Contemporary Cardiology
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Michael H. Davidson
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Kevin C. Maki *Editors*

Therapeutic Lipidology

Second Edition

Humana Press

Contemporary Cardiology

Series Editor

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ISSN 2196-8969 ISSN 2196-8977 (electronic)
Contemporary Cardiology
ISBN 978-3-030-56513-8 ISBN 978-3-030-56514-5 (eBook)
https://doi.org/10.1007/978-3-030-56514-5

This Humana imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

It has been 13 years since the first edition of *Therapeutic Lipidology* was published. During this time, the field of clinical lipidology flourished. Clinical lipidology has been at the forefront of incorporating such novel therapeutic approaches as monoclonal antibodies, antisense oligonucleotides, and gene replacement therapy. Newly discovered pathways have allowed us to leverage facets of lipid metabolism in fresh and novel ways. Many more genetic polymorphisms in cell surface receptors, enzymes, nuclear transcription factors, apoproteins, signaling intermediates, and membrane cassette transport proteins impacting lipid metabolism have been identified, characterized, and catalogued. Newer clinical trials have taught us much about which drugs and drug combinations impact risk for cardiovascular events and which ones do not. More technologies are now available to separate lipid and lipoprotein subfractions. Understandably, basic science has also moved forward our understanding of the relationships between specific lipids and lipoproteins and atherosclerosis. The role of inflammation in atherogenesis is now much more well defined and accepted. Our focus on low-density lipoprotein is evolving and we can now reduce this lipoprotein to levels never before thought possible. Given the findings of some recent clinical trials, we realize that triglycerides and remnant lipoproteins also serve as drivers of atherogenesis. We also know more about sphingolipids, cerebrosides, glycolipids, fatty acids, and high-density lipoproteins. It has been exciting to witness the emergence of whole new classes of lipids that control and resolve inflammation (protectins, resolvins, and maresins). It is likely these highly specialized lipids will be investigated for their efficacy in preventing and resolving inflammation in a wide variety of disorders. Certainly, our understanding of lipid metabolism and how specific derangements impact cardiovascular structure and function will only grow more complex but also yield new avenues for prevention and intervention.

The second edition of *Therapeutic Lipidology* is completely rewritten and more comprehensive with numerous new contributors. We have expanded the number of chapters from 22 to 35 in order to incorporate the enormous amount of new information that has emerged in clinical lipidology. Although readers are provided with a strong basic science background throughout, the focus is on providing clinicians with state-of-the-art information that they can apply so as to optimize the care of their patients. We have made every attempt to incorporate the most recent clinical trials and practice guidelines, and to provide ample illustrations of core concepts and study results. Newer

approved drugs, as well as those still in development, are reviewed and their safety and impact on cardiovascular events summarized. Features of dyslipidemia management particular to women, children, and the elderly are comprehensively addressed. There are new chapters on cardiovascular genomics, statin intolerance, nutraceuticals and medical nutrition therapy, remnant lipoproteins, apoprotein B, modalities for imaging atherosclerosis, lysosomal acid lipase deficiency, dyslipidemia management in patients with human immunodeficiency virus infection, and lipodystrophy, among others.

Dyslipidemia remains highly prevalent throughout the world. Dyslipidemia is a modifiable risk factor and its treatment impacts risk for the development of cardiovascular disease. We know that far too many patients go undiagnosed, and many of those diagnosed with dyslipidemia are either untreated or undertreated, leaving them vulnerable to the development of atherosclerotic cardiovascular disease and its clinical sequelae. Our sincerest wish is that this volume will guide healthcare providers in the diagnosis and appropriate treatment of dyslipidemia in all of its forms so as to reduce morbidity and mortality in the millions of patients worldwide afflicted by lipid disorders.

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History of Lipidology

1

Antonio Gotto Jr. and Michael H. Davidson

Discovery of Cholesterol and the Link to Atherosclerosis

In about 1758, the French chemist Francois Poulletier de la Salle isolated crystals from bile in the gallbladder, but it was not until 1815 that another well-known French chemist Michel Eugene Chevereul purified sterols in bile and called it cholesterine. Dr. Chevereul had a long productive life, but his main claim to fame was not related to cholesterol, fatty acids, or other lipids. Instead, he was renowned for his work as head chemist at the Manufacture de Gobelins, where he directed the dyes used in making beautiful carpets and tapestries. He lived to be 102 and was one of the two still alive of the 72 scientists whose names were inscribed on the Eiffel Tower. He is also credited as the founder of gerontology. The linkage of cholesterol to heart disease took another 100 years [1]. In 1833, M.F. Boudet also found the presence of cholesterol in blood.

The major discoveries then shifted to Germany when Rudolph Virchow, the father of pathology, in 1858 described ulcerating plaques in the coronary arteries of victims of fatal heart attacks [2]. Later, Karl Weigart and Karl Huber are credited with coining the term "atherosclerosis" based on the Greek word *atheros*, meaning cheese, and they described the general hardening (sclerosis) of the coronary artery leading to fatal coronary events [3]. However, the linkage of the "waxy" cholesterol to the plaques was not made until 1910 when Adolf Windaus found an accumulation of cholesterol in the atherosclerotic plaques. He noted that the aortas of patients with atherosclerosis had much higher levels of cholesterol than normal aortas [4]. Windaus went on to win the Nobel Prize in 1928 for his work on sterols and their relations to vitamin. He helped elucidate the several steps required to transform cholesterol into vitamin D3. Windaus was one of the few German scientists to openly oppose the Nazi regime, and he helped protect Jewish students during World War II.

Elucidation of the Correct Structure of Cholesterol in 1932

The most significant breakthrough in linking cholesterol to atherosclerosis occurred in 1913 when Nikolai Anitschkow, a young student under the direction of the prominent histologist Alexander Maximal at the Military Medical Academy in St. Petersburg, Russia, found through a series of feeding experiments in rabbits that

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_1

cholesterol led to atherosclerosis. He first fed the rabbits whole eggs, then egg yolks, and finally just purified cholesterol from the egg yolks dissolved in sunflower oil; all feedings resulted in atherosclerosis. The purified cholesterol dissolved in the sunflower oil caused atherosclerosis, while the sunflower oil alone did not. He went on to make a make a number of seminal discoveries that has stood the test of time; he first described fatty streaks and drew foam cell-rich lesions in the rabbit aortas as the earliest manifestations of atherosclerosis. Anitschkow's dictum was "No atherosclerosis without cholesterol" even though he was aware that other factors could exacerbate the disease process and used the term "combination theory" to explain the phenomenon. Unfortunately, his work was largely ignored by the global medical research community. His experiments could not be replicated in dogs or rats, which are resistant to cholesterol-induced atherosclerosis because their plasma cholesterol is predominately high-density lipoproteins (HDLs); therefore, most experts believed that cholesterol-induced atherosclerosis was exclusively a phenomenon in rabbits. In addition, the prevailing view at the time was that human atherosclerosis was part of the inevitable process of aging. After many years of promoting his cholesterol hypothesis through publications and lectures throughout the world, he died in 1964 at age 79 of myocardial infarction. His initial mentor Alexander Maximal, following the Russian Revolution, immigrated to the United States and became a professor of anatomy at the University of Chicago, dying in 1928 at age 54 of severe coronary atherosclerosis. Anitschkow was studying in Freiburg under Aschoff when he was performing his studies in 1913. He was arrested and put in prison in 1914 when the war broke out. Aschoff helped him get out and escape through Sweden to return to Russia [5].

The Norwegian physician Carl Müller first associated the physical signs and high cholesterol levels with autosomal dominant inheritance in 1938. In his seminal paper in Acta Medica Scandinavica, he referred to Fritz Harbitz describing xanthomas in 1925 and the Norwegian medical literature describing 8–10 cases of patients with xanthomas, in which five died suddenly of "paralysis of the heart." In cases in which necropsy was performed, the cause of death proved to be "vessel changes, viz. deposits of xanthomatous masses in the aorta, on the aortic valves and in the coronary arteries." He went on to confirm the autosomal dominance pattern of xanthomatosis or hypercholesterolemia in 76 cases from 17 Norwegian families. This was the first linkage of severe hypercholesterolemia to atherosclerosis derived from a genetic cause and paved the way four decades later for the discovery of the low-density lipoproteins (LDL) receptor [6].

Elucidation of the Cholesterol Synthetic Pathway

Rudolph Schoenheimer is credited with the initial application of stable isotopes that inaugurated the study of metabolic pathways in which the cholesterol synthetic pathway was elucidated for over more than a 20-year period. Dr. Schoenheimer, born in Berlin as a Jew, was forced to emigrate Nazi Germany and was offered a position at Columbia University in New York in 1933. In the Department of Chemistry was Harold Urey, who discovered deuterium which led to his award of the Nobel Prize in 1934. Schoenheimer attended a seminar by Urey and recognized the potential of an isotope of hydrogen to elucidate biochemical transformations. Utilizing deuterium as a tracer, Schoenheimer and his colleagues in 1937 were able to demonstrate that cholesterol was undergoing degradation at some rate and being resynthesized at a comparable rate to maintain a steady state. Konrad Bloch, another Jewish emigrant from Nazi Germany, joined Schoenheimer's lab at Columbia and focused primarily on using isotopes to understand the cholesterol synthetic pathway [7]. Tragically, Schoenheimer committed suicide in 1941, but Bloch and his colleague David Rittenberg continued the isotope research in cholesterol metabolism. In 1942, they showed that acetate contributes in a major way to both the side chain and ring structures of cholesterol.

Moving to the University of Chicago, he was able to demonstrate in 1950 that all the individual 27 carbon atoms in cholesterol were derived from acetate. Over the next few years, Blochin in collaboration with R. Langdon discovered that acetate over many steps first makes squalene and then converts it to cholesterol in rats.

The exact steps by which three acetate units gave rise to a six-carbon intermediate followed by the loss of one carbon to generate a five-carbon isopentenyl precursor remained elusive. The breakthrough came through the discovery at Merck of mevalonic acid while looking for a nutritive substitute for acetate. Following this discovery, the pathway to mevalonate via acetoacetate and hydroxymethylglutarylCoA (HMGCoA) was quickly demonstrated by Feodor Lynen working independently from Bloch in Germany. In 1964, Bloch and Lynen were jointly awarded the Nobel Prize in Medicine for the mechanism of cholesterol synthesis. Lynen died at age 68 in 1979. During a routine medical check, Lynen was found to have an abdominal aortic aneurysm at a time when he was asymptomatic. Following medical advice, he underwent surgical resection of the aneurysm. However, he died 6 weeks later following complications [8]. Bloch went on to also discover that bile and estrogen were made from cholesterol, which led to the recognition that all steroids are made from cholesterol. He died at age 88 in 2000 of congestive heart failure [9]. Many of the intermediates in cholesterol synthesis and their complex stereochemistry were elucidated by John Cornforth and Popjak in the MRC in the UK. Cornforth received the Nobel Prize for his brilliant work on stereochemistry.

Discovery of LDL and the Birth of Lipidology

Lipids, such as cholesterol, its ester, and triglycerides, are insoluble in water and are transported in plasma and blood as emulsions. Their solubility is made possible by combining with phospholipids and proteins called apolipoproteins to form stable emulsified macromolecules. Lipoproteins were first described by Machboef, a Frenchman, in 1928 in his doctoral thesis. They were subsequently classified based on their flotation rates in the ultracentrifuge or by their migration on electrophoresis. The very-low-density lipoproteins (VLDL) are secreted by the liver and converted to intermediate lipoproteins and then low-density lipoproteins (LDLs) in the circulation. The HDLs are also secreted by the liver while chylomicrons are secreted by the intestine.

In the early 1950s, Dr. John Gofman from the University of California at Berkeley used ultracentrifugation to separate plasma lipoproteins and described an association of increased CHD risk with elevations of LDL and decreased risk with elevations of HDL [10]. The Framingham Heart Study from Framingham, Massachusetts, confirmed these findings through epidemiologic studies [11]. The Framingham investigators referred to elevated cholesterol (LDL-C), hypertension, and cigarette smoking as 3 major risk factors for CHD. Diabetes was subsequently added to this list. Controlling plasma cholesterol with diet and/or drugs became a national priority. In 1965 or 66, Fredrickson, Levy, and Lees published a series of landmark papers in the NEJM in which they proposed a system of classification of the lipoprotein disorders based on which lipids and lipoprotein families were elevated [12]. They used the Roman Numerals I through V to classify the disorders. Fredrickson et al. used electrophoresis on albuminated paper strips for qualitative assessment, ultracentrifugation, called beta quantification, to measure LDL-C and heparin precipitation to quantify HDL-C. Subsequently, Friedewald collaborated with Fredrickson and Levy to develop a simplified equation to quantify LDL-C [13].

$$\text{LDL} - \text{C} = \text{Total cholesterol} - \text{HDL} - \text{C} - \left(\text{Triglyceride} / 5\right).$$

A national diet-heart study was proposed and was deemed to be too expensive, and attention was given to drugs. Rudolf Altschul showed that nicotinic acid, or niacin, reduced cholesterol in the mid-1950s [14]. At this time, it was the only drug available that lowered the levels of both cholesterol and triglyceride in blood. However,

large gram quantities were required in order to reduce cholesterol and LDL. Triglycerides were reduced by about 30%, LDL by 15%–20%, while HDL was increased by 20%–25%. In the Coronary Drug Project, nicotinic acid reduced non-fatal cardiovascular events but failed to decrease total mortality, the primary endpoint [15]. However, long-term follow-up showed a decrease in total mortality in the nicotinic acid group [16]. Despite these positive results, the use of niacin was limited by flushing in most patients using this drug.

Developing Drugs to Lower Cholesterol

With the elucidation of the pathway of cholesterol synthesis by Konrad Bloch and others [17], pharmaceutical companies became interested in finding an inhibitor of the cholesterol synthesis. The first such inhibitor was MER-29 [18], known as triparanol. Triparanol inhibited the final step in cholesterol biosynthesis and led to accumulation of a precursor of cholesterol desmosterol. Unfortunately, the accumulation of this drug resulted in cataract, hair loss, and other side effects. It was withdrawn in the early 1960s due to these adverse reactions. This experience made the pharmaceutical companies cautious about the development of an inhibitor of cholesterol synthesis. The next class of lipid-lowering drugs to be developed were called fibrates or fibric acid derivatives. The mechanisms by which fibrates lower blood cholesterol levels are still uncertain. They are most effective for reducing cholesterol and LDL-C in individuals who have elevations in cholesterol and LDL-C but with normal triglyceride levels. The first such fibrate, clofibrate, Atromid-S, was approved by the FDA in the 70s, and it decreased LDL-C by about 10%–15% and raised HDL by about 10%, but decreased TG by 30% or more. In the World Health Organization Study of clofibrate, there was a decrease in non-fatal MIs but an increase in overall mortality due mainly to adverse events in the gastrointestinal tract, including GI malignancies [19]. These results provided further caution to pharmaceutical companies in the development of cholesterol-lowering agents. In the meantime, a Dow ion exchange resin was used as a bile acid sequestrant called cholestyramine, which was used in the Coronary Primary Prevention Trial [20]. This was sponsored by the National Heart, Lung, and Blood Institute. It was difficult to recruit patients and the drug had limited patient acceptance due to common gastrointestinal side effects, including bloating and constipation. Participants took only ½ the prescribed dose of the drug. In this 7-year trial, LDL was lowered by 12.6%, HDL was increased by 3%–5%, and there was a significant reduction in CHD by 19%.

The Coronary Primary Prevention Trial was the first definitive trial to test the lipid hypothesis, which aimed to reduce total cholesterol, LDL cholesterol, and coronary events? This was an important trial, even though it did not lead to widespread use of bile acid sequestrants, and cholestyramine did not receive FDA approval for an indication to reduce coronary events. Nonetheless, the Coronary Primary Prevention Trial was an important milestone in that it led to the adoption/establishment by the NIH of the National Cholesterol Education Program and subsequently, to a series of cholesterol guidelines over the years, continuing to the present.

NHLBI also supported a trial using ileal bypass surgery called POSCH, which resulted in reduction of LDL cholesterol and decrease in myocardial infarctions [21]. The POSCH study demonstrated this benefit of ileal bypass surgery, which, like bile acid sequestrants, decreased the absorption of bile acids, resulting in an upregulation of LDL receptors in the liver.

Beginning in the 1980s, removal of LDL by apheresis became available. In apheresis, patient's blood is filtered through a column which binds LDL and apoB-containing proteins. The process takes 2–4 hours per treatment and must be repeated on a weekly or biweekly basis. However, it is quite effective in reducing LDL and apoB-containing proteins for individuals for whom drug therapy is not available or effective.

The Statin Era Begins

In the 1970s, Dr. Akira Endo spent time in a laboratory at Albert Einstein Medical College in New York studying microbial metabolism and subsequently returned to Japan and joined the Sankyo Company. He began pursuing the hypothesis that fungal organisms could produce inhibitors of cholesterol synthesis in order to ward off parasites which could destroy them. The target of this research was to find an inhibitor of the rate-limiting step in cholesterol biosynthesis, which was known to be the conversion of HMGCoA to mevalonic acid. This was also being pursued in a number of other laboratories around the world and the responsible enzyme was known as HMGCoA reductase. After extensive research with many different fungal isolates, Dr. Endo isolated a substance called citrinin, which strongly inhibited HMGCoA reductase but was abandoned due to the toxicity to the kidney. In approximately 1973, Dr. Endo isolated another substance from *Penicillium citrinum*, which he called mevastatin or compactin, and showed that it was a powerful competitive inhibitor of HMGCoA reductase [22, 23]. Compactin produced a significant reduction in cholesterol and LDL-C, much more than what had been previously achieved by a drug. When compactin was administered in humans with familial hypercholesterolemia, marked reductions of cholesterol and LDL-C were observed [24]. Dr. Endo and his collaborators published their results describing the properties of compactin [23]. They showed that compactin reduced cholesterol in several animal models. They later, in collaboration with a physician, treated a patient with severe hypercholesterolemia [24]. This experiment was a resounding success, following which Sankyo initiated Phase 1 and Phase 2 trials in patients with familial hypercholesterolemia. Additional reports described excellent efficacy and safety. However, subsequently, Sankyo stopped all development of compactin because of toxicities which have never been described. Thus, following experience with triparanol, compactin became another cholesterol inhibitor to be abandoned, in this case, in the late stage of development due to toxicity. In the meantime, the FDA severely restricted the use of clofibrate following publication of the World Health Organization Study.

By 1979, Dr. Endo had isolated another statin from the cultures of *Aspergillus* mold called monacolin, now known as lovastatin [25–27]. He presented these results at the International Atherosclerosis Society Symposium in Houston, Texas, in 1979. Alberts and his collaborators at Merck at approximately the same time also isolated monacolin from a different fungus and began studying its properties [28]. However, all these studies were suspended and drug development halted on statins from approximately 1980 to 1983 after a report that compactin caused unacceptable adverse events in dogs.

Michael Brown and Joe Goldstein had discovered the LDL receptor in 1973 [29] and subsequently showed that lovastatin increased LDL receptor activity in dogs. Their studies provided a rationale for statins by upregulating the LDL receptor activity in the liver. In the meantime, Mabuchi [30] and others used the combination of statin and cholestyramine to cause a large reduction of LDL cholesterol in patients, as large as 50%–60% in patients with familial hypercholesterolemia.

In 1983, Merck, partially in response to strong encouragement from the community of lipid scientists and investigators in the US and elsewhere, restarted the development of lovastatin and undertook large clinical trials. During the course of this work, they were able to show that not only fungal metabolites but also purely synthetic statins could produce similar reductions in cholesterol and LDL.

The safety and efficacy of lovastatin, or Mevacor, were established and in 1987, it became the first statin to be approved by the FDA. The drug caused reversible elevations in liver enzymes at high doses but these were thought to be related to its primary mechanism action, namely the drug inhibition of HMGCoA reductase and was not an off target effect. The side effect of myopathy or extremely rare condition of rhabdomyolysis was described after the drug was released. It was seen primarily in combination with other drugs when used in combination with gemfibrozil or in high

statin doses with cyclosporine or nicotinic acid. Subsequently, lovastatin and simvastatin, which are semi-synthetic drugs, have been shown to interact with gemfibrozil, resulting in large increases in the statin blood level due to interference with its glucuronide formation. In the meantime, Sankyo isolated a different statin from a fungal metabolite and obtained a patent for it in 1980. This statin was called pravastatin, or pravacol, and was the second statin to be approved by the FDA in 1991. Pravastatin contains a hydroxyl group on its ring structure and is more water soluble than lovastatin or simvastatin. Throughout the late 80s and early 90s, the so-called "statin wars" debated as to whether hydrophilic (pravastatin) or hydrophobic statins were superior or safer [31]. Ultimately, it was shown that, given the same amount of LDL-C, they are equally efficacious and safe. These studies with lovastatin, pravastatin, and simvastatin encouraged other pharmaceutical companies who were hesitant after triparanol and compactin experiences and restriction of clofibrate use, to proceed with statin development.

Merck sponsored a large trial, the 4-S trial, to demonstrate the safety and efficacy of simvastatin, which became a landmark study and showed the efficacy of statins in reducing cardiovascular events [32]. The 4-S study was a secondary prevention study published in 1994 in which the drug was administered to individuals with elevated LDL levels and pre-existing cardiovascular disease. There was a reduction in both fatal and non-fatal MI, as well as total mortality. This was a true game-changing event in the "LDL / cholesterol hypothesis" as cardiologists had viewed total mortality as the holy grail in risk reduction and it had been achieved.

In two primary prevention trials, the West of Scotland [33] and the AFCAPS/TEXCAPS [34] study, there was a significant reduction in cardiovascular events with pravastatin and lovastatin, respectively. AFCAPS/TEXCAPS was a double-blind study in which participants had a baseline LDL-C of 150 mg/dL, but an HDL-C of <50 mg/dL. The subjects were very healthy, on a diet and exercise program, and free of disease at the beginning of the study. This study showed that even healthy subjects benefited from statins and that subjects with low HDL-C especially benefited. Over a 5-year period, there was a 37% decrease in fatal and non-fatal MIs, admission for unstable angina, and revascularization. The West of Scotland Study showed benefit from pravastatin in higher risk groups than AFCAPS. In 1996, atorvastatin, or Lipitor, was approved and became highly successful on a commercial basis. This drug was approved for a wide range of doses from 10 to 80 mg/dL and decreased LDL-C by approximately 40% at the starting dose of 10 mg/dL.

Benefits of statins have been seen in both men and women, diabetics and nondiabetics, and those with and without pre-existing cardiovascular disease. Initial studies did not enroll a sufficient number of women for results to be statistically significant, although there were favorable trends for women. Subsequent studies did enroll more women. The Heart Protection Study [35] with simvastatin and the JUPITER [36] study with rosuvstatin had enough women to be able to demonstrate statistically significant cardiovascular benefit, regardless of gender. In the Heart Protection Study, patients with diabetes had a similar cardiovascular benefit as those given simvastatin in the overall group. The Treat to New Target study with atorvastatin enrolled individuals with previous cardiovascular event who were treated with either 80 mg of atorvastatin or 10 mg of atorvastatin. Those who were on 10 mg of atorvastatin achieved an average cholesterol level of about 100 mg/dL, while those on 80 mg achieved an average cholesterol level of about 70 mg/dL. Those administered with the larger dose achieved greater LDL reduction and had a corresponding greater reduction in cardiovascular events. In the PROVE-IT study and the JUPITER study, the lower the level of achieved LDL, the greater the reduction in cardiovascular events. In the PROVE-IT study comparing pravastatin and atorvastatin, atorvastatin showed greater LDL reduction and superior cardiovascular event reduction. In the JUPITER study, participants were required to have an hsCRP of >2. The greatest event reduction was seen in individuals who achieved the lowest levels of LDL and

hsCRP. Overall, these studies provided evidence that the lower the LDL level, the better.

A group called the Cholesterol Treatment Trialists collaborators reported a meta-analysis with 90,000 subjects in Lancet 2005 [37]. A reduction in LDL cholesterol of 1 mmol or 39 mg/dL was associated with a 21% reduction in major vascular events. The results were similar and statistically significant in individuals with or without a history of diabetes, prior cardiovascular disease, and in males and females. In subjects from primary prevention trials, statins were associated with a decreased risk for mortality of 14%, for major coronary events of 27%, for stroke of 22%, and revascularization by 38%. There was no evidence of excess of adverse events in the studies analyzed. The CTT collaborators published a further meta-analysis in Lancet 2012 on individuals who were deemed to be at low risk for cardiovascular disease, namely, a risk of an event less than 10% over a 5-year period [38]. In this meta-analysis, there was a significant benefit that was greater than any known hazards of statin therapy, including an increase in risk of developing new-onset diabetes.

Beginning in 1988, the NHLBI began publishing a series of cholesterol guidelines called the Adult Treatment Panel Cholesterol Guidelines. The most recent sets have been prepared and published by the American Heart Association and the American College of Cardiology [39]. These emphasize the importance of diet and lifestyle, the cornerstone of therapy, with strong evidence for the benefit of statins as primary drug therapy. The most recent guidelines recommend the use of statins in primary prevention with a 10-year risk score of greater than 7.5%.

In high-risk categories, after diet therapy, if LDL is greater than 70 mg/dl, statin therapy is recommended. Studies with statins plus ezetimibe or with PCSK9 inhibitors have shown additional benefits of LDL reduction achieving extremely low levels of LDL. Since the reduction in events is proportional to the absolute magnitude of LDL reduction, individuals who benefit the most are those with the higher levels of LDL. However, benefit is seen in LDL reductions even with starting levels of LDL cholesterol in the 60s.

Clinical trials with statins and PCSK9 inhibitors have established the three main principals of LDL-C reduction: 1) LDL-C is causal for ASCVD, 2) the lower the LDL-C, the better the cardiovascular outcomes, and 3) the longer the duration of treatment to lower LDL-C the greater the absolute reduction in major adverse cardiovascular outcomes.

The story does not end here. New therapies are currently being tested, such as inclisiran [40], an siRNA inhibitor of PCSK9, apo C-III antisense RNA for elevated triglyceride, bempedoic acid (Nexiflex) for elevated LDL-C, inhibitors of angiopoietin-3 [41], and many more. Indeed, lipidology has reached the point of development where there is support for recognizing it as a subspecialty.

So the history of lipidology does not end here but the end is far from sight.

Appendix

List of Important Discoveries

Anitschkow: Discovery of cholesterol inducing atherosclerosis in rabbits

Discovery of cholesterol by a Frenchman in the eighteenth century and rediscovery by Chevreul

Michel Macheboeuf: Discovery of plasma lipoproteins

Carl Müller: Identification of the physical signs, high cholesterol levels, and heritable nature of familial hypercholesterolemia

John Gofman: Use of analytical ultlracentrifugation to identify LDL with increased risk and HDL with decreased risk of coronary heart disease

Conrad Block and Feodor Lynen: Discovery and elucidation of the structure of cholesterol

Framingham Heart Study identifying cholesterol and increased risk of coronary artery disease

Fredrickson and Levy: Classification of plasma lipoprotein disorders

Brown and Goldstein: Discovery of LDL receptors

Coronary Primary Prevention Trial: First demonstration that lowering LDL cholesterol with drugs reduces risk of CAD: establishment of LDL hypothesis

Endo: Discovery of statins

Demonstration of reduction and CAD risk by statins in clinical trials in all populations including male, female, diabetics, elderly, young, etc. CTT Cholesterol Treatment Trialists: Meta-analysis: 1 mM of LDL cholesterol reduction gives 22% reduction in cardiovascular events

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2 Atherogenesis and Vascular Biology

Peter P. Toth

Atherogenesis

Introduction

Atherosclerotic disease is highly prevalent throughout the world and is the principal cause of morbidity and mortality in Western nations for both men and women [1]. Atherosclerosis is pathophysiologically complex and begins at an early age (fatty streaks can be found in children and adolescents), with anatomically apparent coronary disease frequently becoming apparent in the third decade of life though it tends to remain clinically silent until the sixth or seventh decade [2]. Atherogenesis is driven by highly evolved networks of histologic, rheologic, autoimmune, oxidative, inflammatory, and thrombotic responses to vascular injury. These networks engage in extensive cross talk. Once established, the rate of disease progression is influenced by numerous risk factors, including age, multiple forms of dyslipidemia, hypertension, sympathetic tone, cigarette smoking, obesity, sedentary lifestyle, chronic kidney disease, the intensity of underlying inflammation, depression, insulin resistance, diabetes mellitus, urbanization, air pollution, and perhaps some forms of infection [3, 4].

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In the past six decades, an enormous range of scientific, epidemiologic, and clinical research has shown that the control of modifiable risk factors through lifestyle adjustments and pharmacologic therapies slows or even reverses the trajectory of atherosclerosis [5, 6]. Statin treatment is known to improve endothelial function, reduce inflammation, stabilize established atherosclerotic plaque, and reduce risk for such complications as myocardial infarction (MI), transient ischemic attack and stroke, claudication and peripheral arterial disease, cardiovascular and all-cause mortality, and the need for revascularization via angioplasty/stenting or bypass grafting [7, 8]. Early identification and treatment of risk factors are tantamount to the long-term prevention of atherosclerotic disease given the fact that the number of risk factors, their severity, and the duration of exposure determine lifetime risk [9–11]. Consequently, evaluating global cardiovascular risk burden, quantifying 10-year or lifetime risk, and treating each identified risk factor (e.g., dyslipidemia, hypertension, diabetes mellitus) to current guideline targets are of the essence before the onset of such clinical signs and symptoms as angina pectoris or claudication [12]. Unfortunately, little progress has been made in primordial prevention as guideline writing bodies are hesitant to make recommendations for treating adolescents and young adults [13, 14]. In a very real way, we typically wait until patients develop coronary or peripheral vascular disease

M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_2

before risk factors are identified and treated. Risk factor goal attainment rates even in patients with advanced, unstable disease tend to be distressingly low [15–18]. There continues to be considerable clinical inertia in the treatment of risk factors. *Undertreatment of risk factors does not constitute appropriate or adequate treatment of risk factors,* especially if the therapeutic goal is the prevention of disease.

The arterial system is not a simple tubular conduit network. Arteries are histologically and biochemically complex, dynamic structures that are highly responsive to their milieu. They are endowed with a wide variety of receptors along the endothelium and smooth muscle cells that regulate vasomotor tone (i.e., the capacity to regulate vasoconstriction and vasorelaxation as demanded by physiological circumstances). The coronary and cerebral vasculature are tightly regulated and fine-tuned to the oxygen delivery needs of the myocardium and the brain via local pressor effects (nitric oxide, prostacyclin, endothelin-1) as well as sympathetic and parasympathetic inputs. The coronary and peripheral vasculature (cerebrovasculature and lower extremity arteries) are continually exposed to multiple atherogenic stimuli that act additively to potentiate the pathophysiology underlying atherogenesis in the majority of persons. Atherosclerosis is a diffuse disease that, when left untreated, tends to progress throughout life.

Arterial Structure

Arteries are highly evolved, responsive conduit vessels for blood and, one of its most important constituents, oxygen. Oxygen must be available to aerobic cells in order to function as a terminal electron acceptor for mitochondrial oxidative phosphorylation. During embryological vasculogenesis, the arterial wall differentiates into three layers with distinct cellular and connective tissue constituents: these are the intima, media, and adventitia. The intima is composed of (1) an endothelial cell monolayer that interfaces with blood and (2) the lamina propria which contains smooth muscle cells, fibroblasts, collagen, and intercellular matrix that comprised glycosaminoglycans (hyaluronate, heparin/heparan sulfate) [19]. The media is composed of smooth muscle cells which regulate arterial tone and blood pressure by either contracting or relaxing in response to a variety of vasoactive molecules (e.g., nitric oxide, catecholamines, prostacyclin, bradykinin, endothelin-1, angiotensin II). The media is separated from the intima and adventitia by the internal and external elastic membranes, respectively. During atherogenesis, smooth muscle cells in the media can undergo activation via platelet-derived growth factor or cell surface lipoprotein binding proteins, rearrange their actin cytoskeleton, extend pseudopodia, and migrate into the intima where they are incorporated into atheromatous plaques [20]. The smooth muscle cell is able to migrate by releasing proteases into its surroundings which hydrolyze the intercellular matrix and the internal elastic membrane. The adventitia contains fibroblasts, elastin, and collagen. The *vasa vasora* and sympathetic and parasympathetic nerve fibers are contained in the adventitia. The arterial wall is a highly dynamic and responsive environment with the various cellular constituents of different layers communicating through complex signaling circuits. Arteries undergo a staggering series of changes, both biochemically and physiologically, during all stages of atherogenesis.

