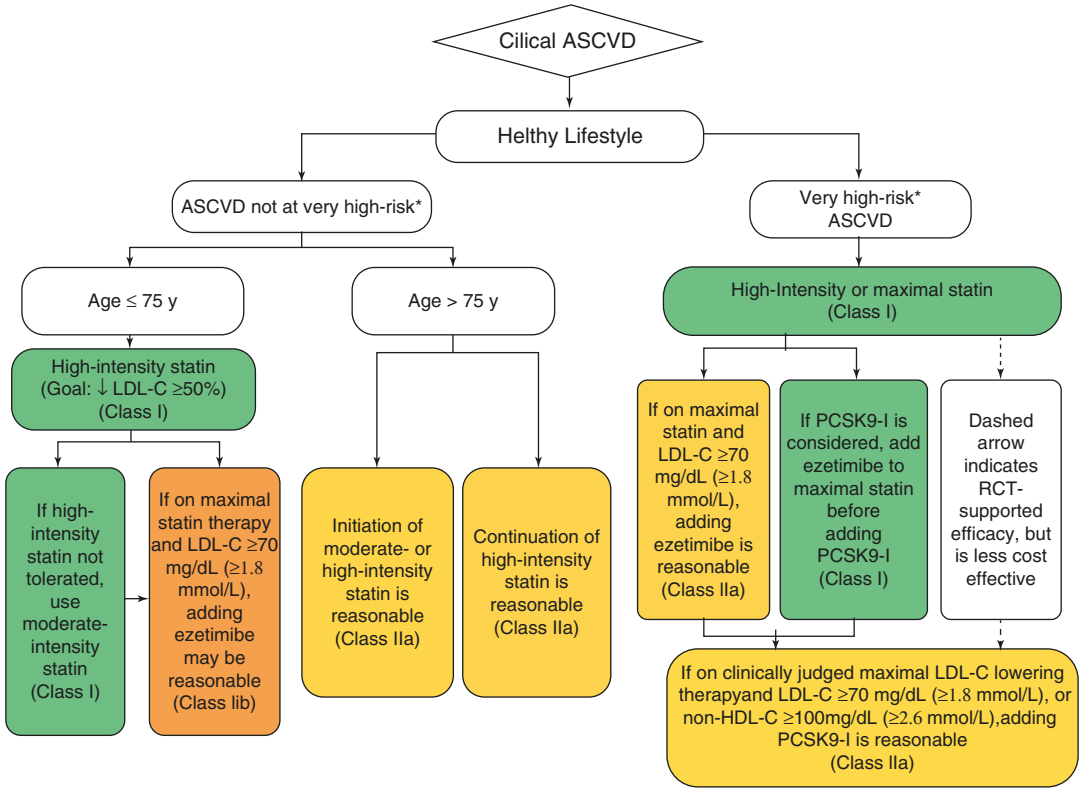

Fig. 13.1 Secondary prevention in patients with ASCVD in ACC/AHA guidelines 2019. Adapted with permission from Circulation, Copyright American Heart Association [8]. ASCVD, atherosclerotic cardiovascular disease; ESC, European Society of Cardiology; EAS, European Atherosclerosis Society; LDL-C, low-density lipoprotein cholesterol; PCSK9-I, Proprotein convertase subtilisin/Kexin type 9 inhibitor; RCT, randomized controlled trials

13.3 ACC/AHA Guidelines

After nearly 10 years of waiting for ATP IV, ACC/AHA guidelines were published in 2013 [5]. In these guidelines, LDL-C target levels were eliminated, and patients 40 to 75 years of age with cardiovascular disease, an LDL-C level ≥190 mg/dL, and patients with diabetes 40–75 years of age with a cardiovascular disease risk level of at least 7.5% were included in the four "statin benefit groups." Those who fulfilled these criteria were recommended for statins with at least moderate intensity by classifying statins into three groups of high, moderate, and low intensity. These guidelines also suggested a new risk calculator, the pooled cohort equations, to assess risk severity [10]. However, these guidelines were not readily endorsed by many associations because they did not consider ethnic differences, in that most cited studies were conducted in Caucasian populations, LDL-C target levels were eliminated, and mention of recommendations for non-statin treatments was extremely limited.

The ACC/AHA guidelines revised in 2018 made many changes to the recommendations for primary prevention, such as suggestions for assessing ASCVD risk in different age groups and the reappearance of LDL-C target levels, which indicated that a subsequent treatment could be added if an LDL-C level of 70 mg/dL could not be achieved despite appropriate statin administration [9] (Fig. 13.1). Considering the recent mega trials on Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), Further Cardiovascular

Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) and Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab (ODYSSEY) trials, the primary treatment recommendation was statin, followed by ezetimibe and then proprotein convertase subtilisin/Kexin type 9 (PCSK9) inhibitors [11–13].

13.4 ESC/EAS Guidelines

The first European joint guidelines that experts from the EAS and ESC worked together to develop was released in 2011 [6]. The aim was to keep pace with emerging data and provide contemporary treatment advice for a wide range of dyslipidemias, including diabetes and MS. In this guideline, the SCORE system, based on European data, was used to stratify patients into very high, high, moderate, or low CV risk as a basis for treatment decisions. Those who had established CVD, type 2 diabetes mellitus or type 1 diabetes with target organ damage, moderate to severe chronic kidney disease, or a SCORE 10-year CV risk ≥10% were categorized in the very high-risk group. The high-risk group included those with markedly elevated single risk factors, 10-year CV risk ≥5 to <10%, and the moderate-risk group included those with a 10-year CV risk >1 to ≤5%. They recommended clinicians should aim for LDL-C levels below <3.0 mmol/L (115 mg/dL) in moderate-risk patients, <2.5 mmol/L (100 mg/dL) in high-risk patients and <1.8 mmol/L (70 mg/dL) and at least 50 percent reduction in levels or both if the target cannot be reached in very high-risk patients.

The guidelines emphasize that lipid-modifying treatment needs to be tailored to patients according to their total CV risk. Lifestyle interventions, including smoking cessation, improving diet, exercising sufficiently, and moderating alcohol consumption, should be the crucial initial steps for managing lipids in all patients. If lipid targets are not met with lifestyle modifications alone, statins are the treatment of choice for lowering LDL-C. The choice of statin should be based on consideration of the extent of LDL-C lowering required and the individual's total CV risk. High TG often responds well to diet and reduced alcohol intake. Drug options include fibrates, niacin, and *n*-3 polyunsaturated fatty acids (PUFAs), alone or in combination with a statin. In combined or atherogenic dyslipidemia, the combination of statin plus niacin or fibrate (avoiding gemfibrozil) may be considered.

In the ESC/EAS guidelines published in 2016, risk groups were changed [7]. The very high-risk category included only patients with severe chronic kidney disease and patients with diabetes and target organ damage, whereas those with diabetes but without target organ damage were classified as high-risk. TG has been omitted from the therapeutic target recommendations. Treatment targets were the most awaited section due to the release of the 2013 ACC/AHA guidelines that omitted lipid target levels. However, European guidelines did not follow the ACC/AHA guidelines and kept the LDL-C targets. Recommendations were made to adjust the LDL-C target value to <100 mg/dL and 70 mg/dL for high-risk and very high-risk groups, respectively.

In the new guidelines published in 2019, patients with diabetes were stratified according to disease duration with type 1 diabetes, with a disease duration of ≥20 years as the very high-risk group, and patients with type 2 diabetes for >10 years as a high-risk group [8] (Table 13.1). LDL-C target levels were recommended for further reduction. In very high-risk patients, LDL-C goals were recommended to be lowered to <55 mg/dL (1.4 mmol/L) and at least 50% reduction from baseline LDL-C levels. In high-risk patients, the LDL-C goal was recommended to be lowered to <70 mg/dL (<1.8 mmol/L) and at least a 50% reduction from baseline LDL-C levels (Table 13.2). Patients with a history of ACS were recognized as at a very high risk of recurrent events. If patients experienced a second vascular event within 2 years on maximally tolerated statin therapy, an LDL-C goal of <1.0 mmol/L (<40 mg/dL) was indicated for consideration.

Table 13.1 Cardiovascular risk categories in ESC/EAS dyslipidemia guidelines 2019

Risk categories	
Very high-risk	– Documented ASCVD, either clinical or unequivocal on imaging – DM with target organ damage (microalbuminuria, retinopathy, or neuropathy), or at least three major risk factors, or early onset T1DM or long duration (>20 years) – Severe CKD (eGFR <30 mL/min/1.73 m^2) – A calculated SCORE ≥10% for 10-year risk of fatal CVD – FH with ASCVD or with another major risk factor
High-risk	– Markedly elevated single risk factors, in particular TC >8 mmol/L (>310 mg/dL), LDL-C >4.9 mmol/L (>190 mg/dL), or BP ≥180/110 mmHg FH without other major risk factors DM without target organ damage, with DM duration ≥10 years or another additional risk factor Moderate CKD (eGFR 30–59 mL/min/1.73 m^2) A calculated SCORE ≥5% and < 10% for 10-year risk or fatal CVD
Moderate-risk	Young patients (T1DM <35 years; T2DM <50 years) with DM duration <10 years, without other risk factors. Calculated SCORE ≥ 1% and < 5% for 10-year risk of fatal CVD
Low-risk	Calculated SCORE <1% for 10-year risk of fatal CVD

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ASCVD atherosclerotic cardiovascular disease, *DM* diabetes mellitus, *TC* total cholesterol, *CKD* chronic kidney disease, *T1DM* type 1 diabetes mellitus, *T2DM* type 2 diabetes mellitus, *FH* familial hypercholesterolemia, *SCORE* Systemic Coronary Risk Evaluation, *GFR* glomerular filtration rate, *BP* blood pressure, *LDL-C* low-density lipoprotein cholesterol, *CVD* cardiovascular disease

Table 13.2 Recommendations for treatment goals for LDL-C in ESC/EAS guidelines 2019. Adapted with permission from European Heart Journal, Copyright Oxford University Press [8]

Recommendations	Class	Level
In secondary prevention for patients at very high-risk, an LDL-C reduction of ≥50% from baseline and an LDL-C goal of <1.4 mmol/L (<55 mg/dL) are recommended	I	A
In primary prevention for individuals at very high-risk but without FH, an LDL-C reduction of ≥50% from baseline and an LDL-C goal of <1.4 mmol/L (<55 mg/dL) are recommended	I	C
In primary prevention for individuals with FH at very high-risk, an LDL-C reduction of ≥50% from baseline and an LDL-C foal of <1.4 mmol/L (<55 mg/dL) should be considered	IIa	C
For patients with ASCVD who experience a second vascular event within 2 years (not necessarily of the same type as the initial event) while taking maximally tolerated statin-based therapy, an LDL-C goal of <1.0 mmol/L (<40 mg/dL) may be considered	IIb	B
In patients at high-risk, an LDL-C reduction of ≥50% from baseline and an LDL-C goal of <1.8 mmol/L (<70 mg/dL) are recommended	I	A
In individuals at high-risk, an LDL-C goal of <2.6 mmol/L (<100 mg/dL) should be considered	IIa	A
In individuals at low-risk, an LDL-C goal <3.0 mmol/L (<116 mg/dL) may be considered	IIb	A

ESC/EAS European Society of Cardiology/European Atherosclerosis Society, *ASCVD* atherosclerotic cardiovascular disease, *FH* familial hypercholesterolemia, *LDL-C* low-density lipoprotein cholesterol

With the recommendation of these new lower LDL-C goals for patients at very high-risk and high-risk, the ESC/EAS guidelines emphasized the importance of combination treatment; first statins with ezetimibe, and then a PCSK9 inhibitor to achieve these targets. In patients with ACS, adding a PCSK9 inhibitor early after the event (during hospitalization if possible) should be considered. In these patients, if the LDL-C goal is not achieved after 4–6 weeks despite maximally tolerated statin therapy and ezetimibe, a PCSK9 inhibitor is recommended.

While statin treatment remains the first choice for managing high TG (>200 mg/dL or 2.3 mmol/L), new guidelines have taken into account evidence from the Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial (REDUCE-IT) and recommended the consideration of a combination of *n*-3 PUFAs (particularly icosapent ethyl 2 × 2 g daily) in high-risk patients with persistently elevated TG levels (135–499 mg/dL or 1.5 and 5.6 mmol/L) despite statin treatment [14]. In

high-risk patients with LDL-C goals with TG >200 mg/dL or >2.3 mmol/L, fenofibrate or bezafibrate may be considered in combination with statins.

13.5 Conclusions

The recent trends in dyslipidemia clinical practice guidelines are (1) focusing on LDL-C as the main target, (2) assessing the risk category of the patient is important before initiating treatment, (3) placing relatively less emphasis on non-LDL-C targets, such as TG and HDL-C, (4) increasing emphasis on the usage of risk calculators, and (5) incorporating novel strong lipid-lowering agents such as ezetimibe and PCSK9 inhibitors. The application of the guidelines between ethnic groups remains inappropriate; therefore, each region will need to develop tailored dyslipidemia guidelines based on population demographics.

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Part IV

Management of Dyslipidemia in Clinical Practice of Stroke

14 Practical Management of Dyslipidemia in Stroke Patients

Jong S. Kim

Abstract

A strategy involving a low target concentration appears to be superior to one involving a moderate circulating LDL-cholesterol (LDL-c) reduction for the prevention of cardio-cerebrovascular disease in patients with coronary disease and in those with carotid artery disease. However, it remains uncertain whether statins are beneficial, and if they are, what would be the most appropriate LDL-c target concentration in stroke patients without carotid artery disease. This chapter will focus on the evidences to elucidate the appropriate strategies in patients with stroke.

14.1 Use of Lipid-Lowering Agents in Patients Who have Experienced a Stroke

Randomized controlled trials have clearly shown that the lowering of circulating LDL-cholesterol (LDL-c) concentration using statins is associated with a lower risk of recurrent cardiovascular events in patients with coronary heart disease. It has also been shown that further lowering of LDL-c is associated with more effective prevention [1]. However, the benefits of statin use in stroke patients have been studied less intensively. The Stroke Prevention by Aggressive Reduction in Cholesterol Level (SPARCL) trial was the first to evaluate the efficacy of statin therapy in stroke patients who did not have a history of coronary heart disease [2]. The investigators enrolled 4732 patients who had experienced stroke or transient ischemic attack (TIA) within the preceding 1–6 months and randomized them to receive atorvastatin 80 mg or placebo. They found that atorvastatin use was associated with a significant reduction of 16% in the risk of cerebrocardiovascular events. Although abnormal serum liver enzyme activities were more frequent in the atorvastatin (2.2%) than in the placebo (0.4%) group, atorvastatin 80 mg was generally well tolerated in stroke patients. In particular, post-hoc analysis revealed that compared with patients who showed no change or an increase in LDL-c, those patients who demonstrated a ≥50% reduction in LDL-c showed a 31% reduction in the outcome measure (hazard ratio (HR), 0.69; 95% confidence interval (CI), 0.55–0.87; $P = 0.0016$), which was higher than the overall reduction in risk of 16% [3]. On the basis of these results, it has been suggested that high dose statin with a low LDL-c target may be the most appropriate strategy for stroke patients.

In a recent multinational (French and Korean) Treat Stroke to Target (TST) study, the investiga-

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tors enrolled patients who had experienced a stroke within the preceding 3 months or a TIA within the preceding 15 days showed evidence of cerebrovascular or cardiac atherosclerosis [4]. The participants were randomly assigned in a 1:1 ratio to groups in which the target LDL-c concentration was <70 mg/dL or 100 ± 10 mg/dL. Statins were the primary therapy, and ezetimibe was added when necessary. The primary outcome was a composite of ischemic stroke, myocardial infarction, new symptoms requiring urgent coronary or carotid/cerebral revascularization, and vascular death. A total of 2860 patients were enrolled, and the median follow-up period was 3.5 years. The mean LDL-c concentrations achieved were 65 and 96 mg/dL, respectively. The primary composite endpoint occurred in 121 (8.5%) and 156 (10.9%) participants, respectively (adjusted HR, 0.77; 95% CI, 0.61–0.98; $P = 0.035$), but the incidence of intracranial hemorrhage did not significantly differ between the groups.

After these findings were published, investigators separately analyzed the data relating to the French participants. One thousand and seventy-three participants were assigned to the <70 mg/dL group and 1075 to the 100 ± 10 mg/dL group. After a median follow-up period of 5.3 years, the average LDL-c concentration achieved was 66 mg/dL in the low target group and 96 mg/dL in the higher target group. The primary endpoint occurred in 9.6% and 12.9% of the participants, respectively (HR, 0.74; 95% CI, 0.57–0.94; $P = 0.019$). Myocardial infarction or urgent coronary revascularization occurred in 1.7% and 2.5% of the participants, respectively (HR, 0.66; 95% CI, 0.37–1.20; $P = 0.18$), and cerebral infarction or urgent cerebral revascularization occurred in 6.7% and 9.1%, respectively (HR, 0.73; 95% CI, 0.54–0.99; $P = 0.046$). Intracranial hemorrhages occurred in 1.2% and 1.0%, respectively (HR, 1.17; 95% CI, 0.53–2.62; $P = 0.70$). Other details are shown in Table 14.1. Thus, these TST trials showed that LDL-c concentration <70 mg/dL, rather than 100 mg/dL, would be the appropriate target for patients who have experienced stroke with evidence of atherosclerosis.

However, the benefit of the "lower target" was not evident in Korean patients. Regarding the Korean patients, 307 patients were assigned to <70 mg/dL and 355 were assigned to 100 ± 10 mg/dL. After a median follow-up of 2.0 years, the achieved average LDL-c level was 66 mg/dL in the lower target group and 96 mg/dL in the higher target group, respectively. The primary endpoint occurred in 19 (5.3%) and 21 (5.9%) of patients, respectively (HR, 0.94; 95% CI, 0.50–1.76; $P = 0.84$). Myocardial infarction or urgent coronary revascularization occurred in 2 (0.6%) and 10 (2.8%) patients, respectively (HR, 0.2; 95% CI, 0.04–0.92; $p = 0.04$) whereas cerebral infarction or urgent cerebral revascularization occurred in 17 (4.8%) and 7 (2.0%), respectively, (HR, 2.51; 95% CI, 1.04–6.06; $p = 0.04$). Intracranial hemorrhages developed in 4 (1.1%) and one (0.3%), respectively, (HR, 3.96; 95% CI, 0.44–35.40; $P = 0.22$). Other details are shown in Table 14.1. Thus, it seems clear that a lower LDL-c target is of benefit in the prevention of coronary heart disease in stroke patients regardless of ethnicities. However, the appropriate LDL-c target for the prevention of stroke remains still unclear.

The Korean data appear to be consistent with those obtained during the previous SPARCL trial. Out of the 4731 SPARCL participants, a subset of 1007 participants had carotid artery disease. Although the risk of the primary outcome was 16% lower in the intervention group in this study, there was a 33% reduction in participants who had carotid artery disease. By contrast, statin administration had a minor effect in participants who did not have carotid stenosis [5]. Therefore, it appears that statin therapy may be less effective in patients who do not have a carotid disease, i.e., those with intracranial atherosclerosis (ICAS) or small vessel disease (SVD). It has been shown that ICAS and SVD are more prevalent in Asian stroke patients than in Caucasian counterparts. Thus, the differences in the findings made in the French and Korean patients may be attributed to differences in the stroke subtype between the two countries. However, it should be noted that the Korean TST study had some limitations: the overall numbers of participants and outcome

Table 14.1 Comparison between French and Korean TST data

Outcome	France				Korea			
	<70 mg/dL (n = 1073)	100 ± 10 mg/dL (n = 1075)	Hazard ratio (95% CI)	P Value	<70 mg/dL (n = 357)	100 ± 10 mg/dL (n = 355)	Hazard ratio (95% CI)	P Value
Primary outcome								
Major cardiovascular events	103/1073 (9.6)	139/1075 (12.9)	0.74 (0.57–0.95)	0.019	19/357 (5.3)	21/355 (5.9)	0.94 (0.50–1.76)	0.84
Death from cardiovascular causes	17/1073 (1.6)	22/1075 (2.0)			0/357 (0.0)	4/355 (1.1)		
Fatal cerebral infarction or undetermined stroke	3/1073 (0.3)	6/1075 (0.6)			0/357 (0.0)	1/355 (0.3)		
Fatal myocardial infarction	1/1073 (0.1)	1/1075 (0.1)			0/357 (0.0)	1/355 (0.3)		
Other vascular death	7/1073 (0.7)	5/1075 (0.5)			0/357 (0.0)	1/355 (0.3)		
Sudden death	6/1073 (0.6)	10/1075 (0.9)			0/357 (0.0)	1/355 (0.3)		
Nonfatal cerebral infarction or undetermined stroke	65/1073 (6.1)	89/1075 (8.3)			17/357 (4.8)	7/355 (2.0)		
Nonfatal acute coronary syndrome	15/1073 (1.4)	22/1075 (2.0)			0/357 (0.0)	1/355 (0.3)		
Urgent coronary revascularization required	3/1073 (0.3)	3/1075 (0.3)			2/357 (0.6)	9/355 (2.5)		
Urgent carotid revascularization required	3/1073 (0.3)	3/1075 (0.3)			0/357 (0.0)	0/355 (0.0)		
Secondary outcome								
Myocardial infarction and urgent coronary revascularization	18/1073 (1.7)	27/1075 (2.5)	0.66 (0.67–1.20)	0.18	2/357 (0.6)	10/355 (2.8)	0.20 (0.04–0.92)	0.04
Cerebral infarction and urgent carotid or cerebral artery revascularization	72/1073 (6.7)	98/1075 (9.1)	0.73 (0.54–0.99)	0.046	17/357 (4.8)	7/355 (2.0)	2.51 (1.04–6.06)	0.04
Cerebral infarction or TIA	103/1073 (9.6)	125/1075 (11.6)	0.83 (0.64–1.08)	0.16	21/357 (5.9)	13/355 (3.7)	1.67 (0.83–3.32)	0.15
Any revascularization procedure (urgent or elective)	90/1073 (8.4)	87/1075 (8.0)	1.01 (0.75–1.36)	0.94	4/357 (1.1)	10/355 (2.8)	0.40 (0.12–1.26)	0.12
Carotid	17/90	22/87			0/357	0/355		
Coronary	41/90	41/87			2/357	9/355		
Peripheral	32/90	24/87			2/357	1/355		
Vascular death	22/1073 (2.1)	29/1075 (2.7)	0.76 (0.44–1.32)	0.32	0/357 (0.0)	4/355 (1.1)	0.02 (0.00–48.86)	0.31
All-cause death	86/1073 (8.0)	86/1075 (8.0)	1.0 (0.74–1.35)	0.99	2/357 (0.6)	7/355 (2.0)	0.29 (0.06–1.42)	0.30
Cerebral infarction or intracranial hemorrhage	80/1073 (7.5)	112/1075 (10.4)	0.72 (0.54–0.96)	0.023	21/357 (5.9)	8/355 (2.3)	2.70 (1.19–6.09)	0.01
Intracranial hemorrhage	13/1073 (1.2)	11/1075 (1.0)	1.17 (0.53–2.62)	0.70	4/357 (1.1)	1/355 (0.3)	3.96 (0.44–35.40)	0.22
Newly diagnosed diabetes†	87/1073 (8.1)	66/1075 (6.1)	1.33 (0.97–1.84)	0.076	6/357 (1.7)	9/355 (2.5)	0.64 (0.23–1.80)	0.40
Primary outcome or intracranial hemorrhage	111/1073 (10.3)	146/1075 (13.6)	0.75 (0.58–0.96)	0.021	25/357 (7.0)	23/355 (6.5)	1.11 (0.63–1.95)	0.73

Modified from Stroke, Copyright American Heart Association [21]

events were small, and more importantly, the follow-up period was shorter than that of the French study (median 2.0 vs. 5.3 years, respectively). Therefore, the findings of the Korean study require confirmation in a larger study with a longer follow-up period.