Endothelial Cell Function and Dysfunction

Endothelial cells line the luminal surface of blood vessels, provide barrier functions to control what enters and exits the arterial wall, and carry out a number of other specialized roles. Endothelial continuity and barrier function are established by tight junctional complexes between cells [21]. These "gap" junctions also facilitate communication between endothelial cells [22]. The endothelium controls vascular tone by producing nitric oxide. Nitric oxide (NO) is produced by endothelial nitric oxide synthase (eNOS) using arginine as a nitrate donor. Nitric oxide production is activated by bradykinin, acetylcholine, and substance P [23]. Once formed, NO diffuses down along a

concentration gradient into the media and activates soluble guanylate cyclase, an enzyme that catalyzes the production of cyclic 5′-guanylate monophosphate (cGMP) [23]. As intracellular cGMP levels increase, smooth muscle cells relax, thereby promoting vasodilatation. Endothelial cells produce other vasodilatory substances as well, including prostacyclin (prostaglandin I_2) and endothelium-derived hyperpolarizing factor [24]. It is not yet established how much each of these molecules contributes to vasodilatory input at any given time or in response to local physiologic or pathophysiologic change.

Under normal conditions, the endothelium establishes an antithrombotic surface by producing (1) tissue plasminogen activator (tPA), an enzyme that converts plasminogen to plasmin, a thrombolytic enzyme that hydrolyzes fibrin [25], and (2) thrombomodulin and heparin sulfate, both of which antagonize the activity of thrombin. Prostacyclin and NO inhibit platelet activation and aggregation along the endothelial surface [26].

When endothelial cells are exposed to increased levels of atherogenic lipoproteins, elevated systemic resistance, tobacco-derived toxins, inflammatory mediators, oxygen free radicals, increased serum concentrations of glucose, oscillatory shear stress, or turbulent blood flow, they become dysfunctional [27–29]. Endothelial cell dysfunction (ECD) is a truly systemic disorder [30] and is characterized by a number of pathophysiological changes:

1. Nitric oxide production decreases [31].
2. The endothelial surface becomes more prothrombotic because the production of tPA and prostacyclin decreases and biosynthesis of plasminogen activator inhibitor (PAI; an inhibitor of tPA and fibrinolysis) increases [32].
3. The barrier function becomes impaired as the tightness of junctional complexes is adversely impacted [33].
4. Production of the vasoconstrictor endothelin-1 increases which not only increases vascular resistance but also induces adverse remodeling of the vessel wall [34].
5. The expression of adhesion molecules increases [35–37].

Adhesion molecules promote the binding, rolling, and transmigration of inflammatory white blood cells, such as monocytes and lymphocytes, along the endothelial surface and include vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and a variety of selectins (e.g., E, P, and L) [38, 39] (Fig. 2.1). As monocytes bind to the luminal surface of endothelial cells, they can gain access into the subendothelial space by homing in on a gradient of monocyte chemoattractant protein-1 (MCP-1) [40–42]. Monocytes can cross the endothelial barrier by either (1) rearranging their cytoskeleton and changing their shape (*diapedesis*) in between adjacent endothelial cells (*paracytosis*) or (2) moving directly through an endothelial cell (*transcytosis*) [38, 43, 44]. Monocytes taken up into the vessel wall can then take up residence in the subendothelial space, transform into macrophages, and create an inflammatory nidus within the arterial wall (Fig. 2.2). Different subpopulations of macrophages (M1 or M2) can then either scavenge lipids or phagocytose apoptotic debris, generate cytokines that potentiate or inhibit inflammation, or engage in other specialized functions as needed during atheromatous lesion initiation, progression/expansion, or regression [45, 46].

In addition to promoting vasodilatation, NO is critical to the inhibition of several mechanisms fundamental to atherogenesis. Nitric oxide decreases the adhesion of platelets to endothelium [47]. In addition to promoting thrombus formation, platelets stimulate intravascular inflammation by functioning as a source of such inflammatory mediators as a platelet-derived growth factor, thrombospondin, platelet factor 4, and transforming growth factor-β, among others [48]. Nitric oxide also inhibits (1) the migration of smooth muscle cells from the media into the subendothelial space, an early event in atherogenesis, and (2) intercellular matrix synthesis and deposition [49]. The intercellular matrix material is believed to be responsible for lipoprotein trapping in the subendothelial space [50, 51]. Reduced NO production is highly correlated with atherogenesis [52].

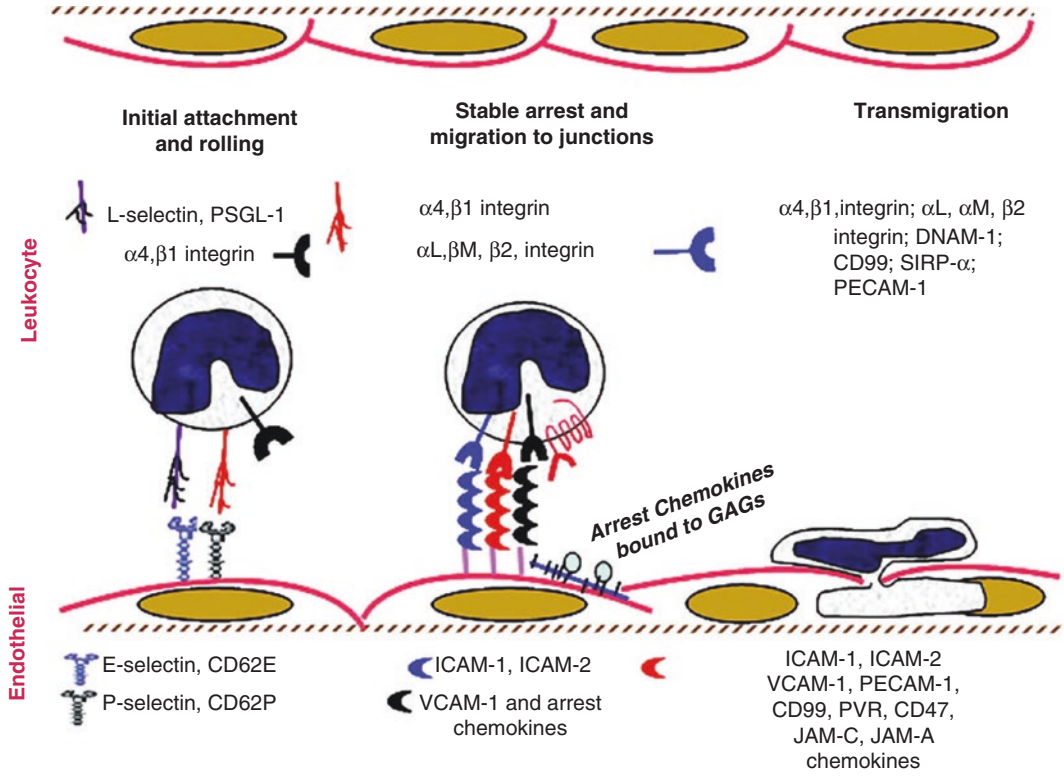

Fig. 2.1 Leukocyte recruitment from blood into the subendothelial space. Complex orchestration of cell attachment and diapedesis with sequential expression of different integrins, selectins, and adhesion molecules. Initial attachment and rolling, arrest, and migration to cell-cell borders and transmigration across the vascular endothelium of a monocyte. Monocytes attach via selectin-mediated mechanisms along with contributions from the *a*4 and ß2 integrins binding to their ligands VCAM-1 and ICAM-1, respectively. The next step is stable arrest; ß2 integrins become activated by arrest chemokines and trigger cell arrest at or near cell-cell junctions. Monocytes then migrate to junctions and transmigrate across the vascular endothelium at both junctional and non-junctional locations. The symbols used to represent adhesion molecules in endothelial cells are identified below each component of the figure. (From Rao et al. [38]. Reproduced with permission)

Angiotensin II (AII) is an important mediator of hypertension and is produced from angiotensin I (AI) via proteolytic hydrolysis by angiotensin-converting enzyme (ACE). Dysfunctional endothelium increases its expression of the AT1 receptor, the binding site for AII. Activation of AT1 by AII increases the activity of such enzymes like xanthine oxidase and NAD(P)H oxidase [53, 54]. These enzymes increase oxidative stress by increasing the production of reactive oxygen species (ROS), such as superoxide anion, hydroxyl ions, and hydrogen peroxide [55, 56] (Fig. 2.3). The ROS are directly toxic to the endothelium, quench NO (forming peroxynitrite anions), and can oxidize and peroxidize the lipids and phospholipids in lipoproteins, thereby rendering them more atherogenic. AII also promotes smooth muscle cell proliferation and migration as well as increased fibroblast collagen production and deposition. This addition of collagen leads to the loss of compliance/reduced elasticity of the artery. Endothelial cell dysfunction as measured by impaired vasoreactivity in response to an acetylcholine or methylcholine challenge [57, 58] and increased expression of PAI-1 are indicators of worse prognosis in patients at risk for cardiovascular events [59]. Endothelial function is improved by increased exercise [60] as well as

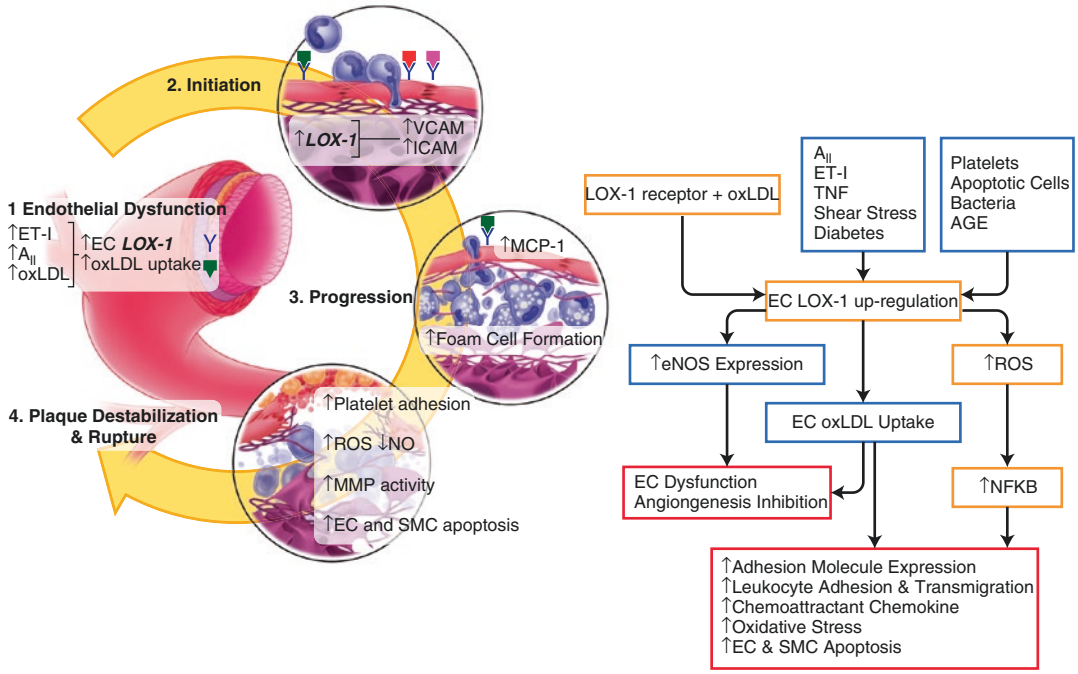

Fig. 2.2 LOX-1 and inflammation. LOX-1 plays a role in the initiation, progression, and destabilization of atherosclerotic plaques. The steps in atherogenesis it impacts are shown on the left and summarized in greater detail on the right. LOX-1 binding and signaling initiate a series of molecular and histologic events that end in vascular occlusion and ischemic injury. Abbreviations: ET-1 endothelin-1, AII angiotensin II, VCAM-1 vascular cell adhesion molecule, MCP-1 monocyte chemoattractant protein-1, MMP Matrix metalloproteinase, NO nitric oxide, oxLDL oxidized low-density lipoprotein, TNF tumor necrosis factor, NFKB nuclear factor kappa B, EC endothelial cells, SMC smooth muscle cells, ROS reactive oxygen species, eNOS endothelial nitric oxide synthase, and AGE advanced glycation end products. (From Szmitko et al. [79]. Reproduced with permission)

pharmacologic intervention with statins (3′-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor) [61] and angiotensin-converting enzyme (ACE) inhibitors [62].

Receptors of Advanced Glycosylated End Products

Patients with insulin resistance, metabolic syndrome, and diabetes mellitus have impaired glucose tolerance and hyperglycemia. Hyperglycemia correlates with increased formation of arterial advanced glycosylated end products (AGEs) [63]. The AGEs represent the nonenzymatic modification of lysine residues in enzymes, proteins, and lipoproteins with the formation of glucose adducts [64]. The formation of AGEs activates the inflammatory cascades regulated by nuclear factor Kappa-B and activator protein-1 [63, 65]. In addition, the formation of AGEs also correlates with the following:

1. Lipoprotein glycosylation (rendering LDL particles more atherogenic and compromising high-density lipoprotein particle function)
2. Endothelial dysfunction with reduced nitric oxide availability, increased adhesion molecule expression, increased procoagulant production, and heightened oxidative tone
3. Increased collagen cross-linking, leading to reduced vessel wall compliance
4. Increased subendothelial intercellular matrix deposition, increasing likelihood of atherogenic lipoprotein trapping
5. Increased leukocyte infiltration and inflammatory mediator expression, among other effects [66]

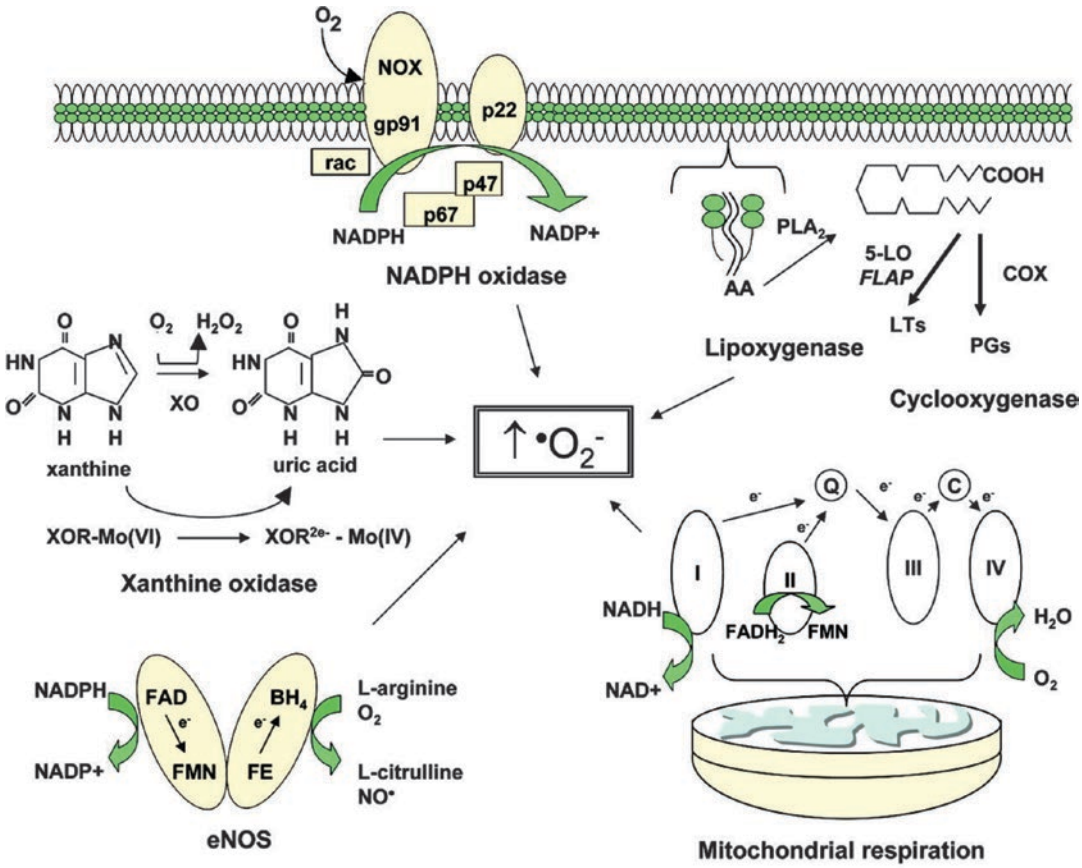

Fig. 2.3 Metabolic and enzymatic sources of superoxide anion in the vasculature. Superoxide anion (•O2⁻) is formed by several metabolic and enzymatic sources within the cell. NADPH oxidase is composed of multiple membrane-bound and cytoplasmic subunits. The enzyme is activated when the cytoplasmic subunits p67 and p47 and the small G-protein Rac assemble with the membrane-bound NOX (vascular homolog of gp91phox) and p22phox. NADPH oxidase uses NADPH as a substrate and, in vascular cells, is considered an important source of reactive oxygen species (ROS) generation. The lipoxygenases and cyclooxygenases (COX) generate ROS indirectly by promoting the formation of inflammatory mediators. Arachidonic acid (AA) that is cleaved from the cell membrane by phospholipase A2 (PLA2) is then metabolized by 5-lipoxygenase (5-LO) in the presence of its accessory protein (FLAP) to form leukotrienes (LTs). AA is also metabolized by the cyclooxygenases to form members of another family of inflammatory mediators, the prostaglandins (PGs). Mitochondria also generate superoxide as electrons are transferred from complex I to cytochrome oxidase during normal cellular respiration. Xanthine oxidase (XO), which converts hypoxanthine and xanthine to uric acid, is an additional source of ROS. As xanthine is converted to uric acid, two electrons are donated to molybdenum (Mo) at the active site of the enzyme, thereby reducing it from Mo(VI) to Mo(IV). Finally, endothelial nitric oxide synthase (eNOS), when substrates or cofactors are not replete, uncouples to generate superoxide in preference to NO. Abbreviations: Q coenzyme Q, C cytochrome C, NAD nicotinamide adenine dinucleotide, FAD flavin adenine dinucleotide, FMN flavin mononucleotide, FE heme iron, BH4 tetrahydrobiopterin. (From Leopold and Loscalzo [56]. Reproduced with permission)

The hyperglycemic milieu is particularly injurious and stimulates a broad swath of proatherogenic influences. In the setting of insulin resistance, there is increased visceral organ steatosis, especially in the liver, pancreas, and epicardium [67]. Epicardial fat pad volume expansion in the setting of insulin resistance loads the epicardium with dysfunctional fat surrounding coronary arteries. This dysfunctional fat is a source of interleukins, cytokines, and growth factors that shower the coronary tree and increase risk for atherosclerotic disease [68].

The Role of Monocytes and Lymphocytes

Monocytes that have become resident in the subendothelial space can undergo several histologic transitions. When exposed to macrophage colony-stimulating factor (M-CSF), the monocyte converts into a macrophage. Macrophages are one of the earliest histologic substrates of atherogenesis. Macrophage egress from the vessel wall can be inhibited by the neural guidance factor netrin-1 and VCAM-1; egress can be potentiated by lymphatic channels and high-density lipoprotein particles [69]. The fatty acids and phospholipids of low-density lipoprotein particles can undergo oxidation via such enzymes as myeloperoxidase [70], NADPH oxidase, and a variety of lipoxygenases [71] (Fig. 2.4). These oxidized phospholipid species are complex and potentiate inflammation and oxidation (Fig. 2.5). In addition, in the setting of hyperglycemia, lipoproteins can undergo glycation [72].

Exposure to oxidatively modified or glycated low-density lipoprotein particles trapped by intercellular matrix proteins in the subendothelial space [73] stimulates macrophages to upregulate the expression of a number of scavenger receptors on their surface [74]. There are a large number of these scavenger receptors and include multiple types of scavenger receptor A (types I-III) [75], CD36 [76], lectin-like oxidized LDL receptor-1 (LOX-1) [77], and scavenger receptor for phosphatidylserine and oxidized LDL (SR-PSOX) [78], among others. LOX-1 is also expressed by endothelial cells and smooth muscle cells. In the setting of increased oxidized LDL (oxLDL) exposure, endothelial cells upregulate LOX-1; the uptake of oxLDL is toxic and potentiates endothelial dysfunction and adhesion molecule expression [79].

The oxidation of phospholipids in LDL particles generates the formation of oxidation specific epitopes recognized by scavenger receptors [80, 81]. These include oxidized sn-2 fatty acids that terminate in γ-hydroxy-α, β-unsaturated carbonyl groups or 1-palmitoyl-2-(5′-oxovaleroyl)-sn-glycero-3-phosphocholine (Fig. 2.5). Scavenger receptors promote the binding and uptake of atherogenic lipoproteins into the intracellular space of the macrophage. As more and more lipids are taken up, the macrophage develops lipid inclusion bodies and becomes a "foam cell." [82] Foam cells produce a variety of cytokines, matrix metalloproteinases, ROS, and tissue factors [83]. Smooth muscle cells can undergo transformation into macrophages and, as they scavenge lipid and lipoprotein, can also form foam cells [84]. Smooth muscle cell transformation occurs secondary to reduced expression of myocardin and the microRNAs miR143/145 [85]. Oxidized phospholipids can also stimulate the hyperphosphorylation of VE-cadherin, a critical protein for maintaining endothelial gap junctions [86]. Gap junction function deteriorates as the VE-cadherin dissociates from the proteins β-catenin and paxillin [87].

Tissue factor is a procoagulant that promotes platelet aggregation on the surface of ruptured atheromatous plaques [88]. The MMPs can destabilize atheromatous plaque by hydrolyzing the matrix proteins which reinforces its structural integrity (Fig. 2.6). As MMPs degrade extracellular matrix material, such degradation products as integrin-binding fibronectin, hyaluronan, and heparan sulfate can trigger immune and proinflammatory responses [81, 89, 90]. Smooth muscle cells also produce MMPs as they break down the internal elastic lamina in order to access the intima [20]. Ultimately, foam cells can coalesce to form fatty streaks. As fatty streaks increase in volume and more cellular debris accumulates, a frank atheromatous plaque evolves.

Foam cells possess measures of self-defense. Macrophages are capable of effluxing excess intracellular lipids into the extracellular space. Intracellular cholesterol can be mobilized and exported onto HDL particles via scavenger receptor B-I (SR-BI), or two ATP-binding membrane cassette transport proteins termed ABCA1 and ABCG1 [91]. In addition, the macrophage can produce and secrete apoprotein E (apoE) which, when externalized, can bind to ABCA1 and drive cholesterol externalization, apoE lipidation, and lipoprotein biogenesis [92, 93]. If these defenses are overwhelmed by excess lipid trapped in the subendothelial space, then foam cell develop-

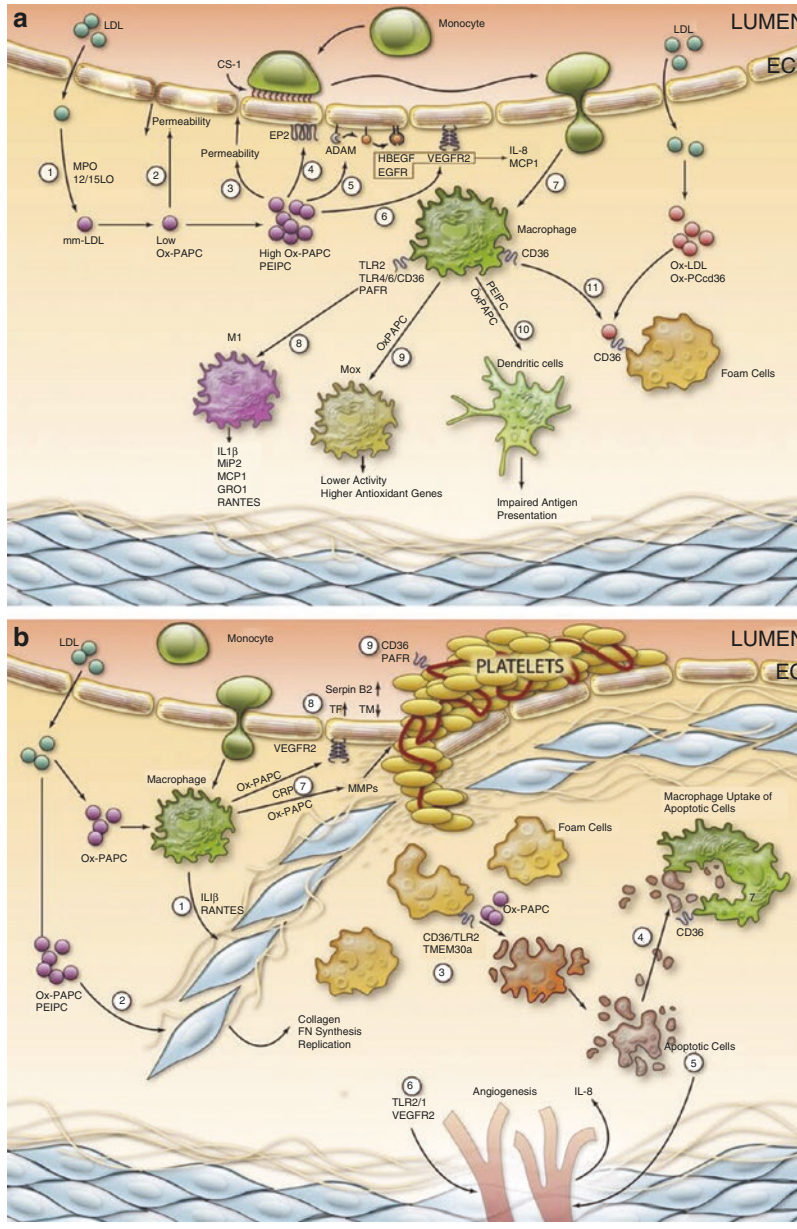

Fig. 2.4 Model of oxidized phosphatidylcholine-containing phospholipids (Ox-PL) regulation of atherosclerosis. (**a**) Early lesions: (1) LDL enters the vessel wall and is oxidized by myeloperoxidase (MPO) and 12/15 lipoxygenase (LO) to form modified LDL which contains oxidation products including oxidized 1-palmitoyl-2-arachidonyl-sn-glycero-3-phosphocholine (Ox-PAPC). (2) Low doses of Ox-PAPC decrease the permeability of the endothelial cell (EC) monolayer by forming adherens junctions. (3) Higher levels of Ox-PAPC cause a strong increase in monolayer permeability because of junction breakdown and stress fiber formation, resulting in increased entry of LDL into the vessel wall. (4) Ox-PAPC binds to the E-type prostaglandin receptor (EP2) receptor, causing the deposition of connecting segment 1 (CS-1) fibronectin on the apical surface which binds monocytes. (5) Ox-PAPC activates specific a disintegrin and metalloproteinases (ADAMs) to cause the release of active heparin-binding epidermal growth factor (HBEGF) and activation of epidermal growth factor receptor (EGFR), leading to interleukin (IL)-8 and monocyte chemotactic protein (MCP)-1 synthesis. (6) Ox-PAPC also activates vascular endothelial growth factor receptor 2 (VEGFR2), leading to IL-8 and MCP-1 synthesis. (7) These chemokines facilitate the entry of monocytes into the vessel wall. (8) Oxidized phosphatidylcholine-containing phospholip-

(continued)

ids (Ox-PL) acting on Toll-like receptor (TLR)2, TLR4/6/cluster determinant 36 (CD36), and platelet-activating factor (PAF) receptor cause some monocytes to differentiate into M1 macrophages producing chemokines. (9) Ox-PAPC causes differentiation of some macrophages into Mox, which have high levels of antioxidant enzymes and lower chemokine syntheses. (10) Ox-PAPC causes the differentiation of some monocytes into dendritic cells with an impaired presentation of lipid antigens. (11) Macrophages further oxidize LDL to form Ox-LDL. Ox-PCCD36 acting on CD36 in the presence of Ox-LDL causes foam cell formation. (**b**) Advanced lesions: (1) In the presence of Ox-PAPC and PAF-like lipids, macrophages make IL-1 beta (IL-1β) and regulated upon activation, normal T-cell expressed, and secreted (RANTES). (2) These chemokines and a direct effect of Ox-PAPC on smooth muscle celsl (SMC) cause the migration and proliferation and matrix production of SMC. These SMC cover the foam cells that accumulate under the endothelium. (3) The interaction of Ox-PAPC with CD36/TLR2 and with unfolded protein response (UPR) activators and the interaction of PAF-like lipids with transmembrane protein 30A (TMEM30a) cause macrophage apoptosis. (4) Oxidized phosphatidylserine-containing phospholipids (Ox-PS)/ PCCD36 in the apoptotic cell membrane bind to CD36 in macrophages, leading to macrophage uptake of the apoptotic cells. (5) Some apoptotic fragments stimulate EC to make IL-8, an angiogenic cytokine. (6) CEP activation of TLR2/TLR1 causing integrin activation and Ox-PAPC acting to increase VEGFA cause angiogenesis of adventitial vessels into the media and intima. (7) C-reactive protein (CRP) and Ox-PAPC interacting with CD36 stimulate macrophage production of metalloproteinase. This weakens the plaque and can lead to plaque rupture. (8) Ox-PAPC activation of VEGFR2 increases tissue factor synthesis in the endothelium. Ox-PAPC also causes increases in Serpin B2 and a decrease in thrombomodulin. (9) Ox-PCCD36 acting on CD36 and PAF-like lipids acting on PAFR cause increased aggregability of platelets. (From Lee et al. [86]. Reproduced with permission)

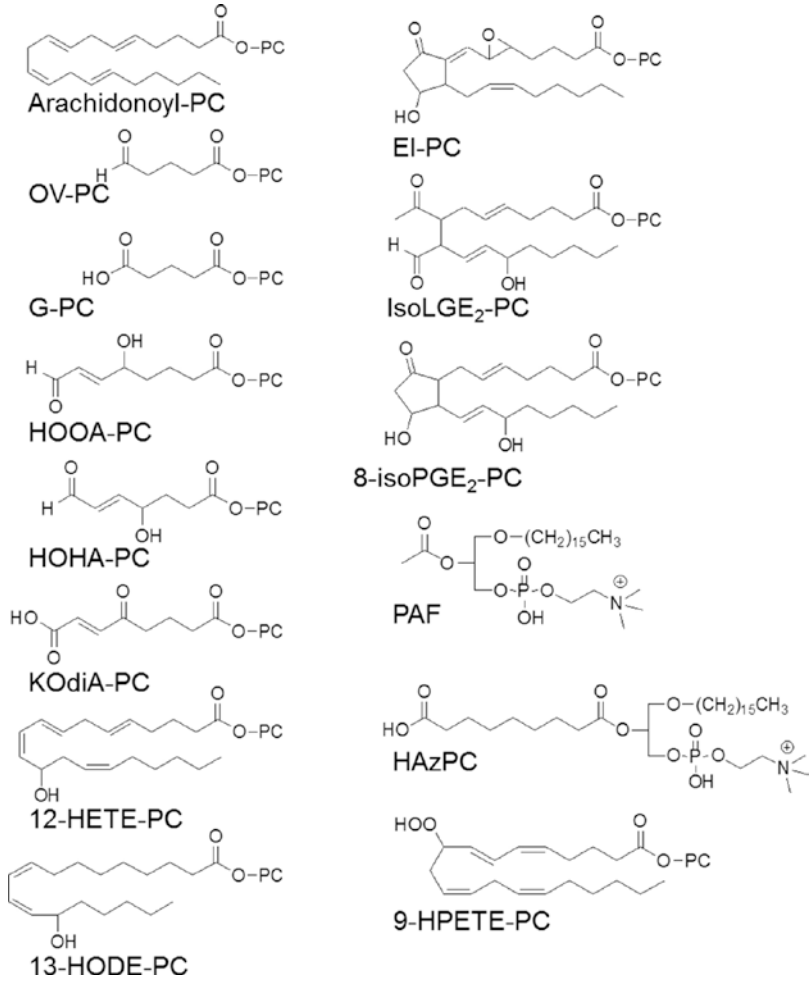

Fig. 2.5 Oxidized phosphatidylcholine-containing phospholipids (OX-PL) lipids**.** PC, 1-acyl-2-lyso-sn-glycero-3phosphatidylcholine. Only the sn-2 position composition is shown for all Ox-PL except those forming an ether bond at the sn-1 position. Abbreviations: PAF platelet-activating factor, HAz-PC hexadecylazelaoyl PC, 13-HODE-PC 1-palmitoyl-2-(13(S)-hydroxy-(9Z,11E) octadeca-9,11-dienoyl)-sn-glycero-3-phosphocholine. (From Lee et al. [86]. Reproduced with permission)

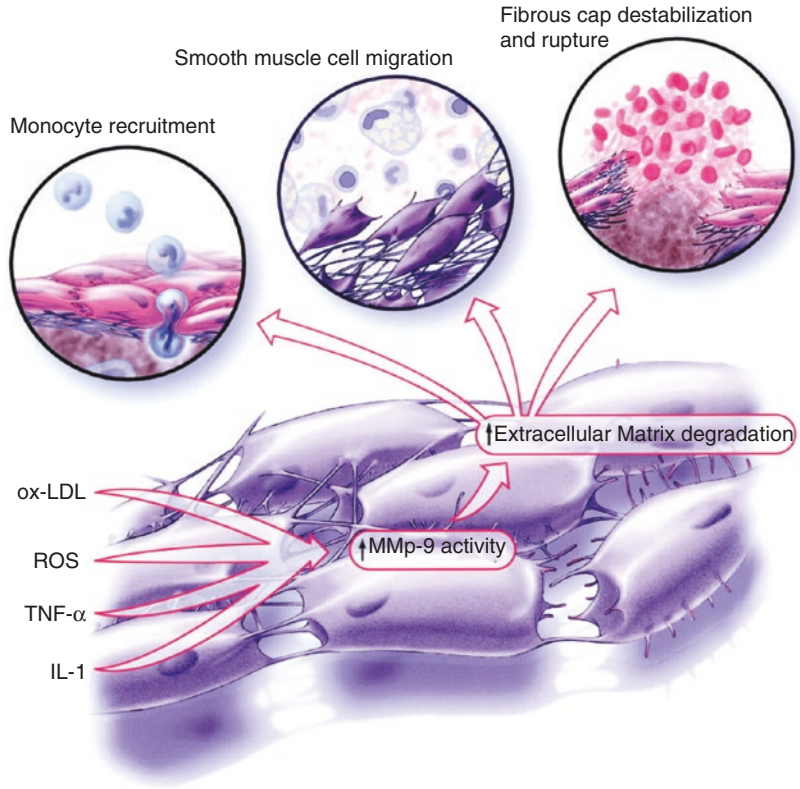

Fig. 2.6 MMP-9, from plaque progression to destabilization. MMP-9 degrades the basement membrane of the arterial wall to facilitate monocyte migration into the plaque and SMC migration to form the fibrous cap. Excessive MMP-9 activity eventually leads to the degradation of the fibrous cap, plaque instability, and plaque rupture. Abbreviations: IL-1 interleukin-1, ROS reactive oxygen species, TNF-α tumor necrosis factor alpha, MMP-9 matrix metalloproteinase-9, oxLDL oxidized low-density lipoprotein. (From Szmitko et al. [79])

ment progresses with increasing lipid inclusion body volume and resultant toxicity [94].

T lymphocytes and mast cells also participate in vascular inflammation and atherogenesis. T cells follow a gradient of chemoattractants (inducible protein-10, interferon-inducible T-cell α-chemoattractant, and monokine induced by interferon-γ) into the subendothelial space [41, 95]. These chemoattractants can bind to CXCR3, a chemokine receptor on the surface of T cells. When a T cell binds oxidatively modified LDL to an antigen receptor it can undergo differentiation into T helper cells, such as TH1 and TH2. TH1 cells potentiate inflammation by producing interleukin-1, interferon-γ, and tumor necrosis factor. TH2 cells produce anti-inflammatory cytokines, such as interleukins −4 and −10. TH1 cells predominate in atheromatous plaques and stimulate inflammation. Following antigen binding and presentation, T cells stimulate macrophage production of MMPs and cytokines.

Lymphocytes also infiltrate and become organized in the vascular adventitia [96, 97]. Adventitial aortic tertiary lymphoid organs (ATLOs) and T cell aggregates associate with more severe atherosclerotic plaques. An ATLO is composed of a nodular center composed of B lymphocytes and dendritic cells surrounded by T lymphocytes. B lymphocytes can be activated to produce antibodies after antigen presentation by dendritic cells, thereby mounting an immune response. There is significant communication between the endothelium and adventitia, and it is believed that ATLOs and organized T cell aggregates play a significant role in atherogenesis [98]. The vasa vasora and small medial conduits mediate the transfer of immune cells, cytokines, and interleukins between the intima and adventitia (Fig. 2.7).

Activated mast cells contribute to atherogenesis and enter the subendothelial space in response to eotaxin exposure [99]. Mast cells secrete two serine peptidases, tryptase and chymase [100]. Chymase catalyzes the intravascular conversion

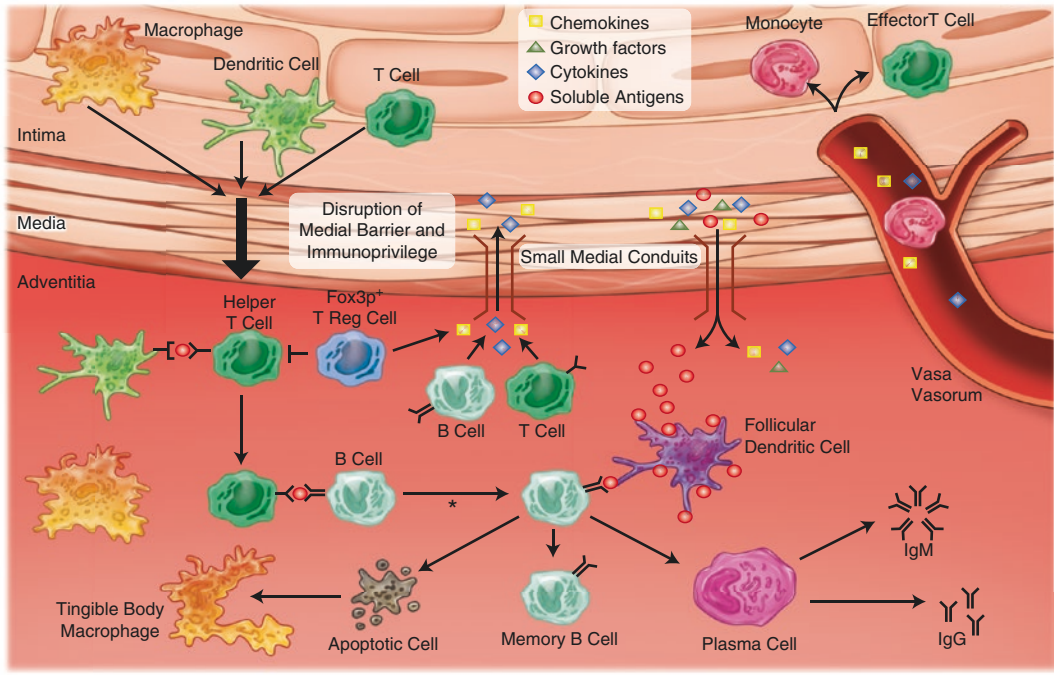

Fig. 2.7 Arterial adventitia and its role in atherogenesis. Lymphocytes, macrophages, dendritic, cells, and plasma B cells can be organized in the adventitia of arteries. Small medial conduits facilitate the passage of cytokines, chemokines, soluble antigens, and growth factors from the adventitia into the media. The vasa vasora can facilitate communication between cells of the adventitia and those of the intima, including endothelium. These communication patterns can promote atherogenesis by stimulating inflammatory and phagocytic cell recruitment, smooth muscle cell migration, and the mounting of an innate immune response. (From Campbell et al. [98]. Reproduced with permission)

of AI to AII, and tryptase activates MMPs. Both enzymes thus not only contribute to early events in atherogenesis but also can induce instability in established plaque. Mast cells also secrete histamine, which promotes increased vascular permeability.

Role of Neutrophils

Neutrophils have evolved a broad-based capacity for biochemically combating infectious organisms by secreting proteases, ROS, and antimicrobial proteins. However, recent investigation also supports multiple roles for neutrophils in atherogenesis. Within the subendothelial space, neutrophils can produce an array of collagenases, elastases, and other matrix metalloproteinases that can hydrolyze and degrade the intercellular matrix material of plaque and its fibrous cap, thereby weakening them and rendering them more prone to rupture [101]. Neutrophils also elaborate myeloperoxidase and ROS in the subendothelial space which are cytotoxic and oxidize trapped lipoproteins [102]. Neutrophils entering the subendothelial space also potentiate injury by releasing (1) four different subsets of granules containing preformed proteases, pro-oxidative enzymes, and cytokines whose release is precisely timed in response to conditions in the prevailing histologic milieu and (2) and leukotrienes such as LTB4, a potent chemoattractant [103].

A more recently elucidated pathway by which neutrophils can promote atherogenesis is by forming neutrophil extracellular traps (NETs) [104] (Fig. 2.8). NETs are produced by suicidal neutrophils and represent an extruded reticular structure composed of decondensed chromatin as

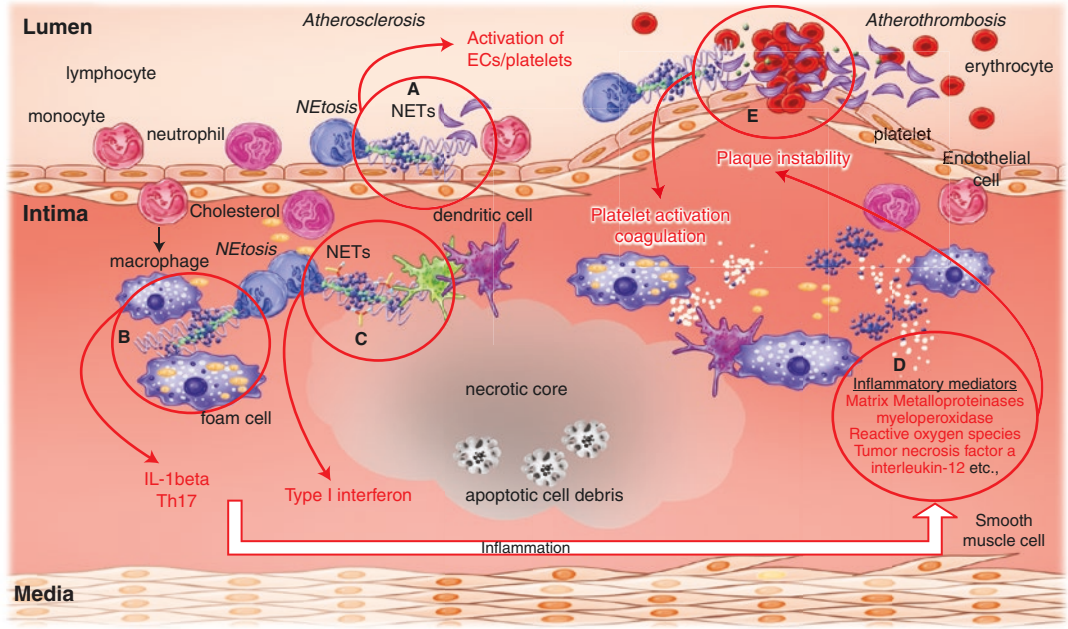

Fig. 2.8 Neutrophil extracellular traps (nets) in atherosclerosis and atherothrombosis. (**a**) Neutrophils netting in the arterial lumen along the endothelial surface activates endothelial cells, platelets, and other leukocytes, inducing an inflammatory nidus and endothelial dysfunction. (**b**, **c**) NETs may stimulate T helper cells to secrete IL-1β and potentiate a type I interferon response, which boosts leukocyte activation and the intensity of inflammation. (**d**, **e**) The proinflammatory milieu promotes plaque instability and rupture. In the setting of acute plaque rupture, NETs can participate in thrombus formation by activating the coagulation cascade with overlying thrombus formation and arterial occlusion. (From Doring et al. [104]. Reproduced with permission)

well as nuclear, granular, and cytosolic proteins. NETs represent a type of mechanism by which endothelial cells can be exposed to sudden, very high concentrations of proinflammatory mediators. NETosis or the process of NET formation can be induced by ROS, cytokines, cholesterol crystals, and activated platelets [105–108]. In addition to nucleic acids, the molecular constitution of NETs contains a complex proteome which includes histones, proteases, lysosomal cathepsins, α-defensins, and myeloperoxidase, among other proteins and enzymes [104]. NETS are prothrombotic and cytotoxic.

Role of Platelets

Platelets are nonnucleated cells arising from parent megakaryocytes and mediate clot formation in concert with coagulation pathways. Platelets potentiate atherogenesis in multiple ways. Platelet α-granules contain a host of cytokines, chemokines, growth factors, and enzymes that can be mobilized and secreted in response to extracellular stimuli [109]. Platelets interact with endothelial cells and leukocytes according to the following mechanisms:

1. Platelets adhere to dysfunctional endothelial cells by binding to either (a) ICAM-1 via the glycoprotein 2b/3a receptor and fibrinogen or (b) selectin P via glycoprotein 1b [109, 110].
2. Thrombus formation along the endothelial surface is modulated in a bidirectional manner. Endothelial cells secrete nitric oxide and prostacyclin, which inhibits platelet activation and aggregation. Endothelial cells can also attenuate ADP availability by releasing CD39 ectonucleotidase, which hydrolyzes ADP to AMP and phosphate. Platelets can secrete nitric oxide which inhibits endothelial P-selectin expression, reduces platelet

recruitment for clot propagation, and promotes platelet dissociation [111].
3. Despite being nonnucleate, platelets effectively store messenger RNAs (mRNAs) for subsequent protein translation. In the setting of heightened inflammation, platelets can boost the inflammatory response by releasing IL-1β, among other inflammatory mediators [112, 113].
4. Inflammation can induce the coactivation of platelets and neutrophils, which leads to increased production of human neutrophil peptide-1 (HNP-1) and regulated activation of normal T cell expressed and secreted (RANTES). RANTES and HNP-1 facilitate monocyte adhesion to endothelial cells and recruitment into the arterial wall [114].
5. In addition to signal transmission by cell surface receptors and granule release, platelets can interact with endothelial cells and leukocytes by direct bilateral mRNA transmission, thereby boosting local molecular biosynthetic capacity and an inflammatory response [115].
6. Platelet microparticles also upregulate the inflammatory response. These microparticles secrete microRNAs (miRNA), which are noncoding RNAs that regulate posttranscriptional gene expression. For example, platelet-derived miRNA-320b decreases surface expression of endothelial ICAM-1 and miRNA-223 stimulates increased phagocytic activity by macrophages resident in the subendothelial space [116–118].

Clearly, the interactions of platelets with endothelial cells and other histologic components of the arterial wall and atherosclerotic plaque are complex and highly orchestrated. Much remains to be learned about these processes and how they might be therapeutically modulated.

Role of MicroRNAs

As noted above, miRNAs are noncoding RNAs that regulate posttranscriptional gene expression. MicroRNAs are highly conserved and bind to the 3′ untranslated region of messenger RNA (mRNA) transcripts, resulting in "RNA silencing." [119] They are produced and secreted by a large variety of cells. MicroRNAs secreted into the circulation are resistant to the activity of plasma RNases, and they can impact the expression of molecules in target cell types. MicroRNAs can be transported in the plasma on microparticles, HDL particles, or bound to the protein Argonaute2 [120]. Distinct patterns of circulating miRNAs have been characterized in the setting of myocardial infarction, heart failure, and diabetes mellitus [120–123]. Specific molecular signatures of miRNAs are also apparent in the setting of CAD [124]. The miRNAs do not unexpectedly have a very complex relationship with atherogenesis, with numerous miRNAs that can either stimulate or inhibit expansion of the vasa vasora, macrophage cholesterol efflux, vascular remodeling, smooth muscle cell proliferation and migration, endothelial cell activation, and monocyte and T-cell differentiation and activation, among other functions [125] (Fig. 2.9). Much is yet to be learned about the role of miRNAs in atherogenesis and how the modulation of these regulators of gene expression might be put to therapeutic use.

Role of Increased Oxidative Tone

Myeloperoxidase, lipoprotein-associated phospholipase A2, xanthine oxidase, NADPH oxidase, cyclooxygenase, and 5′-lipoxygenase are all found in atheromatous plaque and promote ROS production and oxidative lipoprotein modification [56, 126, 127]. The ROS include superoxide anion [128], hydroxyl radicals, peroxynitrite radicals, and hydrogen peroxide [129] (Fig. 2.3). Enzymes such as glutathione peroxidase, the thioredoxins, paraoxonase, and superoxide dismutase are responsible for metabolizing ROS to less reactive species. Deficiencies in anti-oxidative enzymes can be associated with increased atherogenesis. All the major cardiovascular risk factors (dyslipidemia, cigarette smoking, hypertension, diabetes mellitus) increase oxidative tone by upregulating the speciation of ROS [128]. The ROS not only can be directly cytotoxic but also

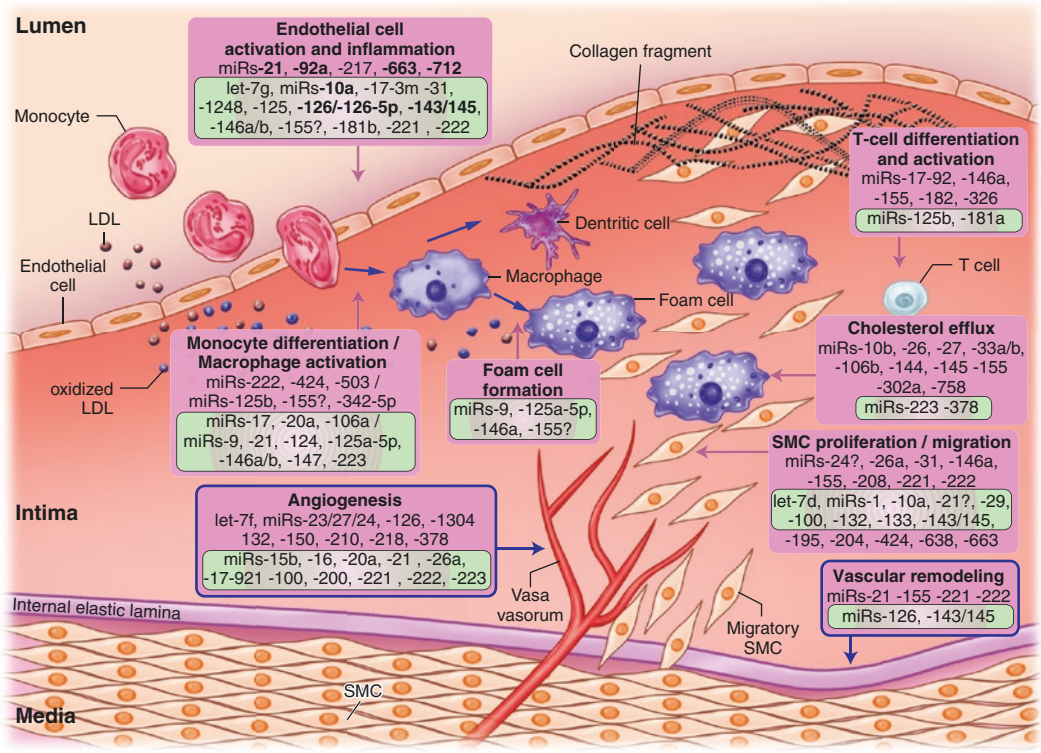

Fig. 2.9 MicroRNAs implicated in atherosclerosis. Positive/atheroprotective (in green frame) or negative/atherogenic (in red frame) effects of miRNAs on atherogenesis. Question marks indicate controversial or contradictory evidence for specific miRNAs. miRNAs in bold are regulated by blood flow/shear stress. Abbreviations: LDL low-density lipoprotein, SMC smooth muscle cell. (From Andreou et al. [125]. With permission from Elsevier)

are responsible for oxidizing and peroxidizing lipid and phospholipid within LDL particles. Lipid peroxidation products (e.g., malondialdehyde, 4-hydroxynonenal, phosphocholine of oxidized phospholipids, γ-ketoaldehydes, and 2-(ώ-carboxyethyl) pyrrole) are highly reactive [130, 131]. For example, proteins can be rendered immunogenic when they form adducts with γ-ketoaldehydes, resulting in the activation of T cells and dendritic cells [132].

Atheromatous Plaque

During the initial phases of atherogenesis, macrophage foam cells that undergo programmed cell death and turn into apoptotic bodies are efficiently cleared by macrophage dependent phagocytosis. This orderly clearance process does not promote inflammation. However, as the rate of foam cell formation and accumulation increases, the milieu within the vessel wall changes [133]. More cellular apoptosis and oncosis (ischemic death) ensues [134]. Phagocytic capacity is eventually exceeded, and the balance between foam cell apoptosis and clearance is lost, leading to progressive accumulation of lipid and apoptotic debris (Fig. 2.10). Fatty streaks progressively enlarge forming an atheromatous plaque which organizes with a lipid core and fibrous cap. More advanced lesions can have a necrotic core and can undergo calcification via the activity of a variety of osteogenic factors, including bone morphogenetic protein, osteonectin, and osteocalcin, among others [135]. Plaque that is not yet fibrosed or calcified retains some degree of plas-

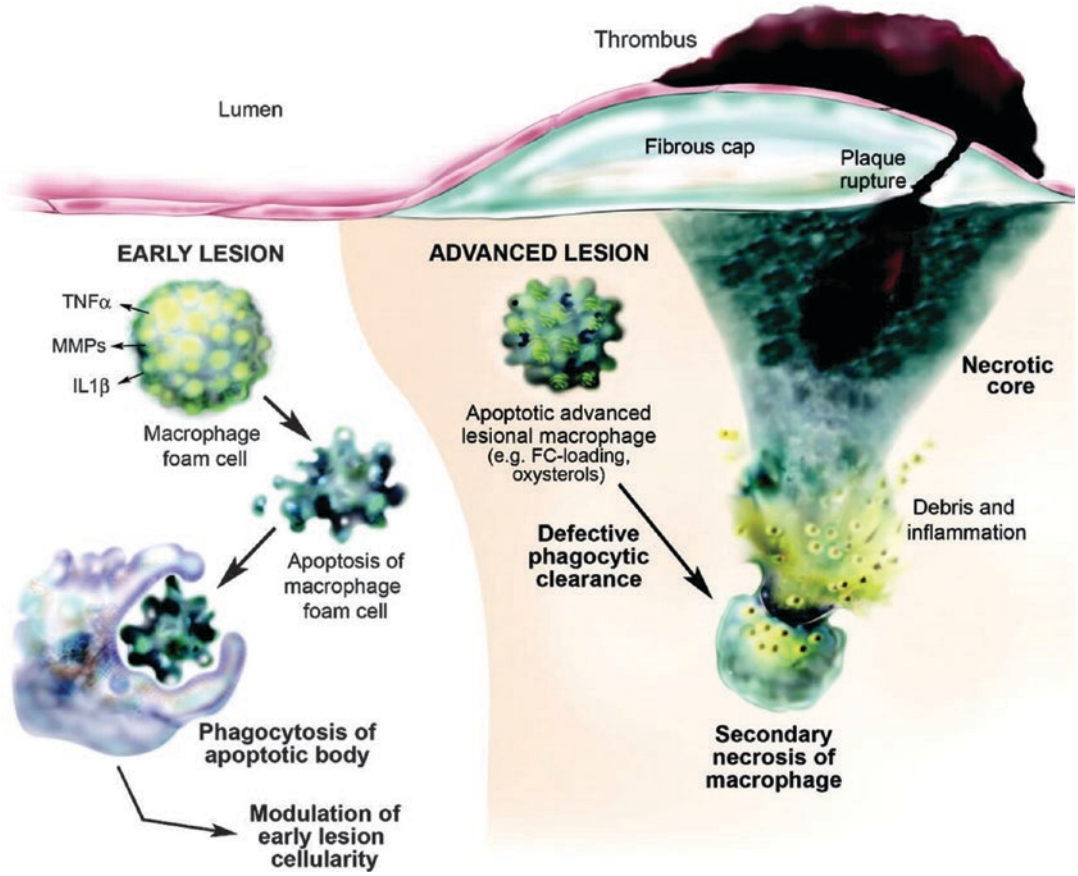

Fig. 2.10 The so-called "volcano" model of atherosclerotic plaque formation. In early atherosclerotic lesions (left), macrophage foam cells undergo apoptosis and are efficiently phagocytosed and cleared by other macrophages. This process controls lesion cellularity and rate of disease progression. However, in later lesions (right), apoptotic macrophages are not engulfed and cleared as efficiently resulting in a net accumulation of apoptotic and necrotic macrophages with the generation of a necrotic core. This leads to the mounting of an inflammatory response which can lead to plaque instability and eventual rupture. (From Tabas [133]. Reproduced with permission)

ticity, as evidenced by the observation that multiple therapeutic interventions can induce plaque regression in target lesions [136–138].

The phagocytosis of apoptotic cells and apoptotic bodies is tightly orchestrated. Apoptotic cells express a variety of "find me" (e.g., lysophosphatidylcholine, sphingosine-1-phosphate, the fractalkine CX3CL1, and adenosine 5′-triphosphate and uridine-5′-triphosphate) and "eat me" (e.g., phosphatidylserine, altered ICAM-1 epitopes on the cell surface, increased calreticulin exposure) molecules that promote phagocytic cell attraction and migration, target cell discovery, and engulfment/clearance [139, 140]. Apoptotic neutrophils express neutrophil-borne pentraxin-3 which promotes their recognition and removal by macrophages [141]. Lactadherin functions as a coupling molecule that facilitates the binding of apoptotic cell phosphatidylserine to vitronectin on phagocytic macrophages [142]. It is possible that deficiencies in these molecules may lead to impaired apoptotic cell clearance. An example of this is a deficiency in the receptor tyrosine-protein kinase MER which is associated with rapid progression and enlargement of the necrotic core in experimentally induced plaques [143].

As an atheromatous plaque evolves, the arterial wall reorganizes in a way that maintains luminal diameter and blood flow [144], a process known as positive or "Glagovian" remodel-

ing [145]. Plaque initially develops in an outward direction, producing vessel wall ectasia. It is only in the later stages of atheromatous plaque evolution that there develop progressive luminal obstruction and, ultimately, physiologically significant reductions in blood flow and oxygen delivery. Within the plaque, cellular necrosis promotes increased inflammation which accelerates atherogenesis and destabilizes plaques [146, 147]. As an illustration of just how important inflammation is in atherosclerosis, suppressing inflammation in humans with a monoclonal antibody directed against IL-1β results in a reduction of acute cardiovascular events independent of any change in serum lipoprotein levels [148, 149].

Maintaining the architectural stability of a plaque is essential to preventing acute cardiovascular events. Unstable plaques typically have large lipid cores, high inflammatory tone (characterized by increased macrophage density and increased inflammatory mediator expression), and reduced smooth muscle cell density [150]. In contrast, stable plaques are characterized by increased smooth muscle cell density, low inflammatory tone, small macrophage infiltrates, and a small lipid core. Calcification of plaque also tends to render it more stable [151, 152].

Superficial surface erosions, plaque ulceration, and frank plaque rupture expose the lipid core to blood [147, 153–156]. This exposed lipids as well as tissue factors and collagen promote platelet degranulation and aggregation, resulting in the propagation of an overlying thrombus [157]. If the thrombus completely occludes the arterial lumen, the patient experiences acute tissue ischemia. A thin fibrous cap provides less structural and tensile strength opposing plaque fracture and opening in response to a sudden stressor, such as vasospasm or hemorrhaging into the base of a plaque from injured or leaky *vasa vasora*. Hemorrhaging into the base of a plaque is an important cause of atheromatous plaque rupture. A sudden rise in the volume of a plaque can lead to the loss of architectural integrity. In addition, repetitive low volume hemorrhages into the base of a plaque secondary to leaky *vasa vasora* can lead to cumulative trauma, increased entry of leukocytes, and increased deposition of cholesterol and other lipids in the core of the plaque [158]. As erythrocytes are cleared from the plaque's interior, cholesterol from cell membranes is left behind and functions as a substrate for expansion of the plaque's lipid core (Fig. 2.11). Over time, this too can lead to plaque destabilization. The plaques that are least likely to rupture are the ones that are calcified and fibrotic.

In the statin era, it is apparent that the percentage of ACS secondary to plaque erosion rather than acute plaque rupture has been increasing [159]. Eroded plaques are described as having been denuded of endothelium and have increased neutrophils (and myeloperoxidase activity), decreased macrophage and T-cell constituents, small lipid cores, and large numbers of smooth muscle cells with dense proteoglycan and glycosaminoglycan intercellular matrix material [160–162].

A variety of coronary imaging studies suggest that culprit lesions giving rise to ACS have (1) large plaque volume, (2) large necrotic core, and (3) positive remodeling compared to plaques that remain stable [163]. Among patients suffering sudden death, more than 70% of ruptured plaques were characterized as having >75% luminal narrowing. In contrast, 5% of these cases were due to culprit lesions with <50% luminal narrowing [164]. Among patients with ST-segment elevating MI, the average luminal obstruction is 66% [165]. Typically, there is significant, rapid progression of plaque volume prior to its rupture, which can also be quite unpredictable [166]. Identifying vulnerable plaque that will eventually rupture or fissure remains a significant unsolved issue in contemporary cardiology [167].