14.2 Effects of Hyperlipidemia and Statin Use in Patients with Various Stroke Subtypes

There is some evidence that the effect of blood cholesterol concentration differs in patients with different stroke subtypes. First, it is recognized that although ischemic stroke is associated with hypercholesterolemia, hemorrhagic stroke is associated with hypolipidemia, and the risk of hemorrhage in hypolipidemic patients is higher in patients with concomitant hypertension than in those without [6]. Furthermore, even in patients who experience an ischemic stroke, the effect of hyperlipidemia may also differ. In the Atherosclerosis Risk in Communities (ARIC) cohort study of 14,175 middle-aged people who were free of clinical cardiovascular disease, baseline measurements were made that included plasma lipid concentrations and subsequent cardiovascular disease endpoints were recorded [7]. Over an average follow-up period of 10 years (142,704 person-years at risk), clinical ischemic stroke was documented in 305 participants, and after multivariable adjustment for stroke risk factors, only a weak and inconsistent association was identified between LDL-c concentration and ischemic stroke. Thus, this relationship does not resemble the well-characterized association of LDL-c with coronary heart disease. This difference may be attributed to differences in the relationships of LDL-c with the various stroke subtypes. In the ARIC study, approximately one-quarter of the ischemic strokes were considered to be due to "lacunar infarctions" (LIs) defined by their anatomic location and size. However, this categorization was not made on the basis of underlying vascular status. The presence of Lis might have accounted for the weak association between LDL-c and stroke in the ARIC cohort. Atherogenesis in intracranial arteries, and particularly in the smaller arteries and arterioles, might differ from atherogenesis in the coronary arteries [8]. Although the relationship between circulating lipid and the extent and/or severity of cerebral atherosclerosis has been previously identified, most of the previous studies focused on carotid artery disease rather than ICAS [8].

In a cross-sectional study conducted in China, the investigators analyzed the clinical characteristics of 1982 patients who had experienced an acute ischemic stroke and were admitted to the Peking University First Hospital between 2007 and 2014 [9]. In this study, the cause of the ischemic stroke was classified as large artery atherosclerosis (LAA), LI, cardioembolism (CE), or undetermined, and 1207, 566, 173, and 36 of the participants were diagnosed with each. When the risk factors for each etiology were identified using multivariate logistic regression analysis, hypertension (odds ratio (OR), 1.832) and white matter hyperintensity (WMH), a marker of SVD (OR, 1.865), were found to be more strongly associated with LI than LAA, whereas LDL-c (OR, 0.774) was more strongly associated with LAA than LI. *In another study*, the investigator*s compared the risk factors in 573 participants with a single LI with those of 122 participants with recurrent LIs and found that h*ypertension was more prevalent in the recurrent-LI group than in the single-LI group (81% vs. 70%, respectively; $P = 0.01$) whereas hyperlipidemia was less prevalent (15% vs. 24%; $P = 0.025$) [10]. Although it is difficult to draw solid conclusions based on the findings of this retrospective study, the authors suggested that hypertension may be a risk factor for recurrence of LIs, whereas hyperlipidemia is not.

These differences can be explained by the nature of the different pathologies involved. LI, a small, deep infarction, is caused by deep perforating arterial disease, which is pathologically characterized by lipohyalinosis or fibrinoid degeneration, whereas LAA is associated with atherosclerosis, with lipid deposits in the vessel wall. Recent studies have also shown that the pathology of "LI," when defined using imaging

criteria, may be heterogeneous. The local thrombus or atheroma in patients with ICAS, may obliterate the orifice of the perforator and produce LI associated with clinical "lacunar syndromes," which is called branch atheromatous disease (BAD). Imaging methods such as magnetic resonance angiography (MRA) and computed tomographic angiography (CTA) can identify such cases. However, the ICAS producing BAD may be mild or diffuse, such that MRA and angiography may be unable to detect it. High-resolution MRI may be able to reveal diffuse atherosclerosis in intracranial arteries, and this so-called BAD or "branch occlusion" is more likely to be associated with atherosclerosis than classical LI caused by small artery pathology.

Developing this idea further, Nah et al. attempted to determine whether indicators of SVD and atherosclerosis in patients with small subcortical infarction (SSI, subcortical or brainstem infarct of diameter ≤20 mm) differ according to the location of the lesion, and the presence of parent artery disease [11]. They assessed 449 patients who had experienced an SSI in the perforator territory of the middle cerebral artery (n = 244), basilar artery (n = 141), and vertebral artery (n = 64) using diffusion-weighted imaging (DWI) within 48 h of stroke onset. The SSIs were characterized as proximal SSIs (dSSIs) if the lesions abutted the main artery, and distal SSIs (dSSIs) if they did not. The SSIs were also classified according to the location of the lesion and whether parent artery atherosclerotic disease (PAD) was present: (1) SSI with PAD (SSI + PAD); (2) proximal SSI without PAD (pSSI-PAD); and (3) distal SSI without PAD (dSSI-PAD) (Fig. 14.1). Then, the prevalence of indicators of SVD (leukoaraiosis and microbleeds) and atherosclerosis (cerebral atherosclerosis and coronary heart disease) were compared among the groups. The investigators found that the SSI + PAD group had the highest prevalence of atherosclerosis indicators and the lowest prevalence of SVD indicators, whereas the dSSI-PAD group had the lowest prevalence of atherosclerosis indicators and the highest prevalence of SVD indicators. The pSSI-PAD group showed intermediate prevalence of each. These results suggest that the pathogenesis of SSI is heterogeneous, varying according to lesion location and the presence of PAD. It was also shown that compared with pSSI, dSSI is more closely associated with WMH and microbleeds, and is marginally associated with hypertension (P = 0.08) but less frequently associated with diabetes (P = 0.003). In this study, the prevalence of hyperlipidemia did not differ between the groups. However, in a study conducted in Asia, LDL-c was more closely associated with pSSI than with dSSI in older patients (>65 years old) [12].

In another study, the investigators hypothesized that when stroke recurs, patients with pSSI

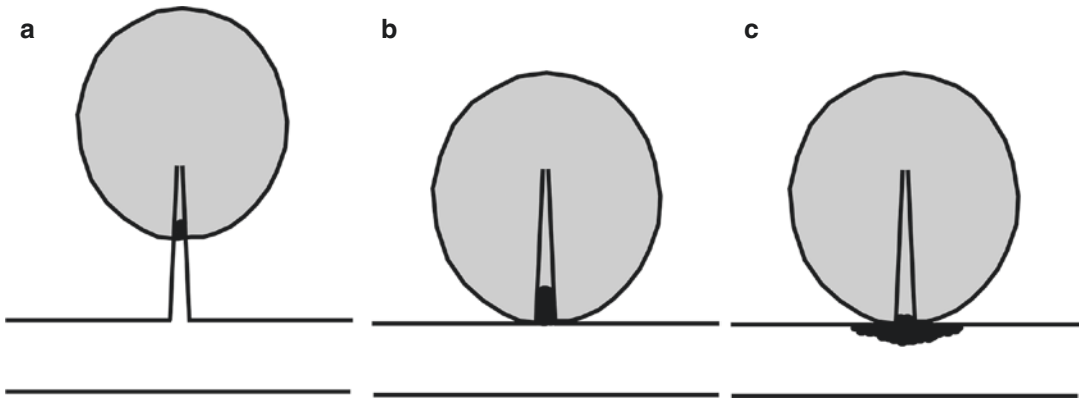

Fig. 14.1 Classification of small subcortical infarction (SSI) according to the extension of the lesion and whether parental arterial disease (PAD) is present. (**a**) Distal SSI without PAD; (**b**) proximal SSI without PAD; (**c**) SSI associated with PAD. Adapted from Stroke, Copyright American Heart Association [11]

are more likely to develop atherosclerotic cerebral infarction than those with dSSI [13]. They enrolled patients who had experienced an ischemic stroke and who had a past history of LAA or SVD. Furthermore, they classified LAA as ICAS or extracranial atherosclerosis (ECAS), and SVD was classified as LI or BAD, based on MR findings. BAD was diagnosed when infarcts were visible on at least four axial MRI slices with a thickness of 5 mm in the lenticulostriate territory, or if the infarcts extended to the basal surface of the pons in the paramedian pontine arterial territory; other SVD lesions were classified as LIs. The relationship between past and recent strokes was analyzed. Two hundred and two patients were enrolled, of which the LAA group ($n = 111$) comprised 64 cases of ICAS and 47 cases of ECAS, and the SVD group ($n = 91$) comprised 63 cases of LI and 28 cases of BAD at the time of the initial stroke. Analysis of the subtypes of the recurrent infarcts showed that participants in the LAA group developed LAA-associated strokes most frequently ($n = 99$, 89.2%), whereas those in the SVD group developed SVD most frequently ($n = 69$, 75.8%; $P < 0.001$). In addition, participants in the ICAS group were more likely to experience a further ICAS ($n = 46$, 79.3%), whereas those in the ECAS group were more likely to experience a further ECAS ($n = 31$, 75.6%; $P < 0.001$). It was noteworthy that in the SVD group, patients with BAD experienced a subsequent LAA more frequently than those in the LI group ($n = 11$, 39.3% vs. $n = 9$, 14.3%, respectively; $P = 0.022$).

14.3 Effects of Lipid-Lowering Agents in Patients with Various Stroke Subtypes

As discussed above, the role of hyperlipidemia in the etiology of stroke may differ according to subtype. Several Asian studies have investigated this possibility. In the randomized Japan Statin Treatment Against Recurrent Stroke (J-STARS) study, patients aged 45–80 years who had a history of non-cardiogenic ischemic stroke within the preceding 1 month–3 years were enrolled at 123 centers in Japan between March 2004 and February 2009 [14]. At the time of enrollment, all patients had a total cholesterol level between 180 and 240 mg/dL without the use of statins. The 1578 patients were randomly allocated to the pravastatin group (10 mg/day) or the control group. The primary endpoint was the onset of stroke or TIA. Secondary endpoints included the onset of each stroke subtype, myocardial infarction, death, or hospitalization. They concluded that pravastatin treatment showed prevention of stroke in patients with atherothrombotic stroke (HR 0.33; 95% CI, 0.15–0.74; $p = 0.0047$), but not the patients with other stroke subtypes. Actually, there was a tendency for the increasing occurrence of stroke in patients with LI in the pravastatin group, although this was not statistically significant. In the post-hoc analysis of J-STARS, the authors suggest that the optimal LDL-c target to prevent ischemic stroke or TIA maybe 80–100 mg/dL [15].

Although hyperlipidemia is one of the most important risk factors for atherosclerosis, the role of hyperlipidemia in the pathogenesis of ICAS remains uncertain. Studies of the potential risk factors of ICAS and ECAS have shown that hyperlipidemia is less closely associated with ICAS than with ECAS [16, 17]. Furthermore, it has been shown that animals that consume an atherogenic diet develop atherosclerosis significantly later in intracranial vessels than in extracranial arteries. It was suggested that the composition of the glycocalyx on luminal endothelial cells may be such that it inhibits the trapping of chylomicrons and very-low-density lipoprotein, resulting in less deposition of apolipoproteins in the intima of intracranial vessels [18].

There have been very few trials of lipid-lowering therapies specifically for ICAS. In one recent study, the investigators enrolled statin-naïve patients who had developed symptoms of acute ischemic stroke ≤7 days earlier, and who had symptomatic ICAS (>50% stenosis) in the proximal portion of the middle cerebral artery or basilar artery, or in the intracranial portion of the internal carotid artery [19]. They were adminis-

tered high doses of atorvastatin (40–80 mg) or rosuvastatin (20 mg) for 6 months and underwent high-resolution MRI (HR-MRI) before and after the statin treatment. Prespecified endpoints were measured after 6 months of statin treatment: (1) degree of stenosis, (2) remodeling index, (3) wall area index, and (4) enhancement volume of atherosclerotic plaque on HR-MRI. A total of 77 participants (mean age, 62.6 ± 13.7 years; 61.0% women) were enrolled, and their LDL-c concentrations at the initial and follow-up assessments were 125.8 ± 35.7 and 61.0 ± 19.3 mg/dL, respectively. Overall, statin treatment significantly reduced the accumulation of plaque (32.07 ± 39.15 mm^3 vs. 17.06 ± 34.53 mm^3, $P = 0.013$), wall area index (7.50 ± 4.28 vs. 5.86 ± 4.05, $P = 0.016$), and degree of stenosis (76.47% ± 20.23% vs. 64.05% ± 21.29%, $P < 0.001$), but not the remodeling index ($P = 0.195$). This suggests that high doses of statins may be of benefit for patients with symptomatic ICAS. However, the study had several limitations. First, it is uncertain whether such MRI changes are clinically meaningful. Second, because there was no control group, it may be that the MRI changes were not the result of statin administration. Third, it is unclear whether the dose used is the most appropriate one for ICAS patients. Finally, despite intensive statin therapy, 35% of the participants showed no changes in the sizes of the plaque or the degree of stenosis. Thus, there may be subtypes of ICAS that respond to statin therapy and others that do not. It also is possible that patients with other non-atherosclerotic diseases (e.g., moyamoya disease or dissection) were mistakenly included in the study.

There has only been one controlled study of asymptomatic ICAS. In a single-center prospective study, 71 asymptomatic patients with ICAS were treated with rosuvastatin, and their vascular stenoses were evaluated using transcranial color-coded sonography (TCCS) before and after the treatment. The therapeutic target was a reduction in LDL-c concentration of ≤1.8 mmol/L or a ≥50% reduction from baseline over 2 years, and the participants were allocated to an intensive statin treatment (IST) group or a standard statin treatment (SST) group. A total of 104 stenotic intracranial arteries were identified in 51 participants in the IST group and 47 in 20 participants in the SST group. In the first year of the study, there was a larger decrease in LDL-c concentration in the IST group than in the SST group (1.48 ± 0.26 mg/dL vs. 2.20 ± 0.58 mg/dL, respectively; $P = 0.000$). However, the percentage regression of the ICAS lesions in the IST group was not significantly larger than that of the SST group (26.3% vs. 5.9%, $P = 0.052$). Forty-nine lesions in 25 participants in the IST group and 16 lesions in seven participants in the SST group were reassessed after 2 years. At this time, the LDL-c concentration had decreased more in the IST group than in the SST group (1.55 ± 0.29 vs. 2.36 ± 0.77, $P = 0.048$), but in addition, the percentage regression of ICAS lesions in the IST group was significantly greater than that in the SST group (34.7% vs. 6.3%, $P = 0.017$). This suggests that the degree of stenosis in ICAS can be ameliorated within 2 years using intensive statin therapy. Therefore, as for carotid diseases, ICAS may improve with intensive statin therapy. However, this study also had some limitations: the number of participants who were followed for 2 years was small, and the stenosis was assessed using TCCS, rather than standard methods, such as MRA or conventional angiography.

Thus, although statins seem to be of benefit for patients with ICAS, further studies are required that include a control group, assess clinical endpoints, and compare the effect of statin therapy in patients with ICAS and ECAS. The changes in the vessel stenosis should also be assessed more meticulously, as in previous studies of carotid diseases [20].

14.4 Summary

The SPARCL and TST studies have shown that statins are beneficial for patients who have experienced an ischemic stroke and that a low target low-density lipoprotein-cholesterol (LDL-c) concentration may maximize this benefit. A strategy involving a low target concentration appears to be superior to one involving a moderate LDL-c

reduction for the prevention of coronary disease in these patients and in those with carotid artery disease. However, it remains uncertain whether statins are beneficial for patients with ICAS or SVD, and if they are, what would be the most appropriate LDL-c target concentration for these patients. Based on small pieces of evidence, it would be advisable to administer a statin to patients with ICAS, although the dosage would depend upon the clinical situation. There are no guidelines for patients with SVD, but a statin may be used in some instances, such as when patients have concomitant cerebral or cardiac atherosclerosis or if they do not have severe WMH or microbleeds. More studies are needed to elucidate the appropriate strategies in patients with ICAS or SVD. The utility of lipid-lowering agents other than statins should also be evaluated in stroke patients.

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15 Practical Dyslipidemia Management in Stroke-Specific Situations

Jin-Man Jung and Woo-Keun Seo

Abstract

Management of dyslipidemia, which implies elevated cholesterol levels, especially low-density lipoprotein cholesterol levels, is essential for stroke prevention. Over the past several decades, remarkable developments in controlling dyslipidemia and a distinct decrease in subsequent cardiovascular events (CVD) have been achieved. This monumental change is due to the use of "statins" or "3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors," which are primarily prescribed to treat dyslipidemia. There is strong evidence regarding the beneficial effects of statins on CVD prevention based on randomized controlled trials, and clinical practice guidelines have been updated to reflect this evidence. However, compared to clinical trials, much less attention has been focused on investigating the direct benefits of lipid-lowering agents, especially in patients with stroke. Therefore, there are many unanswered questions about the management of dyslipidemia in these patients. We focus on the management of dyslipidemia using lipid-lowering agents, especially statins, in general stroke care according to clinical practice guidelines and stroke-specific situations, including the acute period after index stroke, hemorrhagic stroke, and atrial fibrillation-related stroke.

Stroke is at high risk of morbidity and mortality worldwide. Managing dyslipidemia, especially high levels of low-density lipoprotein cholesterol (LDL-C), is considered crucial to reducing the development of stroke. The efficacy of statin 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, as a mainstream treatment for dyslipidemia, has been evaluated and proven in many clinical trials. However, clinicians and stroke neurologists can face diverse clinical situations. A strong body of evidence is needed for the management of patients with stroke, although the role of statins has been increasing in the treatment of atherosclerotic cardiovascular diseases, including stroke. Therefore, in this chapter, we have reviewed important clinical trials and current guidelines based on these trials with regard to dyslipidemia management in stroke patients. In addition, we have summarized the current evidence and relevant recommenda-

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tions regarding the diverse clinical presentations in patients with stroke.

15.1 Clinical Practice Guidelines and Landmark Trials for the Management of Dyslipidemia in Patients with Stroke

Many clinical guidelines for the management of dyslipidemia have been issued by different countries since the first Adult Treatment Panel for National Cholesterol Education Program was published in 1988 [1–15]. In this guideline, an LDL-C targeted level <120 mg/dL in primary prevention was first suggested in terms of the prevention of cardiovascular disease (CVD). Since then, the absolute LDL-C level treatment goal has decreased, and the goal has been to achieve ≥50% reduction in LDL-C levels with an absolute LDL level of <70 mg/dL (Fig. 15.1). Stroke has been included as one of atherosclerotic CVD (ASCVD) in the American College of Cardiology guidelines published in 2013, followed by the 2018 American College of Cardiology/American Heart Association (ACC/AHA) guidelines [16]. Further, patients with stroke as well as a transient ischemic attack (TIA) have been categorized in the very high-risk groups. However, since the recent 2019 European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) guidelines, a more intensified new target with an LDL-C reduction of ≥50% from baseline and an LDL-C level goal of <55 mg/dL has been suggested [14].