A more recently elucidated mechanism by which plaque can rupture is from the formation of cholesterol crystals within the plaque. Recent investigation shows that cholesterol can crystallize within lesions as well as perforate the plaque surface, leading to core expansion, intimal injury, and plaque instability [168, 169] (Fig. 2.12). Oxidized LDL scavenged by the macrophage cell surface receptor CD36 correlates with cholesterol crystallization [170, 171]. Cholesterol crystals augment plaque inflammation by activating

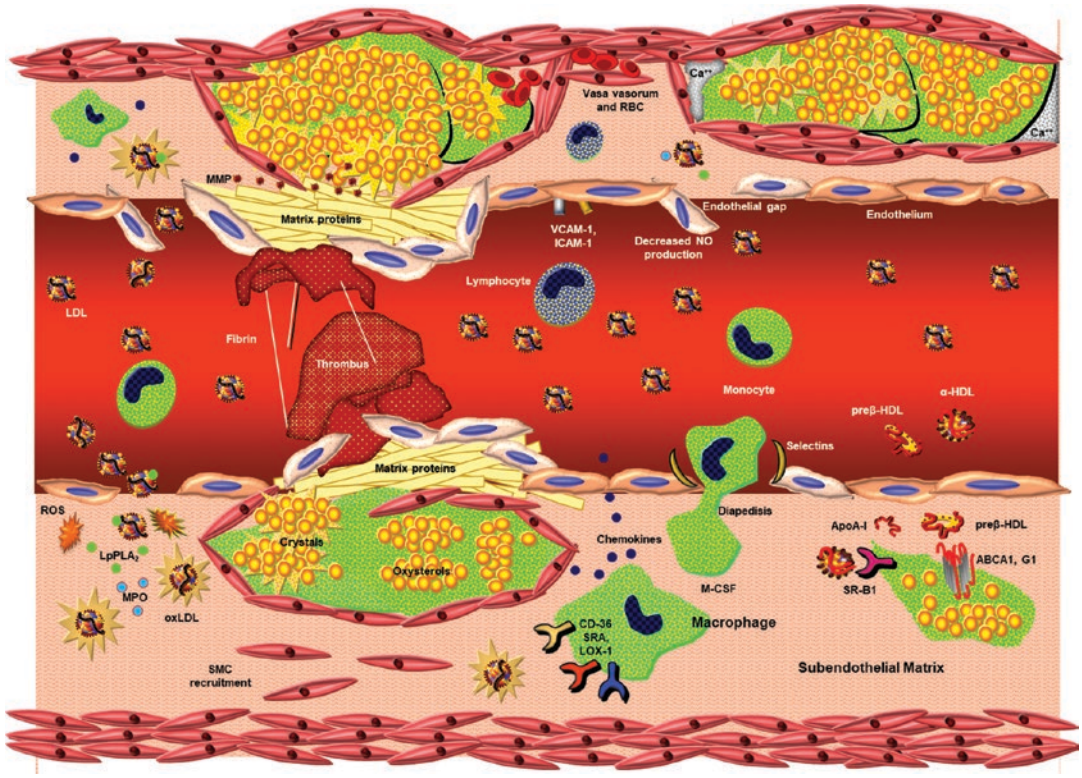

Fig. 2.11 Schematic of atherogenesis and atherosclerotic plaque rupture. *Lower right quadrant*. Monocyte in serum binds to selectins (ICAM-1, VCAM-1, selectin-P) on dysfunctional endothelium. The monocyte then reorganizes its actin cytoskeleton and traverses loosened gap junctions between endothelial cells in response to monocyte chemoattractant protein-1 (MCP). Once in the subendothelial space, it can secrete interleukins and cytokines to mount an inflammatory response. As the monocyte takes up residence, it converts to a macrophage, of which there are multiple populations. By expressing such cell surface receptors as CD36, scavenger receptor A (SRA), and lectin-like oxidized low-density lipoprotein receptor (LOX-1), macrophages scavenge oxidized low-density lipoproteins (LDL) and remnant lipoproteins. As intracellular cholesterol content increases, the macrophage becomes a progressively more lipid-enriched foam cell. In order to offload cholesterol, macrophages express the transmembrane cholesterol transport proteins ATP-binding membrane cassette transport proteins (ABC) A1 and G1, which can lipidate apoprotein A1 and spherical HDL particles, respectively. Early during atherogenesis, smooth muscle cells from the tunica media are recruited for transmigration into the intima. *Upper right quadrant*. Lymphocytes also bind to cell surface adhesion molecules and function as antigen presentation cells and a source of inflammatory mediators. LDL particles enter the subendothelial space by traversing dysfunctional endothelium. Foam cells coalesce to form fatty streaks, and as lipid and cellular debris increase in volume, an atheromatous plaque forms with a lipid core. As larger amounts of cellular debris accumulate that are no longer cleared by phagocytic macrophages, a necrotic core forms. *Upper left quadrant*. A mature atherosclerotic plaque can be rendered unstable by bleeding into the base of the plaque via disrupted vasa vasora coursing through the tunica adventitia. The sudden increase in blood volume at the base of the plaque raises intra-plaque pressure and can induce plaque rupture, exposing collagen and releasing adenosine 5′-diphosphate and calcium, all of which activate platelets, leading to the formation of overlying thrombus and arterial luminal occlusion. The surface of the plaque may be more prone to rupture because surface matrix proteins have been degraded by matrix metalloproteinases (MMP). *Lower left quadrant*. LDL particles can be oxidized by reactive oxygen species (ROS: superoxide anion, peroxynitrite, hydrogen peroxide) produced by myeloperoxidase (MPO) and a variety of lipoxygenases. Oxidized LDL particles are scavenged by macrophages. Lipoprotein-associated phospholipase A2 (LpPLA2) hydrolyzes phospholipids into lecithin and a free fatty acid, both of which promote inflammation. Scavenged cholesterol can be stored as either pools of oxysterol or as cholesterol crystals. Cholesterol crystals can pierce through plaque surface area and promote platelet activation and thrombus formation. As atherosclerotic plaque becomes more inflamed and less stable, it can rupture, also potentiating platelet activation and thrombus formation. (With permission from Dr. Thomas Dayspring)

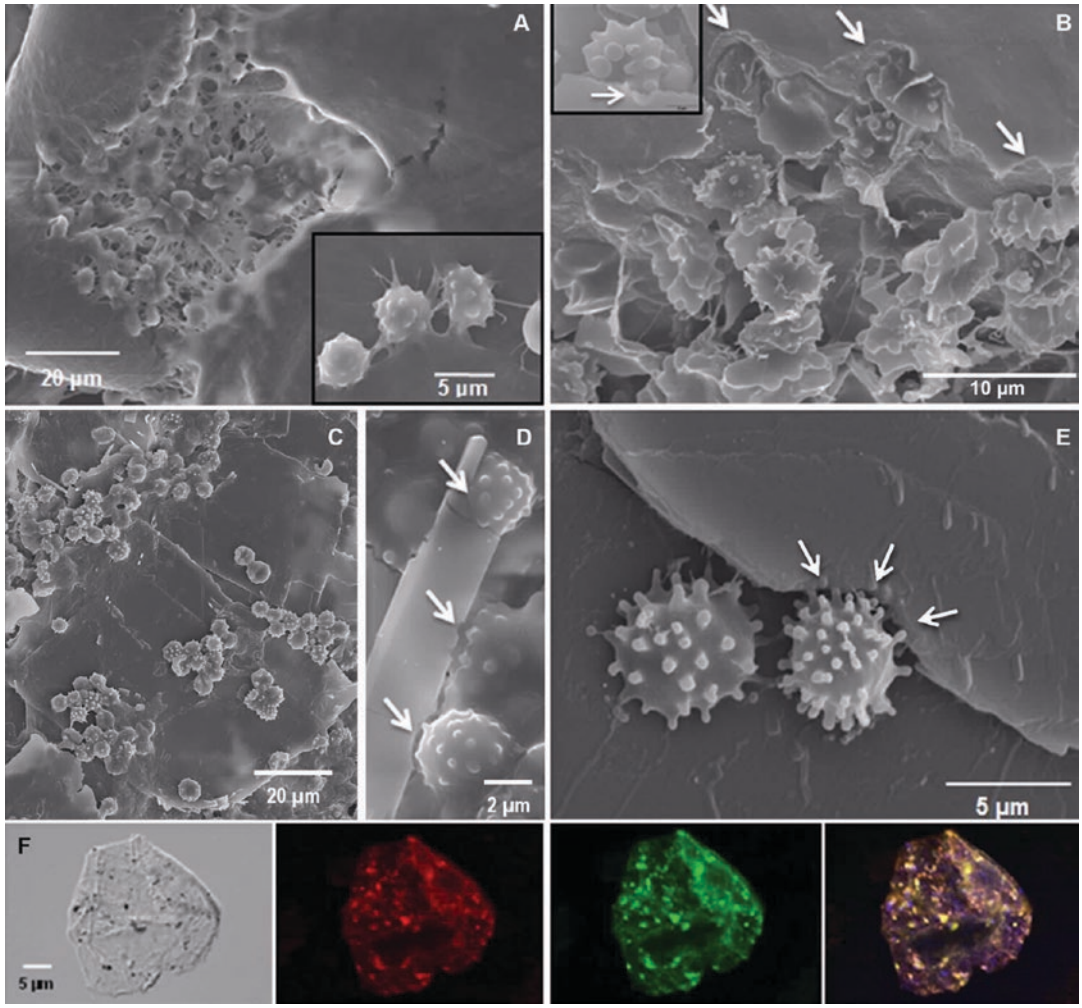

Fig. 2.12 Cholesterol crystals and atherosclerotic disease. Macrophages from coronary aspirates appear to be eroding cholesterol crystals. (**a**–**e**) Scanning electron micrographs demonstrate macrophages engaging cholesterol crystals with notched crystal matrix (arrows). Inserts demonstrate macrophage gummy attachment to the crystal edges and etching (arrow) of the crystal surface. (**f**) Confocal fluorescence microscopy de1monstrates cholesterol aggregates suggestive of crystalline cholesterol (yellow-green particles stained with cholesteryl Bodipy-C12) within the cytoplasm of aspirated macrophages. The orange-red fluorescence is a specific marker for macrophages. Cholesterol deposits can be detected in the cytoplasm using differential interference contrast (shown in gray) and fluorescence microscopy (red, green, and composite image). The unstained control did not exhibit fluorescence (not shown). (From Abela et al. [172]. With permission from Elsevier)

nucleotide-binding domain leucine-rich repeat-containing family, pyrin domain-containing 3 inflammasome that in turn stimulates IL-1β production [170]. Clusters of cholesterol crystals can also be released from culprit plaques during an acute MI and correlate with increased arterial narrowing and reduced reflow subsequent to percutaneous coronary intervention [172].

Conclusions

1. Atherosclerosis is an arterial disease of enormous complexity, whose trajectory is determined by a plethora of genetic and environmental determinants.
2. Atherogenesis encompasses every histologic component in all layers of the arterial

wall (endothelium, intima, media, and adventitia).

3. Atherosclerosis is not simply a process of passive accumulation of apo B-containing lipoproteins in the subendothelial space over time; lipid accumulation and plaque formation are the end result of a highly orchestrated and tightly synchronized network of interlacing pathways involving inflammation, oxidation, and reorganization.
4. Endothelial dysfunction is an early transition point in atherogenesis and is a response to the toxic effects of dyslipidemia, hypertension, smoke exposure, impaired glycemic control, insulin resistance, and a myriad of other risk factors.
5. Endothelial dysfunction is associated with adhesion molecule expression, reduced nitric oxide production, a more thrombogenic surface, and reduced gap junction function.
6. An atherogenic milieu is characterized by heightened oxidative and inflammatory tone; an influx of inflammatory white blood cells, alterations in intravascular cell migration patterns, and an expansion of the vasa vasorum.
7. As plaque evolved and becomes more complex, it can become unstable due to a variety of architectural alterations, among them most notably thinning of the fibrous cap, surface erosions, and leaky adventitial vasa vasora.
8. Acute coronary syndromes are the result of plaque rupture with the formation of overlying thrombus, leading to arterial luminal obstruction, ischemia, and tissue necrosis.
9. There is evidence that at least some atherosclerotic plaques can be reversed, though it is not clear if the arterial wall can be "healed" once a plaque has formed.
10. Despite the fact that we still have much to learn about this disease, using such pharmacologic agents as statins, inhibitors of the renin-angiotensin-aldosterone axis, aspirin, eicosapentaenoic acid, P2Y12 inhibitors, and some antiglycemic agents have all been shown to beneficially impact the course of this dreaded and highly prevalent disease and, most importantly, reduce risk for acute cardiovascular events.

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3 Genetic Disorders of Lipoprotein Metabolism

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Diseases Affecting Primarily Low-Density Lipoprotein

Familial Hypercholesterolemia

Introduction

Familial hypercholesterolemia (FH) is a disorder encompassing a group of genetic defects resulting in severely elevated serum cholesterol concentrations. It is a commonly inherited condition characterized by abnormal regulation of cholesterol metabolism causing severely elevated plasma levels of low-density lipoprotein cholesterol (LDL-C) [1, 2]. Although FH was specifically attributed to mutations of the LDL receptor (LDLR) in the past, the definition has been expanded to include gain-of-function mutations in proprotein convertase subtilisin kexin 9 gene (*PCSK9*), as well as mutations in the apolipoprotein (Apo) B gene (*APOB*) (Fig. 3.1) and the more recently described LDLR adaptor protein 1 gene (*LDLRAP1*) [3–5]. In the Fredrickson classification, most patients will fall under type IIa, with predominantly elevated LDL-C levels and normal triglycerides (TGs); other forms such as type IIb have been described less frequently [6].

Prevalence

FH is the most common autosomal dominant genetic disorder, recognizing that non-dominant forms do exist, but are rare. The prevalence in the general population is between 1:200 and 1:500 depending on which criteria are used for the diagnosis [1, 7, 8]. Certain populations, such as French Canadian, Dutch Afrikaner, Ashkenazi Jewish, and Lebanese Christian, have a preva-

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_3

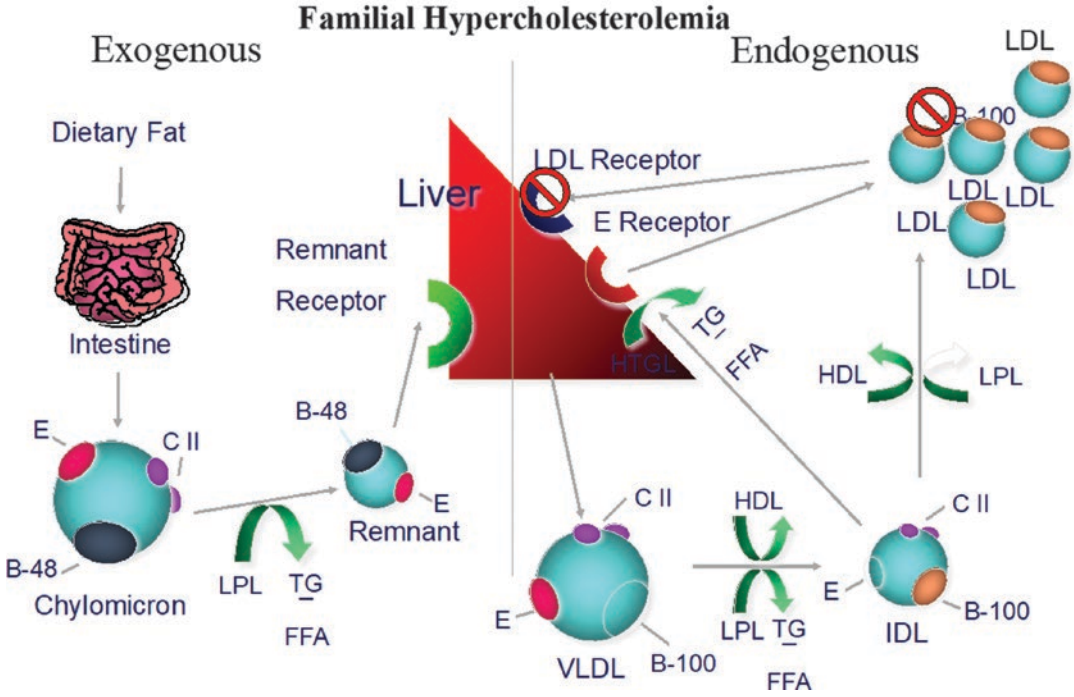

Fig. 3.1 Familial hypercholesterolemia is most commonly due to a mutation in the gene for the LDL receptor, causing receptor dysfunction. More rarely, a mutation in ApoB can cause the disorder due to poor binding affecting of LDL to the LDL receptor. A third etiology, a gain-of-function mutation in the gene for PCSK9 can lead to receptor destruction, which is extremely rare. All three etiologies lead to inability to clear LDL from the circulation and marked elevation of LDL levels

lence of 1:100 or more [9–11]. In the United States, an estimated 600,000–1000,000 individuals are affected. Among patients with premature coronary artery disease (CAD), the prevalence is much higher, reaching 5%, and increases as the age of the first cardiovascular (CV) event decreases [12–14].

Among diagnosed individuals, around 80% carry an identifiable mutation for specific genes. They comprise 85–90% affecting *LDLR*, 5–10% affecting *APOB*, less than 5% affecting PCSK9, and less than 1% affecting *LDLRAP1* [15–22]. FH without an identifiable mutation comprises 20% of all cases [23].The majority is autosomal dominant in transmission, and most patients carry the heterozygous form, with LDL-C levels in the range of 350–550 mg/dL. The homozygous form is extremely rare, occurring in approximately 1 out of a million individuals. It is also much more severe, with LDL-C levels ranging between 650 and 1000 mg/dL [7, 24]. All forms of FH are associated with a significantly elevated risk of premature CAD (around 20-fold in untreated cases), but in homozygous mutations that risk is exceedingly high. In general, "compound homozygous FH" (individuals carrying a different mutation on each allele) may have less risk than true homozygous FH where both alleles carry the same mutation [25–27].Studies suggest that FH is underdiagnosed in up to 80% of cases, and even confirmed cases are frequently undertreated. In the cases of homozygous children, a delay in treatment can be catastrophic; the therapeutic window for intervention may be missed before an individual develops their first CV event [15, 25].

Genetics

LDLR:

The LDLR is responsible for removing LDL-C from the plasma circulation. This process requires synthesis of adequate amounts of protein followed by appropriate transport to the Golgi appa-

ratus, with subsequent expression on the cell surface. The LDLR protein needs to have the capacity to interact with LDL-C (through ApoB receptors), and internalize with the bound particles before being recycled back to the hepatocyte surface in order to maintain adequate LDL-C clearance [28].

The gene for LDLR resides on the short arm of chromosome 19. Any genetic defect affecting the quantity or functional properties of the LDLR protein can consequently lead to significantly increased levels of plasma LDL-C. Genetic defects may result from nonsense, missense, insertion, deletion, or splicing mutations [29]. In total, there are more than 3000 recognized variants of the *LDLR* gene, with over 1600 pathogenic mutations identified to cause FH [30–32]. Phenotypically, allele mutations, and their resultant protein abnormality, can be divided into 5 classes [33–35].

- Class I mutations, also known as "null mutations," result in complete lack of LDLR synthesis.
- Class II mutations give rise to transport abnormalities with partially or completely retained protein within the endoplasmic reticulum.
- Class III mutations are associated with defective binding and consequent inability to interact with ApoB100 on the LDL surface.
- Class IV mutations do not permit proper endocytosis, interfering with LDL-LDLR complex internalization.
- Class V mutations produce proteins with defective recycling.

ApoB (Familial Defective APOB):
The ApoB100 protein is normally expressed on the surface of LDL and serves as a ligand for LDLR. Pathogenic mutations of the *APOB* gene can result in a defective ApoB100 protein, interfering with LDL particle-receptor interaction, and effectively decreasing LDL-C clearance [15, 18, 36]. The Arg3500Gln mutation is the most common cause of FH associated with defective ApoB and is frequently seen in northern European populations. Other pathogenic variants exist as well, and *ApoB* mutations are considered the second most common after *LDLR*, accounting for 5–10% of the cases [17]. Typically, LDL particle levels increase by two- or three-fold, as opposed to more significant elevations seen in *LDLR* mutations.

PCSK9:
PCSK9 is a serine protease, which gained much recognition in the early 2000s. It binds LDLR and forms an LDLR-PCSK9 complex that undergoes endocytosis followed by destruction within hepatocytes. This process does not allow for LDLR recycling and leads to diminished availability of active LDLR, thus causing increased levels of circulating LDL particles [21].

PCSK9 variants can be associated with loss-of-function or gain-of-function mutations, with the latter resulting in more active protein [20, 37]. Thus far, more than 30 variants of gain-of-function mutations have been identified, with associated FH as a consequence [37]. On the other hand, loss of function is more commonly encountered in the general population with important clinical effects. Less active PCSK9 protein allows for more available LDLR, and subsequently more clearance of LDL particles [38]. This implication has been utilized clinically with the advent of a new class of medication (PCSK9 inhibitors) that is currently used in the treatment of FH. Collectively, FH resulting from *PCSK9* gain-of-function mutations are not very common and represent less than 5% of all documented FH cases in many studies [16].

LDLRAP1:
Since the 2011 National Lipid Association Expert Panel Executive Summary, newer genetic mutations have been identified within the *LDLRAP1* sequence that affect the LDL-LDLR interaction. LDLRAP1 protein mediates LDL-LDLR complex internalization by allowing for proper clathrin-coated endosome formation. The end result of dysfunctional endocytosis is limited clearance of LDL particles and increased plasma levels [5]. This entity forms the fourth recognized defect to cause FH and differs by being an autosomal recessive disorder requiring the presence of two abnormal alleles before FH is manifested phenotypically. Overall, it accounts for less than 1% of all cases [4].

Pathophysiology

All forms of FH are associated with elevated LDL-C levels in plasma. As described previously, this may be the result of decreased LDLR quantity or function, decreased ApoB activity, increased LDLR destruction secondary to increased PCSK9 activity or through impaired LDL-LDLR complex internalization. Elevated levels of LDL-C are strongly connected to the development of early atherosclerosis, which may manifest as CAD, stroke, or peripheral vascular disease (PVD) [39–42].The mechanism of atherosclerosis itself is complex, as described elsewhere in this textbook.

Screening

FH is a relatively common disorder, with severe consequences for affected patients if left untreated. Therefore, all clinicians, especially primary healthcare providers and cardiologists, should be aware of the recommended screening guidelines put in place to increase detection in susceptible individuals. It also follows that affected patients should undergo further risk stratification and be started on appropriate therapy as soon as possible.

Universal screening at age 9–11 y with a fasting lipid profile or non-fasting non-high-density lipoprotein cholesterol (non-HDL-C) measurement is recommended in all children [43]. If a non-fasting non-HDL-C concentration of >145 mg/dL is detected, then a follow-up fasting lipid profile should be obtained. If a positive family history for hypercholesterolemia or premature CAD exists, then screening should be performed at an even younger age, beginning at age 2. Collectively, all individuals should be screened by age 20 [44].

FH should be suspected in children, adolescents, and young adults less than 20 y with an untreated fasting LDL-C ≥160 mg/dL or non-HDL-C ≥190 mg/dL. The same applies for adults older than 20 y of age, but the threshold is LDL-C ≥190 mg/dL or non-HDL-C ≥220 mg/dL. The probability of FH increases incrementally as LDL-C levels rise and is approximately 80% if LDL-C level is greater than 190 mg/dL under age 20, greater than 220 mg/dL at age 20–29, or greater than 250 mg/dL at age 30 or more [45].

Certain physical examination findings should also prompt consideration for FH. These include tendon xanthomas at any age, particularly on the Achilles tendon and finger extensor tendons, as well as arcus corneae at <45 y, and tuberous xanthomas or xanthelasma at <25 y (Fig. 3.2a, b, and c). Despite the limited sensitivity associated with these physical findings, they should always prompt an initial screening with a fasting lipid profile whenever identified in the clinical setting [46].

In all of the aforementioned patients, once suspicion of FH is established, further history should be obtained regarding any family members (particularly first-degree relatives) with a positive history for premature CAD or carrying a diagnosis of dyslipidemia. Secondary causes of dyslipidemia should also be ruled out, such as hypothyroidism, liver failure, or nephrotic syndrome, while concurrently searching for a diagnosis of FH [47].

Diagnosis

Initial evaluation should begin with a thorough history. Any symptoms of atherosclerotic cardiovascular disease (ASCVD) such as chest pain, peripheral claudication, or exertional limitations should be explored. Risk factors such as hypertension, smoking, and diabetes mellitus should be noted, and the patient should be questioned regarding any history of CV events. Additionally, family history including the first age of onset for CAD in first-degree relatives must be established.

The physical examination findings associated with FH are insensitive and cannot be used to reliably rule out the diagnosis. However, they are fairly specific and should be carefully looked for during clinical evaluation. Tendon xanthomas occur in <50% of FH cases, but are essentially pathognomonic for the disorder. They present as solid subcutaneous nodules with firm overlying skin. This is most commonly seen on the Achilles tendon, but requires careful longitudinal palpation across the posterior aspect of the tendon in order to be appreciated. They may also form on digital extensor tendons and are seen less frequently within patellar and triceps

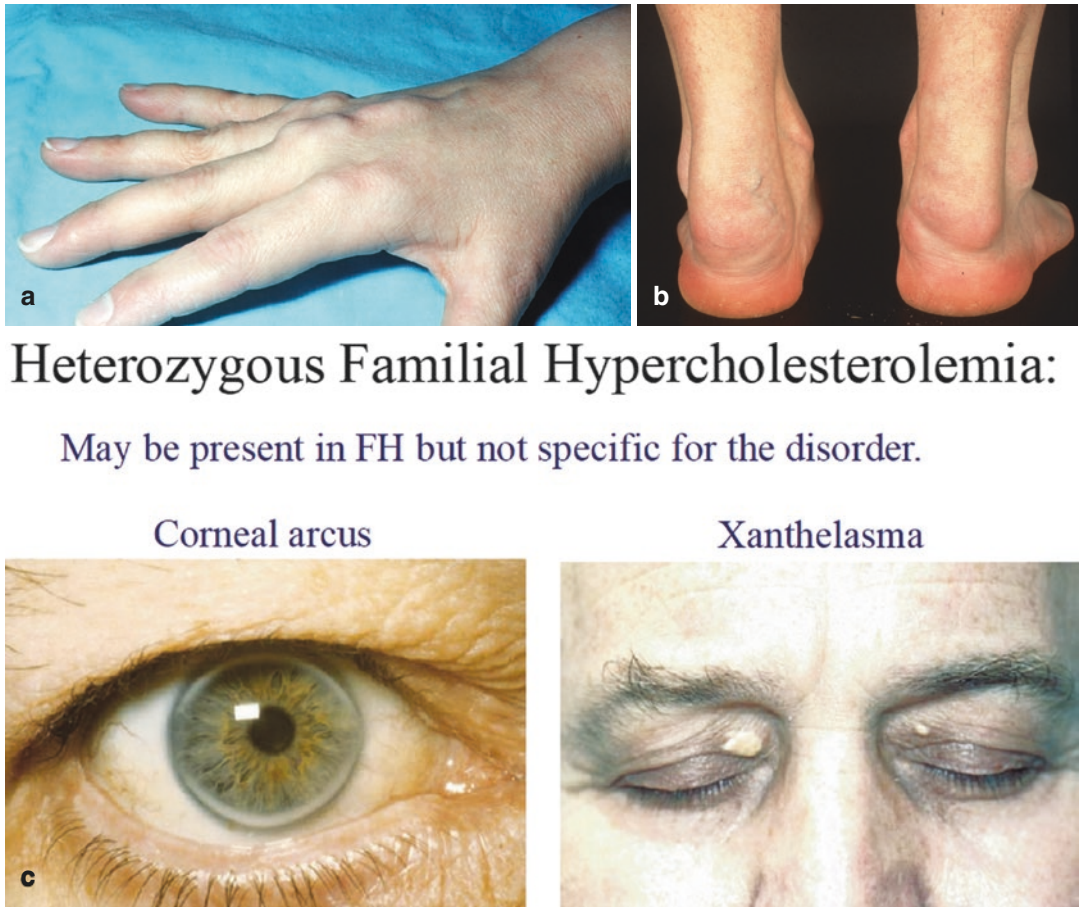

Fig. 3.2 (**a**) Extensor tendon xanthomata of the hand seen in a patient with heterozygous FH. (**b**) Achilles tendon xanthomata in a patient with heterozygous FH. (Courtesy of Dr. Michael H. Davidson, University of Chicago. (**c**) Corneal arcus and xanthelasma in a patient with heterozygous FH. Courtesy of Dr. Jean Davignon, University of Montreal)

tendons [46, 48]. Tendon xanthomas should be differentiated from tuberous xanthomas, which can be seen in other dyslipidemias such as sitosterolemia and cerebrotendinous xanthomatosis [49]. The latter only become specific for FH if they occur in patients younger than age 25. Similarly, xanthelasmas are cholesterol-packed yellow plaque that usually occur on the medial aspects of the eyelids that may occur with FH in the second decade of life. Another manifestation is corneal arcus, which is formed from the deposition of lipid in the peripheral corneal stroma and is frequently seen with advanced age. However, it becomes very suggestive of FH when encountered at <45 y of age.

The FH lipid profile usually shows elevated cholesterol, with high LDL-C. TGs are typically normal, but an elevated level does not rule out FH, as multiple other factors, such as diabetes mellitus or obesity, can induce hypertriglyceridemia [47]. LDL-C levels are typically very high (>190 mg/dL), but may be lower in certain individuals and can be many-fold higher in homozygous FH [50]. More importantly, elevated LDL-C should be correlated with familial history, since only 4% of individuals with LDL-C level above the 95th percentile will have true FH. The remainder suffers from polygenic influences, dietary variations, and other random factors that raise their LDL-C levels and can be mistaken for true

FH [51]. In general, LDL-C levels are expected to be two-fold higher in affected individuals within the same family as compared with non-affected members and somewhat lower LDL-C cutoff limits can be applied to family members of affected patients than the general population. Patients carrying a homozygous mutation will have LDL-C levels higher than 300 mg/dL or even 500 mg/dL. They may demonstrate physical manifestations such as tendon xanthomas within the first decade of life and suffer from premature ASCVD within the second decade of life [7, 25]. Both parents would typically carry a heterozygous mutation, though more often it is two different mutant alleles that get passed to the patient producing "compound homozygous" FH [50].

Multiple clinical definitions of FH have been proposed, but there are three that are commonly used. These include the Dutch Lipid Clinic Network Diagnostic Criteria for FH (see Table 3.1), Simon Broome Register Diagnostic Criteria for FH, and US Make Early Diagnosis Prevent Early Death [52, 53]. All definitions share a requirement for elevated LDL-C levels (with varying cutoff levels) and strong emphasis on familial history of dyslipidemia or premature ASCVD. Clinical parameters such as premature CAD affecting the patient or pathognomonic physical examination findings such as tendon xanthomas also carry significant weight. Once clinically defined, identification of an abnormal mutation affecting *LDLR*, *APOB*, *PCSK9* or *LDLRAP1* genes may be performed to confirm the diagnosis, but is often not necessary. Ordinarily, an FH diagnosis can be made without a positive genetic mutation.

Once a proband is identified, then cascade screening is recommended [54]. This starts by obtaining lipid levels in all first-degree relatives. Since most genetic mutations causing FH are transmitted in an autosomal dominant fashion, 50% of all first-degree relatives are expected to be positive. Cascade screening should then proceed by expanding the screened cohort to involve all first-degree relatives of newly diagnosed individuals, and so forth. If a genetic mutation is identified, then genetic testing can facilitate screening. Overall, cascade screening is considered the most cost-effective method of diagnosing new cases of FH and can directly lower mortality with provision of early therapy [55].

Table 3.1 Clinical Definitions of FH

Dutch lipid clinic network diagnostic criteria	Points
Family history	
Family history of premature[a] ASCVD (or)	1
First-degree relative with LDL-C >95th percentile for age and sex in an adult	
First-degree relative with LDL-C >95th percentile for age and sex age at age <18 (or)	2
First-degree relative with tendon xanthoma or corneal arcus	
Patient clinical manifestations	
Premature[a] CAD	2
Premature[a] PVD and/or CVA	1
Tendon xanthoma	6
Corneal arcus at age <45 y	
LDL-C level	
LDL-C ≥330 mg/dL	8
LDL-C ≥250 and <329 mg/dL	5
LDL-C ≥190 and <249 mg/dL	3
LDL-C ≥150 and <219 mg/dL	1
DNA	
Functional mutation in LDLR, APOB, PCSK9 gene	8
Diagnosis	
Definite FH	>8
Probable FH	6–8
Possible FH	3–5
Unlikely FH	<3

[a]Premature ASCVD (Male <55 or Female <60 y)
DL-C Low-density lipoprotein cholesterol, *FH* Familial Hypercholesterolemia, *ASCVD* Atherosclerotic cardiovascular disease, *LDLR* Low-density lipoprotein receptor, *APOB* apolipoprotein B, *PCSK 9* Proprotein convertase subtilisin/kexin type 9

Treatments

Patients with FH are at increased risk for all forms of ASCVD, including premature CAD, PVD, and stroke with presentations at a younger age than the general population [10]. The mean age of onset for the first CV event in patients with FH is before 45 y for men and before 55 y for women [1]. However, even before age 40, FH is associated with increased risk for premature CAD estimated at more than 20-fold the general population, and homozygous individuals can develop an ASCVD event before age 20. Consequently, all patients carrying a diagnosis

of FH will require medical management beginning with lifestyle modification, and the vast majority will also require drug therapy [56]. If treatment is started early, the risk of premature ASCVD can be substantially reduced and may even equal that of the general population, but will require lifelong regular follow-up [57].

All individuals with FH should be advised regarding lifestyle modification and dietary adjuncts. Dietary modifications include reduced intakes of saturated fats and cholesterol (total fat 25–35% of energy intake, saturated fatty acids <7% of energy intake, dietary cholesterol <200 mg/d), use of soluble fiber 10–20 g/d, and use of plant sterols. Weight loss to target body-mass index (BMI) 18–25 kg/m^2, as well as frequent aerobic exercise should be recommended. All patients should be strongly counseled to stop smoking and limit alcohol intake. Additionally, physicians should aggressively pursue risk factor modification to target blood pressure <130/80 mm Hg and optimal glycemic control in diabetic patients [58, 59].

If after lifestyle modification, LDL-C levels remain ≥190 mg/dL or non-HDL-C ≥220 mg/dL in children or adults, then drug therapy should be initiated. The use of 10-y Pooled Cohort Equation risk calculators has not been validated in FH and is not recommended. In fact, even though elevated LDL-C levels are innately associated with increased risk, individuals with FH have an even higher risk of CV events at any given LDL-C level as compared to the general population [60].

Medications:
Moderate to high intensity statins should be started as first-line agents for most FH patients, with a target LDL-C reduction of >50%. If LDL-C remains ≥160 mg/dL, or an LDL-C reduction of at least 50% is not achieved, then further intensification of therapy and referral to a lipid specialist should be considered [59, 61]. Further intensification of therapy should also be pursued if the following risk factors are present: clinical evidence of ASCVD, family history of premature CAD (male <45 y, or female <55 y), current smoking, lipoprotein (a) [Lp(a)] ≥50 mg/dL using an isoform insensitive assay, or the presence of two or more traditional ASCVD risk factors other than smoking, such as diabetes mellitus and hypertension. In high-risk individuals, the target is LDL-C <100 mg/dL or non-HDL-C <130 mg/dL [62].

Most heterozygous FH patients will have 50% functional LDLR, and even some homozygous mutations allow for residual function of LDLR protein. Thus, statins are effective as initial therapy since they act through LDLR upregulation on hepatocytes by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis (as described elsewhere in this book). Despite the lack of prospective placebo-controlled trials specifically studying statin use in FH, large observational studies in Europe support statin efficacy in this subpopulation [63]. For instance, a retrospective analysis of the U.K. Simon Broome database showed ASCVD risk in FH patients who used statins to be similar to that of the general population [53]. Low intensity statins, such as fluvastatin and lower dose pravastatin, reduce LDL-C levels by no more than 30% and are considered inappropriate for most FH patients. Moderate intensity statins can reduce LDL-C by 30–50%, while high intensity statins, such as atorvastatin and rosuvastatin, can reduce LDL-C by up to 60%. Meta-analysis also showed that high intensity statins reduce events more than moderate intensity statins regardless of LDL-C levels, making them ideal as a first agent for the treatment of FH [58, 64].

Numerous studies suggest that statins are safe and well tolerated even in children, with only occasional side effects reported such as myopathy or liver enzyme elevations [65, 66]. Baseline liver function testing is recommended in all patients, but routine testing of liver function and creatine kinase is also recommended in children and adolescents, while not necessary in asymptomatic adults [67]. If patients develop symptoms suggestive of myopathy or liver involvement, drug use should be discontinued, and liver function as well as creatine kinase levels should be checked. If patients cannot tolerate one statin, then it is recommended to try a different one or change the dosing to every-other-day to assess for tolerability before completely abandoning statin therapy. Finally, if the target LDL-C is not achieved despite use of

maximally tolerated statin dose, then guidelines recommend combination therapy as the next step with PCSK9 inhibitors, ezetimibe, niacin, or a bile acid sequestrant [10, 68].