However, only <10% of the enrolled patients in most of the major clinical trials had a history of stroke; hence, the effect of statins on the primary or secondary prevention of stroke has not been sufficiently evaluated. Moreover, limited clinical trials have assessed the effect of lipid-lowering therapy in patients with stroke or TIA. The Heart Protection Study (HPS) is a randomized clinical trial investigating the effect of simvastatin 40 mg versus placebo in a high-risk group of 20,536 patients with coronary artery disease, other occlusive arterial disease, or diabetes mellitus [17]. Contrary to other trials, 3280 (15.9%) patients with prior stroke were analyzed using post hoc analysis in the HPS. Statin treatment reduced the risk of major vascular events, but it did not affect the development of recurrent stroke (relative risk [RR], 0.99; 95% confidence interval [CI], 0.81–1.21) [18]. The Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) was the first landmark trial that enrolled patients with only recent stroke or TIA [19]. A total of 4731 patients with LDL-C levels ranging from 100 to 190 mg/dL were randomly allocated to take high-dose atorvastatin (80 mg, daily) or a placebo. During a median follow-up period of 5 years, the atorvastatin group had a lower risk of recurrent total stroke (hazard ratio [HR], 0.84; 95% CI, 0.73–1.03), major cardiovascular events (HR 0.65; 95% CI, 0.49–0.87), and fatal stroke (HR, 0.57; 95% CI, 0.35–0.95) than the placebo group. However, the benefit of atorvastatin treatment in reducing the risk of stroke was relatively small (absolute risk reduction [ARR] of 1.9% for 5 years, with a number needed to treat of 52) and mainly attributed to the reduction of fatal stroke (RR reduction = 43%; $P = 0.03$), and it did not reduce the risk of nonfatal stroke ($P = 0.11$). Furthermore, the survival benefit from high-dose atorvastatin was not obvious and the concern that high-dose administration of atorvastatin could increase the risk of hemorrhagic stroke compared to the placebo (2.3% vs. 1.4%) was raised [16, 20]. Nevertheless, intensive reduction of the LDL-C level (≥50% of baseline) was shown to reduce the risk of combined fatal and nonfatal strokes with a nonsignificant increase of hemorrhagic stroke [21]. Many clinical practice guidelines have established statin treatment and the target LDL-C level in patients with stroke or TIA for secondary prevention based on the results of SPARCL.

In another clinical trial, the Japan Statin Treatment Against Recurrent Stroke, Asian patients with ischemic stroke were randomly assigned to a low-intensity pravastatin (10 mg) group and a non-statin group [22]. This trial was early stopped after enrolling 1589 patients without attaining the enrollment of the target population of 3000. During the mean follow-up of 4.9 years, the pravastatin group had slightly improved lipid profiles compared to the non-statin group, but the reduction in the risk of stroke

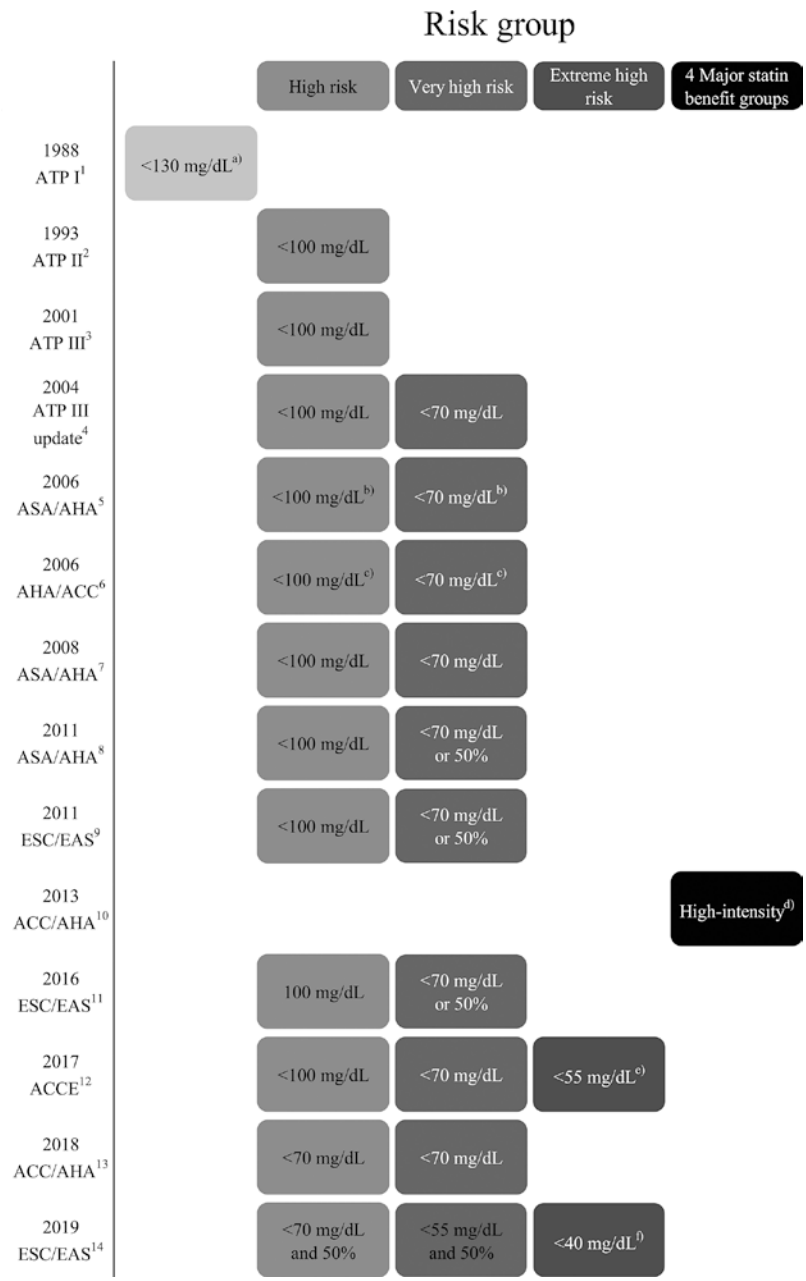

Fig. 15.1 Changes in target low-density lipoprotein cholesterol (LDL-C) goals for secondary stroke prevention in the major clinical practice guidelines. Modified from Precision and Future Medicine, Copyright Sungkyunkwan University School of Medicine [16]. *ATP* Adult Treatment Panel, *ASA* American Stroke Association, *AHA* American Heart Association, *ESC* European Society of Cardiology, *EAS* European Atherosclerosis Society, *ACC* American College of Cardiology, *ACCE* American College of Clinical Endocrinology. [a)]Borderline-high level; [b)]Statin treatment was recommended to reduce the risk of vascular events; [c)]Statin treatment was recommended to reduce the risk of stroke and cardiovascular events; [d)]High-intensity statin is defined as a daily dose that can lower LDL-C by approximately 50% or more from baseline; [e)]This guidelines first addressed 'extreme high-risk group' and LDL-C target <55 mg/dL; [f)]For patients with atherosclerotic cardiovascular disease who experience a secondary vascular event within 2 years while taking maximally tolerated statin-based therapy, an LDL-C goal of <40 mg/dL may be considered. These guidelines are first recommended considering LDL-C target <40 mg/dL

and TIA as the primary endpoint was comparable in both groups. However, in a subgroup of patients with atherothrombotic stroke, the pravastatin group had a reduced risk of recurrent stroke and TIA compared to the non-statin group. Therefore, more attention and focus are needed on well-designed clinical trials to investigate and prove the direct benefits of lipid-lowering agents, especially in patients with stroke.

15.2 Role of Early Lipid-Lowering Therapy During Acute Stroke Period

Experimental and clinical studies have indicated that statin treatment after stroke could improve functional outcomes and survival [23–25]. In particular, statin treatment during the hyperacute and acute post-stroke period (for example, <48~72 h from symptom onset) after index stroke has attracted many clinicians' and researchers' attention because this period is associated with a higher ischemic burden than the risk of bleeding. Many experimental studies have proven the neuroprotective effect of statins in animal stroke models. Statins can reduce the final infarct volume, enhance cerebral blood flow, and finally improve neurological outcomes after cerebral ischemia. In addition to lipid-lowering effect, they have pleiotropic effects, including vasodilatory, antithrombotic, anti-inflammatory, and antioxidant actions [25]. Based on these experimental evidences of statins' effects, observational human studies and randomized trials have investigated this issue. A meta-analysis, based on non-randomized observational studies, published in 2013 demonstrated that early statin treatment (within 72 h of stroke), including a continuation of pre-stroke statins and initiation of de novo statins, was associated with good functional outcomes (modified Rankin scale [mRS] score, 0–2) at 30 and 90 days after discharge (pooled odds ratio [OR], 1.9, 95% CI, 1.59–2.27 and 1.84, 95% CI, 1.37–2.48, respectively; all $P < 0.001$) [25]. Statin treatment in the acute post-stroke period could also consistently reduce the risk of mortality at 30 days, 90 days, and 1 year (pooled OR, 0.15, 95% CI, 0.07–0.31; pooled OR, 0.29, 95% CI, 0.19–0.45; and pooled OR, 0.18, 95% CI, 0.14–0.24, respectively; all $P < 0.001$). The beneficial effect of statins on functional outcomes and short-term mortality has been replicated in a similar direction and magnitude in subsequent observational studies and an updated meta-analysis [26–28].

Several randomized control trials (RCTs) have investigated the beneficial effects of acute statin therapy in terms of clinical outcomes and prevention of recurrent stroke. However, the results of these RCTs have not been consistent across clinical trials and appear to be disappointing compared with the results of preclinical and observational studies. A meta-analysis [25] based on RCTs showed no beneficial effect of acute post-stroke statin treatment on the 90-day functional outcome (pooled OR, 1.57, 95% CI, 0.88–2.81; $P = 0.12$) or fatality (pooled OR, 1.71, 95% CI, 0.74–3.97; $P = 0.2$); however, it included a small number of patients (<200 patients). Subsequently, one RCT (Administration of Statin on Acute Stroke Patient, ASSORT trial) was conducted at multiple centers in Japan to compare functional outcomes between early (<24 h) and delayed statin treatment (at 7 days) [29]. This trial failed to show any difference in 3-month functional outcome in 257 patients with non-cardioembolic acute stroke. In addition, the rate of mortality at 90 days (1.5% in the early group, 0.8% in the delayed group) and recurrent stroke (6.9% vs. 4.0%) were comparable between the two groups. The overall negative results might be attributed to the administration of intermediate-intensity statin therapy during the trial (atorvastatin 20 mg/day, pitavastatin 4 mg/day, or rosuvastatin 5 mg/day) although positive result limited to atherothrombotic stroke patients. Thus far, two RCTs (one on rosuvastatin at 20 mg and one on atorvastatin at 80 mg) have investigated the effect of high-intensity statin on clinical outcomes. The effects of very early use of high-intensity rosuvastatin in preventing recurrence of ischemic stroke were examined in patients with acute atherosclerotic stroke within 48 hours of onset [30]. The trial was ended early and inconclusively after enrolling 316 patients without attaining the preventive effect of statin regarding

new ischemic lesions as primary endpoint (RR, 0.83; 95% CI, 0.53–1.30). The event number of secondary outcomes defined as stroke or TIA were more common in the placebo group (4.4%) than in the rosuvastatin group (0.6%); however, this difference was not statistically significant (P = 0.067). Surprisingly, early high-intensity rosuvastatin was significantly associated with a lower risk of hemorrhagic transformation compared to control (4.4% vs. 14.5%, P = 0.007). The authors suggested a neuroprotective effect for early statin therapy against microvascular injury [16, 30]. In a small RCT, the potential clinical implication of early high-dose atorvastatin at 80 mg was suggested in large artery atherosclerotic stroke [31]. In this RCT, 42 patients were allocated to the atorvastatin 80 mg group (n = 22) and no treatment (n = 20) group. Statins were administered after symptom onset within a mean time of 12 ± 4.8 h. High-dose atorvastatin improved the levels of inflammatory markers and short-term functional outcomes, measured by the National Institutes of Health Stroke Scale and mRS scores at 7 days. However, caution is needed while interpreting the results due to the small sample size, the study design, which lacked a placebo in the control group, and the uncertainty of blinded capture of outcomes. From now on, there has been a lack of strong evidence supporting the benefit of acute statin therapy, but harmful effects from such treatment during the acute period seems to be minimal. Therefore, there is no reason to delay the administration of high-intensity statin in patients with acute stroke, especially those with atherosclerotic stroke [16].

15.3 Management of Dyslipidemia in Atrial Fibrillation-Associated Stroke

Atrial fibrillation (AF) is an important cause of ischemic stroke and accounts for approximately a quarter of total ischemic stroke cases. The prevalence of stroke attributed to or associated with AF has been increasing as the proportion of the elderly population has been increasing gradually. AF shares most of the important determinants, including left ventricular hypertrophy, obesity, and hypertension, with ASCVD in coronary, carotid, and peripheral vascular beds. Furthermore, each component of the CHA_2DS_2-VASc as a stratification tool for accessing thromboembolic risk in AF is also a risk factor for ASCVD. In fact, patients with AF frequently have vascular risk factors, and the use of statins has been increasing in such patients. However, there are no RCTs or clear guidelines regarding statin treatment for stroke attributed to AF.

In this situation, several observational studies and a recently published meta-analysis have demonstrated the beneficial effects of statin therapy on the clinical outcomes in patients with AF-related stroke [32–39]. This meta-analysis included studies on pre-stroke and post-statin groups according to the prescription time of statins [39]. Pre-stroke statin use was associated with a lower risk of poor short-term functional outcomes than post-stroke statin use (OR, 0.63; 95% CI, 0.47–0.85). Post-stroke statin therapy reduced the risk of all-cause mortality compared to pre-stroke statin therapy (HR, 0.63; 95% CI, 0.55–0.74). This beneficial effect was maintained regardless of the intensity of statin therapy. However, a reduction in the risk of recurrent ischemic stroke, acute coronary events, or composite vascular events was not observed in the post-statin group. The role of post-stroke statins in cardioembolic stroke, where AF might be a major etiology, has been investigated in some observational studies [40, 41]. The benefit of statins is consistent and sustained in terms of mortality and composite vascular events, but not in terms of stroke recurrence. The exact mechanism or action by which statins can reduce mortality in patients with AF or cardioembolic stroke is unclear. The survival benefits in the aforementioned studies could be attributed to systemic pleiotropic statin-induced effects (including anti-inflammatory and antioxidant effects), improvement in endothelial function, and angiogenesis and they may contribute to reduced mortality [42]. It is presumed that the preventive effects on stroke recurrence in patients with cardioembolic sources, mainly AF, are sustained by long-term anticoagulation and

not statins. Therefore, statin treatment could be considered an add-on treatment for long-term survival in stroke patients with cardioembolic sources, mainly AF.

15.4 Management of Dyslipidemia in Hemorrhagic Stroke

A meta-analysis based on large-scale population studies demonstrated that the serum levels of total cholesterol and LDL-C are inversely related to the risk of hemorrhagic stroke [43]. A post hoc analysis of SPARCL showed a significant increase in the incidence of hemorrhagic stroke in the high-dose atorvastatin group and primarily in elderly men with a history of hemorrhagic stroke [20]. The SPARCL trial raised major concerns about the relationship between low LDL-C levels after intensive statin treatment, or statin itself, and intracerebral hemorrhage (ICH). This could be attributed to two mechanisms—(1) alterations in cellular membrane integrity due to a statin-mediated reduction in cholesterol levels and (2) the pleiotropic effects of statins, including antithrombotic effects, via inhibition of platelet aggregation and the coagulation cascade [44, 45]. However, subsequent meta-analyses and reviews have revealed that the mechanism by which statins may increase the risk of hemorrhagic stroke is unknown and that there has been no evidence that statin treatment, or lower achieved LDL-C levels increase the risk of intracerebral hemorrhage [46–49]. In contrast, several observational studies and a meta-analysis have shown that pre-stroke or in-hospital early statin treatment can ameliorate survival and functional outcomes [50, 51]. In terms of mortality, withdrawal of statins during hospitalization for ICH can be harmful, and continuing or initiating statin therapy after ICH can be beneficial [52–55]. The beneficial effects of statins on ICH may stem from the potential neuroprotective and recovery enhancement effects afforded by statins in the acute or subacute stage after ICH [45]. These clinical and preclinical results support the use of statin treatment after ICH despite the lack of high-level evidence from well-designed RCTs evaluating the efficacy of acute statin therapy in ICH. Nevertheless, when prescribing statins after ICH to reduce any possible risk of ICH, attention must be paid to patients with a high risk of hemorrhagic strokes such as those with hypertension, specific ApoE genotype, or cerebral microbleeds, especially those at a lobar location [20, 56].

15.5 Conclusions

Management of dyslipidemia through the administration of statins as lipid-lowering agents is fundamental for preventing ASCVD, including stroke. Statin therapy can be adjusted according to the diverse clinical situations that patients with stroke might encounter, possibly because of the pleiotropic effects of statins. However, a large and strong body of clinical evidence is needed because of the lack of sufficient data and inconsistent results reported in clinical trials.

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Dyslipidemia in Women: Etiology and Management

16

Nazanin Rajai and Francine K. Welty

Abstract

This chapter summarizes the current knowledge regarding the prevalence of dyslipidemia in women, different response to therapy, and strategies to prevent and treat dyslipidemia during pregnancy and in postmenopausal women. Cardiovascular disease (CVD), particularly coronary heart disease (CHD), is the leading cause of death among women aged 60 and older. Appreciation of the differences between men and women in CHD risk factors and presentations can assist in treatment decisions. Some factors are unique to women, including reproductive status and menopause that increase the risk of dyslipidemia and consequently CVD in women. Menopause is associated with an elevation in LDL-cholesterol level in addition to threefold increase in the risk of CVD. Total cholesterol, very-low-density lipoprotein (VLDL) cholesterol, and triglyceride increase markedly after menopause. Dyslipidemias in postmenopausal women are particularly atherogenic and tend to cluster with other metabolic and non-metabolic risk factors. Randomized trials of statins for primary and secondary prevention of coronary heart disease suggest that statins have been effective in reducing the morbidity and mortality of CHD and should be considered as a first-line therapy for lipid lowering. In addition, pregnancy, known as an insulin resistance state, is associated with elevation of both cholesterol and triglyceride. Statins are contraindicated during pregnancy but omega-3 fatty acids may be used for hypertriglyceridemia. Those with genetic lipid disorders should consider consulting a clinician with lipid expertise before starting the pregnancy. This is particularly important due to the narrowed therapeutic options of lipid management which are available for pregnant women.

Cardiovascular disease (CVD), particularly coronary heart disease (CHD), is the major cause of mortality among women older than 60 years [1, 2]. Globally, a total number of 17.8 million (95% confidence interval (CI), 17.5–18.0 million) deaths attributed to CVD were estimated in 2017, accounting for nearly one-third of all-cause mortality [3]. An estimated 3.4 million women die each year from CHD worldwide [3]. Nearly 299,578 women died of CVD in the USA in 2017 accounting for about one in every five female deaths [4]. CHD is the leading cause of disability in women in the USA, accounting for more than

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one-third of hospitalizations of women aged older than 55 years [5]. These trends are particularly worrisome, considering the fact that women are 4–8 times more likely to die of CVD than of any other disease [6]. The prognosis is even worse in African–American women: given that the age-adjusted CHD mortality rate is 69% higher for ages 35–74 years than that of white women of the same age [7]. Despite the dramatic decline in men since 1980, the mortality rate of CVD continued to increase in women, as from 1984, annual CVD mortality among women has exceeded that of men by about 50,000 deaths a year [6]. While CHD rates in women increase markedly with age [8], the increasing number of CHD deaths among US women aged 35–54 years, believed to be due to the increasing prevalence of obesity is of particular concern [9]. Therefore, early identification and aggressive management of modifiable risk factors are essential to reduce the overall burden of CHD in women [10]. Elevated levels of low-density lipoprotein cholesterol (LDL-C) and triglyceride and lower levels of high-density lipoprotein cholesterol (HDL-C) are major modifiable risk factors for CHD in both men and women; thus, lipid-lowering agents are effective in reducing risk of CVD.

Women develop coronary artery disease (CAD) relatively late in life, with menopause (natural or surgical) marking an abrupt rise. The rate of CAD is 2–3 times higher in postmenopausal women compared to premenopausal women of the same age [11]. With each decade of life, the CAD mortality rate among women increases three- to five-fold [2]. Between ages of 45 and 64 years, 1 in every 9 women develop some form of CVD. In contrast, the prevalence rises substantially to 1 in every 3 after age 65 [2]. Menopause predisposes women to a significant elevation in serum cholesterol levels in addition to a three-fold increase in the risk of CVD [5, 12]. These changes have been explained in part due to declining levels of estrogen after menopause. However, recent evidence from prospective clinical trials has not supported protection against CVD for hormone replacement therapy (HRT) [13]. Concerning the population growth and aging, both the number of older women and the prevalence of CVD are expected to rise; thus, it is important to pay special attention to the primary and secondary prevention to minimize the burden of CVD in women. In this review, we summarize the current knowledge regarding the prevalence of dyslipidemia in women, differential response to therapy, and treatment strategies during pregnancy and postmenopause in women.

16.1 Prevalence of Dyslipidemia in Women

16.1.1 CHD Risk Factors in Women

A high level of LDL-C is considered a strong risk factor for CHD risk in women younger than 65 years and to a lesser extent in women aged 65 years and older [14]. Total cholesterol, very-low-density lipoprotein (VLDL) cholesterol, and triglycerides increase markedly after menopause, predisposing women to an increased risk of CHD [15, 16]. LDL-C levels are typically lower in women than in men until menopause. In four national surveys conducted between 1960 and 1991, mean LDL-C and triglyceride levels increased from 117 mg/dL and 113 mg/dL, respectively, in the 35- to 44-year-old women to 145 mg/dL and 168 mg/dL, respectively, in those 55–64 years old [17].

Low HDL-C level is a stronger predictor of CHD mortality in women compared to men, particularly in those aged 65 years and older [18]. In the cross-sectional National Health and Nutrition Examination Survey (NHANES) and Framingham Heart studies, mean HDL-C levels in women were approximately 10 mg/dL higher than men and the difference did not change with age [19, 20]. However, in two smaller longitudinal studies, HDL-C decreased in postmenopausal women [21, 22]. According to the study of Stevenson et al., the HDL-2 cholesterol subfraction, which is considered to be more cardioprotective than HDL-1 or HDL-3, exhibited a marked drop after the onset of menopause [12].

In a study of 2500 women aged 71 years and older, in those with HDL-C levels less than 0.9 mmol/L (35 mg/dL), the relative risk of CHD

mortality was double that of women with HDL-C levels of 1.6 mmol/L (60 mg/dL) or more [23, 24]. Previous studies indicated that each increment in the ratio of total to HDL-C is consistent with a marked increase in the risk of coronary events. In the Framingham Heart Study, the 8-year risk of CHD increased from 7% for women with a total/HDL-C ratio of less than 5, to 12% for those with ratios of 5 to 7 and reached 20% in those with ratios greater than 7 [25].

Elevated triglyceride concentrations are an important risk factor in women, particularly when the HDL-C level drops below 1.03 mmol/L (40 mg/dL) while average or high HDL-C levels make up for the triglyceride-associated increased CHD risk [26, 27]. Elevated serum triglycerides in older women may reflect an increase in VLDL, a triglyceride-rich lipoprotein involved in atherogenesis and the major carrier of triglyceride in plasma [28, 29]. Greater CHD risk in postmenopausal women may be attributable to the precipitous rise in triglyceride levels that are now considered as an accepted independent risk factor [26, 30]. Several behavioral, metabolic, and genetic risk factors are associated with hypertriglyceridemia [31]. In a pooled data analysis of 17 prospective, population-based studies including approximately 11,000 women, those with elevated triglyceride levels had a 75% increase in CHD risk in women compared to a 30% increase in men [31]. The most important predictor of small dense LDL is an elevated triglyceride level. Therefore, small dense LDL particles increase in postmenopausal women as triglyceride level increases. An increase in small, dense LDL particles is associated with a three-fold increase in the risk of myocardial infarction (MI) [32, 33].

High triglyceride level (>150 mg/dL) and low HDL-C (<50 mg/dL in women) along with increased small, dense LDL particles are the typical dyslipidemic feature of the metabolic syndrome. The guideline of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III defined diagnosis of the metabolic syndrome in women as the presence of at least three of the following risk factors: increased waist circumference (>102 cm in men and >88 cm in women), treated hypertension or systolic blood pressure >130 or diastolic blood pressure ≥85, HDL-C <50 mg/dl in women, triglyceride level ≥150 mg/dl and glucose ≥100 mg/dl [34]. The metabolic syndrome has a greater association with the incidence of CVD in women than in men [35] and postmenopausal women are more prone to develop the syndrome, with the highest prevalence in women's seventh decade [33]. According to a recent report from the third NHANES (NHANES III), metabolic syndrome is present in more than 40% of women aged 60 and older, and approximately 22.6% of US women have risk factors for developing metabolic syndrome. It has been reported that premature CHD in women (<65 years old) is more likely to occur in those with multiple risk factors of the metabolic syndrome [32].

As risk factors aggregate, women's premenopausal cardioprotective benefits shrink, and the risk of coronary events dramatically rises [5]. Consequently, screening for dyslipidemia in postmenopausal women to determine appropriate interventions is needed to reduce the risk of CHD.

16.1.2 Diabetes Mellitus

Cardiovascular disease is the leading cause of mortality in diabetic people of both sexes, especially in women. Diabetes offsets any cardiovascular advantage of premenopausal women so that their mortality rate from CHD approaches that of men [36]. Based on the evidence, mortality rates for CHD, stroke, and peripheral vascular disease are much higher in diabetic than in non-diabetic women [37, 38].

Hypertension and dyslipidemia are commonly seen with diabetes [39]. The characteristic dyslipidemic pattern in type 2 diabetes is a low level of HDL-C together with high VLDL-C, which is the cholesterol carried in the triglyceride-rich VLDL particle [36]. A population-based longitudinal study including 944 diabetic and non-diabetic adults (mean age, 61 years) with a follow-up period of 16 years revealed that dyslipidemia contributed substantially to the ischemic heart disease mortality in diabetic women. Thus,

the combination of diabetes, low HDL-C and high VLDL-C levels identifies those who would likely benefit from aggressive intervention [37].

Diabetes also reduces women's life-expectancy advantage [40]. Women with diabetes have a CHD-related mortality rate 3–7 times higher than that of non-diabetic women, whereas men's CHD mortality rate is 2–4 times higher than that of non-diabetic men [41]. The reasons for this sex disparity are not well understood but may be attributed to differences in lipid levels, as HDL-C level of 1.3 mmol/L (50 mg/dL) or less and a VLDL-C level of 0.5 mmol/L (20 mg/dL) or more result in a higher CHD risk in diabetic women than in men with diabetes [42].

16.2 Different Response to Therapy in Women

There is insufficient data regarding the benefits of cholesterol-lowering therapy in women due to a lack of sex-specific research and the fact that most studies have included only men or a small number of women. The large, long-term statin trials that included women are summarized in Table 16.1 (primary prevention) and Table 16.2 (secondary prevention). Women comprised 19% of the total population of the four earliest major statin trials (1994–1998). More recent trials as the HPS study were designed with a specific focus on women. In these trials for both primary and secondary prevention, women benefited from statin treatment to at least the same degree as did men; with decreased rates of cardiovascular events by 11–46% [42]. Notably, women older than 65 years benefited from utilizing statin use no less than younger women [40, 43]. Studies of statins including women are summarized below.

16.2.1 Statins: Primary Prevention

Four primary prevention trials included a large number of women without CVD (Table 16.1). The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) investigated the effect of statins in primary prevention, enrolling men and women with no clinical CAD, mean total cholesterol of 221 ± 21 SD mg/dL; mean LDL-C of 150 ± 17 mg/dL (3.9 mmol/L); mean HDL-C of 36 ± 5 mg/dL for men; and mean of 40 ± 5 mg/dL (1.03 mmol/L) for women. Participants were randomized to receive lovastatin 20–40 mg per day or placebo. At 1-year follow-up, lovastatin reduced LDL-C level by 25%, total cholesterol level by 18%, and triglyceride level by 15% and increased HDL-C level by 6%. After an average of 5.2 years of follow-up, the risk of a first major acute coronary event (fatal or nonfatal MI, unstable angina, or sudden cardiac death) was reduced by 37% in the lovastatin group compared with those taking placebo ($P < 0.001$). Although there was a difference in relative risk reduction (RRR) between women and men (46% vs. 37%, respectively), it was not statistically significant for women as only 20 out of the 997 women in the trial had coronary events (7 in the lovastatin group vs. 13 in the placebo group). Moreover, due to insufficient power, CHD mortality or total mortality could not be assessed [44].

In the Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA study) of 7832 individuals in a Japanese population (5356 women and 2475 men), those randomized to 10–20 mg pravastatin had reductions in total cholesterol and LDL-C by 11.5% and 18%, respectively, compared to the control group (2.1% and 3.2%, respectively). At 5.3 years of follow-up, the risk reduction in cardiovascular disease was similar between women and men although significant for men (hazard ratio (HR), men: 0.63 (95% CI, 0.42–0.95) but not for women: 0.71 (95% CI, 044–1.14, $P = 0.71$). The lack of significance in women is most likely due to the low number of women [45].

The Heart Outcomes Prevention Evaluation (HOPE)-3 study was another multicenter randomized control trial conducted on the effect of rosuvastatin in primary prevention in an ethnically diverse population. Of 12,705 participants (5874 women and 6831 men) with an intermediate risk of CVD (defined as estimated annual risk of major cardiovascular events of approximately 1%), the incidence of cardiovascular events (car-

Table 16.1 Characteristics of statin trials in primary prevention

Study	N	Age	LDL-C (mean)	Statin	Follow-up	Outcome
AFCAPS	997	55–73	130–190, HDL < 47	Lova 20,40	**1° endpoint**: First acute major coronary event (fatal or nonfatal MI, unstable angina, sudden cardiac death)	Women: RR 0.54; 95% CI: 0.22 to 1.35; *P* = 0.183
MEGA	5356	40–70	TC 220–270	Prava 10,20	**1° endpoint**: Composite of first occurrence of CHD (fatal and nonfatal MI, cardiac and sudden death, coronary revascularization procedure, and angina)	Women: HR: 0.75; 95% CI: 0.45–1.25; *P* = 0.27
JUPITER	6801	≥60	<130, CRP >2	Rosuva 20	1.9 years First major cardiovascular event (MI, stroke, hospitalization for unstable angina, arterial revascularization, cardiovascular death)	HR: 0.54; 95% CI: 0.37–0.80; *P* = 0.002 All-cause death HR: 0.77; 95% CI: 0.55–1.06 Nonfatal stroke: Women: HR: 0.84; 95% CI: 0.45–1.58 Revascularization/unstable angina HR: 0.24; 95% CI: 0.11–0.51
HOPE-3	Placebo: 6344 Rosu: 6361	Men ≥55 and women ≥65 without cardiovascular disease, at least 1 additional risk factor and women ≥60 with 2 additional risk factors	Mean LDL-C 127.8	Rosuvastatin 10	**1° endpoint**: CV death, MI, stroke, cardiac arrest, revascularization, heart failure, angina	Hazard ratio (95% CI): Male: 0.72 (0.58–0.90) Female: 0.83 (0.64–1.09) *P* = 0.427

Table 16.2 Characteristics of statin trials in secondary prevention

Study	N	Age	LDL-C (mean)	Statin	Follow-up	Outcome
Scandinavian Simvastatin Survival Study (4S)	3617 men and 827 women	35–70	Lowered 37.4%/mean LDL-C 4.86 mmol/L (188 mg/dL)	Simvastatin, 20 mg/day (titrated to 40 mg/day if necessary	5.4 years/combined endpoint (all-cause mortality, CHD death, nonfatal MI, or resuscitated cardiac arrest)	Total: Reduced by 34%. CHD and total mortality were significantly reduced in men. No significant reduction in CHD mortality and no benefit on total mortality in women (RR, 1.16; 95% CI, 0.68–1.99). Lower total and CHD mortality of 6% and 4%, respectively, in women compared with 13% and 8%, respectively, in men
Cholesterol and Recurrent Events (CARE)	3583 men and 576 postmenopausal women	21–75	3.59 mmol/L (139 mg/dL)	Pravastatin sodium, 40 mg	5-year follow-up/risk of CHD death or nonfatal MI	Reduction in nonfatal MI: female: 51%; male: 15% Risk of CHD death or nonfatal MI was about twice as great in women randomized to receive pravastatin as in men (43% vs. 21%). The need for PTCA or CABG was decreased by 48% and 39%, respectively, in women compared with 17% and 24% in men
Long-term Intervention With Pravastatin in Ischemic Disease (LIPID)	7498 men and 1516 women	31–75		Pravastatin sodium, 40 mg/day	6.2 years follow-up	26% reduction in CHD death or nonfatal MI in men (95% CI, 17%–35%) but a non-significant 11% reduction in women. Mortality data for women were not reported.

Study	N	Age	LDL-C (mean)	Statin	Follow-up	Outcome
Canadian Coronary Atherosclerosis Intervention	62 women		5.69 and 7.76 mmol/L (220 and 300 mg/dL)	Lovastatin	2 year	Lowered LDL-cholesterol level by 32% and total cholesterol level by 24%. Progression of coronary atherosclerosis: 28% lovastatin versus 59% placebo, $P = 0.031$ New coronary lesions: lovastatin 4% versus 45% placebo, $P < 0.001$
Women's Atorvastatin Trial on Cholesterol (WATCH)	318		Women with established CVD: the upper 10% of the distribution for age-matched North American women and without CVD: top 5% of the LDL-C distribution for age-matched women in this population	Atorvastatin 10 mg/day titrated to 80 mg/day to reach the NCEP LDL-cholesterol goal	16 weeks	Enabled 87% of women with two or more CHD risk factors and 80% of women with documented CHD to reach their LDL-cholesterol goals. The trial showed that lowering LDL-cholesterol level to the NCEP goal of 2.59 mmol/L (100 mg/dL) or less is feasible for the majority of women with dyslipidemia and CVD
Heart Protection Study	20,536	Aged 40–80 years		Simvastatin	5 years	Reduced the risk of a first major vascular event in women compared with placebo (17.7% vs. 14.4%, respectively)
Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE)	1600 people (including 344 women)			Atorvastatin 10 mg/day titrated to 80 mg/day to reach the NCEP LDL-cholesterol goal of less than 100 mg/dL	3 years	Reduced total mortality by 43% ($P = 0.002$), coronary mortality by 47% ($P = 0.0016$), coronary morbidity by 54% ($P < 0.0001$), and stroke by 47% ($P = 0.0018$). Women in the atorvastatin group had a significant 54% reduction in relative risk of all events compared with those in the "usual care" group ($P = 0.0038$)

diovascular cause-specific mortality, fatal MI, and nonfatal stroke) was 3.7% in the rosuvastatin 10 mg treated group and 4.8% in the placebo group (HR 0.76; 95% CI, 0.64 to 0.91; $P = 0.002$). Subgroup analysis showed a significant response to therapy in men (HR 0.72; 95% CI, 0.58–0.90), but not in women (HR for women: 0.83 (95% CI, 0.64–1.09)). There was also no significant gender heterogeneity for rate of cardiovascular events ($P = 0.427$) [46].

Beyond their lipid-lowering properties, statins have been reported to protect against CVD in part due to anti-inflammatory effects. In the large multicenter Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER trial), 17,802 people with no history of CVD, LDL-C less than <130 mg/dL, and high-sensitivity C-reactive protein levels>2 mg/l were randomized to receive rosuvastatin 20 mg daily or placebo [47]. At median follow-up of 1.9 years, rosuvastatin reduced the rate of major cardiovascular events by approximately 50% from 1.36 to 0.77 per 100 person-years of follow-up (HR for rosuvastatin, 0.56; 95% CI, 0.46 to 0.69; $P < 0.00001$). The absolute CVD rates in women for rosuvastatin and placebo (0.57 and 1.04, respectively) were lower than those in men (0.88 and 1.54, respectively), although the relative risk reduction associated with rosuvastatin was similar in women and men (HR 0.54; 95% CI, 0.37–0.80; $P = 0.002$ vs. HR 0.58; 95% CI, 0.45–0.73; $P < 0.001$). The corresponding rate between men and women did not differ significantly ($P = 0.82$, HR for women: 0.54; 95% CI, 0.37–0.80; $P = 0.002$ and for men: 0.58; 95% CI, 0.45–0.73; $P < 0.001$) [47].

16.2.2 Meta-analyses of Primary Prevention Statin Trials

A meta-analysis of 27 trials on primary prevention with statins (n = 174,149, 27% women) showed similar benefit for women and men as 39 mg/dL (1 mmol/L) reduction in LDL-C was associated with a 16% (rate ratio 0.84; 99% CI, 0.78–0.91) reduction in major vascular events in women, comparable to the 22% (rate ratio 0.78, 99% CI, 0.75–0.81) reduction observed in men (P for heterogeneity = 0.33) [48]. Further analysis of five studies comparing more versus less intensive statin therapy revealed that major vascular events were reduced by 25% (rate ratio 0.75, 99% CI, 0.58–0.97) per 39 mg/dL (1 mmol/L) reduction in LDL-C in women receiving more versus less intensive statin therapy, compared with 29% (rate ratio 0.71, 99% CI, 0.63–0.80) in men [48]. A meta-analysis of 18 randomized controlled trials of statins in primary prevention found that statin intervention was associated with a lower cardiovascular event rate versus control and was similar in women and men (odds ratio [OR] 0.81; 95% CI, 0.75–0.89; $P < 0.0001$ vs. OR 0.77; 95% CI, 0.71–0.83; $P < 0.0001$, respectively) [49] (Fig. 16.1).

16.2.3 Statins: Secondary Prevention

In the Scandinavian Simvastatin Survival Study (4S), 3617 men and 827 women with a history of angina or previous MI and mean LDL-C level of 4.86 mmol/L (188 mg/dL) (total cholesterol level ranged from 5.48 to 7.99 mmol/L [212 to 309 mg/dL]) were randomized to receive simvastatin 20 mg/day (titrated to 40 mg/day if necessary to lower total cholesterol level to <5.17 mmol/L [<200 mg/dL]) and placebo [50]. At a median follow-up of 5.4 years, simvastatin reduced LDL-C levels by 37.4% and the need for CABG or PTCA by 49% in women (95% CI, 0.30–0.86), and the risk of the composite endpoint (all-cause mortality, CHD death, nonfatal MI, or resuscitated cardiac arrest) by 34% in total cases. However, there was a differential response to treatment in men (relative risk [RR] 0.66; 95% CI, 0.53–0.80), while no significant reduction was observed in women (RR 1.12; 95% CI, 0.65–1.93), (6% and 6.6% in women compared to 8.5% and 12.8% in men, respectively). One potential reason for the lack of benefit in women is that chest pain syndromes in women are less likely to be associated with significant epicardial coronary disease compared to men (37% vs.

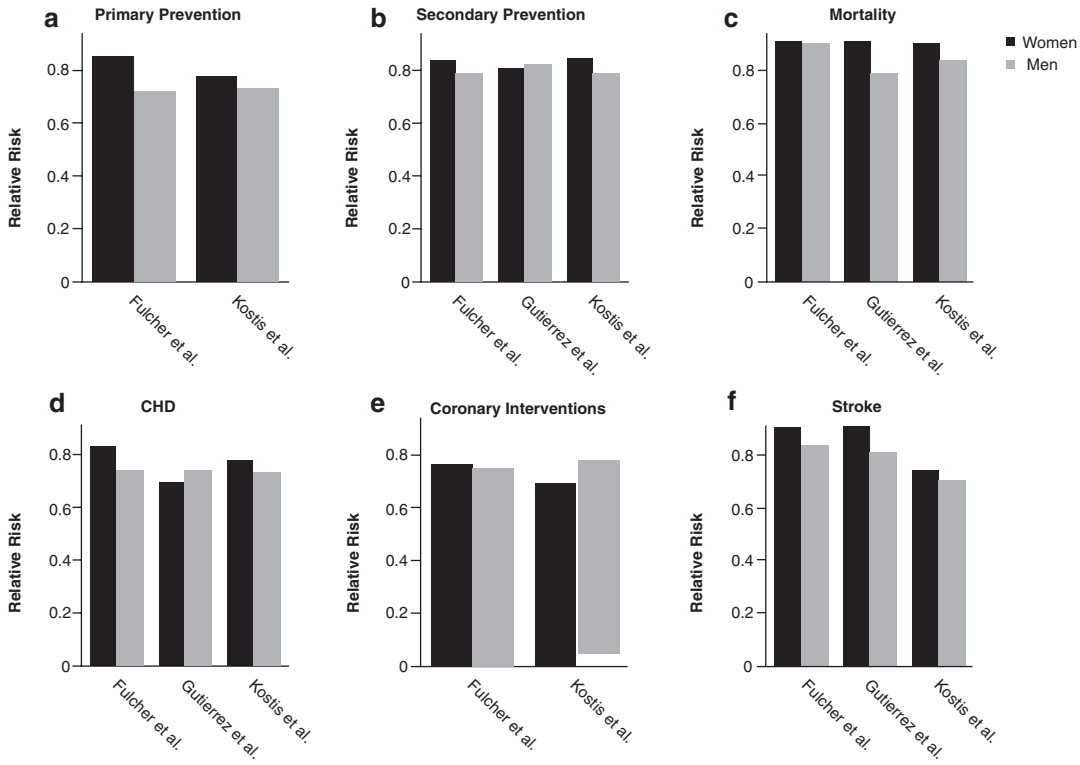

Fig. 16.1 Sex-specific results of three meta-analysis on the effect of statin trials on primary and secondary prevention. (**a**) Role of statins in major coronary events (CHD, coronary death, stroke, or coronary interventions) in primary prevention. (**b**) Role of statins in major cardiovascular events in secondary prevention. Panels (**c**) through (**f**) are for primary and secondary prevention combined. (**c**) Role of statins in cause-specific mortality. (**d**) Role of statins in coronary heart disease. (**e**) Role of statins in coronary revascularizations. (**f**) Role of statins in Stroke. *CHD* coronary heart disease

17%). These natural sex differences in clinical symptoms must be taken into consideration when results of clinical trials are interpreted based on a history of angina rather than MI or diagnostically confirmed CHD [50] (Table 16.2).

The Cholesterol and Recurrent Events (CARE) trial included 3583 men and 576 postmenopausal women with a history of MI and a mean LDL-C level of 3.59 mmol/L (139 mg/dL) randomized to receive pravastatin sodium, 40 mg, or placebo. After 5 years of follow-up, women experienced a greater reduction in risk of the primary endpoint compared to men (CHD death or nonfatal MI) (43% (95% CI, 4–66%) vs. 21% (95% CI, 4–35%)), nonfatal MI (51% (8–74%) vs. 18% (−5–40%)), need for percutaneous coronary intervention (48% (8–71%) vs. 18% (−1–34%) or CABG (39% (−17–69%) vs. 24% (5–39%) [51] (Table 16.2).

In the Long-term Intervention with Pravastatin in Ischemic Disease (LIPID) trial conducted in Australia and New Zealand, 7498 men and 1516 women with previous MI or unstable angina were randomized to receive pravastatin sodium, 40 mg/day, or placebo. At an average follow-up of 6.2 years in men and women combined, pravastatin was associated with a total mortality reduction of 22%; CHD death, 24%; MI, 29%; CHD death or nonfatal MI, 24%; stroke, 19% (P = 0.048); and revascularization, 20%. However, there was a sex-specific difference in CHD death or nonfatal MI reduction with a 26%

decrease in men (95% CI, 17–35%) but a non-significant 11% (95% CI, −18–33%) reduction in women [52] (Table 16.2).

In a quantitative coronary angiographic trial, the benefits of statin drugs in women were confirmed. The Canadian Coronary Atherosclerosis Intervention Trial (CCAIT) enrolled women with diffuse coronary atherosclerosis, various coronary risk factors, and total cholesterol levels between 5.69 and 7.76 mmol/L (220 and 300 mg/dL) to either lovastatin or placebo and performed quantitative coronary angiography at baseline and 2-year follow-up. The results indicated that lovastatin lowered LDL-C level by 32% and total cholesterol level by 24%. Women taking lovastatin had slower progression of coronary atherosclerosis (narrowing of coronary lumen diameter assessed by serial quantitative angiography) and fewer new lesions compared to placebo [23 out of 165 patients in lovastatin and 49 out of 166 patients in placebo ($P = 0.001$)] [53] (Table 16.2).