Ezetimibe is a cholesterol absorption inhibitor that has been studied in combination with statins and showed clinical efficacy and safety. In the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) trial, patients were followed up for 9 y on combination ezetimibe-simvastatin vs. simvastatin monotherapy. The results showed incremental lowering of LDL-C and improved CV outcomes without a significant mortality benefit. Ezetimibe produces an LDL-C reduction of 15–20% when given alone, or in combination, and has relatively few reported side effects [69]. It may be added to multiple lipid-lowering drugs or used as a first-line agent for patients who fail statin therapy before escalation to PCSK9 inhibitor use, though it is almost never sufficient as monotherapy for management of FH [70, 71].

Recently, the combination therapy of PCSK9 inhibitors and statins has been strongly advocated after the landmark Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) and ODYSSEY Outcomes (Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome) trials showed that PCSK9 inhibitors can produce significant LDL-C reduction and decreased major adverse CV events when added on a background of statin therapy [72, 73]. The addition of evolocumab to statin therapy resulted in LDL-C reduction by 59% after 48 weeks, whereas the LDL-C reduction was 55% for alirocumab after a median follow-up of 2.8 y. Both medications were also able to achieve a meaningful reduction in CV events.

In the FOURIER trial, evolocumab addition to statin therapy significantly reduced the risk of the primary end point of major CV events as compared to patients receiving only statin therapy (9.8% vs. 11.3%; hazard ratio 0.85; 95% confidence interval [CI] 0.79 to 0.92; $p < 0.001$) [72]. Similarly, in the ODYSSEY Outcomes trial that followed patients who had an acute coronary event within the last year, alirocumab was associated with reduced all-cause mortality (3.5% vs. 4.1%; $p = 0.026$) and ischemia-driven coronary revascularization (7.7% vs. 8.8%; $p = 0.009$) in addition to meeting the primary endpoint of reduced major adverse CV event (9.5% vs. 11.1%; $p < 0.001$) [73].

Bile acid sequestrants (colesevelam, cholestyramine, and colestipol) are now used less frequently in the advent of PCSK9 inhibitors, but remain a viable alternative as third-line therapy should LDL-C target not be met. They exert their effects through binding intestinal bile salts and interfering with enterohepatic recirculation. This can effectively lower LDL-C levels by 10–20%, but their use is associated with significant gastrointestinal side effects such as constipation, as well as drug-drug interactions [74]. In general, colesevelam has more tolerability, with fewer gastrointestinal side effects and drug interactions. It is also the only bile acid sequestrant with a pediatric indication (for boys and postmenarchal girls, age 10–17) and can be used as monotherapy or combination therapy with statins [75]. It is also approved by the Food and Drug Administration (FDA) for treatment of diabetes mellitus. Therefore, colesevelam is the bile acid sequestrant of choice for FH patients.

Fibric acid derivatives (gemfibrozil, fenofibric acid, and fenofibrate) are used primarily to lower TGs and may have the unintended side effect of increasing LDL-C. They also interact with statins, and particularly gemfibrozil-statin combination use has been associated with increased risk for myopathy [76]. Consequently, they are not routinely used in the treatment of FH, though fenofibric acid is approved by the FDA for use with low- or intermediate-intensity statins.

Lastly, extended-release niacin, dosing not to exceed 2 g/d, may be added to statin therapy for additional LDL-C reduction [77]. Use is, however, limited by side effects such as worsened glycemic control, gout, flushing or hot flashes, and potential for liver toxicity.

LDL Apheresis:

LDL apheresis is recommended for patients who have ongoing symptomatic disease, or who do not reach target LDL-C after 6 months on maxi-

mal medical therapy. Additionally, for any patient population, non-HDL-C levels more than 30 mg/dL above the set LDL-C targets are considered an indication for LDL apheresis. A single session can remove at least 60% of all ApoB-containing lipoproteins, but it needs to be repeated every 1–2 weeks [78]. Over the long-term, apheresis has been shown to reduce LDL-C levels by 20–40% [79]. It is also the only treatment currently available that has been proven to decrease Lp(a) levels by more than 50% [80]. All patients with an indication for apheresis should be referred to a lipid specialist. An adequate response is defined as LDL-C <300 mg/dL for homozygous FH, LDL-C <300 mg/dL for heterozygous FH with 0–1 risk factors, LDL-C <200 mg/dL for heterozygous FH with ≥2 risk factors or Lp(a) ≥50 mg/dL, and LDL-C <160 mg/dL for heterozygous FH with very high risk such as established ASCVD.

Autosomal Recessive Hypercholesterolemia

Autosomal recessive hypercholesterolemia is an extremely rare disorder. It is caused by the inheritance of mutations affecting both alleles for the gene responsible for LDLRAP1. This protein is responsible for endocytosis of LDLR and binds LDL into hepatocytes and anchors the base of the receptor to the clathrin-coated pit [22]. The disorder is distinguishable from FH in that, since it is a recessive trait, the parents do not exhibit the phenotype of heterozygous FH. These patients often present with severe hypercholesterolemia, with LDL-C levels often greater than 500 mg/dL, suggestive of a phenotype more like homozygous FH. The disorder is difficult to treat and often poorly responsive to traditional lipid-lowering therapy [81].

Hereditary Sitosterolemia

Hereditary sitosterolemia is a rare autosomal recessive disorder that clinically is sometimes confused with heterozygous FH. The reason for this confusion is that the disorder presents with physical findings that include Achilles tendon xanthomas as well as xanthomas on the extensor tendons of the hands. LDL-C levels, however, are often only mildly to moderately elevated and usually do not reach the markedly elevated levels seen with FH. Hereditary beta sitosterolemia is due to mutations into alleles affecting either the ATP-binding cassette subfamily G member 5 (ABCG5) or ABCG8 transporter proteins, which are expressed in the intestine and liver [82]. These proteins transport plant sterols out of the intestinal cells and back into the gut lumen as well as transport potentially toxic plant sterols out of the hepatocyte and into bile. In patients with this disorder, plant sterols cannot be transported out of intestinal cells and hepatocytes; thus, they are incorporated into lipid particles causing high levels of serum phytosterols leading to increased atherosclerosis. The disorder is associated with a significantly elevated risk for premature CAD. Current pharmacologic treatment includes ezetimibe, which blocks absorption of plant sterols through its effect on inhibiting the Niemann-Pick C1-Like 1 (NPC1L1) transport protein [83].

Take-home points and differential diagnosis for elevated LDL syndromes:

- **Heterozygous FH** is an extremely common disorder associated with a markedly elevated risk for premature coronary atherosclerosis. Three different mutations can be responsible for the phenotype, but by far the most common is a mutation in the gene for LDLR. More rarely, a mutation in the gene for ApoB can present with the same phenotype, as can gain-of-function mutations for the PCSK9 gene, which are extremely rare. Since this is an autosomal dominant disorder, one parent will express the phenotype.
- **Homozygous FH** has a one in four chance of occurring in the children of two parents who each have heterozygous FH. The incidence is therefore quite rare. Individuals with this disorder often have symptomatic CVD by the first decade of life. Response to statin therapy is often modest at best, and, hence, more

aggressive therapies, such as LDL apheresis, or newer medication such as lomitapide and mipomersen (currently no longer on the market), which are described elsewhere in this chapter, are required. PCSK9 inhibitors can produce up to a 30% LDL-C reduction in some patients.

- **Autosomal recessive hypercholesterolemia** is due to a mutation in the two alleles responsible for ABCG5 or ABCG8 causing increased blood levels of toxic plant sterols. It can present with a similar phenotype to homozygous FH, but, because it is a recessive trait, the parents will not exhibit the phenotype of heterozygous FH as would occur with homozygous FH. Both disorders, however, can be relatively difficult to treat with poor responsiveness to traditional LDL-lowering therapies.

Diseases Leading to Elevated Triglycerides

Familial Combined Hyperlipidemia

Familial combined hyperlipidemia (FCHL) is one of the most common inherited lipid disorders. The prevalence data suggest the disorder affects 1 to 2% of the population. It has traditionally been defined as an autosomal dominant trait, but more recently has been considered an oligogenic disorder with variable penetrance [84]. It presents with a variable phenotype and affected individuals have lipid profiles that may fluctuate from one reading to the next and may present with high LDL-C, high TGs, or both. It has been said that when a patient has multiple lipid profiles that are different, suggesting laboratory error, FCHL should be suspected. Affected relatives can present with elevated LDL-C while others present with elevated TGs and still others have both. The disorder is associated with a marked increased risk for ASCVD. Though the disease appears to be inherited in an autosomal dominant manner, it is likely that multiple genes may be responsible for the lipid disorder. Certain mutations in the genes for lipoprotein lipase (LPL), ApoA5, ApoE, and ApoC3 have been implicated [85]. The single-nucleotide polymorphism (SNP) rs3737787 has been associated with differences in the expression of the target genes for upstream transcription factor 1(*USF1*) in adipose tissue and lymphoblasts, as well as higher TG concentrations in certain populations, and is the SNP most consistently associated to FCHL. Genome-wide association studies have revealed that patients with FCHL have high polygenic lipid scores for associated LDL-C and TG variants consistent with a polygenic origin for the disorder [86].

The increased risk for coronary disease likely stems from increased production of very low-density lipoprotein (VLDL), due to increased ApoB production or decreased clearance of ApoB-containing lipoproteins, leading to increased atherogenic particles as well as small dense LDL particles (Fig. 3.3) [87]. The disorder tends to develop in adolescence, which helps to differentiate it from FH or familial chylomicronemia syndrome (FCS), both of which are evident from childhood. The dyslipidemia is exacerbated by secondary causes, such as obesity and diabetes mellitus, and patients with FCHL have a high incidence of concomitant insulin resistance and development of type 2 diabetes. Patients with FCHL have been shown to have common pathophysiological mechanisms with type 2 diabetes. These include muscle and adipose tissue insulin resistance, as well as impaired insulin-mediated suppression of hepatic production of VLDL [88]. The diagnosis is suspected if one first-order relative has markedly elevated lipid levels, whether LDL-C, TGs, or both. Commonly, there is a strong family history of premature CAD. The LDL-C levels do not achieve the level seen in FH, and the TG levels, though often elevated, are not as high as those seen in FCS. CAD, though common in FCHL, is relatively rare in patients with FCS and familial hypertriglyceridemia, where the primary morbidity for the latter two is pancreatitis. Because FCHL is not a pure monogenic disorder, the role of genetic testing is not extremely helpful at present, but helps to rule out other causes of

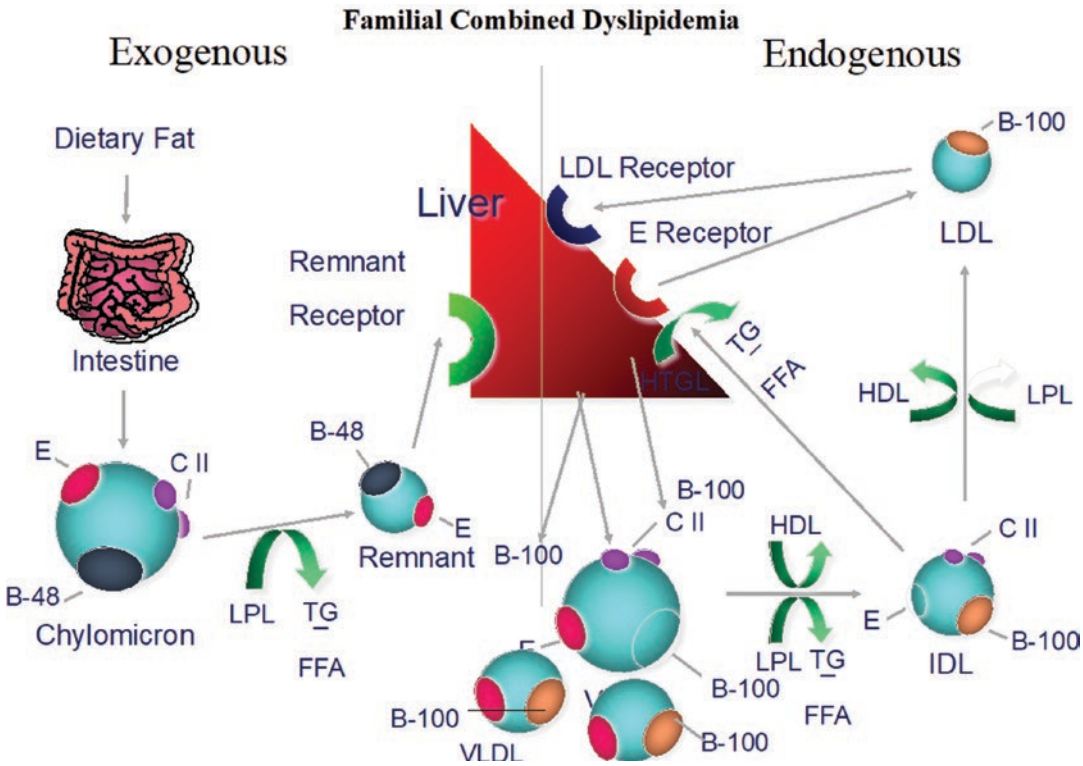

Fig. 3.3 Familial combined dyslipidemia is an autosomal dominant disorder characterized by an overproduction of ApoB leading to increased numbers of atherogenic lipoproteins. Some individuals may present with high levels of VLDL while others may have increased LDL. Family members therefore can have elevated LDL-C, elevated TG, or both, in their lipid profiles

dyslipidemia. Elevated ApoB levels support the diagnosis, with ApoB levels usually greater than simultaneously obtained LDL-C levels in these patients [86, 89, 90].

Treatment of this FCHL includes aggressive control of secondary causes starting with a low carbohydrate and low saturated fat diet, as well as statin therapy to reduce the risk of CVD. Abstaining from alcohol and smoking, and management of obesity are also necessary. A 5% weight loss in patients with FCHL has been shown to significantly reduce TG and non-HDL-C levels in 3 to 6 months [91]. Statins are first-line therapy for these patients because of the markedly elevated risk for CAD. If TGs are significantly elevated despite diet and statin therapy, the addition of omega-3 fatty acids and/or fenofibrate is sometimes necessary for control of TG levels. It is also very important to aggressively control secondary disorders such as diabetes mellitus [92, 93].

Familial Hypertriglyceridemia

Familial hypertriglyceridemia syndrome is an autosomal dominant inherited trait characterized by moderately to markedly elevated TGs. These patients have low to normal LDL-C levels and normal HDL-C levels. The prevalence of this disorder is thought to be 5 to 10% of the population. Mutations in the gene for ApoA5 have been implicated, but the exact genetic etiology remains unclear. Likely, the combination of an underlying genetic predisposition plus superimposed environ-

mental factors leads to its expression. Though traditionally, this disorder did not express itself until puberty or early adulthood, the prevalence in children is thought to be increasing due to the increasing incidence of obesity in childhood. Unlike FCHL, affected family members all have elevated TGs and fluctuations in the lipid profile are less common. Since the disorder is thought to be due to the production of large VLDL particles, the ratio of TG to cholesterol in the lipid profile is approximately 5:1 (Fig. 3.4). The risk of coronary atherosclerosis is quite low and only slightly higher than average, in contrast to FCHL, which has an extremely high risk for coronary disease. For this reason, most family members are affected mainly by pancreatitis without a strong family history for premature atherosclerosis in the absence of other risk factors. In addition, unlike FCS, treatment with TG-lowering medications such as fenofibrate and omega-3 fatty acids is quite effective [94].

Familial Dysbetalipoproteinemia (Type III Hyperlipoproteinemia)

Familial dysbetalipoproteinemia or type III hyperlipoproteinemia, also known as broad beta disease, is a genetic disorder of lipoprotein metabolism that results in accumulation of remnant lipoproteins in the plasma with development of premature atherosclerosis [95]. The incidence of this disease is unknown and the prevalence varies from 1:5000 to 1:10,000. Men are predominantly affected in about a 2:1 ratio, manifesting at 25–40 y of age. The disease is rare in children and premenopausal women [96].

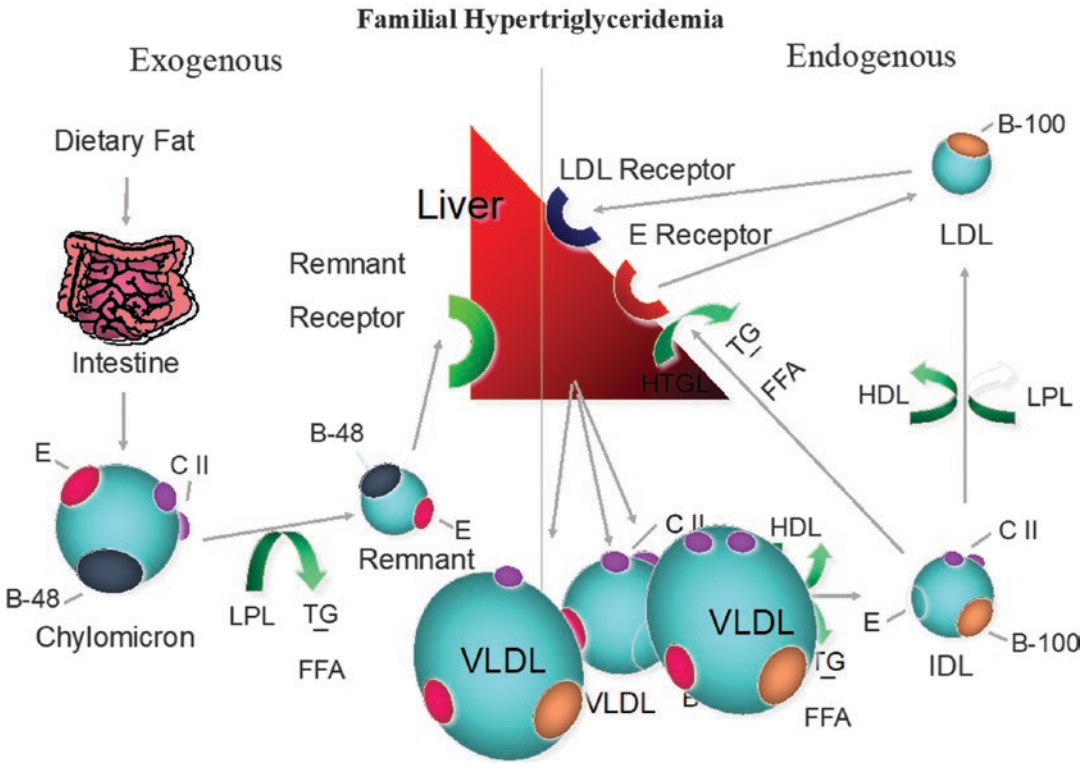

Fig. 3.4 Familial hypertriglyceridemia is an autosomal dominant disorder leading to production of large VLDL particles. Several genes have been implicated including ApoA5. Other polygenic mutations likely contribute. Patients have minimal increased risk for atherosclerosis, but moderate to marked hypertriglyceridemia and episodic pancreatitis. All affected family members have elevated TG and usually normal levels of LDL-C and HDL-C

Genetics

Familial dysbetalipoproteinemia results from mutations in the *APOE* gene (19q13.31) encoding ApoE. ApoE is a multifunctional 299-amino-acid glycoprotein located on the surface of TG-rich lipoproteins and it is essential for their metabolism. There are three common ApoE isoforms: ApoE2, ApoE3, and ApoE4. The E3:E3 variant is considered wildtype, as it is the most frequent genotype in humans, found in 50–70% of the population [97, 98]. Homozygosity for ApoE2 (E2:E2) is the least common, occurring in about 1% of the North American population, and is the hallmark of dysbetalipoproteinemia [95]. However, only 5–15% of ApoE2 homozygotes develop the disease (about 1 per 1000 individuals) [96, 99]. Carriers of only one E2 allele generally do not develop a lipid disorder; thus, dysbetalipoproteinemia is inherited as a genetically autosomal recessive disease. However, about 10% of patients with dysbetalipoproteinemia have a mutation in ApoE with a dominant or codominant inheritance pattern [100, 101]. The ApoE2 differs from ApoE3 by a single amino acid substitution Arg158Cys located near the LDLR recognition site. Thus, the ApoE2 exhibits impaired binding to the receptor, resulting in an inability to promote clearance of TG-rich lipoprotein remnant particles and leading to their accumulation in the blood [102].

Pathophysiology

Impaired clearance of TG-rich remnants is the basis of the pathophysiology of the disease. Remnant lipoproteins are atherogenic particles derived from the lipolytic processing of intestinal chylomicrons and hepatic VLDL. Chylomicron remnants and about half of the VLDL remnants are cleared directly by the liver through an ApoE-mediated process with the remaining half of VLDL remnants converted to LDL particles, as shown in Fig. 3.5 [3, 95, 103–106].

Two critical steps are required for the development of familial dysbetalipoproteinemia, an ApoE gene mutation and a second "hit" with the condi-

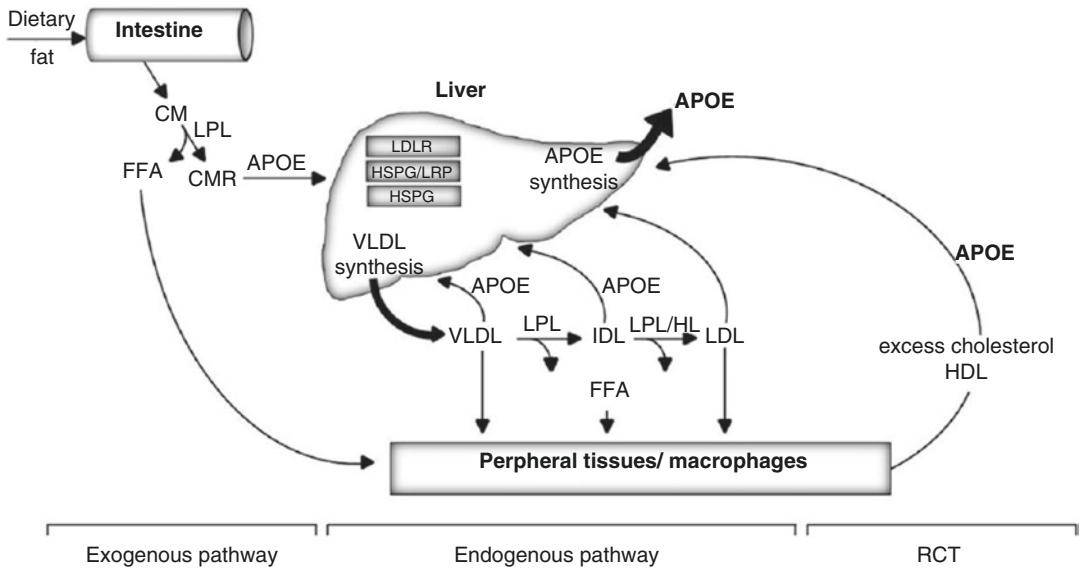

Fig. 3.5 Chylomicron (CM) particles synthesized in the intestines and VLDL particles synthesized in the liver are lipolyzed in the circulation by lipoprotein lipase (LPL) to form remnant particles. ApoE on such particles mediates their uptake in the liver by the LDL receptor or the LDL receptor-related protein and heparan sulfate proteoglycan (HSPG) pathways. The LDL particles are the end product of VLDL catabolism and only ApoB-100 on their surface is required for uptake by LDL receptor. (Taken from Dose et al. [106])

tions causing overproduction of ApoB particles and/or reduction of LDLR activity (Fig. 3.6). ApoE is a high affinity ligand that mediates the binding, uptake, and plasma clearance of TG-rich lipoproteins by LDLR, LDLR-related protein, and cell-surface heparin sulfate proteoglycans. Thus, a receptor-binding defect of ApoE in familial dysbetalipoproteinemia results in impaired remnant clearance, causing their accumulation in the blood [101]. Additionally, there is a significant decrease in the hepatic lipase activation by ApoE2 compared to ApoE3 or ApoE4, which leads to a reduction of hepatic lipase-mediated lipolysis of IDL to LDL, and a decrement in the processing of chylomicron remnants [107]. In addition, an increase in ApoE on the surface of lipoprotein particles in dysbetalipoproteinemia leads to displacement or masking of ApoC2, causing inhibition of lipolysis of TG-rich particles. Moreover, elevation in ApoE on the surface of lipoprotein particles causes stimulation of hepatic VLDL production [99, 108].

Accumulated remnants in the circulation become cholesterol-enriched as they acquire excess cholesterol ester due to core lipid exchanges mediated by the cholesterol ester transfer protein (CETP). Consequently, these abnormal remnant particles have a high cholesterol/TG ratio, a high cholesterol/ApoB ratio, and abnormal electrophoretic properties. Since VLDL particles are not converted in the usual proportion to LDL particles, the ratios of VLDL-ApoB/LDL-ApoB and VLDL-ApoB/total ApoB become greater than normal [109, 110].

Familial dysbetalipoproteinemia is a critical exception to the rule that the lipoprotein-related risk of CVD is directly related to plasma ApoB concentration [111]. Cholesterol-enriched chylomicron and VLDL remnants (collectively known

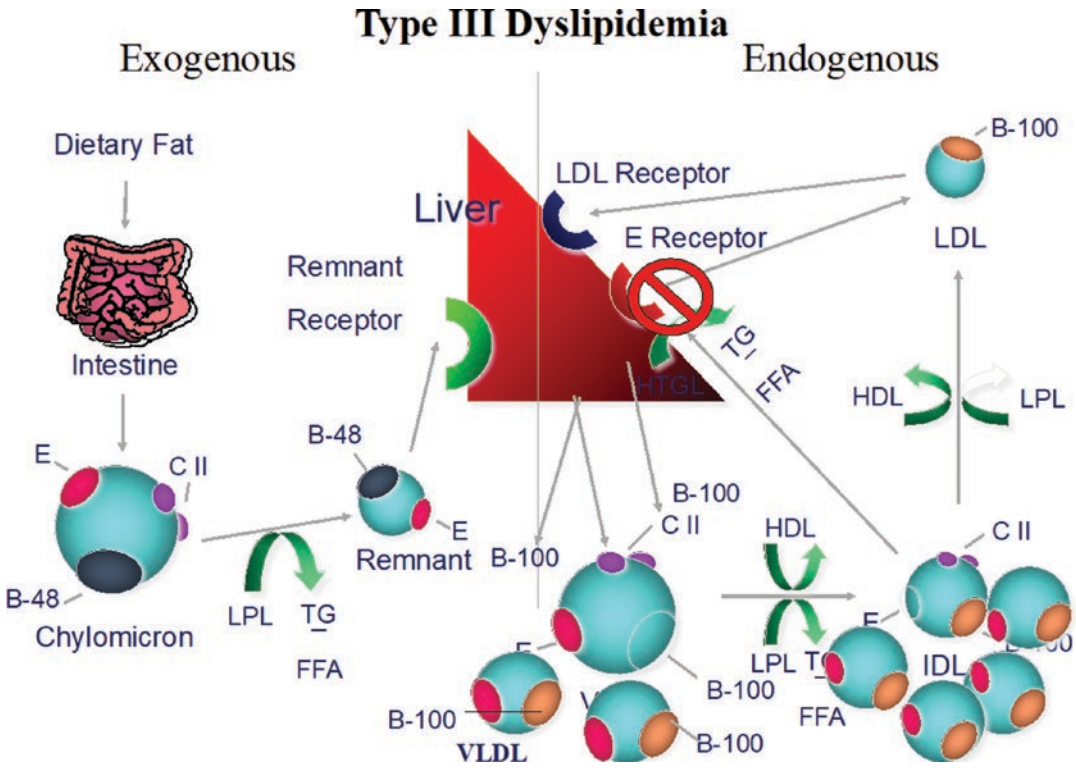

Fig. 3.6 Familial dysbetalipoproteinemia (type III dyslipidemia) is due to inheritance of Apo E2:E2 genotype with superimposed increased production of VLDL. The disorder causes a decrease in binding of remnant particles to the hepatic receptors, and, in combination with overproduction, leads to increased remnant particles in the circulation

as β-VLDL) in the circulation have a propensity for uptake by macrophages in peripheral tissues. As a result of massive cholesterol loading, these macrophages become plaque-forming foam cells in the atherosclerotic lesions [95, 99, 108, 109].

Despite impaired remnant clearance, they can still be removed from the circulation through hepatic receptors that also bind, with less affinity, to ApoB100 on VLDL remnants and ApoB48 on chylomicrons remnants. Therefore, in the absence of additional genetic, hormonal, or environmental factors, remnants do not accumulate to a degree sufficient to cause hyperlipidemia. Remnant removal disease results when an ApoE defect (almost always the E2:E2 genotype) occurs in conjunction with a second genetic or acquired defect that causes either overproduction of VLDL (i.e., FCHL or diabetes) or a reduction in LDLR activity (i.e., heterozygous FH or hypothyroidism) [112].

Clinical Presentation

Despite low LDL-C concentration, dysbetalipoproteinemia is highly atherogenic and carries increased risk of CAD and PVD [96]. In addition to atherosclerosis, the disease can manifest as palmar xanthomas, tuberoeruptive xanthomas, tendinous xanthomas, and pancreatitis. Palmar xanthomas (xanthoma striata palmaris) are considered pathognomonic of dysbetalipoproteinemia, but present in only 20% of cases (Fig. 3.7a, b, and c). These are yellow-orange depositions in the palm creases [113]. These may be confused with planar xanthoma seen in cholestatic diseases, which are usually white plaques that extend beyond the palmar creases [114]. Less commonly, tuberoeruptive xanthomas can be found at pressure points on the elbows, buttocks, and knees. Tendinous xanthomas, seen on the extensor tendons of the hands and feet as well as the Achilles tendons, may occur [112]. Finally, accumulation of TG-rich lipoproteins might precipitate pancreatitis [115, 116].

Diagnosis

Diagnostic tests are based on either the demonstration of remnant accumulation or ApoE genotyping. A frequently cited observation in the literature is a total cholesterol/TG (in mg/dL) ratio of close to 1, consistent with accumulation of mostly IDL particles. However, this observation becomes of no diagnostic value with the eventual accumulation of VLDL and chylomicrons [111, 117, 118]. Analysis of VLDL particles isolated from plasma using preparative ultracentrifugation demonstrates cholesterol-enriched VLDL (VLDL cholesterol/TG ratio >0.3; normal <0.2). Despite elevated total cholesterol and TG values, ApoB concentrations are lower than in patients without this diagnosis, yet the disease is associated with high CV risk. Therefore, ApoB and lipid measurements are complementary in diagnosing this condition. The ApoB/total cholesterol ratio is a simple and effective screening test for dysbetalipoproteinemia in

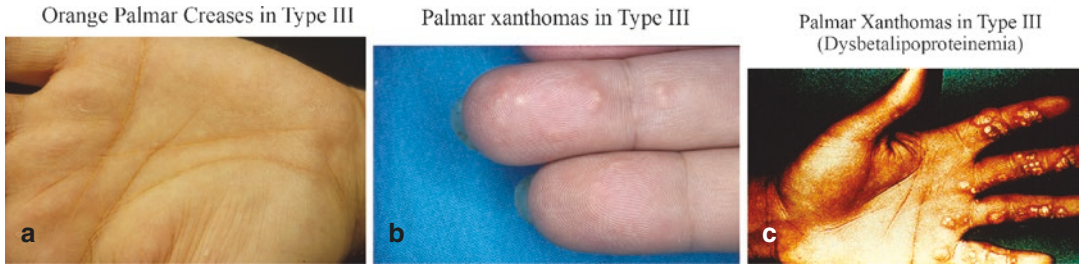

Fig. 3.7 Type 3 dyslipidemia has the distinguishing feature of physical findings on the palmar surface of the hand including orange palmar creases (**a**) and palmar xanthomata (**b** and **c**). (Courtesy of Dr. Michael H. Davidson, University of Chicago)

patients with mixed hyperlipidemia. An ApoB/total cholesterol ratio less than 0.15 identifies dysbetalipoproteinemia with a sensitivity of 89% and a specificity of 97% [119]. The non-HDL-C/ApoB ratio has been shown to help distinguish dysbetalipoproteinemia from other hyperlipidemias, such as FCHL and dyslipidemia of hypothyroidism. Dysbetalipoproteinemia patients have higher values of non-HDL-C/ApoB ratio (>2.6), consistent with a greater elevation of cholesterol per lipoprotein particle [120]. De Graafet al. proposed an algorithm (Fig. 3.8) based on total cholesterol, TG, and ApoB that can guide the diagnosis of familial dysbetalipoproteinemia by virtually any modern clinical chemistry laboratory [110, 111].