The Heart Protection Study (HPS) is one of the largest trials of statin therapy especially in women (Table 16.2). The HPS was unique in its inclusion criteria which consisted of people aged 40–80 years at high risk for CHD whose history would have excluded them from previous trials: these included people with diabetes, intermittent claudication, and baseline blood total cholesterol of 3.5 mmol/l. Among total participants of 20,536 UK adults, simvastatin (40 mg) significantly reduced the risk of a first major vascular event compared with placebo (19.8% vs. 25.2%, respectively, $P < 0.0001$) after an average of 5 years of follow-up. A similar trend in the incidence of vascular events was observed in both men (21.6% simvastatin vs. 27.6% in placebo) and women (simvastatin 14.4% compared to placebo 17.7%) subgroups with no sex-specific difference ($P = 0.76$) [54].

The Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) randomized 1600 people (including 344 women) with established CHD to either "usual care" or atorvastatin 10 mg/day titrated to 80 mg/day to reach an LDL-cholesterol target of less than 100 mg/dL. At a mean follow-up of 3 years, atorvastatin significantly reduced total mortality by 43% ($P = 0.002$), coronary mortality by 47% ($P = 0.0016$), coronary morbidity by 54% ($P < 0.0001$), and stroke by 47% ($P = 0.0018$) compared to "usual care." All subgroups of participants, including women, showed significant benefit. Women had a significant 54% reduction in relative risk of all coronary events compared with those in the "usual care" group ($P = 0.0038$) [55] (Table 16.2).

16.2.4 Meta-analysis of Secondary Prevention Statin Trials

A meta-analysis compared the effect of statins in secondary prevention of CVD in men and women in 11 trials [56] (Fig. 16.1). Statin therapy (atorvastatin, fluvastatin, lovastatin, pravastatin, or simvastatin) was shown to be associated with a reduced risk of cardiovascular events in all outcomes for women (risk ratio 0.81; 95% CI, 0.74–0.89) and men (risk ratio: 0.82; 95% CI, 0.78–0.85). However, there was no benefit with regard to stroke and all-cause mortality in women, although there was in men. The 2015 meta-analysis by the CTT Collaboration showed no heterogeneity by gender for the risk of major vascular events with statin therapy in participants with a history of vascular disease [57].

In summary, statins demonstrated promising effects in women in both primary and secondary prevention trials: up to a 46% reduction in major coronary events in AFCAPS/TexCAPS, together with improving lipoprotein profiles [58–62]. These randomized trials suggest that statins are at least as effective for reducing cholesterol levels and cardiovascular events in women as in men with CHD; thus, statins are recommended as first-line therapy in postmenopausal women with elevated LDL-C levels along with those who have established CHD.

16.3 Lipid Management in Pregnancy

Pregnancy is considered as a state of insulin resistance. The serum level of total cholesterol and triglyceride begin to rise from week 6 of

gestation and gradually increase during each trimester [63]. Eventually, there is a two- to four-fold increase in triglyceride and cholesterol level up to 42% mostly in the third trimester [64]. These changes could be attributed to a rising level of estrogen throughout pregnancy which leads to an increase in hepatic production of VLDL and inhibition of hepatic and adipose lipoprotein lipase that lipolyzes the triglyceride of VLDL. This physiologic increase in lipid and lipoprotein profile helps in maintenance of fetal development. Cholesterol plays an essential role in different aspects of fetal development such as cellular membrane integrity, synthesis of hormones, brain development, and hepatic and lung maturation [65–67]. However, an excess level of lipid and its oxidized products, as seen in familial hypercholesterolemia (FH), could result in both maternal and fetal deleterious effects such as preterm labor, intrauterine growth retardation, premature atherosclerosis, and preeclampsia [68–70].

In terms of management, the initial approach is to determine the medical conditions that predispose pregnant women to dyslipidemia at the very first antenatal visit. Some examples include gestational or overt diabetes, pregnancy-related hypertensive disorder, polycystic ovary syndrome, hypothyroidism, kidney disease (i.e., nephrotic syndrome), alcohol consumption, and genetic susceptibility (e.g., FH), all of which should be treated appropriately before conception [63, 71].

A medication history should be taken and women on medications which worsen the lipid profile such as oral contraceptives, estrogen, glucocorticoids, selective serotonin reuptake inhibitors, valproate and retinoic acid should be discontinued or switched to safer alternatives [71] (Table 16.3).

Management of hyperlipidemia in pregnancy predominantly relies on lifestyle modifications and glycemic control. Close follow-up and monitoring of weight gain during pregnancy, especially in pregnant women with metabolic disorders is highly recommended [63]. However, in women with a higher level of cholesterol and triglyceride, medical management might be indicated. To minimize the potential adverse effects on fetal development, it is important to discontinue all lipid-lowering drugs except fish oil, omega-3-fatty acids, or bile acid sequestrates. All

Table 16.3 Effects of selected drugs on triglyceride and cholesterol levels

Drug	Triglycerides	LDL-C	HDL-C
Alcohol[a]	Increased	No effect	Increased
Estrogens, estradiol[a]	Increased	Decreased	Increased
Androgens, testosterone	Increased	Increased	Decreased
Progestins	Decreased	Increased	Decreased
Glucocorticoids[a]	Increased	No effect	Increased
Cyclosporines	Increased	Increased	Increased
Tacrolimus	Increased	Increased	Increased
Thiazide diuretics[a]	Increased	Increased	Decreased
Beta-blockers[a]	Decreased	Increased	No effect
Sertraline[a]	Decreased	Increased	No effect
Protease inhibitors[a]	Increased	No effect	No effect
Valproate and related drugs[a]	Increased	No effect	Decreased
Isotretinoin[a]	Increased	No effect	Decreased
Clozapine; olanzapine[b]	Increased	No effect	Decreased

[a]Alcohol, estrogens, estradiol, glucocorticoids, thiazide diuretics, beta-blockers, sertraline, protease inhibitors, valproate and related drugs, and isotretinoin can cause severe hypertriglyceridemia and the chylomicronemia syndrome in patients with a familial form of hypertriglyceridemia. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein

[b]Second generation anti-psychotics: clozapine and olanzapine have most effect; risperidone and quetiapine have intermediate effects and aripiprazole and ziprasidone have least effect

statins are currently contraindicated in pregnant women, primarily as a result of a 2004 series of cases of first-trimester statin exposure reported to the FDA, which showed 20 cases of malformation, including 5 severe defects of the central nervous system and 5 unilateral limb deficiencies [72]. In all cases of adverse birth outcomes, the statin used was lipophilic. No malformation was identified in the 14 infants exposed to pravastatin (hydrophilic). Since this case series, cohort studies of statin exposure in pregnancy did not show an increase in teratogenic risk [73–75] and in fact, the safety of pravastatin is under study for the prevention of preeclampsia in high-risk pregnant women [76]. In a meta-analysis of six studies of pregnant women exposed to statins, no increased risk of birth defects was observed compared with control subjects. However, there was an increased risk of miscarriage in the statin-exposed women versus controls [77]. Furthermore, in a recent retrospective cohort study that used time-to-event analysis as a covariate, the adjusted hazard ratio of spontaneous pregnancy loss in the statin-exposed group was increased (HR, 1.64; 95% CI, 1.10–2.46) [78]. The increase in miscarriages may be related to confounders, such as older age, CVD risk factors, and other medications. Statins should be stopped 1–2 months before pregnancy is attempted. When pregnancy is unplanned, statin therapy should be stopped promptly and not restarted until after pregnancy and breastfeeding are completed. Cholesterol levels rise in pregnancy, with a similar percentage rise in normal women and those with heterozygous FH. Women with FH do not appear to have a higher risk of preterm delivery or of having infants with low birth weight or congenital malformations than unaffected women, but undetected bias cannot be ruled out [79]. An experienced lipid specialist should be consulted for women with homozygous FH.

Levels of triglyceride rise progressively with each trimester, and women with triglyceride levels ≥500 mg/dL (5.6 mmol/L) at the onset of pregnancy may develop severe hypertriglyceridemia during the third trimester of pregnancy, which can lead to pancreatitis [80]. Advising patients on lifestyle (including both diet and physical activity), optimally managing diseases like diabetes mellitus and hypothyroidism, and choosing medications that are less likely to raise triglycerides can reduce levels of triglyceride before pregnancy begins.

Hypertriglyceridemia, often greater than 500, can be treated safely with a combination of a low-fat diet and omega-3-fatty acids [81, 82]. Table 16.4. outlines a management approach to hypertriglyceridemia. Fenofibrate and gemfibrozil should only be prescribed if the benefits outweigh the risk (Class C) [71, 83]. Severe

Table 16.4 Management approach to hypertriglyceridemia (Goal <150 mg/dL)

TG 150–199 mg/dL: Counseling on diet, 30 min of daily aerobic exercise and ideal body weight
TG 200–499 mg/dL: Goal < 150 is secondary target after LDL-C goal reached
1. Review Medications (see 3) – Change to lipid neutral or favorable agents when possible (e.g., alpha blockers, biguanides, thiazolidnedione) – Lower doses of or stop drugs that increase triglycerides such as beta-blockers (particularly nonselective agents), glucocorticoids, diuretics (thiazide and loop), ticlopidine and estrogens when indicated clinically.
2. Laboratory Studies Exclude secondary disorders of lipid metabolism: – Fasting blood glucose – Serum BUN and creatinine – Thyroid function studies (TSH)
3. Diet and exercise – Weight loss – Avoid concentrated sugars and simple carbohydrates – Reduce saturated fat – Reduce or eliminate alcohol – Increase omega-3 fatty acid intake through fish consumption – Aerobic exercise minimum of 3 h weekly
4. Recheck lipid profile in 3–6 months (give enough time for adequate weight loss) *Primary prevention* If LDL-C goal not reached in 3–6 months with steps 1–3 above, consider adding statin therapy Once LDL-C goal reached, if TG > 150 mg/dL: Reinforce lifestyle changes. If TG not <150 mg/dL, consider: – Fish oil (omega-3 fatty acids) up to 3.2 g EPA and DHA daily. – Repeat labs 6–8 weeks after dose adjustments. *Secondary prevention* In addition to following steps 1–3 above, statin therapy should be utilized to reach LDL-C goal with repeat labs 6–8 weeks after dose adjustments

Table 16.4 (continued)

TG 500 mg/dL–999 mg/dL
Weight loss; increased exercise—follow steps 1–3 above Consider very low-fat diet (<15% of caloric intake) Remember that LDL-C cannot be estimated when TG > 400 mg/dL Consider fibrate therapy (monitor INR if on warfarin) • Fenofibrate – Fenofibrate micronized 160 mg or 200 mg/day—must be taken with dinner – Nanocrystallized—145 mg/day; taken without regard to meals • Gemfibrozil (Lopid) 600–1200 mg/day (usually 600 mg bid) 30–60 min before meals. Gemfibrozil raises level of statin drug; therefore, if the patient is on a statin, fenofibrate is preferred over gemfibrozil
TG ≥1000 mg/dL
Follow steps 1–3 above Initiate fibrate therapy—monitor serum creatinine With acute pancreatitis • Very-low-fat diet (10%–15% of energy intake) • Cessation of alcohol • Insulin, if indicated for glycemic control • Admit patient to hospital if necessary – Nothing by mouth: IV fluid replacement – Plasma exchange has been used

hypercholesterolemia, as seen in familial hypercholesterolemia, can be treated with bile acid sequestrates (preferably colesevelam) and in homozygous familial hypercholesterolemia, mipomersen (Class B) [84]. There are also a few novel therapeutic interventions for gestational hyperlipidemia with only limited supporting evidence. Some examples are niacin, medium-chain triglyceride, plasma apheresis, and gene therapy all of which are at the stage of research trials, and their clinical implications yet need to be investigated [85]. Treatment of severe hypertriglyceridemia during pregnancy requires consultation with an experienced lipid specialist.

16.4 Lipid Management in Postmenopausal Women

16.4.1 Menopause and Dyslipidemia

Natural menopause is associated with a threefold increase in CHD risk [86]. In the Nurses' Health Study cohort, women who had undergone surgical menopause with bilateral oophorectomy had up to an eightfold increase in the risk of CHD [87]. A contributory factor may be due to declining levels of estrogen, which subsequently leads to down-regulation of hepatic LDL receptors leading to a significant elevation in serum cholesterol levels [88–90]. By the age of 55 years, almost half of all women have total cholesterol levels of 6.2 mmol/L [240 mg/dL]) [29] (Fig. 16.2). In contrast, in men, cholesterol levels remain constant after age 50 years whereas in women, LDL-C levels increase an average of 0.05 mmol/L (2 mg/dL) per year between ages 40 and 60 years [91].

Considering the role of estrogen deficiency in increasing CHD risk, a meta-analysis of 30 observational studies of estrogen therapy exhibited a significant reduction in CAD risk as much as 44% [92]. However, recent randomized trial results provide no evidence regarding the protective effects of hormone replacement therapy against CAD but even harm in some cases [93]. Therefore, HRT is prescribed only for women with symptomatic hot flashes.

In a large trial of the Heart and Estrogen/progestin Replacement Study (HERS), 2763 postmenopausal women with CAD (≥80 years) were evaluated for the possible protective effect of HRT [94]. At 4-year follow-up, HRT (conjugated equine estrogen 0.625 mg + medroxyprogesterone acetate 2.5 mg) showed no significant benefit in the incidence of primary CAD events (RR: 0.99; 95% CI, 0.8–1.22), CAD mortality (RR: 1.24; 95% CI, 0.87–1.75), or nonfatal MI (RR: 0.91; 95% CI, 0.71–1.17) as compared to control group. Surprisingly, HRT resulted in a significant increase in the risk of heart attack, stroke, and pulmonary embolism during the first year of the study (relative hazard for year 1: 1.52; 95% CI, 1.01–2.29 vs. year 4 and 5: 0.67; 95% CI, 0.43–1.04; $P = 0.009$) [95]. Although the estrogen plus progestin group demonstrated an 11% reduction in LDL-C and a 10% increase in HDL-C levels, the explanation for the early adverse outcomes observed in HERS might be due to the prothrombotic effects of estrogen, as indicated by a threefold increase in the risk of venous thromboembolic events in the HRT group compared to placebo.

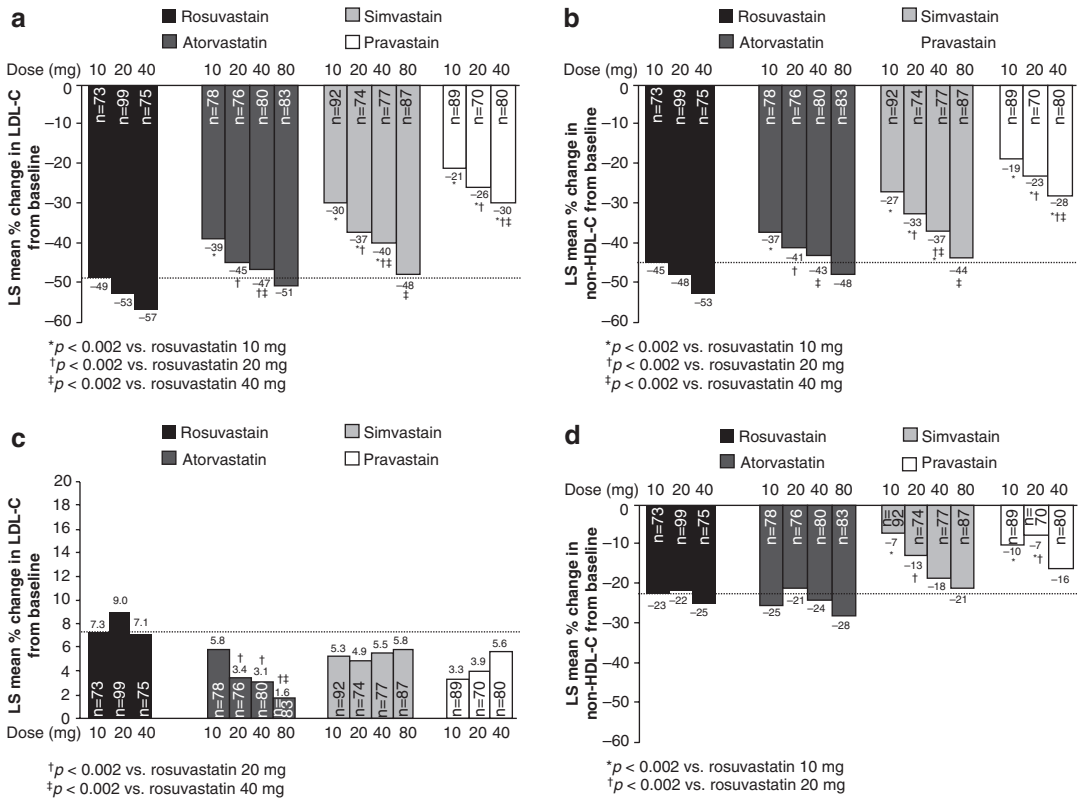

Fig. 16.2 (**a**) LS mean % change from baseline in LDL-C at week 6 (LOCF). (**b**) LS mean % change from baseline in non-HDL-C at week 6 (LOCF). (C) LS mean % change from baseline in HDL-C at week 6 (LOCF). (D) LS mean % change from baseline in triglyceride at week 6 (LOCF). The dashed line refers to the least-squares mean percentage change for rosuvastatin 10 mg. *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *LOCF* last observation carried forward, *LS* least-squares

In the Estrogen Replacement and Atherosclerosis (ERA) trial of postmenopausal women (mean age 65.8 years) with at least one coronary artery with 30% stenosis, 309 cases were randomized to receive 0.625 mg conjugated equine estrogen, conjugated equine estrogen 0.625 mg + medroxyprogesterone acetate 2.5 mg, or placebo [95]. A total of 48% of the women had a prior MI and 47% had undergone prior percutaneous transluminal coronary angioplasty. At a mean follow-up of 3.2 years, women receiving estrogen or estrogen plus progestin demonstrated a significant reduction in LDL-C levels (16.5 ± 21.8%, as compared with 1.3 ± 21.5% in the placebo group, $P < 0.001$) and increases in HDL-C levels (14.2 ± 17.1% compared to 6.8 ± 15.6%; $P < 0.01$ in placebo). However, neither therapy caused a significant change in angiographically assessed coronary disease compared with placebo (change in minimal coronary artery diameter, −0.12 ± 0.02 mm vs. −0.9 ± 0.02 mm, $P = 0.38$) [95].

The Women's Health Initiative (WHI) was a large, randomized trial of 27,000 women with no clinical CAD which raised concerns about the safety of HRT. After a 2-year follow-up period, women on estrogen monotherapy or combination of estrogen and progestin demonstrated increased rates of CHD (1.29; 95% CI, 1.02–1.63), stroke (1.41; 95% CI, 1.07–1.85), or pulmonary embolism (2.13; 95% CI, 1.39–3.25) compared with placebo [96].

Based on the results from HERS, ERA, and the WHI trial, women who are considering using

estrogen, either as monotherapy or in combination with progestin, should be fully informed of the potential benefits and possible risks. As might be expected, prescribing HRT in women with established CAD should not be recommended to prevent subsequent cardiovascular adverse events.

16.4.2 Patient Screening

An initial laboratory screening for dyslipidemia should include a fasting lipoprotein profile including total, LDL-C, HDL-C, triglyceride, and glucose level at the age of 20 [97]. The fasting blood glucose level is to assess for metabolic syndrome and rule out diabetes as a secondary cause. To rule out secondary causes of dyslipidemia, thyroid function should also be evaluated [67].

The 2018 AHA/ACC cholesterol guidelines identified hypertensive disorders during pregnancy, preeclampsia, gestational diabetes mellitus, delivering a preterm or low-birth-weight infant [98, 99] and premature menopause [age < 40 years] [100–102] as factors shown to increase the risk of atherosclerotic cardiovascular disease (ASCVD) [97]. They included preeclampsia and premature menopause (age < 40 years) as risk-enhancing factors for statin therapy because they appear to increase ASCVD risk in the same range as other risk-enhancing factors. However, they did not include preterm birth as a risk-enhancing factor for statin therapy since the mechanism or cause of preterm birth is often unknown. Furthermore, if gestational diabetes mellitus predisposes a woman to metabolic syndrome or diabetes mellitus, these are already identified as major ASCVD risk factors. After pregnancy and throughout the life course of every woman, a thorough pregnancy history should be obtained, and risk factors and risk-enhancing factors should be identified. Interventions should include aggressive lifestyle counseling to reduce ASCVD risk and when appropriate, statin therapy.

16.4.3 Nonpharmacologic Therapy

The first approach in managing dyslipidemia is aggressive risk factor modification and lifestyle changes including tobacco cessation, being physically active, healthy weight maintenance, and consumption of a diet low in saturated fat and dietary cholesterol, rich in fruits and vegetables, and elimination of trans fat [43]. To reduce the risk of developing ASCVD, clinicians should recommend a dietary pattern such as the Mediterranean diet consisting of minimally processed foods such as fruits, vegetables, nuts/seeds, beans/legumes, whole grains, fish/seafood, and unsaturated fats to replace saturated fats and trans fats) [103, 104]. The contents of the Mediterranean diet are shown in Table 16.5. Adults who would benefit from LDL-C lowering should be advised to consume a dietary pattern such as the Mediterranean diet or DASH dietary pattern that emphasizes intake of vegetables, fruits, and whole grains; includes low-fat dairy products, low-fat poultry (without skin), fish/seafood, legumes, non-tropical vegetable oils and nuts; and limits intake of sweets and sugar-sweetened beverages and encourages healthy-alternatives [103, 104]. To specifically lower LDL-C, the diet should contain reduced calories from saturated fat and dietary cholesterol and eliminate trans-fat [103, 104]. Regular physical activity should include five sessions a week of moderate to vigorous intensity physical activity averaging 30 min per session. Most health benefits occur with at least 150 min per week of moderate intense physical activity or 75 min of vigorous physical activity [105].

The Mediterranean Diet, which is high in polyunsaturated fatty acids, lowered risk of CVD in both primary and secondary prevention trials. Adhering to the Mediterranean diet is strongly recommended because it has been shown to lower both CHD and total mortality. The Prevention with Mediterranean Diet (PREDIMED) study was the first large randomized controlled trial to show that a Mediterranean diet is able to reduce clinical events in primary cardiovascular preven-

Table 16.5 Approach to nutritional assessment and counseling

Height: _____ in **Weight:** _____lbs **BMI:** _____ **Usual Body Weight (UBW) :** _____

Change in weight: ☐ stable ☐ increased ☐ decreased
Goal weight : _____ lbs

Risk Factors: ☐ DM type 1 ☐ DM type 2 ☐ high chol ☐ low HDL ☐ high trig ☐ HTN ☐ GERD

Labs	**TC**	**LDL**	**HD L**	**TG**	**HbA 1C**
XX / XX /XX XX / XX /XX XX / XX /XX XX / XX /XX					

Supplements:

Food Allergies:

Exercise:
☐ Currently Exercising on Regular basis: _____
☐ No formal exercise at this time
☐ Plans to include regular exercise

ASSESSMENT

Diet Recall
Breakfast: _____
Snack: _____
Lunch: _ _____
Snack: _____
Dinner: _____
Snack: _____

Beverages: ☐ **soda**_____, ☐ **water** _____, ☐ **alcohol** _____
Other: ☐ **fish**_____, ☐ **red meats** _____
Restaurant frequency: _ _____

Diet History
☐ Follows meal plan
☐ Skips meals _____
☐ Variety and balance:
☐ Excessive calorie intake, snacking
☐ High calorie beverages_____
☐ High intake of: refined carbohydrates, sodium, fat(saturated, trans fat)
☐ Inadequate intake of: fruits, vegetables, w hole grains, fiber, fluids, calcium rich foods

Table 16.5 (continued)

Trans Fats-eliminate	Saturated Fats (<10%)	Unsaturated fats		Fiber (14 g fiber per 1,000 kcal)	Omega-3 fatty acids
		Monounsaturated	Polyunsaturated		
deep fried restaurant foods	Meat	olive oil	sunflower oil	Oatmeal	Salmon
				Oat bran	mackerel
stick margarine	butter	peanut oil	corn oil	legumes	fresh herring
crackers	high-fat cheese	avocados	sesame oil	fruits	bluefish
cookies	milk whole and 2%	canola oil	safflower oil	barley	trout
baked products	condensed or sweetened milk	peanut butter	soybean oil	Metamucil 1 tlbsp daily lowers LDL-C 9%	albacore tuna (packed with water)
	cream	nuts (in moderation)	tub or liquid margarine		sardines (packed in water or mustard)
	ice cream				
	coconut oil				
	palm oil				

Mediterranean Diet Composition	Animal Foods that contain Cholesterol
• vegetables (at least 300-400 g a day) • fruit (at least 4 pieces or 400 g a day) • legumes • whole grains, pasta and/or bread (mostly whole wheat) • olive oil and nuts • water (more than 2 liters per day) • wine during meals (maximum 2 glasses per day) • seafood (fish, shellfish, mollusc), 4-6 times a week • yoghurt, 2 - 4 times per week • cheese, 1 - 4 times per week • milk, 1 - 2 times per week • eggs, 0 - 4 eggs per week (maximum 1 per day) • poultry, 1 - 2 times per week • red meat, 0 - 1 time a week	• meats: beef, pork, veal, lamb (limit; try to choose lean cuts) • whole milk (try to avoid; use low-fat or fat free milk instead) • egg yolks (limit to two yolks per day) • poultry with skin (try to avoid; choose skinless poultry instead) • shrimp, squid (calamari), scallops (in moderation) • liver and other organ meats (try to avoid)

Key recommendations in the 2015-2020 dietary guidelines	• Consuming variety of nutrient dense foods such as dark green, red and orange vegetables; legumes; starchy vegetables, whole • Fruits, grains, at least half of which are whole grains; and low- or fat-free milk, yogurt, cheese and fortified soy drinks. • Protein rich foods such as seafood, lean meats and poultry, eggs, soy, nuts, seeds, as well as plant-based oils are also considered • Healthy choices. **Measurable limits were also set for added sugar, sodium, fats and alcohol:** • Limit added sugar to less than 10% of daily caloric intake • Limit sodium to less than 2300 mg of daily intake, and even less for children and adolescents aged younger than 14 • Limit saturated and trans fat to less than 10% of daily caloric intake • Limit alcohol to up to one drink per day for women and up to two drinks per day for men of legal drinking age • For adults with prehypertension and hypertension, further sodium reduction to 1500 mg per day is recommended for an even • greater reduction in BP

Table 16.5 (continued)

Education material provided:

☐ Heart Healthy Guide, ☐ Lower Triglycerides, ☐ Lower Sodium, ☐ Fish Oil, ☐ Fiber, ☐ DM,

☐ Goals, ☐ Diabetic Diet, ☐ Increasing Fiber, ☐ Weight loss

Nutrition Problem:

Interventions/Recommendations:

☐ Plan a regular schedule for meals and snacks every 3-4 hours
☐ Plan meals ahead of time; cook in bulk and freeze in portion sizes
☐ Portion control at meals- follow plate model: 1/2 plate non-starchy vegetables, 1/4 grain (ideally whole grain more often), 1/4 protein source; use measuring cups if needed for proper portion sizes or use smaller plates/bowls
☐ Reduce calorie-density of meals- choose low calorie/high nutrient foods like fruits, vegetables, lean proteins, and whole grains. Limit foods high in calories that offer little nutrition (sweets, butters, dressings, etc)

Fiber

14 g fiber per 1,000 kcal
Good sources of fiber include oatmeal, oat bran, legumes, fruits, barley, Metamucil

☐ Increase fiber: add more fruits (aim for 2 servings per day); vegetables (aim for 1/2 your plate to be vegetables or 3 servings per day); whole grains/starches; beans, lentils

Carbohydrates

☐ Limit portion size of refined carbohydrates
☐ Low fat/fat-free milk and dairy

Fats

Intake of saturated fats should be limited to less than 10 percent of calories per day by replacing them with unsaturated fats and while keeping total dietary fats within the age-appropriate AMDR

☐ Decrease saturated /trans fats- limit processed foods (chips, sweets, butter, etc), fast food, fried foods, limit red meats to 6 oz per week, trim fats, also limit cheese and added fats.
☐ Use apple sauce or canola oil
☐ Read labels for fat content and look for 'partially hydrogenated' in ingredients list on label for hidden trans fat
☐ Increase omega 3 fatty acids: 1 gram per day recommended: via increased intake of herring, salmon, mackerel, sardines (in water), trout, tuna; goal is >2 servings week; other sources include soybeans, canola oil, walnuts and flaxseed (ground up)

Beverages

consumption of added sugars to less than 10% of calories per day
sodium intake should not exceed 2,300 mg/day; Intake below this level is recommended for children younger than 14 years old and people who have prehypertension or hypertension (i.e., high blood pressure).

☐ Reduce sweetened beverages: less juice, soda, sweetened teas, sports drinks; if you choose juice dilute 4oz juice with 4 oz water or choose diet
☐ Limit alcoholic beverages
☐ Increase water/fluid intake; aim for 64oz/day
☐ Limit sodium intake: limit salt when cooking and at table. Use natural herbs and spices, Mrs. Dash. Buy fresh/frozen vegetables when able-if buying canned, buy 'low sodium' varieties and rinse with water
☐ Limit meals ordered out
☐ Read label for sodium amount (per serving)-sauces, condiments, gravies, marinades, dressing, cheese; Watch portion sizes of added salt foods

Snacks

☐ Snacks: limit snacks to 100-200 calories; Pair a carbohydrate (whole grain) or vegetable/ fruit with a protein such as nuts (1/4 cup/day) or nut butter (2tbsp/day) or low fat cheese or hummus greek yogurt
☐ Increase exercise
☐ Plan a regular schedule for meals and snacks every 3-4 hours
☐ Plan meals ahead of time; cook in bulk and freeze in portion sizes
☐ Portion control at meals- follow plate model: 1/2 plate nonstarchy vegetables, 1/4 grain (ideally whole grain more often), 1/4 protein source; use measuring cups if needed for proper portion sizes or use smaller plates/bowls

Table 16.5 (continued)

☐ Reduce calorie-density of meals-choose low calorie/high nutrient foods like fruits, vegetables, lean proteins, and whole grains. Limit foods high in calories that offer little nutrition (sweets, butters, dressings, etc)
MONITORING AND EVALUATION:
Expected compliance: ☐ Excellent ☐ Good ☐ Fair
Comments:

tion [106]. Conducted in Spain from 2003 to 2011, 7447 men and women at high CVD risk were randomized into one of three diets: (a) Mediterranean diet supplemented with extra-virgin olive oil, (b) Mediterranean diet supplemented with nuts, or (c) control diet (advice to follow a low-fat diet). Those randomized to a Mediterranean diet with either extra-virgin olive oil or nuts had multivariable adjusted hazard ratios of 0.70 (95% CI, 0.54–0.92) and 0.72 (95% CI, 0.54–0.96), respectively, for the primary end point of MI, stroke or death from cardiovascular causes when compared to a low-fat diet [106]. These event reductions are comparable to those of statin drugs.

In the Lyon Diet Heart Study, individuals post-MI randomized to a Mediterranean diet had a 72% reduction in cardiac death and nonfatal MI and a 56% reduction in total mortality at 4-year follow-up compared to an AHA Step I diet with total fat <30% [107]. The Mediterranean diet was further shown to be beneficial in secondary prevention in the Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy, which showed significant reduction in major adverse cardiovascular events in patients with high-risk stable CAD [108].

Fatty fish are a source of omega-3 PUFA which include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The Greenland Inuit, who ingest whale omega-3 fatty acid have an MI rate 1/10 that of North Americans [109, 110]. Japan has the highest ingestion of fish and the lowest risk of CHD in the world. In the Diet and Reinfarction Trial, men post-MI randomized to increased fish intake had a 29% reduction in total mortality and a 32% reduction in CHD death compared to increased intake of cereal or decrease in total fat to 30% [111]. In the Multi-Ethnic Study of Atherosclerosis and Coronary Health Study, higher levels of EPA and DHA were associated with a 51% lower rate of incident CVD and 27% reduction in total mortality (HR, 0.73; 95% CI, 0.61–0.86; *P*-trend 0.008), respectively [110, 112]. In the Nurses' Health Study, two or more servings of fish per week were associated with a 30% lower risk of CHD in women [113]. A recent meta-analysis of 127,477 subjects in omega-3 fatty acid trials reported a significantly lower risk for MI, CHD death, total CHD, CVD death, and total CVD [114]. These epidemiologic findings support a beneficial relationship between EPA and DHA levels and CVD risk and could be a potential reason for the beneficial effects of the PREDIMED and Lyon clinical trials since they emphasize intake of fatty fish. Over 4,833,042 person-years of follow-up, higher adherence to plant-based diets of healthful complex carbohydrates was independently inversely associated with CHD (HR, 0.75; 95% CI, 0.68–0.83; *P*-trend <0.001) whereas unhealthy plant-based diets with refined foods were positively associated with CHD (HR, 1.32; 95% CI, 1.20–1.46; *P*-trend <0.001) [115]. Changes in diet toward whole grains, vegetables, fruit, and fish or

other omega-3 fatty acids were associated with decreased all-cause mortality [116]. An improved quality of diet (toward Mediterranean or Dietary Approaches to Stop Hypertension) was significantly associated with a reduction in total mortality of 8–17% and a 7–15% reduction in CVD death. Both of these important studies emphasize the value of complex carbohydrates, fruits, and vegetables as the fundamental components of an optimal diet.

Improvement in diet is a public health priority that can lead to a significant population level reduction in CVD morbidity and mortality. Therefore, it is important that clinicians understand evidence-based dietary recommendations to counsel their patients on heart-healthy diets. The nutrition counseling tool we use is provided in Table 16.5 to support efficient nutrition assessment and evidence-based dietary counseling during an office visit.

The metabolic syndrome is a secondary target of therapy, after the primary target of LDL-C [67]. In the aim of treating the metabolic syndrome, LDL-C should be lowered first with lifestyle modifications, since at any given level of LDL-C, the metabolic syndrome increases CHD risk [67]. Once LDL-C is reached to the target, weight reduction and enhanced physical activity will improve risk factors of the metabolic syndrome [67].

Although lifestyle approaches are the first step for primary prevention of CHD, medication may be indicated in some cases including in women at higher CHD risk, those with multiple risk factors or poor response to 6 to 12 weeks of lifestyle changes [67].

16.5 Pharmacologic Therapy

16.5.1 Statins

Statins lower LDL-C more than the other classes of lipid-lowering agents with the exception of the proprotein convertase subtilisin/kexin type 9 (PCSK-9) inhibitors; thus, statins are considered as a treatment of first choice in lowering LDL-C in postmenopausal women with CHD and at risk for CHD [117]. Statins are also beneficial in decreasing triglyceride levels. The clinical trials supporting the use of statins are reviewed in the section above on different response to therapy in women.

Benefit on HDL-C levels in women was reported in the Statin Therapies for Elevated Lipid Levels Compared Across Doses to Rosuvastatin (STELLAR) trial (clinical-trials.gov identifier: NCT00654537). The STELLAR study compared rosuvastatin treatment with three other widely used statins (atorvastatin, simvastatin, and pravastatin) on both LDL-C and HDL-C levels and triglyceride levels in a hypercholesterolemic patient population [118] (Fig. 16.2). The STELLAR trial was a randomized, parallel group, open-label, comparator-controlled multicenter trial in 2431 hypercholesterolemic patients including 1145 women (51%). After a 6-week dietary lead-in period, men and women aged 18 years or older with fasting LDL-C >160 mg/dL and <250 mg/dL (>4.1 mmol/L and < 6.5 mmol/L) and triglycerides <400 mg/dL (<4.5 mmol/L) were discontinued from all cholesterol-lowering drugs and dietary supplement and randomized to 6 weeks of treatment with one of four statins: rosuvastatin 10, 20, or 40 mg; atorvastatin 10, 20, 40, or 80 mg; simvastatin 10, 20, 40, or 80 mg; or pravastatin 10, 20, or 40 mg. Figure 16.2 shows that the mean reduction in LDL-C was 49% with rosuvastatin doses of 10 mg or higher, 39% with atorvastatin 10 mg or higher, 30% with simvastatin 10 mg or higher, and 21% with pravastatin 10 mg or higher. Moreover, statin therapy was generally well tolerated in the women included in this study. These results suggest that substantial improvements in lipid levels are achievable with moderate- to high-intensity statin therapy. Of note, atorvastatin 20 and 40 mg lowered HDL-C whereas rosuvastatin raised HDL-C.

Data on the impact of statin treatment on carotid intima-media thickness have been reported. A small study of 51 postmenopausal women aged ≥55 years with dyslipidemia showed that women who received rosuvastatin

2.5 mg per day for 12 months had significantly lower carotid intima-media thickness values when compared with women who received no statin therapy [119]. These changes were in conjunction with significant decreases in LDL-C and high-sensitivity C-reactive protein.

Overall, statins have proved to be generally well tolerated in most patients [120, 121]. Safety data from a number of large-scale statin trials, when analyzed by gender, indicate that statins are generally well tolerated in women with only minor adverse effects [49, 56]. The most common complications of statins include liver damage and myopathies indicated by elevation of liver function tests and creatine phosphokinase, respectively. However, only a minor proportion of people may exhibit complications. As was seen in the HPS trial, there were no significant differences in the prevalence of statin adverse effects in those randomized to simvastatin versus placebo for the total study group [122]. Furthermore, in the meta-analysis of 44 randomized, placebo-controlled trials (9416 participants) of atorvastatin (10–80 mg once daily) with a duration of treatment from 2 weeks to 18 months, hepatic transaminase elevation did not significantly differ in those randomized to atorvastatin (any dose) compared with placebo (0.96% vs. 0.45%, respectively) [123]. There was also a lack of differences for men and women indicating no gender difference regarding drug safety. Taken together, the available efficacy and safety data indicate that dyslipidemic women should receive appropriate statin therapy to reduce LDL-C levels and thereby reduce risk of CVD.

Despite the superior efficacy of statins, many women do not achieve LDL-C goal, in part because they receive inadequate therapy [124]. In 2763 postmenopausal women with CVD enrolled in the Heart and Estrogen/progestin Replacement (HERS) Study, even though 47% of participants were taking lipid-lowering medication, the LDL-C level was not lowered to target, which was less than 100 mg/dL at the time of the trial, in 91% of participants [124].

16.5.2 PSCK-9 Inhibitors

The proprotein convertase subtilisin Kexin type 9 inhibitors (PCSK-9i) are a relatively new class of treatment for reducing LDL-C level alone or in combination therapy with statins. They are human monoclonal antibodies that act via disinhibiting the LDL-C receptor recycling. Multiple large clinical trials have demonstrated incremental benefit of PCSK-9i in attenuation of low LDL-C level and consequently major adverse cardiovascular events. In phase 3 of the Durable Effect of PCSK9 Antibody Compared with Placebo Study (DESCARTES) trials, additional therapy with evolocumab, a PCSK-9i, (at the dose of 420 mg monthly) among 901 patients, showed a least-square mean LDL-C reduction of 55.7 ± 4.2% in patients who were treated with diet alone, 61.6 ± 2.6% with atorvastatin 10 mg, 56.8 ± 5.3% with atorvastatin 80 mg, and 48.5 ± 5.2% with atorvastatin 80 mg plus ezetimibe 10 mg ($P < 0.0001$ for all between groups differences) [125].

The GLAGOV (Global Assessment of Plaque Regression with a PCSK9 Antibody as Measured by Intravascular Ultrasound) trial recruited 968 (27.8% women) individuals who had at least 1 coronary stenosis of 20% or greater and randomized them to receive a subcutaneous injection of evolocumab 420 mg monthly or placebo in combination with statin. After 78 weeks follow-up, serial intravascular ultrasonography revealed a significant reduction in percent atheroma volume in the evolocumab treated group (−0.95%) compared to 0.05% increase in placebo (difference, −1.0%; 95% CI, −1.8% to −0.64%; $P < 0.001$). The subgroup analysis showed similar changes in both women (treatment difference, −1.45%; 95% CI, −2.15% to −0.76%) and men (treatment difference, −0.86%; 95% CI, −1.29% to −0.43%) with no significant sex-specific heterogeneity ($P = 0.017$) [126].

The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial was designed to evaluate the cardiovascular outcomes in 27,564

patients (24.6% women) with evident atherosclerotic CVD (defined as history of MI, nonhemorrhagic stroke, symptomatic peripheral artery diseases) who were randomized to receive either evolocumab (140 mg every 2 weeks or 420 mg monthly) or placebo [127]. At 48 weeks, the mean absolute reduction of LDL-C level was 56 mg/dL (95% CI, 55 to 57) and the incidence of major adverse cardiovascular diseases (including cardiovascular death, MI, stroke, hospitalization for unstable angina, or coronary revascularization) was 9.8% in evolocumab and 11.3% in placebo (HR, 0.85; 95% CI, 0.79–0.92; $P < 0.001$). Unfortunately, the study did not provide the sex-specific difference regarding the evolocumab cardiovascular benefits. When measured per mmol/L reduction in LDL-C, treatment with evolocumab reduced the risk of the primary outcome by 11.0% (HR 0.89, 95% CI: 0.84–0.94) per mmol/L reduction in LDL-C.

The ODYSSEY OUTCOMES trial (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) was conducted among 18,924 patients (25.1% women) to evaluate the effectiveness of Alirocumab as secondary prevention [128]. In patients with a history of acute coronary syndrome who were undergoing standard lipid-lowering therapy with statins, alirocumab administration (75 mg every 2 weeks) was associated with a lower incidence of the primary end point 9.5% as compared to 11.1% in placebo. The hazard ratio showed no significant difference in sex-specific analysis (Overall, 0.85; 95% CI, 0.78–0.93; women, 0.91; 95% CI, 0.77–1.08) and men 0.79 (95% CI, 0.68–0.91), *P*-value for comparison between men and women = 0.35).

Although sex-specific data have been limited with PCSK9i, they are recommended for statin-intolerant women or when LDL-C <70 mg/dL has not been achieved with statin and/or ezetimibe in women with established vascular disease [97].

16.5.3 Cholesterol Absorption Inhibitors

Ezetimibe is the first-in-class of lipid-lowering drugs with the mechanism of inhibiting the intestinal absorption of biliary and dietary cholesterol [129]. Cholesterol absorption inhibitors may be indicated as second-line therapy for those who failed to respond to statin monotherapy or were unable to tolerate higher doses of statin. In a study of 1184 women randomized to colestipol or placebo, at an average follow-up of 2 years, treatment with colestipol lowered cholesterol level by an average of 10% but did not affect CHD mortality (RR, 0.93; 95% CI, 0.38–2.26) due to lack of power [61].

In two large double-blind, placebo-controlled trials in people with hypercholesterolemia, 10 mg/day of ezetimibe alone decreased LDL-C by approximately 18% ($P < 0.01$ vs. placebo) and significantly improved levels of total cholesterol, triglycerides, and HDL-C [130]. When administered as a combination therapy with statin, 10 mg/day of ezetimibe resulted in an additional reduction of 21.4% in LDL-C compared with statin monotherapy ($P < 0.001$) [130].