Electrophoretic techniques, including serum agarose gel electrophoresis, can help identify this condition. Normally, on electrophoresis, chylomicron is precipitated at the point of application, and the second band above the point of application is VLDL (pre-beta band), followed by the LDL (beta band), while the band furthest from the point of application is formed by HDL (Fig. 3.9) [121]. A broad beta band, resulting from the migration of VLDL particles to the beta instead of the normal pre-beta location, is due to cholesterol-enrichment of VLDL particles. This phenomenon explains the term dysbetalipoproteinemia [122], although it is found in less than one-half of the patients [123, 124]. Nonetheless, sample preparation requires ultracentrifugation, which is not readily available in many diagnostic laboratories.

ApoE phenotyping and genotyping methods are not universally available [119]. In cases of a strong clinical suspicion and negative ApoE2 genotyping, sequencing of ApoE for rare dominant mutations can be considered. In addition, genetic hepatic lipase deficiency can cause elevated remnant particles similar to dysbetalipoproteinemia and could be considered in the differential diagnosis [125].

Treatment

Since plasma LDL-C levels are low to normal and HDL-C concentration is normal in familial dysbetalipoproteinemia, the primary treatment target is non-HDL-C rather than LDL-C [115]. Treatment strategies include lifestyle modifications with cessation of alcohol intake, regular exercise, and weight loss. Dysbetalipoproteinemia usually responds well to a low-fat diet. Furthermore, underlying metabolic disorders, such as diabetes, hypothyroidism, and obesity, should be controlled.

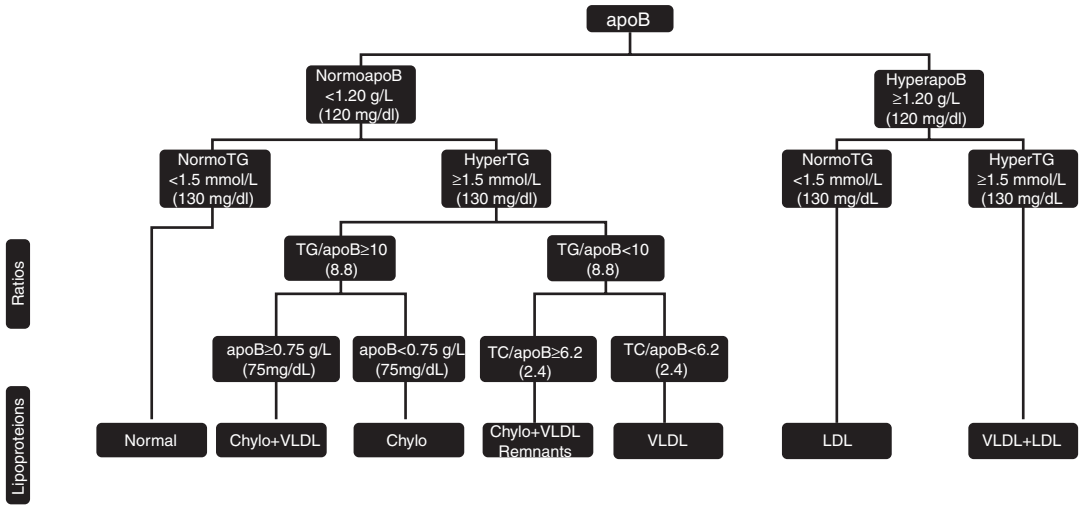

Fig. 3.8 Diagnostic algorithm for ApoB. (Abbreviations: *ApoB* apolipoprotein B, *Chylo* chylomicron, *LDL* low-density lipoprotein, *TC* total cholesterol, *TG* triglyceride, *VLDL* very low-density lipoprotein. Units in brackets are in mg/dL. From Sniderman et al. [111]. Reproduced with permission from Elsevier)

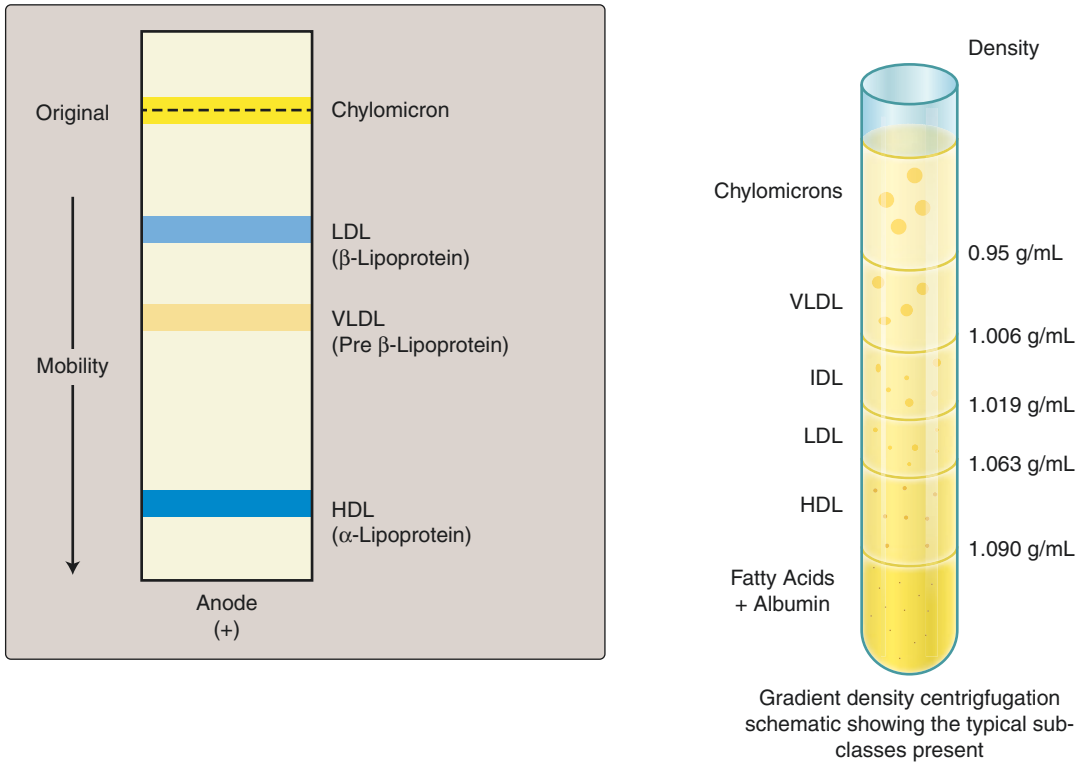

Fig. 3.9 Electrophoretic mobility of plasma lipoproteins: The electrical properties of the lipoproteins are similar to the simple proteins with isoelectric point at about pH 5.5, above which they are negatively charged. Lipoproteins more negatively charged and with highest protein content move closest to the anode, whereas those less negatively charged and with minimum protein content have minimal mobility. Large chylomicron particles do not migrate and form a band at the origin, followed by LDL, VLDL, and HDL particles that form the beta, pre-beta, and alpha bands, respectively. (Adapted from Ferrier et al. [121]. Reproduced with permission from Wolters Kluwer Health, Inc.)

Aggravating medications, such as oral estrogen, corticosteroids, protease inhibitors, and retinoids, should be withdrawn if possible [113]. The extent of response to lifestyle modifications depends on ApoE genotype, such that ApoE2 carriers demonstrate greater reductions of plasma cholesterol and TG levels when embarking on a low-fat diet. Homozygotes for ApoE2 respond with reductions in LDL-C and TG, while those with ApoE4 alleles experience only LDL-C reduction [125]. ApoE2 carriers have better improvements in their lipid profile with exercise when compared to individuals with the E3:E3 genotype. However, a decrease in the size of VLDL particles and reduction of small LDL particles in response to exercise is seen only in the E3:E3 genotype [126].

Pharmacotherapy usually includes HMG-CoA reductase inhibitors (statins), often combined with fibric acid derivatives [95]. Two clinical trials that included a total of 31 familial dysbetalipoproteinemia patients showed that statin/fibrate combination therapy decreased fasting levels of total cholesterol, TG and VLDL-C in 12 patients who remained hypercholesterolemic on monotherapy with either fibrate or statin [127, 128]. Statins increase hepatic LDL uptake, reduce VLDL production, and decrease CV risk but do not improve the delayed postprandial fat clearance in dysbetalipoproteinemia [129]. Fenofibrate has been shown to reduce postprandial TG compared to placebo in patients with type 2 diabetes, but has not been investigated in dysbetalipopro-

teinemia [130–132]. High doses of omega-3 polyunsaturated fatty acids (4.8 g/day of eicosapentaenoic acid and 4.9 g/day of docosahexaenoic acid) lower TG and decrease ApoE levels by about 15% [133]. Extended-release niacin lowers ApoE concentration by 25% in patients with metabolic syndrome and can be considered in familial dysbetalipoproteinemia [134].

Familial Chylomicronemia Syndrome [135][1]

Introduction

Chylomicronemia is a rare but devastating disorder, which is challenging to classify, diagnose, and treat. The disorder has recently gained significant attention by those interested in lipidology [136, 137]. The disease leads to a tremendous burden for affected patients as well as for those responsible for their medical care [137].

Recently, with advances in molecular genetics, the classification of chylomicronemia has gone beyond the Fredrickson or World Health Organization classification scheme [138]. In the Fredrickson classification, both hyperlipoproteinemia type 1 (elevated chylomicrons in the circulation) and hyperlipoproteinemia type 5 (elevated VLDL and chylomicrons) are two disorders that manifest with severely elevated levels of TG [139]. Familial chylomicronemia syndrome (FCS), formerly known as hyperlipoproteinemia type 1, is currently thought to be a monogenic disorder [138]. It is inherited in autosomal recessive fashion, initially manifested in children or adolescents [140]. The disease is commonly associated with failure to thrive, pancreatitis, high TGs, and low levels of other lipid fractions. In addition, FCS is usually refractory to TG-lowering therapy, making treatment challenging [138]. In contrast, polygenic, or "multifactorial" chylomicronemia, formerly known as hyperlipoproteinemia type 5, is a much more prevalent disorder, which includes a range of combined pathologic factors including genetic and environmental factors such as type 2 diabetes, alcohol abuse, estrogen use, etc. [141]. Polygenic chylomicronemia usually manifests later in life in older individuals and is generally responsive to available TG-lowering therapies [142].

In this review, the focus will be on FCS (monogenic), its clinical and genetic features, physiology, diagnostic algorithm, current and novel therapies in development [135]. This disease is one of the most genetically studied and described lipid disorders [143].

Definition and Clinical Features of FCS

TG elevation in the plasma of fasting individuals is defined as mild to moderate if TGs are greater than 150 mg/dL and less than 880 mg/dL and severe if TGs are greater than 885 mg/dL. Severe hypertriglyceridemia is rare and typically associated with the monogenic disorder [132]. In normal individuals, TGs are cleared from plasma 3–4 h after eating. If TG levels greater than 885 mg/dL persist for 12–14 h after a meal, FCS should be suspected [138]. The blood of patients with FCS can have a creamy or milky appearance due to the presence of large amounts of chylomicron particles. The chylomicrons appear as a white supernatant layer in serum after standing at 4 °C for 24 h or after mild centrifugation [141, 144, 145]. Having elevated TG levels, however, is not adequate to make the diagnosis of FCS [138].

Physical findings such as eruptive xanthomas, which appear on the extensor sides of the limbs, buttocks, trunk, and shoulders (usually when TG are >2000 mg/dL), lipemia retinalis (TG >4000 mg/dL) and hepatosplenomegaly are frequently present. A history of recurrent pancreatitis is often present and is a key feature to establish the FCS diagnosis [138, 141]. The lack of responsiveness to traditional TG-lowering therapy is also an important clue. Dyspnea, infectious complications such as perianal abscess, memory impairment, dementia, depression, and psychosocial issues have been reported as well [137, 141, 146]. The most life-threatening complication of FCS is recurrent pancreatitis. Mortality from uncomplicated pancreatitis can reach 5–6%. If pancreatic

[1]This section was previously published as a full chapter in Chyzhyk V, Brown AS. Familial chylomicronemia syndrome: A rare but devastating autosomal recessive disorder characterized by refractory hypertriglyceridemia and recurrent pancreatitis. Trends Cardiovasc Med. 2020 Feb;30:80-5. With permission from Elsevier.

necrosis, abscess formation, and multi-organ failure complicate the disease course, mortality can be as high as 30% [138, 145, 147].

Genetics and Physiology of FCS

FCS is a very rare autosomal recessive disease with an estimated prevalence of 1 in 500,000 to 1000,000 people [141]. Affected individuals are often homozygous or compound heterozygotes for large-effect loss-of-function mutations in genes that regulate catabolism of TG-rich lipoproteins [140]. More than 80% of genetic defects causing FCS are mutations in the genes responsible for LPL (Fig. 3.10). Hundreds of different mutations affecting LPL have been described in the literature [143]. A much smaller proportion of patients have mutations in other genes affecting chylomicron metabolism, namely ApoC2, which is the apoprotein that activates LPL and is the second most common mutation [138]; ApoA5, a cofactor for the interaction of ApoC2 and LPL; lipase maturation factor 1 (LMF1), which is necessary for the production of LPL in adipocytes and myocytes; and glycoprotein-inositol high-density lipoprotein-binding protein 1 (GPIHBP1), which transports LPL from the interstitial space into the capillary lumen [136]. LPL is an enzyme that removes TGs from TG-rich lipid particles (chylomicrons and VLDL) and breaks them down into free fatty acids for use as energy. It is produced in adipose and muscle tissues and secreted into the interstitial space of these tissues [148]. After LPL is synthesized, to further gain functional capacity, it needs co-factors and transport proteins [145]. LMF-1 facilitates secretion of LPL from the endoplasmic reticulum of adipocytes and myocytes in the active form as a homodimer [149]. Following that, GPIHBP1 is required to transfer LPL from the interstitial space across the capillary endothelium and anchor it to the capillary surface [149]. Recent reports showed that autoantibodies to GPIHBP1 can cause an FCS-like clinical picture [150, 151]. To become fully functional, LPL binds to ApoC2

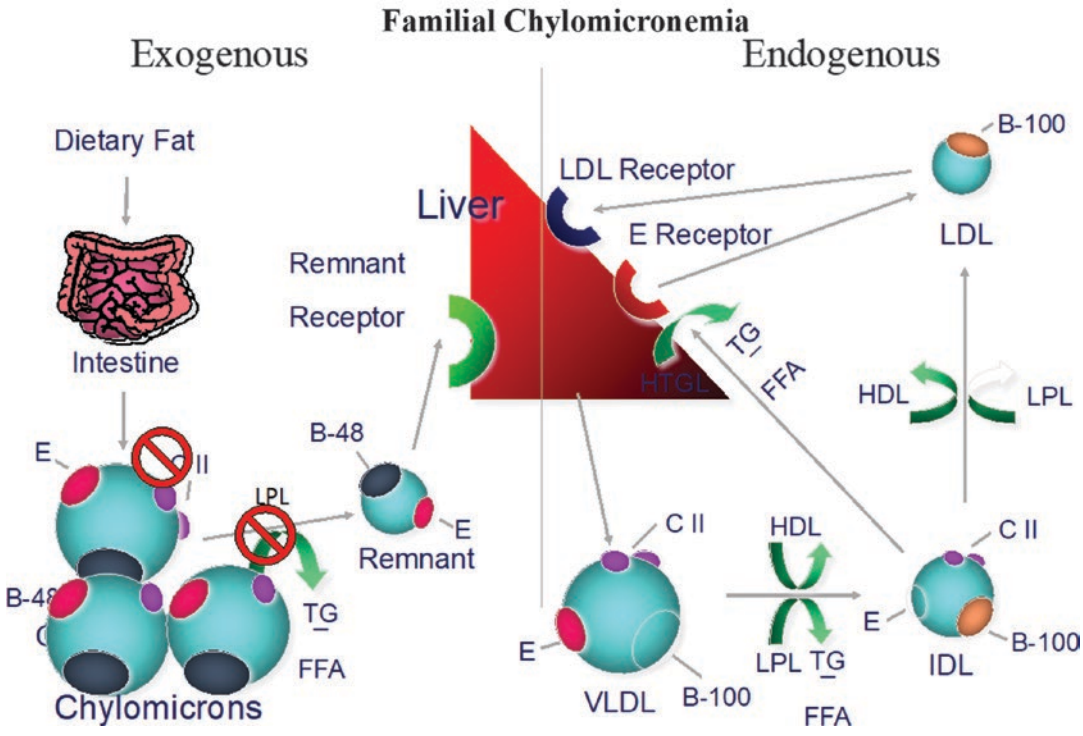

Fig. 3.10 In familial chylomicronemia, a recessive disorder, mutations in both alleles for one of five genes affecting chylomicrons metabolism lead to a marked elevation of chylomicrons in the circulation and hence markedly elevated TGs. The most common mutation is in the gene for LPL

[145, 152, 153]. ApoC5 also enhances LPL activity by acting as a cofactor for the interaction between LPL and ApoC2 [154]. ApoC3, when present on TG-rich particles, acts to inhibit LPL function and also has other functions including inhibition of hepatic TG lipase [155]. Mutations causing failure of LPL production such as LMF-1, LPL transport such as GBIHBP1, or activation of the LPL, such as ApoC2 or ApoC5, can result in impairment of LPL function and chylomicron excess in the circulation, leading to FCS.

Diagnosis

FCS is often undiagnosed or misdiagnosed in the primary care setting, likely because it is such a rare condition [145]. Patients, on average, have been evaluated by five physicians of different specialties before ultimately being appropriately diagnosed with FCS [137]. Pediatricians, neonatologists, family practitioners, endocrinologists, obstetricians, gastroenterologists, and cardiologists have been shown to be lacking in awareness of this condition. Many of them encounter patients with elevated TG and refer them to other colleagues. Collaboration between different medical specialties will likely be the key to making the diagnosis and providing appropriate therapy in a timely manner going forward [145].

Patients with fasting TG levels of >885 mg/dL in three consecutive samplings should be evaluated with a stepwise approach (Fig. 3.11) [135, 138, 145]. A ratio of total TG to total cholesterol of greater than 5 (in mg/dL) suggests a high level of circulating chylomicrons and VLDL. The ratio depends on the relative proportion of VLDL in the circulation as well as the size of circulating chylomicron [145]. To differentiate from polygenic combined hyperlipidemia, which manifests with high levels of TGs and LDL-C, related to increased plasma levels of ApoB100, ApoB should be measured. Patients with FCS have low levels of ApoB [145]. Clinical evaluation should be performed to look for physical features and complications of FCS such as recurrent pancreatitis and TG elevation that is refractory to TG-lowering medications. Secondary factors that lead to TG elevation such as, pregnancy, obesity, alcohol abuse, uncontrolled diabetes, untreated

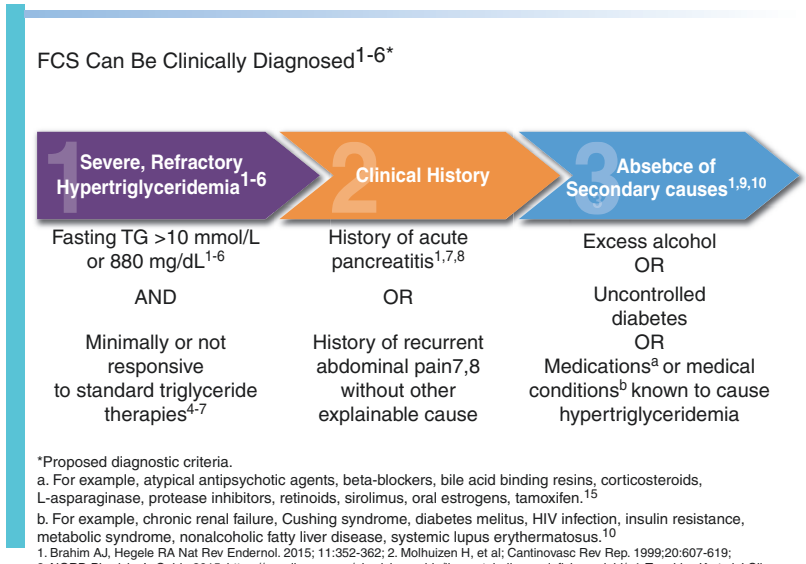

Fig. 3.11 A stepwise approach for the clinical diagnosis of familial hypercholesterolemia syndrome. (aExamples: atypical antipsychotic agents, beta-blockers, bile acid-binding resins, corticosteroids, L-asparaginase, protease inhibitors, retinoids, oral estrogens, and tamoxifen. bExamples: chronic renal failure, Cushing syndrome, diabetes mellitus, HIV infection, insulin resistance, metabolic syndrome, nonalcoholic fatty liver disease, and systemic lupus erythematosus. Adapted from Chyzhyk and Brown [135]. Reproduced with permission from Elsevier)

hypothyroidism, nephrotic syndrome, and poor diet, or use of thiazide diuretics, estrogens, corticosteroids, retinoids, bile acid resins, second-generation antipsychotics, beta-blockers, and antiretroviral agents, should be excluded. These secondary factors, superimposed on milder underlying TG disorders, often exacerbate marked TG elevation but are generally responsive to the treatment of the underlying factors and TG-lowering medications [138, 145]. Following that, gene sequencing of LPL, LMF1, GPIHBP1, ApoC2, and ApoA5 is advised and represents the current gold standard for the diagnosis of FCS [136, 138, 145]. To exclude type III dyslipidemia, also known as dysbetalipoproteinemia, which has an ApoE2:E2 genotype, ApoE sequencing may also be considered [145]. If a mutation suggesting FCS is detected, family screening is also recommended. Knowing the precise disease-causing mutation may help in treatment considerations, especially in light of the development of emerging therapies [138, 145, 155–158].

Current Treatment

The main goal of therapy in the patient with FCS is prevention of pancreatitis and related comorbidities [140]. Because patients with FCS have a markedly reduced ability to metabolize chylomicrons and are therefore resistant to current medical therapies, the basis of treatment is to reduce chylomicron formation by restriction of dietary fat. Fat intake should be limited to 30–50 g/day or less or to 15–25% of the total energy intake [138, 141, 145]. Adherence to such a diet is very difficult for many patients and therefore often negatively impacts their emotional status as well as their ability to attend social events, go to restaurants, and even eat at family gatherings. Many patients have difficulty maintaining employment as well as maintaining relationships due to chronic abdominal pain and recurrent hospitalizations. Unnecessary cholecystectomy is also often common in FCS patients due to misdiagnosis [137, 138].

Infants presenting with FCS are given milk containing medium chain triglycerides (MCTs), which can enter the circulation without being incorporated into chylomicrons, as well as defatted milk. Acute pancreatitis is treated by fasting, using low-calorie total parenteral nutrition, or plasmapheresis [141, 159]. Pregnant patients with FCS are at high risk for morbidity, pancreatitis, and fetal mortality due to aggravation of hypertriglyceridemia during pregnancy. Options for treatment for pregnant patients with FCS include a very strict low-fat diet but may also require total parenteral nutrition throughout the pregnancy or frequent plasmapheresis [159, 160]. Healthy lifestyle, risk factor modification, control of secondary factors, and avoidance of substances and medications that lead to an increase in TGs are extremely important measures for all patients with FCS [138, 145].

Fibrates, niacin, omega-3 fatty acids, and statins are often not effective in significantly lowering TG levels in patients with FCS, which is a clue to the diagnosis. A greater effect is seen in patients with mild to moderate TG elevation who have the polygenic form of hypertriglyceridemia as compared to those with FCS [138, 141, 145]. Fibrates enhance the oxidation of fatty acids in the liver and muscle and reduce the rate of hepatic lipogenesis, decreasing synthesis of VLDL. They also decrease ApoC3, hence removing inhibition of LPL [138, 161]. Niacin also mainly affects VLDL and is not very effective in reducing chylomicrons causing FCS [138]. Statins increase catabolism of chylomicron remnants and mildly decrease chylomicron. Omega-3 fatty acids in doses of 4–6 g/day inhibit VLDL production and increase lipolysis of chylomicrons [138, 140]. Since patients with FCS have predominately ineffective LPL, these medications do little to lower their TG levels.

Novel Therapies

Volanesorsen is a second-generation antisense oligonucleotide drug targeting ApoC3 mRNA [162]. As discussed earlier, ApoC3 reduces the clearance of TG- rich lipoproteins from plasma via a pathway other than LPL, the mechanism of which is not completely clear. Therefore, reduction of ApoC3 would be expected to lower plasma TG by facilitating clearance. Patients treated with volanesorsen had approximately a 70% reduction in TG levels from baseline after 3 months of

treatment. The most common side effects were injection site reactions, flu-like symptoms, hypersensitivity, liver and renal side effects, and thrombocytopenia. Volanesorsen is approved in the European Union as an adjunct to diet in adult patients with genetically confirmed FCS and at high risk for pancreatitis, in whom response to diet and TG-lowering therapy has been inadequate. It currently has expanded access/compassionate use in the USA.

Based on preliminary research data, lomitapide, a microsomal TG transfer protein inhibitor (MTTP), may also be a useful treatment for patients with genetic hypertriglyceridemia and recurrent acute pancreatitis, who are refractory to traditional treatment. However, it is currently only indicated for homozygous FH [138, 142, 156].

Alipogene tiparvovec gene replacement therapy is a nonreplicating adeno-associated viral vector of serotype 1 that was designed to deliver and express the human gain-of-function LPL gene. The drug was delivered by intramuscular injection. Effects of the therapy were short-lived; however, lower incidence of pancreatitis in FCS patients was noted for up to 6 y after treatment. The drug was conditionally approved in Europe, but later discontinued by the manufacturer in 2017 [156, 157].

Mipomersen is an antisense RNA compound that impairs synthesis and secretion of ApoB, including the intestinal form, ApoB48. It is currently FDA approved only for homozygous FH. However, by inhibiting ApoC3, it also increases LPL activity and decreases TGs [138, 163].

Pemafibrate and LY518674 are two new peroxisome proliferator activated receptor-α (PPAR-α) modulators under development. These are more potent alternatives to existing fibrates and may offer an option for FCS patients [164].

Pradigastat, a new diacylglycerol acyltransferase 1 inhibitor, showed efficacy in reducing TGs in FCS patients. An open-label study that administered a 40 mg dose to six patients with FCS showed a 72% reduction in TG levels. A study that randomized 45 FCS patients showed good safety and tolerability, but 72% of participants reported diarrhea. This might complicate further use of pradigastat [164, 165].

Gemcabene calcium, another agent in development, is a salt of a dialkyl ether dicarboxylic acid. In chow-fed male rats, gemcabene reduced LDL-C, TG, and ApoC3 levels, and increased HDL-C levels, apparently through both reduced synthesis and increased clearance of hepatic TG-rich lipoproteins. It may also be a treatment option in the future [164].

Evinacumab is a fully human antibody that blocks angiopoietin-like protein 3. This protein increases TGs, LDL-C, and HDL-C. Evinacumab was evaluated in an open-label study of nine adults with homozygous FH and showed a 49% decrease in LDL-C and a 47% decrease in TGs. This drug may be promising in the future in treatment of FCS patients [166, 167].

In summary, FCS is a rare but devastating disorder, characterized by marked TG elevation, often diagnosed in infancy. Patients suffer episodes of recurrent pancreatitis, abdominal pain, fatigue, and even "brain fog," and are frequently misdiagnosed, leading to frustration, depression, social isolation, unnecessary surgeries, and difficulty maintaining employment. The mainstay of current therapy is to maintain a very low-fat diet to reduce chylomicron formation, often supplemented with appropriate vitamins and MCT oil. Current TG-lowering medications are often ineffective, but newer medications such as volanesorsen [162], which has been shown to be effective in these patients, offer promise of a better life. The importance of making the diagnosis and being aware of the disease for all physicians who treat pancreatitis and patients with severe hypertriglyceridemia, including gastroenterologists, endocrinologists, cardiologists, and primary care physicians, will need to be emphasized to reduce morbidity and mortality in patients with FCS [137].

Take-home points and differential diagnosis for high TG syndromes:

- **FCHL** has autosomal dominant inheritance. It is associated with variability in lipid profile for the patient from one reading to the next. Different family members with the disorder can present with high LDL-C, high TGs, or

both. This syndrome is associated with overproduction or decreased clearance of ApoB-containing lipoproteins, and hence, elevated ApoB levels, and is often seen with concomitant insulin resistance or diabetes. The dyslipidemia is not usually seen until young adulthood. There is usually a strong family history of CAD.

- **Familial Hypertriglyceridemia** is an autosomal dominant trait with all affected individuals having a similar phenotype of moderate to marked TG elevation and relatively normal LDL-C and HDL-C levels. The disorder is due to production of large VLDL particles rather than overproduction or increased numbers of ApoB-containing lipoproteins. Family history is remarkable for pancreatitis with minimal increased risk for CAD. The ratio of TG to cholesterol in the lipid profile is approximately 5:1, reflecting the average ratio of TG to cholesterol in VLDL particles.
- **Type III dyslipidemia**, also known as **familial dysbetalipoproteinemia**, is caused by inheriting the genotype for ApoE in the form of E2:E2. In addition, these patients have concomitant overproduction syndrome of ApoB due to genetic factors or superimposed other disorders such as obesity and diabetes. The ApoE genotype leads to difficulty in clearing remnant particles, which have an average cholesterol to TG ratio of 1:1. The lipid profile therefore often has cholesterol and TG levels that are roughly equal. This is the only disorder where physical findings are on the palms in the form of palmer xanthomata and orange palmar creases.
- **FCS** is an autosomal recessive disorder most commonly due to a mutation in the gene for LPL, but in rare cases can be due to mutations in ApoC2, ApoA5, GBIHBP1, or LMF-1. There is only a slightly increased risk for coronary atherosclerosis with the major presenting symptom being recurrent pancreatitis. Because chylomicrons are poorly metabolized in patients with FCS, they have markedly elevated TG. Chylomicrons have roughly a 10:1 ratio of TG to cholesterol, and, therefore, the lipid panel in these patients usually shows TG levels approximately 10 times the cholesterol level. Unlike the other genetic syndromes that raise TGs, the usual medications for TG lowering are often ineffective in these patients and the treatment is an extremely low-fat diet. Potentially effective drug therapies are currently in development.

Diseases Causing Low HDL

Familial Hypoalphalipoproteinemia (Familial HDL Deficiency)

Familial hypoalphalipoproteinemia is an autosomal dominant disorder with dose-dependent penetrance. It is most commonly associated with a mutation in one allele for the *APOA1* gene. A single allele mutation in the gene for ATP-binding cassette subfamily A member 1 (*ABCA1*) can give a similar syndrome, as can a mutation in the gene coding for alpha lecithin-cholesterol acyltransferase (*LCAT*). Tangier disease, which will be discussed later in this section, occurs when mutations are present in both alleles for *ABCA1*.