In the IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), 18,144 patients (24.3% women) with post-acute coronary syndrome were randomized to ezetimibe/simvastatin (10/40 mg) vs. simvastatin alone. Those receiving ezetimibe/simvastatin had a better outcome regarding reduction of the incidence of CVD death, nonfatal MI, re-hospitalization for unstable angina or coronary revascularization versus placebo/simvastatin (RR, 0.91; 95% CI, 0.85–0.97; $P = 0.007$). Sex-stratified analysis showed an absolute reduction rate of major cardiovascular events of 12% in women (HR, 0.88; 95% CI, 0.79–0.99) compared to 5% in men (HR, 0.95; 95% CI, 0.90–1.01); however, the difference between sexes did not reach statistical significance (*P* value for interaction = 0.26) [131].

16.5.4 Fibrates

Because fibrates have the greatest effect on triglyceride levels, they are an appropriate choice in people with very high triglyceride levels (≥500 mg/dL) [84]. However, in women trying to conceive or already pregnant, fish oil capsules are the only safe option for reducing elevated triglyceride levels. Fibrates only moderately decrease LDL-cholesterol levels and may increase LDL levels in people with hypertriglyceridemia [84]. Side effects of fibrate therapy are mild and include gastrointestinal pain and nausea. Combination therapy using fibrates and statins should be used with caution as it may increase the risk of myositis and rhabdomyolysis [132]. When combination therapy is necessary, using a hydrophilic statin as pravastatin or rosuvastatin is less likely to increase risk of rhabdomyolysis [133, 134].

16.5.5 Omega-3 Fatty Acids

In the Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT), add-on therapy of 4 mg/day icosapent ethyl (a derivative of eicosapentaenoic acid) to statins significantly reduced the primary endpoint (composite of cardiovascular death, nonfatal MI, nonfatal stroke, coronary revascularization, or unstable angina) by 25% in 8179 patients with triglyceride level of 135–499 mg/dl who have established CVD or diabetes with one additional risk factor (17.2% in placebo vs. 22% in the EPA group; HR, 0.75; 95% CI 0.68–0.83; $P < 0.001$) [135]. Subgroup analysis showed a similar pattern in reducing the rate of CVD event in women from 15.6% in placebo to 13.3% with icosapent ethyl (HR, 0.82; 95% CI 0.66–1.01) and in men from 24.7% to 18.8%, respectively (HR, 0.73; 95% CI 0.65–0.82) with no significant sex-specific interaction ($P = 0.33$) [135]. The mechanism for this benefit is not known. Our group has shown in 291 statin-treated subjects with stable CAD and normal triglyceride levels (median 122 mg/dL) randomized to 3.36 g EPA and DHA daily for 30 months in the Slowing HEART diSease With Lifestyle and omega-3 fatty acids (HEARTS) trial, triglyceride was lowered 14.3% in the setting of LDL-C levels <80 mg/dL and hs-CRP levels <1 [136]. Those with an omega-3 fatty acid index ≥4% had prevention of coronary plaque progression compared to those <3.4% who had progression [136]. Therefore, omega-3 fatty acids may reduce CVD events by stabilizing coronary plaque and preventing coronary plaque progression.

The Vitamin D and Omega-3 Trial (VITAL) randomized 25,871 patients (51% women) to receive the combination of 1 g/day of EPA and DHA and 200 IU of vitamin D3 or placebo [137]. After 5.3 years of follow-up, those randomized to 1 g/day of EPA + DHA did not have a significant reduction in the incidence of major CVD events. In a subgroup analysis of major CVD events, HRs for major CVD events between women and men showed no significant difference (women: 0.93; 95% CI 0.76–1.15 and men: 0.91; 95% CI, 0.76–1.10; $P = 0.88$ for interaction) [137]. The lack of benefit in this study may have been due to the low dose of EPA and DHA used.

It should be noted that data regarding the effectiveness of omega-3 in reducing the risk of CVD have been inconsistent most likely due to differences in doses. In fact, a recent meta-analysis examining doses reported that omega-3 fatty acids were associated with a significant reduction in CVD event (RR, 0.90; 95% CI, 0.84–0.97) and meta-regression analysis showed a strong relationship between dosage and the incidence of CVD events and estimated that the risk reduction of CVD events was 5.8% (1.6% to −9.9%) for each additional 1 g/day intake of EPA + DHA [138].

16.6 Conclusions

Although atherosclerosis typically occurs later in women than in men, CVD remains the leading cause of death in women. Statins clearly reduce

ASCVD events in women as well as in men with ASCVD. The 2015 meta-analysis by the CTT Collaboration showed no heterogeneity by gender for the risk of major vascular events with statin therapy in participants with a history of vascular disease. A history of certain pregnancy-related conditions and premature menopause (age < 40 years) has been associated with increased ASCVD risk. However, current best practice emphasizes that statins should not be taken during pregnancy. Thus, women of childbearing age who are on statin therapy and are sexually active should use a reliable form of contraception to avoid pregnancy. When pregnancy is planned, stopping statin therapy 1–2 months before pregnancy is attempted is suggested as reasonable guidance. When an unplanned pregnancy occurs, statins should be stopped immediately when the pregnancy is discovered. Both cholesterol and triglycerides rise with pregnancy, and those with genetic lipid disorders should consider consulting a clinician with lipid expertise before starting the pregnancy.

Despite the high rate of morbidity and mortality from CVD in women, prevention and treatment strategies have drawn less attention in women compared to men. Lowering the risk of CHD requires assessing differences along with the similarities between men and women in the incidence of attributable risk factors.

The incidence of CVD can be reduced by aggressively lowering LDL-C to levels recommended by the 2018 AHA/ACC cholesterol guidelines, which is 2.59 mmol/L (70 mg/dL) or less for documented CHD in both sexes. For those without CHD, an LDL-C level of less than 2.6 mmol/L (100 mg/dL) is recommended although there is little randomized trial data supporting this in women. Although only a few primary and secondary prevention trials have included women until recently, available data assert that women benefit from lipid-lowering drug therapies, particularly statins, if diet and exercise are not effective enough. Based on the clinical trials, statins should be prescribed as a drug of first choice for women with established CHD. Hypercholesterolemic postmenopausal women who require estrogen for menopausal symptoms may derive further lipid-lowering benefits with the addition of a statin drug. Finally, given the population growth and aging, a major public health concern will doubtless ensue in the near future if clinical issues fail to be addressed now.

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17 Safety Considerations of Pharmacological Treatment

Kyuho Kim and Sung Hee Choi

Abstract

In this chapter, the safety of each lipid-lowering drug and drug–drug interaction between lipid-lowering agents is described in detail for considering combination therapy to normalize various lipid deteriorations.

From recent guidelines for treating dyslipidemia, especially in patients with diabetes, in high or very high-risk population for CVD, with established atherosclerotic cardiovascular diseases (ASCVD), has been focused on the use of high-intensity statin therapy. It is so-called the era of high-intensity statin, however we should think about the potential adverse effect of high-intensity statin such as elevation of glucose levels and statin intolerance. Understanding of safety of lipid-lowering drugs can lead us to the most proper treatment for dyslipidemia.

Ezetimibe and PCSK9 inhibitors are emphasized currently in patients with established ASCVD, in familial hypercholesterolemia, in very high-risk patients from recent guidelines, who cannot reach the proper goal of LDL-cholesterol after maximum statin therapy or in case of statin intolerance. We include the possible safety issues of those new drugs in this chapter.

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17.1 Drug Interaction Among Lipid-Lowering Agents

17.1.1 Statin

The 2018 American Heart Association (AHA) guideline recommends moderate- to high-intensity statin therapy in individuals 40–75 years of age with LDL-C 70–189 mg/dL and a 10-year ASCVD risk ≥7.5%, individuals 40–75 years of age with diabetes and an LDL-C 70–189 mg/dL for primary prevention, and recommends high-intensity statin in stroke patients for achieving ≥50% reduction in LDL-C levels with absolute LDL-C level of <70 mg/dL for secondary prevention [1]. Along with antithrombotic and antihypertensive therapies, lipid-lowering therapy is important in stroke treatment, and statins are the cornerstone of lipid-lowering therapy. Therefore, drug interaction with statins must be considered when combination therapy is needed.

Cytochrome P-450 (CYP450) enzyme system and permeability glycoprotein (P-gp) play important roles in pharmacokinetics of statins. Other transport proteins such as organic anion-transporting polypeptides (OATP) 1B1 and

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OATP1B3 should be considered. CYP450 enzymes detoxify chemicals and metabolize drugs. These enzymes are mostly expressed in the liver, but some are also expressed in the small intestine, kidney, and lungs. There are more than 50 CYP450 enzymes, and, among these, CYP2C9 and CYP3A14 have major role in statin metabolism. P-gp belongs to the ATP-binding cassette superfamily, and can actively transport drugs against a concentration gradient. P-gp is located primarily in the gastrointestinal tract, liver, kidney, brain, and the results of drug interactions involving P-gp depend on the location of the interaction. For example, P-gp inhibition can increase drug bioavailability in the gastrointestinal tract, can increase drug penetration at central nervous system in the brain, and can decrease drug elimination in the liver and kidney. OATP1B1 and OATP1B3 are mainly expressed in the liver, and responsible for the hepatic uptake of drugs. Therefore, inhibition of OATP1B1 and OATP1B3 can increase the serum concentrations of drugs which are normally eliminated by hepatic metabolism. Table 17.1 gives common inhibitors and inducers associated with the CYP450 enzymes, P-gp, OATP1B1, and OATP1B3.

Table 17.1 Common substrates, inhibitors, and inducers associated with the CYP450 enzymes, P-gp, OATP1B1, and OATP1B3

Enzyme	Statin Substrates	Inhibitors	Inducers
CYP2C9	Fluvastatin, rosuvastatin (also CYP2C19, minor)	Amiodarone, capecitabine, etravirine, fluconazole, fluvoxamine, fluvastatin, ketoconazole, metronidazole, miconazole, oxandrolone, sulfamethoxazole/ trimethoprim, voriconazole, zafirlukast	Carbamazepine, phenobarbital, phenytoin rifampin
CYP3A4	Atorvastatin, lovastatin, simvastatin	Amiodarone, amlodipine, aprepitant, atorvastatin, bicalutamide, cilostazol, cimetidine, ciprofloxacin, clarithromycin, conivaptan, cyclosporine, diltiazem, erythromycin, fluconazole, fluoxetine, fluvoxamine, grapefruit juice, imatinib, isoniazid, itraconazole, ketoconazole, mibefradil, midazolam, nefazodone, nilotinib, posaconazole, protease inhibitors, ranolazine, sertraline, tacrolimus, telithromycin, ticagrelor, tricyclic antidepressants, verapamil, voriconazole	Aprepitant, bosentan, carbamazepine, cyclophosphamide, corticosteroids, efavirenz, modafnil, nafcillin, nevirapine, phenytoin, pioglitazone, phenobarbital, rifampin, St. John's wort
P-gp	Atorvastatin, lovastatin, pitavastatin, simvastatin	Amiodarone, atorvastatin, azithromycin, captopril, carvedilol, cimetidine, clarithromycin, colchicine, conivaptan, cyclosporine, diltiazem, dipyridamole, dronedarone, erythromycin, felodipine, grapefruit juice, itraconazole, ketoconazole, lovastatin, mefloquine, nicardipine, omeprazole, protease inhibitors, quinidine, ranolazine, reserpine, sertraline, simvastatin, tacrolimus, verapamil	Carbamazepine, phenytoin, rifampin, St. John's wort
OATP1B1	Atorvastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin	Carbamazepine, clarithromycin, cyclosporine, erythromycin, gemfibrozil, protease inhibitors, roxithromycin, rifampin, sildenafil, sacubitril, telithromycin	Unknown
OATP1B3	Fluvastatin, pravastatin, rosuvastatin	Clarithromycin, cyclosporine, erythromycin, rifampin, roxithromycin, rifampin, sacubitril, telithromycin	Unknown

Adapted with permission from Circulation, Copyright American Heart Association [4]
CYP cytochrome P, *OATP* organic anion-transporting polyprotein, *P-gp* permeability glycoprotein

Every statin has different pharmacokinetic profiles of absorption, distribution, metabolism, and excretion, and drug interaction with statin is affected by changes of these profiles. Statins are well absorbed from the gastrointestinal tract, but most statins except pitavastatin have low bioavailability due to extensive first-pass metabolism in the liver. Simvastatin and lovastatin have very low bioavailability (<5%), while pitavastatin has a relatively high bioavailability (>40%) [2]. Distribution of statins depends on the extent of protein binding and lipophilicity. Most statins except pravastatin have high level of protein binding (≥95%), and the free fraction is pharmacologically active. All statins except pravastatin and rosuvastatin have high lipophilicity. Drug interaction with statin is usually not affected by changes in absorption or in distribution. The majority of drug interaction with statin is affected by changes in metabolism. Lipophilic statins are generally metabolized to hydrophilic salts and conjugates by CYP450 enzymes, and excreted from the body. Simvastatin, lovastatin, and to lesser extent, atorvastatin are metabolized by CYP3A4, and fluvastatin, pitavastatin, and rosuvastatin are metabolized by CYP2C9. Pravastatin is the only statin which is not metabolized by CYP450 enzymes. Because CYP3A4 is responsible for the metabolism of more than 50% of all drugs, simvastatin and lovastatin have the highest potential of drug interaction. Concomitant use of CYP450 inhibitors with statins can increase serum concentrations of statins, while concomitant use of CYP450 inducers with statins can decrease one. Statins are mainly excreted into the bile by transporters such as P-gp, ATP-binding cassette G2 (ABCG2), and 90% of fluvastatin and rosuvastatin are excreted into the bile [3]. To a lesser extent, statins are excreted into the urine (2–20%). Inhibition or induction of these transporters by drugs can change excretion rate of statins, thereby inducing drug interaction. Table 17.2 gives pharmacokinetic properties of statins, and Table 17.3 gives summary of the evidence for drug interaction with statins and other drugs in patients with cardiovascular disease [4, 5].

17.1.2 Ezetimibe

The 2018 AHA guideline recommends add ezetimibe to maximally tolerated statin therapy in very high-risk ASCVD patients whose LDL-C remains ≥70 mg/dL [1].

Ezetimibe inhibits intestinal uptake of dietary and biliary cholesterol. Ezetimibe does not inhibit or induce CYP450 enzymes, and instead, is mainly metabolized via glucuronidation in the small intestine and liver [6]. As expected, there have been a lack of clinically important drug interaction between ezetimibe and drugs including statin, fibrate, digoxin, and warfarin. However, concomitant use of cholestyramine with ezetimibe can significantly decrease ezetimibe bioavailability. In addition, concomitant use of cyclosporine with ezetimibe can increase serum concentration of both cyclosporine and ezetimibe.

17.1.3 Fibrate

The 2018 AHA guideline recommends add fibrates or omega-3 fatty acids for patients with persistently elevated severe hypertriglyceridemia (fasting triglyceride ≥500 mg/dL) [1].

Gemfibrozil and its glucuronide metabolite are strong inhibitors of CYP2C8, and inhibitors of OATP1B1- and OATP1B3-mediated hepatic uptake of statin, as well as OATP2B1, Na + −taurocholate cotransporting polypeptide (NTCP/SLC10A1), the organic anion transporter 3 (OAT3), and hepatic glucuronidation of statin. Thus, drug interaction between gemfibrozil and statin depends on pharmacokinetic characteristics of each statin. Pharmacokinetic studies showed that magnitudes of drug interaction between gemfibrozil and lovastatin, pravastatin, and simvastatin are moderate. Therefore, AHA recommended that combination use of gemfibrozil with lovastatin, pravastatin, and simvastatin should be avoided [4]. Although the evidence showing drug interaction between gemfibrozil and warfarin is limited, this combination should be used with caution [7].

Table 17.2 Pharmacokinetic properties of statins

	Absorption	Distribution		Metabolism		Excretion		
	Bioavailability %	Protein Binding, %	Lipophilicity	Major P450 Hepatic Enzyme	Active Metabolites	Urinary Excretion, %	Fecal Excretion, %	$t_{1/2}$, h
Atorvastatin	12	80–90	Yes	CYP3A4	Yes	2	70	15–30
Fluvastatin	19–29	>99	Yes	CYP2C9 (CYP2C8 is minor)	No	NR	90	0.5–2.3
Lovastatin	5	>95	Yes	CYP3A4	Yes	10	83	2.9
Pitavastatin	>60	99	Yes	CYP2C9 (CYP2C8 is minor)	No	15	79	12
Pravastatin	18	55	No	Non-CYP	No	20	71	1.3–2.8
Rosuvastatin	20	88	No	CYP2C9	Minimal	10	90	19
Simvastatin	5	94–98	Yes	CYP3A4	Yes	13	58	2–3

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CYP cytochrome P, *NR* not reported, $t_{1/2}$, drug half-life

Table 17.3 Summary of the evidence for drug interaction with statins and other drugs in patients with cardiovascular disease

Interacting agent	Statin	Effect	Magnitude	Recommendation
Amiodarone	Lovastatin	Increased statin exposure/ increased risk for muscle-related toxicity	Minor 1.8-fold increase in AUC of lovastatin	Combination may be considered
	Simvastatin	Increased statin exposure/ increased risk for muscle-related toxicity	Minor 1.8-fold increase in AUC of lovastatin	Combination may be considered
Amlodipine	Lovastatin	Increased statin exposure/ increased risk for muscle-related toxicity	Minor	Combination may be considered
	Simvastatin	Increased statin exposure/ increased risk for muscle-related toxicity	Minor 1.8-fold increase in AUC of lovastatin	Combination may be considered
Cyclosporine/ tacrolimus/ everolimus/ sirolimus[a]	Atorvastatin	Increased statin exposure through multiple mechanisms Increased risk for muscle-related toxicity	Severe 6- to 15-fold increase in AUC of atorvastatin	Combination may be considered
	Fluvastatin	Increased statin exposure through multiple mechanisms Increased risk for muscle-related toxicity	Moderate two- to fourfold increase in AUC of fluvastatin	Combination may be considered
	Lovastatin	Increased statin exposure through multiple mechanisms Increased risk for muscle-related toxicity	Severe 5- to 20-fold increase in AUC of lovastatin	Combination is potentially harmful
	Pitavastatin	Increased statin exposure through multiple mechanisms Increased risk for muscle-related toxicity	Severe fivefold increase in AUC of pitavastatin	Combination is potentially harmful
	Pravastatin	Increased statin exposure through multiple mechanisms Increased risk for muscle-related toxicity	Severe five- to tenfold increase in AUC of Pravastatin	Combination may be considered
	Rosuvastatin	Increased statin exposure through multiple mechanisms Increased risk for muscle-related toxicity	Severe sevenfold increase in AUC of rosuvastatin	Combination may be considered
	Simvastatin	Increased statin exposure through multiple mechanisms Increased risk for muscle-related toxicity	Severe six- to eightfold increase in AUC of simvastatin	Combination is potentially harmful
Diltiazem	Atorvastatin	Increased statin exposure/ increased risk for muscle-related toxicity	Minor 51% increase in AUC of atorvastatin	Combination is reasonable
	Lovastatin	Decreased metabolism of lovastatin leading to increased concentrations Increased risk of muscle-related toxicity	Moderate 3.6-fold increase in AUC of lovastatin	Combination may be considered
	Simvastatin	Decreased metabolism of simvastatin leading to increased concentrations Increased risk of muscle-related toxicity	Moderate 4.6-fold increase in AUC of simvastatin	Combination may be considered

(continued)

Table 17.3 (continued)

Interacting agent	Statin	Effect	Magnitude	Recommendation
Fenofibrate/ fenofibric acid	Atorvastatin	Potential increase in muscle-related toxicity	Insignificant 1.0-fold increase in AUC of atorvastatin	Combination is reasonable
	Fluvastatin	Potential increase in muscle-related toxicity	Specific data not available but magnitude likely to be minor	Combination is reasonable
	Lovastatin	Potential increase in muscle-related toxicity	Specific data not available but magnitude likely to be minor	Combination is reasonable
	Pitavastatin	Potential increase in muscle-related toxicity	Insignificant 1.2-fold increase in AUC of pitavastatin	Combination is reasonable
	Rosuvastatin	Potential increase in muscle-related toxicity	Insignificant 1.1-fold increase in AUC of rosuvastatin	Combination is reasonable
	Simvastatin	Potential increase in muscle-related toxicity	Insignificant 1.1-fold increase in AUC of simvastatin If taken at same time, 1.05-fold increase	Combination is reasonable
Gemfibrozil	Atorvastatin[b]	Decreased metabolism of atorvastatin leading to increased concentrations Increased risk of muscle-related toxicity	Minor 1.4-fold increase in AUC of atorvastatin	Combination may be considered
	Lovastatin	Decreased metabolism of lovastatin leading to increased concentrations Increased risk of muscle-related toxicity	Moderate two- to threefold increase in AUC of lovastatin	Combination should be avoided
	Pitavastatin[b]	Decreased metabolism of pitavastatin leading to increased concentrations Increased risk of muscle-related toxicity	Minor 1.5-fold increase in AUC of pitavastatin	Combination may be considered
	Pravastatin	Decreased metabolism of pravastatin leading to increased concentrations Increased risk of muscle-related toxicity	Moderate 2.0-fold increase in AUC of pravastatin	Combination should be avoided
	Rosuvastatin[b]	Decreased metabolism of rosuvastatin leading to increased concentrations Increased risk of muscle-related toxicity	Minor 1.6- to 1.9-fold increase in AUC of rosuvastatin	Combination may be considered
	Simvastatin	Decreased metabolism of simvastatin leading to increased concentrations Increased risk of muscle-related toxicity	Moderate two- to threefold increase in AUC of simvastatin	Avoid combination

Table 17.3 (continued)

Interacting agent	Statin	Effect	Magnitude	Recommendation
Ticagrelor	Atorvastatin	Increased statin exposure/ increased risk for muscle-related toxicity	Minor 1.4-fold increase in AUC	Combination is reasonable
	Lovastatin	Decreased metabolism of lovastatin leading to increased concentrations Increased risk of muscle-related toxicity	Unknown but expected to be similar to simvastatin Moderate two- to threefold increase in AUC	Combination may be considered
	Simvastatin	Decreased metabolism of simvastatin leading to increased concentrations Increased risk of muscle-related toxicity	Moderate two- to threefold increase in AUC	Combination may be considered
Verapamil	Lovastatin	Decreased metabolism of lovastatin leading to increased concentrations Increased risk of muscle-related toxicity	Moderate 3.6-fold increase in AUC	Combination may be considered
	Simvastatin	Decreased metabolism of simvastatin leading to increased concentrations Increased risk of muscle-related toxicity	Moderate 2.5-fold increase in AUC	Combination may be considered
Warfarin	Fluvastatin	Increased INR/potential for increased bleeding	Variable	Combination therapy is useful
	Lovastatin	Increased INR/potential for increased bleeding	Variable	Combination is useful
	Rosuvastatin	Increased INR/potential for increased bleeding	Variable	Combination is useful
	Simvastatin	Increased INR/potential for increased bleeding	Up to 30% change in INR	Combination is useful

Magnitude of drug interactions based on AUC increase: >1.25 to <2, minor; ≥2 to 4.9, moderate; ≥5, severe. *AUC* area under the curve, *INR* international normalized ratio

[a]Changes in magnitude of statin AUC are reported with cyclosporine

[b]Use in combination is recommended only when other options have been exhausted

Fenofibric acid is primarily conjugated with glucuronic acid, and then excreted in urine. Fenofibric acid and its prodrug, fenofibrate, do not undergo oxidative metabolism via the CYP450 enzymes. They are not inhibitors of CYP3A4, CYP2D6, CYP2E1, or CYP1A2, but mild to moderate inhibitors of CYP2C9, and weak inhibitors of CYP2C8, CYP2C19, or CYP2A6. Concomitant use of fenofibrate with statin does not affect oxidation, glucuronidation, or serum concentrations of statins [4]. Therefore, when combination use of fibrate with statin is indicated, fenofibric acid or fenofibrate is better choice than gemfibrozil because of lower incidence of drug interaction. While, concomitant use of cholestyramine with fenofibrate can decrease fenofibrate bioavailability. Concomitant use of warfarin with fenofibrate may increase warfarin effects [8]. When it used in patients with renal impairment, the renal function should be carefully monitored.

17.1.4 Niacin

The 2018 AHA guideline does not recommend adding niacin to statin therapy [1].

Combination use of niacin with fluvastatin, pravastatin, or simvastatin was not associated with myopathy [9]. However, the Heart Protection Study 2-Treatment of HDL-C to Reduce the Incidence of Vascular Events (HPS2-THRIVE)

trial showed a higher incidence of myopathy in Chinese patients on concomitant extended-release niacin/laropiprant 2 g/40 mg and simvastatin 40 mg treatment [10]. Concomitant use of alcohol or hot drinks with niacin may increase the side effects of niacin such as facial flushing and pruritus.

17.1.5 Fish Oils

The 2018 AHA guideline recommends add fibrates or omega-3 fatty acids for patients with persistently elevated severe hypertriglyceridemia (fasting triglyceride ≥500 mg/dL) [1].

There has been no significant drug interaction between omega-3 fatty acids and simvastatin, rosuvastatin, and atorvastatin [11]. Although omega-3 fatty acids have the potential for antithrombotic effects, clinical studies showed that combination use of omega-3 fatty acids with aspirin or warfarin did not increase bleeding risk compared with aspirin or warfarin monotherapy [12, 13].

17.1.6 PCSK9 Inhibitors

The 2018 AHA guideline recommends add PCSK9 inhibitor to maximally tolerated statin and ezetimibe therapy in very high-risk ASCVD patients whose LDL-C remains ≥70 mg/dL [1].

Alirocumab and evolocumab are human monoclonal immunoglobulins G1 and G2, respectively, against PCSK9. They are not inhibitors or inducers of any transporter or enzyme, and therefore, no significant drug interactions have been reported to date [14, 15].

17.2 Adverse Drug Reactions

17.2.1 Statin

Statin-associated muscle symptoms (SAMS)
SAMS are the most commonly reported adverse effects of statins. Incidence of SAMS ranges from 10 to 29% in observational studies, but from 1 to 2% in randomized controlled trials (RCTs) [16].

Symptoms include myalgia, cramp, and weakness, and are usually located in proximal muscle groups bilaterally, but usually not accompanied by significant creatine kinase (CK) elevation. Symptoms present shortly after starting statin treatment or increasing the dose, or after starting an interacting drug. Symptoms generally resolve quickly after discontinuation of statin. The incidence of myopathy with CK concentration >10 times the upper limit of normal (ULN) is approximately 1 in 1000 and the incidence of rhabdomyolysis is approximately 1 in 10,000. And, a dose-response relationship for myopathy has been demonstrated with most statins except atorvastatin and fluvastatin [17]. The concomitant use of drugs which affect statin metabolism, alcohol, old age, female sex, hypothyroidism, pre-existing muscle disease, and renal impairment can increase the risk of SAMS.

Diabetes mellitus
The Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial showed an increased risk of diabetes in patients treated with statins [18].

Subsequently, a meta-analysis showed that statin therapy was associated with a 9% increase in the incidence of diabetes [19]. More details will be discussed in 18.3 Statin-induced diabetes section.

Liver enzyme elevation
The Expanded Clinical Evaluation of Lovastatin (EXCEL) trial showed that statins cause asymptomatic, dose-related elevation of liver enzymes >3 times the ULN in about 1% of patients [20]. The Treat to New Targets (TNT) trial showed elevation of liver enzymes >3 times the ULN in 1.2% of patients with atorvastatin 80 mg and 0.2% of patients with atorvastatin 10 mg [21]. Severe hepatotoxicity induced by statin is rare. Analysis of data from the Swedish Adverse Drug Reactions Advisory Committee showed that

statin-related liver injury occurred in 1.2/100,000 users (about 0.001%) [22]. The EXCEL trial showed no cases of severe hepatotoxicity [20].

Hemorrhagic stroke
The Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study showed significant increased risk of hemorrhagic stroke with statin therapy [23]. However, a meta-analysis of 23 randomized trials and 19 observational studies showed no significant association between hemorrhagic stroke and statin therapy [24]. In addition, the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) study showed no increased risk of hemorrhagic stroke with statin and ezetimibe combination therapy compared with statin monotherapy [25]. The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) study showed no increased risk of hemorrhagic stroke with statin and evolocumab combination therapy compared with statin monotherapy [26]. Therefore, there have been a lack of evidence for an increased risk of hemorrhagic stroke by statin.

Cognitive dysfunction
Cognitive dysfunction can be defined as impairment of any of domains of cognitive function. Several case reports and case series showed the possibility of statin-associated cognitive dysfunction [17]. However, based on several studies including randomized trials, the National Lipid Association Statin Cognitive Safety Task Force concluded that evidence supporting a causal relationship between statin and cognitive dysfunction is weak [27]. More details will be discussed in Sect. 17.4.

Peripheral neuropathy
Some observational studies showed that statin therapy was associated with increased risk of peripheral neuropathy [28, 29]. However, other study showed that statin therapy was associated with decreased risk of peripheral neuropathy [30]. In addition, long-term RCT showed not increased risk of peripheral neuropathy with statin therapy [31]. Therefore, there have been a lack of evidence to support the association between statin and peripheral neuropathy.

Cancer
The Cholesterol and Recurrent Events (CARE) trial showed an increased incidence of breast cancer in patients with pravastatin 40 mg daily [32]. The PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) trial showed an increased incidence of gastrointestinal cancer in patients with pravastatin 40 mg daily [33]. However, the Prospective Pravastatin Pooling (PPP) project showed no differences in incidence of cancer between the pravastatin and placebo group [34]. In addition, a meta-analysis of 27 randomized trials including 175,000 patients showed no effect of statin on the incidence of cancer [35]. Long-term follow-up study of the original studies showed no effect of statin on the incidence of cancer [36]. Therefore, there have been a lack of evidence to support the association between statin and cancer.

17.2.2 Ezetimibe

Myopathy/Rhabdomyolysis
Clinical trials showed no increased risk of myopathy or rhabdomyolysis in ezetimibe monotherapy or in ezetimibe-statin combination therapy [37, 38]. In addition, a meta-analysis of 17 RCTs including 4558 patients showed no increased incidence of statin-related myopathy in ezetimibe therapy [39]. However, considering several case reports of myopathy associated with ezetimibe, the possibility of myopathy should be considered when patients report muscle-related symptoms [40, 41].

Diabetes mellitus
A few studies showed an improved insulin resistance by ezetimibe, thereby suggesting a potential benefits of ezetimibe in glucose metabolism [42, 43]. However, other studies showed no effect of ezetimibe on glucose metabolism [44, 45]. A meta-analysis of 16 RCTs showed no evidence of adverse effects of ezetimibe on glucose metabolism, and suggested that ezetimibe with low-dose

statin for more than 3 months may give beneficial effects on glucose metabolism compared with high-dose statin [46]. Taken these together, the effect of ezetimibe on diabetes mellitus is not yet clear.

Liver enzyme elevation

The incidence of liver enzyme elevations >3 times the ULN was similar between ezetimibe (0.5%) and placebo (0.3%) [47]. In studies of ezetimibe-statin combination therapy, the incidence of liver enzyme elevations >3 times the ULN was 1.3% for ezetimibe-statin and 0.4% for statin alone [47]. Ezetimibe is not recommended in patients with moderate to severe hepatic impairment. Combination use of ezetimibe with statin is contraindicated in patients with active liver disease or unexplained persistent elevation of liver enzyme [48].

17.2.3 Fibrate

Myopathy/Rhabdomyolysis

Myopathy and rhabdomyolysis are rare, but considered the most serious adverse effects of fibrate. An epidemiological study showed that the incidence of myopathy associated with lipid-lowering drugs was 2.3/10,000 person-years, and that fibrate was associated with a 5.5-fold increased risk compared with statin user [49]. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study showed that the incidence of myositis rhabdomyolysis was 0.06% for fenofibrate and 0.02% for placebo [50].

Gemfibrozil is associated with a higher risk of myopathy and rhabdomyolysis than other fibrate class drugs [51]. Combination use of statin and fibrate increases the risk of myopathy and rhabdomyolysis. Incidence of myopathy was 0.4% in patients with lovastatin, but increased to 5% in patients with lovastatin and gemfibrozil [52]. Combination use of statin and gemfibrozil resulted in a 33 times higher rate of rhabdomyolysis compared with the use of statin and fenofibrate [53]. Taken together, fenofibrate is better choice than gemfibrozil when combination therapy of statin and fibrate is considered.

Liver enzyme elevation

One RCT showed that the incidence of liver enzyme elevation was 4.5% for gemfibrozil and 0% for placebo, and these were reversible with discontinuation of the drug [54]. Another RCT showed that the incidence of liver enzyme elevation was 1.5% for fenofibrate and 0% for placebo [55]. Severe liver injury by fenofibrate is rare. FIELD study showed that the incidence of serum alanine aminotransferase levels >5 times the ULN was similar between fenofibrate (0.22%) and placebo (0.24%) [50].

Gastrointestinal side effects

Gastrointestinal symptoms such as indigestion, diarrhea, dyspepsia, and abdominal pain are the most common adverse effects of fibrates, and gemfibrozil is more frequently associated with gastrointestinal symptoms than other fibrates [56, 57]. Previous studies showed that fibrates were associated with increased risk of gallstone formation and acute pancreatitis [50, 58].

Skin side effects

The incidence of skin adverse effects including skin eruption, pruritus, rash, photosensitivity, alopecia, erythema multiforme, Stevens–Johnson syndrome was about 2% [59]. Allergic or idiosyncratic reactions may cause these side effects.

Peripheral neuropathy

Previous studies suggested that fibrate was associated with an increased risk of peripheral neuropathy [60, 61]. However, an observational cohort study showed fibrate may protect against the development of peripheral neuropathy [30]. Therefore, the effect of fibrate on peripheral neuropathy is not yet clear.

Hematological effects

Several case reports and case–control studies showed that combination use of fibrate with warfarin was associated with prolonged prothrombin time and increase in the international normalized ratio, and increased risk of bleeding [62, 63]. Therefore, when combination of fibrate with warfarin is used, frequent monitoring of PT and INR

is desirable, and warfarin dose should be adjusted to prevent bleeding complications [64].

Cancer

World Health Organization (WHO) and Helsinki Heart Study (HHS) studies showed higher cancer and total mortality in fibrate-treated groups [65, 66]. However, Veterans Affairs High-density Lipoprotein Cholesterol Intervention Trial (VA-HIT) and FIELD studies showed no difference in cancer incidence between fibrate-treated groups and placebo group [50, 67]. In addition, a meta-analysis showed no difference in cancer prevalence between fibrate-treated groups and placebo groups [68]. Therefore, there have been a lack of evidence to support the association between fibrates and cancer.

17.2.4 Niacin

Flushing develops in almost all patients taking immediate-release niacin, and is the major reason for the discontinuation of niacin.

Niacin can cause serious hepatotoxicity, and this is mostly associated with the use of slow-release formulations, with dose of ≥1500 mg/day. In contrast, an extended-release niacin showed <1% of the incidence of serum aspartate aminotransferase elevations >3 times the ULN, 0% of the incidence of serum alanine aminotransferase elevations >3 times the ULN [69].

Niacin can induce insulin resistance, thereby causing mild hyperglycemia [70]. HPS2-THRIVE study showed that niacin was associated with an increased incidence of disturbed diabetes control (rate ratio, 1.55), and with an increased incidence of diabetes (rate ratio, 1.32) [71]. In addition, a meta-analysis of 11 RCTs including 26,340 non-diabetic patients showed that niacin was associated with an increased incidence of diabetes (risk ratio, 1.34) [72].

Incidence of maculopathy was 0.67% in patients with niacin, typically ≥3000 mg/day, and it generally resolved after discontinuation of niacin [73]. When patients present symptoms such as blurred vision and decreased visual acuity, the possibility of niacin-induced maculopathy should be borne in mind.

17.2.5 Fish Oils

The most common adverse effects of omega-3 fatty acids are gastrointestinal symptoms which occurred in 19% at doses of 2 g/day, 27% at doses of 4 g/day [74]. Omega-3 fatty acids usually do not cause hepatotoxicity [75]. Because omega-3 fatty acids do not have significant drug interaction with statins, the incidence of adverse effects in patients with omega-3 fatty acids and statins was similar to those in patients with statins alone [76].

Although omega-3 fatty acids have the potential for antithrombotic effects, clinical studies showed that combination use of omega-3 fatty acids with aspirin or warfarin did not increase bleeding risk compared with aspirin or warfarin monotherapy [12, 13].

17.2.6 PCSK9 Inhibitors

Alirocumab and evolocumab have a favorable safety profile. A pooled safety analysis of alirocumab from 14 RCTs showed incidence of local injection site reactions (7.4% vs. 5.3% placebo), pruritus (1.3% vs. 0.4% placebo), and upper respiratory tract infection signs and symptoms (2.1% vs. 1.1% placebo) [77]. A pooled safety analysis of evolocumab showed incidence of nasopharyngitis (5.9% vs. 4.8% control), injection site reactions (3.3% vs. 3.0% control), and hypersensitivity reactions (3.2% vs. 2.4% control) [78].

Two meta-analyses showed that PCSK9 inhibitors were associated with an increased incidence of neurocognitive events [79, 80]. However, the Evaluating PCSK9 Binding Antibody Influence on Cognitive Health in High Cardiovascular Risk Subjects (EBBINGHAUS) study and a meta-analysis showed no significant effect of PCSK9 inhibitors on neurocognitive outcomes [81, 82].

17.3 Statin-Induced Diabetes

Since JUPITER trial showed 25% increased relative risk of new-onset diabetes in rosuvastatin 20 mg treatment arm compared to placebo, followed by meta-analysis of statin trials showed that around 9~12% increased risk of diabetes by statin therapy group [18, 19, 83]. This risk for newly diagnosed diabetes was increased according to the higher statin intensity. Most of statin trials and meta-analysis results support incident diabetes is a statin class effect, however, there are some exceptions. For example, the re-analysis of WOSCOPS trial showed that pravastatin group showed hazard ratio of 0.70 compared to placebo arm, showing protective effect of pravastatin in developing diabetes [84]. For supporting this notion that the hydrophilicity of statins can affect differently on both insulin secretion and insulin resistance compared to hydrophobic statin. Recent human studies using pitavastatin showed the neutral or beneficial effect on glucose metabolism [85, 86]. However, we need larger cohort studies whether it is significantly different effect on the glucose alterations by different statin formulations. Various pathophysiological mechanisms have been suggested to explain the increased risk of statin-induced glucose deterioration.

17.3.1 High-Risk Population for Incident Diabetes After Statin Therapy

Pre-existing metabolic abnormalities for developing diabetes such as obesity, prediabetes, high triglycerides, and hypertension were proven risk population for statin-induced diabetes. In TNT and IDEAL studies showed that 6231 subjects who had pre-existing 2–4 above risk factors developed new-onset diabetes in 14.3% of high-intensity statin group and 11.9% in low-intensity statin group. On the contrary, 8825 patients with 0–1 risk factor group did not have increased risk of statin-induced diabetes in high- or low-intensity statin treatment [87, 88]. Other result of high-risk population for statin-induced diabetes seemed to be very similar. The high-risk population for diabetes had more influenced by statin therapy for developing diabetes rapidly. Even they are at high risk for diabetes already, close monitoring is needed when we treat those population with high-intensity statin.

17.3.2 Suggested Mechanisms for Glucose Alterations

The main action site of statin is inhibition of 3 Hydroxy-3-Methylglutaryl-CoA reductase (HMGCR). There is a study to analyze the single nucleotide polymorphism in the HMGCR gene for statin inhibition target (rs17238484 and rs12916). From 43 genetics studies, 223,463 subjects' data were available for metabolic parameter analysis. The rs127238484-G allele and rs12916-T allele were partially associated with increased new-onset diabetes, weight gain, and lowered LDL-cholesterol by statin therapy, which gave us the possibility of HMGCR inhibition itself can deteriorate metabolic parameters [89].

Other cellular mechanisms were suggested that statin down-regulated insulin responsive glucose transporter 4 (GLUT4) and upregulation of GLUT1 in adipocytes. It can cause marked decrease of insulin-stimulated glucose transport into adipocytes and significant increase for insulin resistance. The mevalonate administration reversed GLUT4 decrease in adipocytes [90]. Nakata et al. showed that atorvastatin inhibits adipocytes maturation, SLC2A4 and C/EBPalpha expression. Those inhibitory effects were reversed by mevalonate and geranylgeranyl pyrophosphate, which was inhibited by statin treatment [91]. Thus, we can consider that statin therapy can increase insulin resistance in vitro model. Not only for insulin resistance, experimental data showed that statins inhibit mevalonate pathway and products to make β-cell impairment and decreased insulin secretion [92]. The inhibition of HMGCR and mevalonate pathway can directly be associated with the worsening of metabolic parameters, decreased insulin secretion, and increased insulin resistance, those

are considered important explanation for the statin-induced diabetes.

17.4 Cognitive Impairment

There are several studies that have shown the possible association of statin treatment and cognitive dysfunction [93, 94]. Those studies which showed association between statin therapy and cognitive impairment were small numbers, observational, even among elderly populations who have pre-existing cognitive problems. In addition, the accurate tools for the evaluation of cognitive impairment were lacking. The mechanism for the statin-induced cognitive dysfunction is not clearly understood, but there are some suggestions for this phenomenon. Statin reduced cholesterol synthesis below the certain range that dendritic cells required at least to maintain their function, it resulted in the defect of myelination [95]. In animal study, the lipophilicity is important for maintaining the levels of statin by penetration in brain, and statin inhibits the re-myelination process in chemically damaged neuronal cells [96]. But, we cannot generalize those chemically induced animal model as in human situation. The cholesterol turnover is also very slow in human brain, which cannot explain the acute or subacute cognitive symptoms after several days of statin treatment. In such a short time, statin could not alter the cholesterol synthesis significantly lower in human brain.

Many experts agreed with the results from randomized clinical trials, which are prospective, large numbers, and well-validation for neuropsychological measurement of cognitive impairment: The PROSPER and HPS studies [16]. The PROSPER study showed no difference in cognitive dysfunction for 3 years in pravastatin treatment group vs. placebo group (n = 5804, age 70–82, well-validated cognitive function test with including immediate and delayed memory, processing speed, and executive function) [97]. The HPS also showed that there is no difference in the rate of cognitive impairment in simvastatin group vs. placebo group (n = 20,536, 5 years of treatment in average, systemized telephone interview on memory) [98]. In 2014, the statin cognitive safety task force of National Lipid Association published their opinion based on the thorough analysis of reliable studies and expert opinions about statin and cognitive issues. They addressed that (1) spontaneous reports of adverse cognitive effects have been attributed to statins and its label change by FDA. But, these data do not allow for appropriate causality assessment, (2) the weight of the evidence does not support the contention that statins have a clear or meaningful adverse cognitive effects, (3) if patients has problems in cognition before initiation of statin, a thorough assessment should be conducted to rule out other potential causes. However, general assessment of cognitive function before starting statin is not recommended. If a statin is suspected to alter cognition, the reasonable option is to stop the drug for 1–2 months before considering a re-challenge [27]. By far, statin-induced cognitive impairment does not have clear causality and accurate mechanisms. However, if we have to start statin in patients with pre-existing cognitive dysfunction, or having patients who are suspicious that statin is an inducer of newly onset cognitive impairment, we have to be very careful to stop and to re-challenge drugs and have to perform thorough neuropsychological assessment by the protocol.

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