Traditionally, it was thought that isolated low HDL inherited disorders were primarily due to mutations in the gene for ApoA1, leading to decreased production of HDL. Patients who inherit this disorder have been thought to have significantly increased risk for CAD, often by age 50 [168]. The story is actually more complicated, however. In a recent genetic analysis of families with HDL-C levels less than 20 mg/dL, the most common mutation was seen in the gene for ABCA1 (Fig. 3.12a). These patients were also the most likely to develop premature heart disease. Mutations in the genes for ApoA1 and LCAT were rare, and, if patients were heterozygotes, there did not seem to be an increased risk for CAD. Homozygous mutations for ApoA1 were extremely rare but associated with severe coronary atherosclerosis [169]. Patients with severe ApoA1 abnormalities can have the physical findings of corneal arcus and planar xanthomas (Fig. 3.13). These analyses illustrate the complexity of the connection between HDL lev-

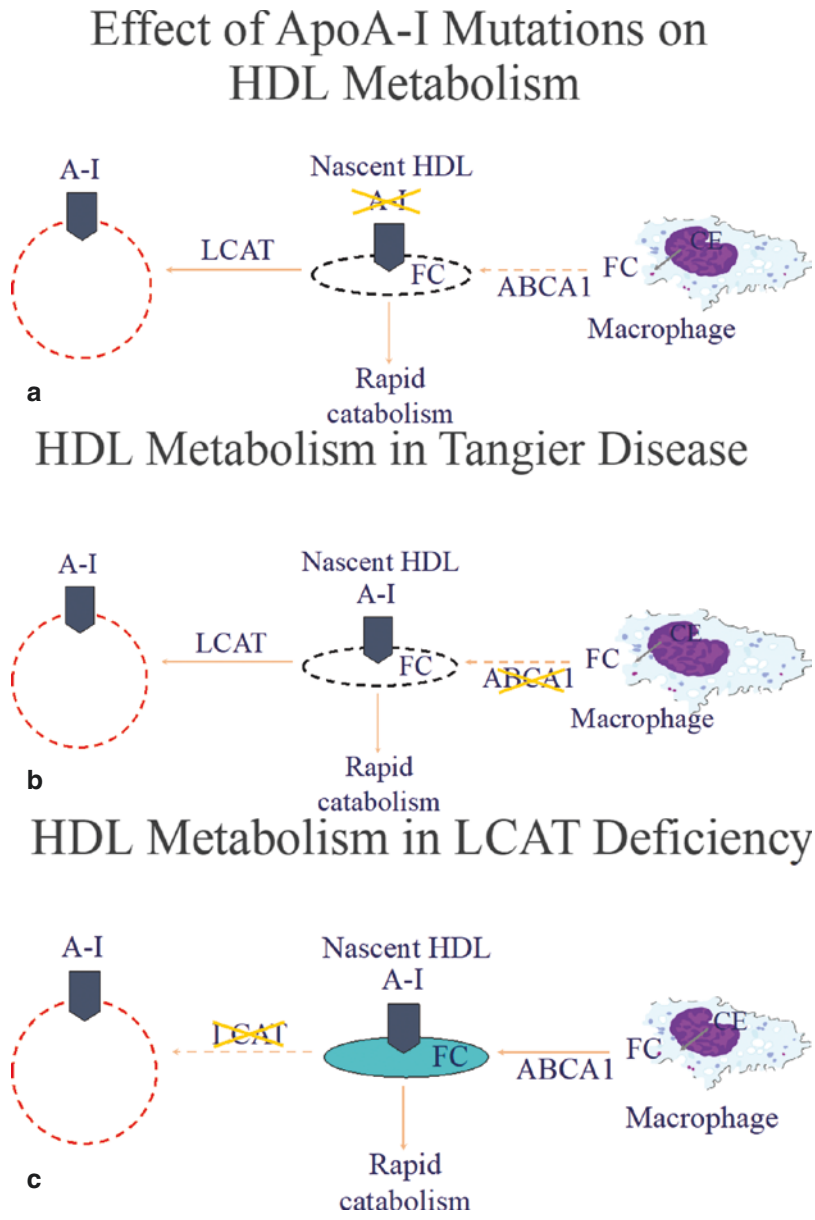

Fig. 3.12 (**a**) ApoA-I is necessary for formation of nascent HDL. ABCA-1 is necessary to move free cholesterol from macrophages to nascent HDL and LCAT is required to esterify free cholesterol to form cholesterol esters, which are necessary for the formation of mature HDL. (**b**) Tangier disease is due to a mutation in the gene for ABCA1, and hence, free cholesterol is unable to efflux from the macrophage to nascent HDL. This leads to rapid metabolism of nascent HDL and a buildup of cholesterol-laden macrophages in multiple tissues. (**c**) LCAT deficiency causes lack of esterification of free cholesterol and therefore inhibits formation of mature HDL particles and causes rapid catabolism of nascent HDL

els and atherosclerosis. The disorder should be suspected in patients with extremely low HDL-C levels, often below 30 mg/dL, in the absence of secondary disorders such as marked hypertriglyceridemia, severe inflammation, diabetes, liver disease, cigarette smoking, or use of anabolic steroids. Affected patients will often have first-order relatives with similar lipid profiles since it is an autosomal dominant effect. LDL-C and TG levels are usually normal or with mild TG elevation. The most important predictor for the risk of CAD in a patient with isolated low HDL-C levels is family history of premature atherosclerosis. The current treatment of patients with isolated low HDL-C and a family history of premature CAD remains healthy lifestyle and statin therapy with a goal of achieving low levels of LDL-C [170].

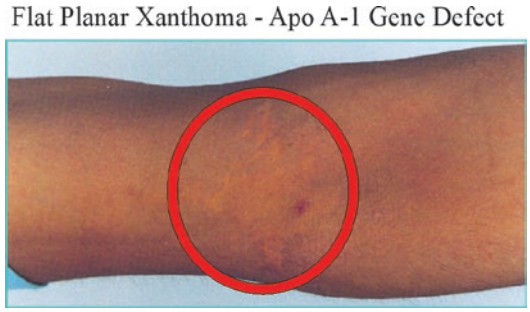

Fig. 3.13 Planar xanthoma in a patient with familial HDL deficiency (ApoA1 gene defect). (Courtesy of Dr. Michael H. Davidson, University of Chicago)

Fig. 3.14 Orange tonsils in a patient with Tangier disease. (From Puntoni et al. [171]. Reproduced with permission from Springer Nature)

Tangier disease

Tangier disease is a rare disease, so named because it was first discovered by Fredrickson and colleagues in two young siblings who lived on Tangier Island in the Chesapeake Bay. Physical findings revealed orange colored, enlarged tonsils (Fig. 3.14) [171], as well as mild corneal opacifications. The lipid profiles revealed very low HDL-C, less than 5 mg/dL, with moderate elevations in TGs and low LDL-C [172, 173]. The disorder is due to autosomal recessive inheritance of a mutation in the gene that codes for the ABCA1 protein, leading to decreased efflux of free cholesterol from macrophages to nascent HDL, resulting in rapid clearance of the nascent HDL particles(Fig. 3.12b). Cholesterol-laden macrophages are found in cells in the tonsils, bone marrow, nerves, and smooth muscle cells of patients with Tangier disease. The parents, who were heterozygous for the mutation, were less severely affected, but had approximately half the normal levels of HDL-C. Homozygous Tangier disease patients have an increased risk of premature coronary disease, usually manifesting by 50–60 y of age. The later onset of coronary disease is thought to possibly be due to the low LDL-C levels seen in these patients [170, 172, 173].

Familial Lecithin:Cholesterol Acyltransferase (LCAT) Deficiency and Fish Eye Disease

Familial LCAT Deficiency (also referred to as "Complete LCAT Deficiency") was first described by Norum and Gjone in 1967. A 33-y-old woman living in Norway presented with marked corneal opacification, hyperlipidemia, anemia, proteinuria, and normal kidney function. A kidney biopsy was performed and revealed foam cells in the glomeruli of the kidney. Plasma cholesterol and TG levels were moderately elevated, but the HDL-C level was extremely low. Interestingly, the cholesterol was predominantly free cholesterol rather than cholesterol ester. Evaluation of LCAT activity revealed a marked decrease in activity and genetic analyses revealed that the patients had a recessive disorder involving a mutation in the gene coding for LCAT. LCAT facilitates the esterification of free cholesterol allowing it to reside inside the mature HDL particle's lipophilic core. The absence of LCAT activity inhibits the formation of mature HDL-C from nascent HDL with rapid catabolism of the nascent HDL particles (Fig. 3.12c). The affected individuals with LCAT deficiency are either homozygous for the mutation or compound heterozygotes [170, 172, 173].

Fish Eye Disease (also referred to as "Partial LCAT Deficiency") was first described by Carlson and Philipson in 1979 in a male Norwegian patient

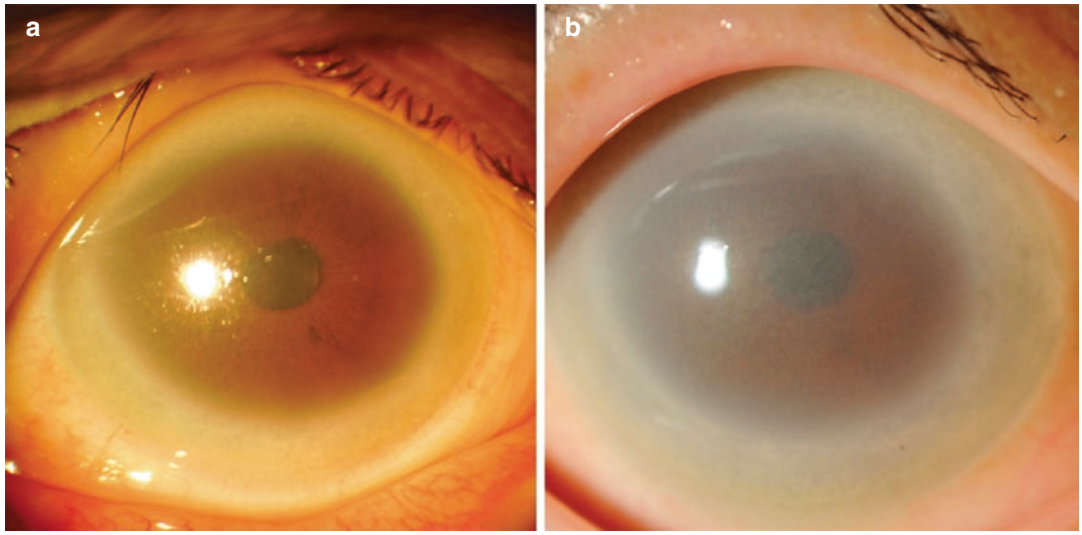

Fig. 3.15 Corneal opacities in Fish Eye Disease (can be quite variable in their presentation). (**a**) A slit-lamp photograph showing the cornea with marked corneal opacification in the peripheral area. (**b**) A slit-lamp photograph showing the cornea of the same patient after 15 years of observation. The corneal opacity has become denser and has progressed to the central area. (From Ono et al. [174])

and his daughters who all presented with marked corneal opacification (Fig. 3.15) [172–174]. The patient and his two daughters were noted to have normal serum cholesterol, elevated TG, VLDL-C, and LDL-C levels, and marked HDL deficiency. They did not have the anemia and proteinuria associated with traditional LCAT deficiency. This disease was characterized as being associated with CVD in later life, visual impairment, and dense corneal opacifications. Findings in these kindreds have led to the understanding that two different LCAT activities exist in normal plasma. One of these activities, referred to as α-LCAT, is specific for HDL, and the other, β-LCAT, is specific for chylomicrons, VLDL, and LDL. Fish Eye Disease is due to an α-LCAT deficiency, in contrast to the classical LCAT deficiency, where patients are deficient in both α- and β-LCAT activities [170].The patients with Fish Eye Disease have elevated TG and LDL-C levels that respond well to statin therapy [175].

ApoA1 Milano

ApoA1 Milano is a mutant form of ApoA1 first described in 2001 after investigating families in a small town near Milan, Italy, called Limone sul Garda. Despite having low HDL-C levels and elevated TGs, they had a remarkably low incidence of CHD. Of the 1000 inhabitants of the town, 40 were heterozygotes for the A1 Milano mutation [176]. The initial mutation was traced by church records back to common ancestors named Giovanni Pomaroli and Rosa Giovanelli from approximately the year 1780.

The mutation appears to confer antioxidant activity and promotes endothelial function improvement, as well as enhanced plaque stabilization in animal models [177]. The low serum level of HDL-C is thought to be due to rapid catabolism rather than decreased production of HDL particles. Subsequent intravascular ultrasound studies in humans with acute coronary syndromes suggested plaque regression after infusion of recombinant A1 Milano protein [178]. Several studies have been undertaken to investigate whether a therapeutic agent with recombinant A1 Milano protein could be developed, but, for a number of reasons, no such therapy has come to market [179]. Considerable interest remains in the A1 Milano mutation, yet its future as a treatment option for atherosclerosis or a preventative agent remains an open question.

Take-home points and differential diagnosis for Low HDL Syndromes:

- **Familial hypoalphalipoproteinemia** is an autosomal dominant trait associated with a mutation in one allele for *ApoA1* or *ABC1*, leading to low levels of HDL-C, usually <20 mg/dL. Affected family members, especially if they are homozygotes, have increased risk for premature coronary disease. The diagnosis should be considered in patients with very low HDL-C, similar lipid results in first-order relatives, and a family history of premature CAD. Planar xanthomas are sometimes present on physical exam.
- **Tangier disease** is an autosomal recessive disease caused by a mutation in the gene coding for the ABC1 protein, leading to inability for the macrophage to facilitate the efflux of free cholesterol and make it available to nascent HDL. This leads to deposition of cholesterol-laden macrophages in the tonsils and other tissues. Orange tonsils are the classic physical finding.
- **LCAT deficiency** is due to mutations causing reduction in both α and β LCAT activities, leading to low HDL, high TGs, corneal opacities, and proteinuria as well as anemia. Mutations affecting α-LCAT deficiency only lead to "Partial LCAT deficiency" or Fish Eye Disease.
- **A1 Milano** is a beneficial mutation in the gene coding for ApoA1. Despite low levels of HDL-C and TG, it appears to provide protection from atherosclerosis due to its beneficial effects on endothelial function and plaque stabilization, as well as its antioxidant effects.

Diseases Causing Low LDL Disorders

Hypocholesterolemias comprise a unique/varied group of genetic lipoprotein disorders defined by low (<5th percentile) or absent LDL-C and ApoB in the plasma [180]. Abetalipoproteinemia (ABL) and familial hypobetalipoproteinemia (FHBL) are the best-known inherited disorders of ApoB-containing lipoproteins that result from mutations in MTTP and ApoB genes, respectively. Chylomicron retention disease results from accumulation of lipid droplets within the enterocytes and the selective absence of ApoB48.

Abetalipoproteinemia

Bassen and Kornzweig first described ABL in 1950 [181]. ABL is a very rare, autosomal recessive hypocholesterolemia, with a prevalence of less than one in one million. It is characterized by virtual absence of ApoB-containing lipoproteins in plasma [182]. ABL is generally diagnosed in infancy due to severe diarrhea, vomiting, and failure to thrive. Additional clinical features include fat malabsorption, steatorrhea, hepatomegaly, neurological and ophthalmologic impairments such as spinocerebellar ataxia and myopathy, loss of night and/or color vision, and acquired atypical pigmentation of the retina [183]. Supportive laboratory findings include marked hypocholesterolemia (~40 mg/dL), absent/extremely low plasma LDL-C, absent/extremely low ApoB, very low plasma TGs, prolonged international normalized ratio, abnormal liver enzymes (aspartate transaminase and alanine transaminase), and low serum concentrations of fat-soluble vitamins [184].

MTTP mutations within chromosome locus 4q23 are responsible for disease presentation [185, 186]. MTTP is a protein involved in the assembly of ApoB-containing lipoproteins in the liver and intestine by forming a heterodimer with protein disulfide isomerase. Missense MTTP pathogenic variants result in the inability of ApoB-containing lipoprotein particles to be secreted.

Diagnosis is made during first months of life on the basis of undetectable vitamin E, severe hypocholesterolemia, and hypotriglyceridemia with no plasma ApoB. In its advanced form, it can result in major liver steatosis and chronic hepatitis [184]. Atypical cases have been described with milder clinical presentation. Supplementation of fat-soluble vitamins (A, D, E, and K) and a low-fat diet, in particular avoidance of long-chain fatty acids, are an efficient way to prevent most complications.

Familial Hypobetalipoproteinemia

FHBL is the most frequent monogenic form of hypobetalipoproteinemia. It is a codominant disorder that can be linked, or not, to the *ApoB* gene with a prevalence of ~1 in 1000 to 3000 [187]. Carriers of *APOB* mutations result in the formation of truncated forms of ApoB, which often are no longer secreted in the plasma (loss-of-function mutation) [188].

Heterozygous FHBL is diagnosed later in life due to its usually more benign clinical phenotype, with most subjects asymptomatic. Affected individuals often become overweight in adulthood. While liver steatosis is observed in both ABL and FHBL, the prevalence of severe liver fibrosis is higher in FHBL (20% vs. 6%; $n = 5/27$ vs. $n = 4/58$) [189]. FHBL subjects less frequently have intestinal fat malabsorption. With regards to laboratory findings, serum TG concentration is higher in FHBL, otherwise the lipid profile is similar between ABL and FHBL [184].

Glueck et al. suggest that individuals with FHBL have an extended life expectancy, between 9 and 12 y, when compared to the general US population, with significantly less nonfatal myocardial infarctions (MIs). The authors speculated that the low ratio of LDL:HDL relates to the prolonged longevity and decreased morbidity from MI in familial hypobetalipoproteinemia [190]. Clinically, it is important to note that patients with ABL are quite ill, while patients with FHBL often present as healthy with very low LDL-C levels and, in the absence of steatohepatitis, do very well with low risk for coronary events.

Loss-of-Function *PCSK9* Mutations

Subjects with loss-of-function mutations in *PCSK9* have decreased LDL-C and ApoB levels, which, in turn, lead to a lifetime lower risk of CVD. This mutation was initially described in African Americans with a prevalence of ~1 in 10,000 [38]. In 2005, Cohen et al. found that two inactivating mutations of *PCSK9* (Y142X and C679X), present in 2–2.6% of Blacks in the Dallas Heart Study, were associated with 30–40% reduction of plasma LDL-C [191]. They proposed that, in humans, loss-of-function mutations of *PCSK9* would likely lead to an increase in the number of liver LDLR and hence increase the receptor-mediated uptake and catabolism of plasma LDL, as observed in *pcsk9*$^{-/-}$ mice. These loss-of-function mutations were soon thereafter shown to confer substantial protection against CHD [38]. This finding was an important factor in pursuing a therapeutic antibody to PCSK9 since it suggested that removal of PCSK9 from the circulation might be a safe and effective way to lower LDL-C with the potential to improve CV outcomes.

Chylomicron Retention Disease

Chylomicron retention disease is a very rare autosomal recessive condition (<1 in one million) caused by mutations in the *SAR1B* gene. SAR1B is a member of the Sar1-ADP-ribosylation factor family of small GTPases that control the intracellular trafficking of proteins [192]. Clinical manifestations are similar to ABL, including fat malabsorption, vomiting, diarrhea, and failure to thrive. Disease presentation shortly after birth is due to accumulation of lipid droplets within the enterocytes and the selective absence of ApoB48-containing particles from plasma. Additional clinical features include transient acanthocytosis, hepatomegaly, and hepatic steatosis. Neurologic impairments such as hyporeflexia and loss of proprioception occur in adolescence, and ataxia, myopathy, and sensory neuropathy occur later in life. Unlike ABL and FHBL, cirrhosis has not been reported. A low-fat diet of polyunsaturated fatty acids is an efficient way to prevent most complications [193].

Lipoprotein(a)

Introduction

In the ever growing opportunity for addressing CV risk factors, a new and unique particle enters our lipid-minded consciousness: lipoprotein(a),

also known as Lp(a). Lp(a) has become a heart risk factor that has entered mainstream media, but physicians know little about it, as described in the New York Times article about the host from biggest loser who had an MI due to an elevated Lp(a) in his early 40s [194]. Elevated Lp(a) levels have been associated with increased CV risk, and clinical trials have been initiated in the last decade to study it as a potential risk stratifier and risk modifier [195]. This section will review the genetics, structure, and function of Lp(a), as well as its association with ASCVD, possible indications for screening, and the available growing therapeutic options. Lp(a) and its association with major adverse CV events, association with calcific aortic valve stenosis (CAVS), and other significant medical disease will also be discussed.

Lp(a) is composed of ApoB100 and a particle analogous to LDL, bound by a singular disulfide bond to Apo(a). Apo(a) traces its evolutionary lineage to the plasminogen gene and has evolved into the central component of Lp(a) [196–200]. The Apo(a) chain comprises an inactive protease domain and "kringles," which are essentially cysteine-rich domains similar to plasminogen. The name kringle comes from the delicious Scandinavian pastry that these structures resemble. These domains have additionally been found in blood clotting and fibrinolytic proteins including plasminogen, hepatocyte growth factor and prothrombin [197]. There are 5 such domains in plasminogen with the fourth kringle being a fibrin-binding domain. Plasminogen is transformed into a fibrinolytic enzyme by urokinase, tissue plasminogen activator through catalytic cleavage [196–198]. Apo(a) contains 10 subtypes of kringle IV (KIV) with 1 copy of KIV1, KV, and KIV3–10; and 1–40 copies of KIV2. Apo(a) is hydrophilic and therefore can bind to vascular endothelium, similar to plasminogen, via exposed lysine residues [196–200]. Lp(a) is a rarity in human proteins as there are at least 40 different isoforms recorded in the literature [196, 197]. These are the reasons for Apo(a) mass variance due to isoform mass variance, which eventually determines the level of plasma Lp(a) [196–200]. The majority of the Lp(a) contribution is due to the small isoform. KIV repeats have an inverse relationship with Lp(a) concentration in the blood with repeats >25 correlating with levels of 12.9 and 13.7 in control and CHD patients, respectively, whereas repeats of 17–19 correlate with levels of 54.5 and 79.2 in control and CHD patients, respectively [199, 200].

Incidence and Genetics of Lp(a)

Estimates of the prevalence of elevated Lp(a) in the top 20% (i.e., Lp(a) >50 mg/dL) include a staggering number of 1.4 billion people globally and about 64 million in the US Prevalence of elevated Lp(a) in the top 5% includes 350 million people globally [201]. These estimates are even higher for secondary CVD prevention patients [202].

Lp(a) levels are solely dependent on the polymorphisms at the Lp(a) gene locus, known as the *LPA* gene [196, 203]. Lp(a) is manufactured in the liver but the complete synthesis and processing of Lp(a) has not been determined [203]. In the liver, Apo(a) is commonly bound to LDL via a disulfide bond between KIV9 of Apo(a) and a newly synthesized ApoB of LDL. Lp(a) levels are inversely proportional to the size of the Apo(a) isoforms. For an individual, two alleles contribute to the Lp(a) level, one received from each parent. The small Apo(a) allele with low KIV repeat contributes more to the isoform-specific Lp(a) concentration whereas the large Apo(a) allele with a high number of KIV repeats contributes less to the Lp(a) level. A combination of these results in the total Lp(a) concentration. In the presence of two variant alleles, extraordinarily high levels of Lp(a) are detected. It is probably the only risk factor that has an odds ratio of 4–8. This underscores the importance of testing for Lp(a) [196].

Statins increase the number of LDLR but do not affect Lp(a) levels, whereas PCSK9 inhibitors work on the same target, yet lower Lp(a) levels [196]. This indicates an alternate undiscovered mechanism for Lp(a) metabolism. Lp(a) is also not influenced by dietary or lifestyle modifica-

tions. The distribution of Lp(a) varies in ethnic and racial groups. A higher risk skew is detected in Blacks, Eastern Asian, and Asian Indian populations [204, 205]. The mean Lp(a) in Blacks is about 35.1 mg/dL, in Whites 12.9 mg/dL, in Chinese 12.9 mg/dL, and in Hispanics 13.1 mg/dL [206]. An important observation from a study by Sandholzer et al. is that the upper limit of the 90% CI in Blacks indicated that 1 out of every 10 Black patients encountered had an Lp(a) concentration >200 mg/dL [206]. Lp(a) originated in Africa and the transfer of the gene to different ethnic populations can be accurately mapped through migration dynamics [207]. African Americans have higher Lp(a) levels and this holds true for any number of kringle repeats as compared to the other ethnicities. There is a disproportionate hazard ratio for clinical outcomes, including CVD, CHD and stroke, among African Americans, consistent with its genetic origin history [208]. This same disproportionate risk is detected in South Asians and Southeast Asians, with odds ratios for MI of 2.14 and 1.83, respectively [209].

Testing

A commercially available assay is used to detect Lp(a) levels independent of kringles. Per the Framingham Heart Study, 39 mg/dL (1.39 μmol/L) is a cutoff for 90th percentile of Lp(a) levels. The threshold for increased Lp(a) from multiple studies is about 50 mg/dL (100 nmol/L), although an increase in statistically significant risk has been detected at levels >30 mg/dL [210, 211]. This has been reported with both mg/dL and nmol/L units, and it is not necessarily easy to convert between them. Some rough conversions conclude 140 nmol/L is ~60 mg/dL and 165 nmol/L is ~70 mg/dL. The majority of the population will be below the 50 mg/dL (100 nmol/L) cutoff for elevated Lp(a), but about 20% of the population for males and females exceed this cutoff [201].

Lp(a) levels by enzyme-linked immunosorbent assay (ELISA) depend on the antibody used; there are three assays available on the market including MAb a-5, MAb a-40, and PAb B-100. The problem with these multiple assays is the high variability in the Lp(a) levels detected by each assay, and particularly the discordance at low KIV repeats (and therefore elevated average Lp(a) levels) [205]. Thus, depending on the antibody used by the laboratory, a significant discrepancy can be noted, which is confusing to physicians and patients alike. A consistent high precision assay is necessary, in addition to agreement on the units, to ensure that treatment of elevated Lp(a) is easily adopted by the medical community [212]. A key insight to note is that since Lp(a) is an ApoB-containing particle, it cross reacts with LDL in most assays. Therefore, the standard lipid panel measures BOTH Lp(a) and LDL-C levels. This is significantly relevant for patients whose LDL-C levels do not fall appropriately with statins or ezetimibe, so called "statin resistance," in which case the possibility of a very high Lp(a) should be considered. There are formulas for adjusting LDL-C levels for Lp(a) mass that are feasible, but not yet standardized [213].

Lp(a) and CVD

A link between excess Lp(a) and CVD was suggested initially in small studies with one of the first reports in the Framingham Heart Study [210]. A large prospective study by Bennet et al. showed an adjusted odds ratio of 1.60 between the upper and lower thirds of baseline Lp(a) levels [214]. This association was confirmed in a 2009 meta-analysis of 120,000 patients with rates of CVD events slightly lower than previous studies (adjusted risk ratio of 1.13) [215]. These studies also showed Lp(a) as a risk factor for CVD events in patients without established CHD. A 2018 meta-analysis evaluated the impact of elevated Lp(a) on the risk of CVD events in patients with high baseline CVD risk [216]. Three categories of Lp(a) levels, 15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL were compared with <15 mg/dL. Results showed a continuous linear increase in CVD risk with increasing Lp(a) values. Increased Lp(a) levels have been noted in

patients with premature CHD. Additionally in an examination of the prevalence of genetic lipoprotein disorders, Lp(a) levels higher than the 90th percentile were present in 18.6% of patients with premature CHD, 12.7% of whom had no other dyslipidemia [211]. Further evidence for the atherothrombotic process was suggested by a study looking at the association of elevated serum Lp(a) levels with angiographically extensive coronary disease and the presence of totally occluded coronary arteries. In a study from 1998, baseline Lp(a) concentrations in patients admitted with acute coronary syndromes were associated with an increased risk of cardiac death, specifically about a 62% increase over 3 y [217]. An Lp(a) concentration of >29 mg/dL was an effective risk discriminator in patients with MI. For patients with unstable angina, however, even lower concentrations (≥7.9 mg/dL or 0.28 μmol/L) of Lp(a) had prognostic significance in predicting cardiac death (relative risk 2.48). In the Bruneck Study, the prognostication of MI and CHD risk and corresponding LPA risk genotypes (LPA KIV-2 repeats, rs3798220 and rs10455872) showed a small but statistically significant degree of improvement in 15-y CVD outcomes and improved CVD risk prediction. There is also some evidence that measurement of Apo(a) isoforms could improve risk prediction [216]. Data from the Copenhagen City Heart Study, showed that at 5–29 mg/dL there was some increased risk, but it was not statistically significant. Beyond 30 mg/dL, there was a significant 60% increased risk. Lp(a) >117 mg/dL had an odds ratio of almost 2.8 [218]. Lp(a) is essentially an MI, CVD, and death marker. Risk of elevated Lp(a) is independent of gender, age, BMI, HDL-C, and TG in this study [196, 219].

Lp(a) and Other Diseases

Aortic Stenosis:
Lp(a) and aortic stenosis share an extraordinary relationship, with Lp(a) being a strong predictor for development and progression of aortic stenosis. In fact, Lp(a) is a strong monogenetic risk factor for CAVS [220]. The SNP rs10455872 has a 58% greater risk of developing a clinical phenotype of CAVS. Patients with mild to moderate aortic stenosis and excess Lp(a) or oxidized phospholipids (OxPL)-ApoB >58.5 mg/dL in the Aortic Stenosis Progression Observation Measuring Effects of Rosuvastatin (ASTRONOMER) Trial progressed quickly, with 20% of the patients requiring aortic valve replacement over 5 y. The rate of aortic stenosis progression was faster in patients with peak aortic jet velocity + 0.26 ± 0.26 vs. +0.17 ± 0.21 m/s/y in the top tertiles of Lp(a). The rates of progression were independent of the valve number, and patients <57 y of age progressed twice as fast and had a higher need for aortic valve replacement [221, 222].

A pooled analysis of the SNP rs10455872 from the Framingham, Reykjavik, Multi-Ethnic Study of Atherosclerosis and Heinz Nixdorf cohorts showed an odds ratio of 2.05 for development of CAVS [208, 223–227]. Studies have now shown an association of Lp(a) with the progression of peak velocity of aortic stenosis. The top tertile in this study had an adjusted event-free survival without aortic valve replacement of only 60% over 5 y [228, 229].

Cerebrovascular Disease:
Two meta-analyses suggested that elevated Lp(a) is a risk factor for incident stroke [215, 230]. This relationship is stronger in men than in women with the range of risk comparing the highest to the lowest tertiles of 1.10–1.22. In a systematic review including six studies that evaluated ischemic stroke as an outcome, the relative risk was 2.14 in patients with smaller Apo(a) isoforms [231].

Hypertension
Elevated Lp(a) was found to strongly associate with increased blood pressure, duration of hypertension, and levels of total cholesterol, LDL-C, ApoB, Lp(a), and fibrinogen; this correlated with the presence and severity of target organ damage. Stepwise multivariate analysis indicated Lp(a) level was the best discriminator of the presence of target organ damage, followed by systolic blood pressure, duration of hypertension and LDL-C. Lp(a) level was related to target organ

damage independent of blood pressure. The association between Lp(a) concentration and severity of target organ damage was observed significantly in higher frequency of low molecular weight Apo(a) isoforms [232].

Venous Thromboembolism

Given the significant homology between Apo(a) and plasminogen, there is a pro-thrombotic aspect to Apo(a) [233]. A meta-analysis of six studies showed a statistically significant odds ratio of 1.77 in patients with elevated Lp(a) and venous thrombosis risk. Lp(a) therefore should be added to diagnostic considerations in patients with unprovoked venous thromboembolism given that Lp(a) is both atherogenic and thrombotic. There are anecdotal data suggesting use of vitamin K antagonists in patients with venous thromboembolism and elevated Lp(a) [233–235].

Diabetes

Examination of quintiles of Lp(a) concentration indicates a paradoxical relationship between Lp(a) and risk of onset of non-insulin dependent diabetes. Patients with low Lp(a) have a higher risk of developing diabetes compared to the higher quintiles. This is not related to the Lp(a) concentration, but rather to the number of actual kringle IV repeats [236].

Biochemistry, Genetics, and Mechanism of Lp(a)-Mediated CVD

Lp(a) contributes to CVD risk via multiple unique mechanisms. Lp(a) is more atherogenic than LDL because, by definition, it is constituted of proatherogenic components of LDL-C and Apo(a). Similar to LDL particles, after entry into the vessel wall, Lp(a) has the proclivity to oxidize, creating a highly immunogenic and proinflammatory particle: oxidized LDL. Apo(a) also forms OxPL, accumulates in the arterial wall via its lysine-binding sites, and inhibits plasminogen activation, leading to its atherothrombotic and antifibrinolytic effects [237]. In most patients at risk for CVD, ApoB-driven risk is due to the surplus of LDL-C compared to Lp(a). Lp(a) facilitates CVD risk in a measurable manner, and has a linear increase in CVD risk with increasing concentration of Lp(a). Lp(a) OxPL is present in the lipid phase as well, or can be bound to Apo(a) [198, 235, 238]. As OxPL-ApoB exhibits the OxPL content of Lp(a), it prognostication matches or is superior to that of Lp(a) [221, 234, 239–244].

In vivo studies in patients with elevated Lp(a) by van der Valk have shown that those with Lp(a) 50–195 mg/dL have increased arterial inflammation and enhanced peripheral blood mononuclear cell trafficking to the arterial wall compared with subjects with Lp(a) 2–28 mg/dL. In addition, monocytes with elevated Lp(a) remain in a primed state, and when stimulated have an increased capacity to transmigrate and produce proinflammatory cytokines. In vitro studies have shown that Lp(a) contains OxPL and augments the proinflammatory response in monocytes derived from healthy control subjects. This effect was markedly dissipated by inactivating OxPL on Lp(a) with a specific antibody or using recombinant Apo(a) constructs lacking OxPL. These findings demonstrate that Lp(a) induces monocyte trafficking to the arterial wall and mediates proinflammatory responses via OxPL [235]. OxPL-Lp(a) also stimulates a complex genetic and biochemical cascade that facilities vessel wall entry via interleukin-8 and monocyte chemoattractant protein-1. Vessel entry and, in fact, aortic valve leaflet entry is further facilitated by Apo(a) lysine-binding sites and lead to inflammation and thrombosis via excess accumulation [245, 246]. These findings show a new pathway by which Lp(a) mediates CVD and CAVS [235]. Pathology from procedures including carotid endarterectomy, renal, and peripheral interventions, and specifically coronary interventions, showed a significant presence of Lp(a)-OxPL in lesions and increased concentrations correlated with increased plaque incidence [247, 248]. Oxidation of phospholipids also generates lysophosphatidylcholine, which is converted to the inflammatory, pro-fibrotic and pro-motility entity, lysophosphatidic acid via autotaxin. Elevated autotaxin along with Lp(a) or OxPL-ApoB drastically increases CAVS with odds

ratios of 3.46 and 5.48, respectively. The progression and severity of CAVS are mediated by accumulation of both autotaxin and OxPL into aortic valve leaflets via Lp(a) and a subsequent biochemical and genetic cascade of inflammation and fibrosis [244, 249].

The Coronary Artery Disease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics (CARDIoGRAMplusC4D) consortium is a collaboration to identify risk loci for cardiac disease. In this consortium looking at numerous genetic studies, 46 loci and 104 unique variants were identified, with the highest preponderance in those linked to lipid metabolism and inflammation [224]. The LPA locus was found to be more robustly associated with cardiac disease when compared to the variants related to LDL, PCSK9, and 9p21 and, in fact, was one of the most potent, if not the most potent, variant overall. Positive associations have also been found in gene association studies showing an association between elevated Lp(a) levels and increased risk of MI.

In one genetic association study, Lp(a) levels, MIs, and the frequency of the number of 5.6-kilobase repeats determined by an *LPA* gene polymorphism showed a hazard ratio of 1.22 (95% CI 1.09-1.37) per doubling of Lp(a) level. There was a statistically significant trend when comparing mean Lp(a) levels in all the quartiles of the number of 5.6 kilobase repeats. A doubling of Lp(a) levels was associated with a 2.2% increase in the risk of MI [218]. Another gene association study compared Lp(a) levels to two common variant chromosomal regions (SNPs) near the gene locus that code for Apo(a), and the presence or absence of CAD. The SNPs rs3798220 (risk allele frequency C 0.02%) and rs10455872 (risk allele frequency G 0.07%) had odds ratios for CAD of 1.92 and 1.70, respectively, while the alleles only had a mild increase in frequency, again showing a strong causative relationship between excess Lp(a) and an increased risk of CAD events [196]. Another study showed three chromosomal regions (6q26-27, 9p21, and 1p13) were strongly associated with the risk of coronary disease. The *LPA* locus on 6q26-27 encoding Lp(a) had the strongest association. The common variant (rs10455872) at the *LPA* locus had an odds ratio for coronary disease of 1.70 [204]. The *LPA* null allele (rs41272114) was genotyped in the Precocious Coronary Artery Disease (PROCARDIS) trial and was associated with decreased circulating Lp(a) levels and decreased CAD risk indicating a potential for therapeutic interventions [250, 251].

Treatment and Management of Lp(a)

Management

The Cleveland Clinic Prevention Database showed similar data to the population studies for Lp(a) concentration, but a slightly higher all-cause mortality. The risk of death is even higher in the secondary prevention cohorts. A study by Bruneck in a 15-y prospective follow-up based on well-validated risk prediction scores (Framingham and Reynolds) allowed for reclassification of about 40% into the low or high-risk categories, and therefore, Lp(a) is an important risk stratifier and should be considered in CVD reduction strategies [252]. A misconception that needs to be addressed is that Lp(a) is not a risk factor when LDL-C is less than 70 mg/dL. Diminishing returns are seen when LDL-C is less than 100 mg/dL as in the IMPROVE-IT, Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides (AIM-HIGH), Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin(JUPITER) and Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID) trials. This suggests the presence of "residual risk." Sub-analyses of these studies showed higher major adverse CV events in the setting of controlled LDL-C but elevated Lp(a) [69, 253–255]. The cost of Lp(a) level testing is an economical $50-$100, and because Lp(a) levels are genetically determined and are not influenced by dietary or environmental factors, checking Lp(a) levels once for diagnostic or screening purposes is enough [204, 215, 221, 256].

Per the European Atherosclerosis Society consensus panel in 2010 and the US National Lipid Association Expert Panel Recommendations, there is a recommendation for Lp(a) screening once in patients who fall into the following categories [132, 210, 255]:

- Premature CVD
- FH
- Family history of premature CVD or Lp(a)
- Recurrent CVD despite statins
- >3% risk of fatal CVD
- >10% 10-y risk of fatal/nonfatal CHD
- For reclassification in subjects with borderline risk (Class IIa, Level C; in the US NLA guidelines)

Treatment

Exercise results in improvement in HDL-C, but Lp(a) is purely a genetic marker. So far, there have been no studies that show it to be a modifiable risk factor. Lifestyle changes have minimal effect on Lp(a) levels. The current armory for pharmacological treatment of Lp(a) consists of:

- Aggressive LDL-C reduction
- Statins
- Niacin
- CETP inhibition – inhibition of Apo(B) lipidation
- PCSK9 inhibition
- Mipomersen
- Aspirin
- Estrogen
- Apheresis
- Apo(a) antisense or SiRNA - decreased Apo(a) synthesis [257]

Aggressive LDL-C reduction in the presence of elevated Lp(a) has not been directly studied. In a post-hoc analysis of the Familial Atherosclerosis Treatment (FATS) study, excess Lp(a) levels were associated with progression of CHD events if there was less than 10% decrease in the LDL-C on therapy [258]. In another post-hoc analysis of the Familial Hypercholesterolaemia Regression Study, a decrease in Lp(a) reduction in patients with LDL-C less than 130 mg/dL did not show supplementary angiographic benefit [259]. These studies were small and were not designed to specifically address elevated Lp(a) levels.

Statins to lower LDL-C are reasonable, but they do paradoxically increase Lp(a) levels. Multiple studies and a meta-analysis have shown a non-inconsequential increase in Lp(a) in patients on statin therapy. The mean Lp(a) increased by 11% (and up to 50% in some studies), and OxPL-ApoB increased by 24% [228]. Therefore, being on a statin does not seem to mitigate the risk of Lp(a), and, in fact, there are placebo-controlled randomized trial data showing there is an increased risk of CVD in patients with elevated Lp(a) on statins [216]. Furthermore statins unfortunately increase the OxPL-ApoB levels by 46%. It is unclear at this stage if this statin-mediated increase in the levels of Lp(a) and OxPL-ApoB worsen the progression of CAVS [260].

Estrogen replacement has been reported to lower Lp(a) levels. In a post-hoc analysis of the Heart and Estrogen/progestin Replacement Study (HERS), estrogen with progestin lowered Lp(a) by 15–20% in postmenopausal women and the greatest advantage was detected in the fourth quartile [261]. Currently, this is not an option in women at risk of atherothrombosis and is contraindicated [262]. Niacin 1–3 g/day lowers Lp(a) by 30–40%, but the effectiveness in reducing events is unknown. The AIM-HIGH trial did not show a clinical benefit despite a modest reduction of 39% in Lp(a) levels in the highest risk quartile on patients on high dose niacin [210].

Mipomersen is an antisense oligonucleotide that prevents the formation of ApoB100, resulting in a decrease in the levels of ApoB, LDL-C, and total cholesterol, but it does not affect production of Apo(a) released into the circulation. Mipomersen was studied in four trials of LDL-C lowering, and showed a 25% reduction in Lp(a) [262].

PCSK9 inhibitors may reduce Lp(a) by 20–30%, but only have a limited indication [263–265]. In a meta-analysis of 12 randomized trials with 6566 patients comparing PCSK9 antibody therapy to no antibody therapy, the Lp(a) level

decreased 26% [266]. A subanalysis of the FOURIER trial showed that evolocumab produced a 25% (36 nmol/L) reduction in Lp(a) levels. This benefit was seen disproportionately in the higher quartiles of Lp(a) [267]. The CETP inhibitor trials showed a 20–30% reduction in Lp(a), yet the patients still had residual risk. These data, along with the AIM-HIGH study, suggest that the impact of Lp(a) reduction might not be significant unless it is greater than 50% reduction without other side effects [254].

Apheresis can significantly reduce Lp(a) levels and may decrease events but it is cumbersome. A historical control study of 120 patients with established CAD received apheresis for a mean duration of 5 y. These patients had a mean Lp(a) reduction from 112 mg/dL to 30 mg/dL, and an absolute risk reduction of 73% for major adverse CV events. The annual CV event rate was decreased by 86% and the annual MI rate was decreased by 97% [268]. In another prospective observational multicenter study of 170 high-risk patients with mean LDL-C 99.0 mg/dL and Lp(a) 104.9 mg/dL who underwent apheresis, there was an approximately 66% reduction in both LDL-C and Lp(a), and reductions in MI, percutaneous coronary intervention, and coronary artery bypass grafting 2 y post-apheresis [269].

A Women's Health Initiative substudy reported that patients with a genetically confirmed SNP for elevated Lp(a) had increased CV risk with a hazard ratio of 2.11. The risk was reduced by the use of aspirin, hazard ratio of 0.44, with a statistically significant interaction between genetic studies and aspirin efficacy [270].

There are multiple approaches to gene silencing including antisense single strand that is used to prevent translation of the mRNA of the protein, siRNA double strand RNA-induced silencing complex (RISC) mechanism—short interrupting RNA which is an RNA based approach and a structured aptamer. The antisense oligonucleotide trials were the first trials to specifically study Lp(a) reduction with controlled randomization. This is significant as there are no approved therapies explicitly indicated for treating elevated Lp(a) levels [271, 272]. *LPA* gene is transcripted producing the Apo(a) mRNA and then a DNA-like single stranded antisense oligonucleotide combines with it, and then is degraded by RNase H1' preventing formation of Apo(a) and hence Lp(a). This antisense oligonucleotide enters the liver and blocks the formation of Apo(a). The hepatocytes continue to produce and transport LDL, but both Apo(a) alleles are inhibited and Lp(a) levels decrease as Lp(a) assembly is disrupted. The liver also does not develop steatosis. In a phase 1 trial of antisense oligonucleotide therapy, the mean change in Lp(a) concentration from baseline was −80% for the 300 mg dose at day 30. The effect persisted for an additional 1–2 months after therapy was discontinued [272]. A similar phase 2 trial showed it to be an effective treatment approach when compared to placebo [271]. Additionally, a significant reduction was noted in OxPL and monocyte-mediated inflammation.

A breakthrough was achieved using advanced antisense technology to enhance drug delivery and improve safety and efficacy utilizing N-Acetyl-galactosamine (GalNAc), a highly efficient ligand for the asialoglycoprotein receptor. GalNAc is derived from galactose and is a well characterized pro-drug that is cleaved and cleared rapidly. Apo(a)-LRX is roughly 30 times more potent than the parent antisense oligonucleotide, leading to more than 10 times lower dose and improved tolerability. The liver is able to actively uptake the antisense oligonucleotide [273], which allows a huge reduction in the dose of the antisense oligonucleotide therapy. The IONIS-Apo(a)-LRX at a dose of 40 mg achieved a 90% reduction in mean Lp(a) level [271]. A phase 2 trial by Tsimikas et al. also showed an effective Lp(a) reduction with antisense oligonucleotide and GalNAc therapy [274].

Lp(a) >60 mg/dL is the threshold for apheresis in Germany and the United Kingdom as it is the threshold dictated by the insurance companies for reimbursement [228].

A brilliant genetic Mendelian randomization study by Ference et al. showed that a 38 mg/dL decrease in LDL-C or about a 22% reduction overall is a meaningful clinical reduction. The same analysis for Lp(a) is about 100 mg/dL. Future treatment studies can aim for this therapeutic threshold [275].

Summary

Per the European Atherosclerosis Society and US National Lipid Association recommendations, backed by a vast amount of robust data, Lp(a) levels <50 mg/dL are considered optimal. An argument can be made, backed by fair data, that levels of Lp(a) >30 mg/dL should be the threshold for therapy. There is a 20–30% reduction in Lp(a) levels with, niacin, estrogen, PCSK9 inhibitors, CETP inhibitors, and mipomersen, whereas statins lead to a 10–20% increase. Apheresis provides a 30–35% decrease in Lp(a) levels and antisense oligonucleotide therapy achieves a 80–90% reduction. With such potent therapies, the next frontier is their utilization in the prevention of major adverse CV events and CAVS in patients on guideline-directed optimal medical therapy. With this high level of cost effectiveness and lack of knowledge about the impact of Lp(a) risk, a strong case can be made to add Lp(a) as a standard part of lipid panel.

Acknowledgement To Sheba Khan for assistance on the Lp(a) section.

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Cardiovascular Genomics

4

Michael J. Wilkinson

What Is Cardiovascular Genomics?

Genomics involves comprehensive analysis of the genetic make-up and variation for a species in order to better understand both normal biology and the relationship between genetic variation and risk of disease. It takes a "wide-view" of the potential impact of genetic variation on health and disease, seeking to understand the potential influence of multiple, concomitant genetic variations (compared to single, high-impact, monogenic variations) on determining the phenotype and health of an organism. Genomics has grown out of efforts to fully define the human genome, beginning with completion of the Human Genome Project in 2003. Multiple efforts to catalog variation in the human genome have included The Single Nucleotide Polymorphism (SNP) Consortium, the International HapMap Project, and the 1000 Genomes Project [1, 2]. The field of cardiovascular genomics involves studying the influence of genetic variation on cardiovascular disease (CVD) risk. Examining the human genome for factors contributing to CVD risk has led to the discovery of SNPs which increase the risk of CVD. Through experiments such as genome-wide association studies (GWAS) and Mendelian randomization, investigators have recently linked particular SNPs to clinical CVD phenotypes. Identification of the links between genetic variants and CVD has also allowed for the development of "risk scores," which estimate the CVD risk resulting from the presence of multiple SNPs. Such studies have also led to the identification of potential targets for pharmacotherapy in lipidology and CVD. Recent advances in cardiovascular genomics have been facilitated by the development of newer tools for gene sequencing. The automated Sanger method has been largely replaced by newer technologies which can perform gene sequencing more quickly and cost-effectively. These newer technologies are collectively referred to as next-generation sequencing (NGS) [3].

Two commonly used methods for analyzing the potential genetic contribution to CVDs are GWAS and Mendelian randomization. GWAS involves measuring the frequency of genetic variations (such as SNPs) in individuals with a disease and comparing these individuals to healthy controls. This method allows for exploring associations between an increased frequency of a genetic variant and a particular disease phenotype, such as CVD. An early example of GWAS in CVD involved analysis of 92,788 SNPs to identify a locus on chromosome 6p21 associated with an increased risk of myocardial infarction [4], while a recent GWAS in a large cohort of 312,571 genotyped participants from the Million Veteran

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_4

Table 4.1 Summary of the relationship between mutations in nine genes related to lipid metabolism and risk of coronary artery disease (CAD)

Gene	Carrier frequency	Intermediate phenotype	CAD risk	Therapy to mimic protective variants
Inactivating mutations confer increased risk				
LDLR	1 in 221 (0.5%)	↑ LDL cholesterol	↑ 320%	Not applicable
LPL	1 in 249 (0.4%)	↑ Triglyceride-rich lipoproteins	↑ 84%	Not applicable
APOA5	1 in 216 (0.5%)	↑ Triglyceride-rich lipoproteins	↑ 120%	Not applicable
Inactivating mutations confer decreased risk				
PCSK9	1 in 50 (2%)*	↓ LDL cholesterol	↓ 88%	Alirocumab, evolucumab (approved by the FDA and EMA)
NPC1L1	1 in 650 (0.2%)	↓ LDL cholesterol	↓ 53%	Ezetimibe (approved by the FDA and EMA)
ASGR1	1 in 120 (0.8%)	↓ LDL cholesterol ↓ Triglyceride-rich lipoproteins	↓ 34%	None
APOC3	1 in 150 (0.7%)	↓ Triglyceride-rich lipoproteins	↓ 40%	Volanesorsen (formerly known as ISIS-APOCIII$_{Rx}$ (phase III trials))
ANGPTL4	1 in 360 (0.3%)	↓ Triglyceride-rich lipoproteins	↓ 53%	REGN1001 (preclinical development)
LPA	1 in 285 (0.4%)	↓ Lipoprotein(a)	↓ 24%	AKCEA-APO(a)-L$_{Rx}$ (phase II trials)

*Prevalence estimate based on individuals of African ancestry. Carrier frequency substantially lower in other racial and ethnic groups. Reprinted by permission from Springer Nature: Khera and Kathiresan [35]

Program examined genetic variants (~32 million) for their association with lipid metabolism and risk of CVD in 297,626 participants with known lipid values [5]. A GWAS in >100,000 individuals identified 95 loci implicated in lipid metabolism, and in some cases, risk of CVD. At the time of publication in 2010, these investigators confirmed the association between lipids and 36 SNPs identified previously and reported associations between lipids and 59 SNPs for the first time (including in the genes *LDLRAP1, SCARB1, NPC1L1, MYLIP,* and *PPP1R3B)* [6]. Such GWASs identify candidate loci to test in Mendelian randomization studies. Mendelian randomization is an experimental design in which outcomes among individuals with a genetic variant of interest are compared with those among individuals without that genetic variation. Like randomized controlled drug trials in which individuals are randomized to exposure vs control, Mendelian randomization is based on the principle that genotypes are randomly assigned during meiosis; individuals are "randomized" to be exposed to the genetic variant of interest vs not (i.e. the control group) and outcomes can be compared between the two groups. This design relies on the quality of gene association studies in order to identify genetic variants of interest to test in these experiments [7]. To date, this approach has led to the identification of many genetic variants implicated in lipid disorders and CVD risk (Table 4.1). This chapter will explore some of the contributions and applications of cardiovascular genomics to the fields of CVD and clinical lipidology through a discussion of the use of cardiovascular genomics to: (1) develop polygenic risk scores, (2) estimate prevalence and CVD risk in familial hypercholesterolemia (FH), (3) define the role of lipoprotein(a) in risk for CVD and aortic valve disease, and (4) identify potential targets for lipid-modifying pharmacotherapy.

Development and Validation of Genetic Risk Scores for Cardiovascular Disease

One clinical application for cardiovascular genomics is the development of "genetic risk scores," which can be used to estimate risk for CVD based on the presence or absence of multi-

ple genetic variants. The development of such risk scores begins by defining the relationship between specific genetic variants and risk of CVD. For example, in the CARDIoGRAM plus C4D Consortium, investigators performed a GWAS meta-analysis from the 1000 Genomes Project, characterizing genetic variants in coronary artery disease (CAD) (evaluating 6.7 million common (minor allele frequency (MAF) >0.05) and 2.7 million low-frequency variants (0.005 < MAF < 0.05)). The investigators confirmed the contribution of multiple known loci to CAD and also identified 10 new loci. The minor allele frequency of the implicated genes was high, which supports the common disease-common variant hypothesis [8]. In another study involving an analysis of risk of coronary heart disease (CHD) in secondary and primary prevention trials (total of 48,427 individuals and 3477 events), patients were stratified by "genetic risk score" (defined by the presence of up to 27 SNPs, known to associate with CHD). The authors defined low, intermediate, and high genetic risk, which predicted CHD events and statin benefit. The greatest odds ratio (OR) for CHD from the panel of SNPs came from SNPs in the gene for lipoprotein(a) (*LPA*): rs3798220 (OR 1.47) and rs10455872 (OR 1.70). After these SNPs in *LPA*, the SNP with next highest OR for CHD in this analysis was rs4977574 at the 9p21.3 locus (OR 1.29) (Fig. 4.1) [9]. Another study examined the relationship between a CAD risk score (based on 50 SNPs) and cardiovascular outcomes in 3 large prospective cohorts. The investigators examined the interaction between genetic risk and lifestyle on outcomes and found that genetic risk can be offset by lifestyle [10]. Another study evaluated a polygenic risk score based on 57 variants in the West of Scotland Coronary Prevention Study (WOSCOPS), Coronary Artery Risk Development in Young Adults (CARDIA), and BioImage cohorts, and found that the greatest risk reduction from statins occurred in the subgroup with the highest genetic risk, despite similar degrees of low-density lipoprotein (LDL) lowering across genetic risk groups. Also, investigators found that a higher genetic risk score was associated with increased coronary artery calcium and carotid artery plaque [11]. A recent report describes the development and testing of a genome-wide polygenic score for CAD, including validation and testing. Here, the CAD risk score is based on >6 million variants and performed well as a risk estimator, and there was a large amount of patients (8%) who were estimated to be at ≥3-fold risk of CAD based on the CAD risk score; many more people affected than with FH [12]. It is likely that the predictive ability of such genetic risk scores will continue to improve as additional genetic variants implicated in CVD are discovered and incorporated into such polygenic risk estimates. As these polygenic risk estimates continue to improve, it is conceivable that their use will soon be translated into

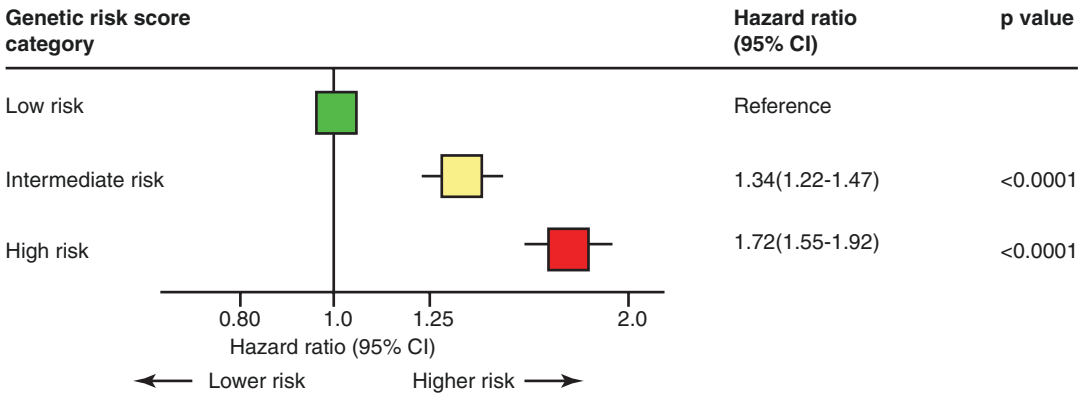


Fig. 4.1 An example of a genetic risk score used to estimate risk for coronary heart disease based on up to 27 single nucleotide polymorphisms (SNPs). (Modified from Mega et al. [9], with permission from Elsevier)

clinical practice and used alongside traditional risk factors (such as plasma lipid levels) to provide clinicians and patients with a more robust estimation of individualized cardiovascular risk.

Exploring Prevalence and Risk of Familial Hypercholesterolemia through Cardiovascular Genomics

FH is an autosomal dominant lipid disorder characterized by elevated LDL-C (low-density lipoprotein cholesterol) and an increased risk for premature CVD [13]. Interestingly, cardiovascular genomics has been used to gain further insight into the prevalence of FH and to examine CVD risk associated with FH. With regard to prevalence of FH, investigators performed genetic testing in a large cohort of patients in a US healthcare system to determine the prevalence of FH based on variants in *LDLR, APOB, PCSK9*, and associated risk of cardiovascular outcomes and patterns of statin use. The authors estimated the prevalence of FH to be 1:256. Only 2.5% of individuals with the severe hypercholesterolemia phenotype (LDL ≥ 190 mg/dL) had an FH variant, and 45% of people with an FH variant did not have an LDL-C ≥ 190 mg/dL [14]. Another study determined the prevalence of certain FH mutations in 98,098 individuals from the Copenhagen General Population Study as a way of estimating the prevalence of FH in the general population. Based on mutations in *LDLR* and *APOB*, the estimated prevalence of FH in the population was predicted to be 0.46% (1:217) [15]. Another study analyzed a large cohort (n = 20,485) to assess for the prevalence of FH mutations (in *LDLR*, *PCSK9*, and *APOB*). Overall, the prevalence of an FH mutation in those with LDL-C ≥ 190 mg/dL was low at 1.7%. However, at any given LDL-C level, the risk of CAD was increased in those with an FH mutation. For example, compared to a group with no FH mutation and an LDL-C < 130 mg/dL, those with high LDL-C (≥190 mg/dL) but without an FH mutation had a sixfold increased risk for CAD (OR 6, 95% CI 5.2–6.9), while those with both an FH mutation and LDL-C ≥190 mg/dL had a 22-fold increase in the odds of CAD (OR 22.3, 95% CI 10.7–53.2). This likely represents the effect of lifetime exposure to high LDL-C in those with an FH mutation, compared to those in whom exposure to LDL-C may be limited to a shorter portion of their lifetime [16]. Another study examined the contribution of monogenic risk (FH) vs polygenic risk in the odds of myocardial infarction (MI) in patients ≤55 years old (n = 2081), vs controls without history of MI (n = 3761). Monogenic risk was based on the presence of mutations in *LDLR*, *PCSK9*, and *APOB*; however, only mutations in *LDLR* were observed. The polygenic risk score was based on 6.6 million common DNA variants, which have been previously studied and implicated in risk for MI. Patients in the top 5% of polygenic risk had an increased odds by 3.73-fold (95% CI 3.06–4.56, $p < 0.0001$) for premature MI relative to controls and their mean LDL-C was 130 mg/dL (compared to mean LDL-C of 122 mg/dL for those with neither monogenic or polygenic risk). Patients with monogenic risk had a similarly increased odds for premature MI by 3.76-fold (95% CI 2.12–6.82, $p < 0.0001$), but mean untreated LDL-C was higher at 202 mg/dL. This suggests that despite similar risk for premature MI conferred by polygenic and monogenic factors, unlike in patients with FH, LDL-C may not serve as a biomarker to identify those at high polygenic risk for premature MI [17].

The Lipoprotein(a) Story: Establishing Links to Cardiovascular Disease through Genome-Wide Association Studies and Mendelian Randomization

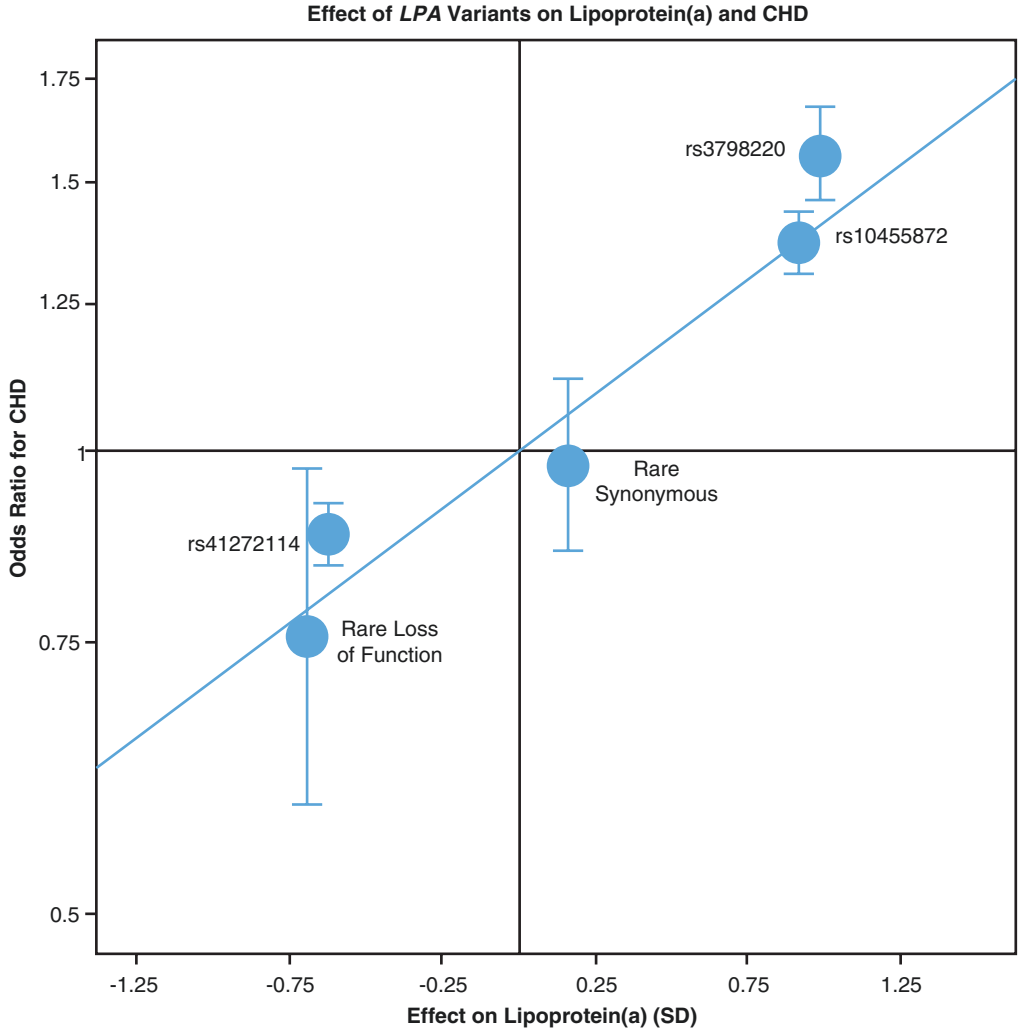
A powerful example of the use of cardiovascular genomics to advance our understanding of risk factors for CVD is in the story of lipoprotein(a) (Lp(a)). Elevated blood levels of Lp(a) are associated with an increased risk of CVD and calcific aortic valve stenosis (CAVS), and Lp(a) levels are genetically determined. To date, in addition to extensive preclinical and mechanistic research, our understanding of the link between genetic

variants in the Lp(a) gene (*LPA*), Lp(a) blood levels, and risk of CVD and CAVS is largely a result of epidemiologic observations and cardiovascular genomics, namely GWAS and Mendelian randomization [18]. The use of GWAS and Mendelian randomization has rapidly advanced our understanding of the importance of Lp(a) in CVD and CAVS risk and has culminated in the development of targeted therapies to lower Lp(a), which are currently in clinical trials (antisense oligonucleotide (ASO) AKCEA-APO(a)-LRx (completed phase 2: NCT03070782), now called TQJ230 with planned phase 3 cardiovascular outcomes trial: NCT04023552, and small interfering ribonucleic acid (siRNA) therapy (AMG890, phase 1: NCT03626662)). In 2009, a GWAS-based case-control study using NGS to detect multiple SNPs associated with CVD included 40 SNPs in the region on chromosome 6 containing the *LPA* gene (6q26–27). Two of the SNPs identified in the *LPA* gene (rs10455872 and rs3798220) were found to account for a significant degree (36%) of the variation in Lp(a) levels, and both associated with Lp(a) with a small number of Kringle-IV type 2 repeats. When controlling for Lp(a) level, the association between these SNPs and CVD was no longer observed, suggesting that Lp(a) is a causal link between these SNPs and CVD [19]. In a study by Emdin and colleagues, genetic variants associated with lower Lp(a) resulted in reduced risk of CHD, peripheral vascular disease, aortic stenosis, stroke, and heart failure, and were also associated with better kidney function (an increase in estimated glomerular filtration rate) (Fig. 4.2) [20]. The link between genetically increased Lp(a) and risk of stroke was further demonstrated in a recent analysis which found that the *LPA* SNP rs10455872 increased risk of ischemic stroke (risk ratio 1.27 (95% CI 1.06–1.51)) in a large Danish cohort [21]. Additional evidence supporting the importance of genetic variants in *LPA* and risk of peripheral arterial disease (PAD) comes from a recent GWAS from the Million Veteran Program. The authors of this study identified 19 SNPs associated with risk of PAD, and the *LPA* SNP rs118039278 had the strongest association with PAD compared with the other 18 variants based on an OR of 1.26 (95% CI 1.22–1.30, $p = 1.57 \times 10^{-43}$). Significant risk of PAD was also observed for SNPs in lipoprotein lipase (*LPL*) and *LDLR*, which relate to triglyceride and LDL metabolism, respectively [22]. With regard to CAVS, Thanassoulis and colleagues performed GWAS using existing SNP data from large cohorts to evaluate for associations between SNPs and calcification of the aortic valve by computed tomography, presence of aortic valve (AV) stenosis, and need for aortic valve replacement. Only 1 SNP was significantly associated with AV calcification in the discovery cohort: rs10455872 in *LPA*. The relationship between rs10455872 and risk of aortic valve calcification was verified in a replication cohort, and the risk was no longer observed when controlling for Lp(a), suggesting Lp(a) as a causal link between the SNP and development of the phenotype [23]. Substantial data from genetic studies are now available to support a relationship between mutations in *LPA*, lipoprotein(a) plasma levels, and risk of CAVS [24]. Thus, due in part to such GWAS and Mendelian randomization studies, the link between Lp(a) and CVD is clear [18].

Use of Cardiovascular Genomics to Identify Potential Targets for Lipid-Modifying Pharmacotherapy

Lipoprotein(a)

Clinical trials of Lp(a)-lowering therapies are currently underway, and interestingly, recent studies have used Mendelian randomization in an attempt to predict the magnitude of Lp(a) lowering by a drug which would be required to translate into CVD risk reduction. For example, Burgess and colleagues used Mendelian randomization to predict the impact of Lp(a) reduction on CHD risk, compared with LDL-C reduction, based on the presence of 43 *LPA* SNPs. They performed genotyping to determine the presence of SNPs in large databases with CHD outcomes data. In their conclusions, the authors suggest that a 101.5 mg/dL reduction in Lp(a) should provide the same degree of CHD risk



Variant rsID	Variant Class	Protein Change	Minor Allele (Frequency)	Normalized Lp(a) Beta	CHD Odds Ratio	P Value (CHD)
rs3798220	Missense	Ile4399Met	C (0.051)	0.98	1.57	3.0E-35
rs10455872	Intronic	--	G (0.022)	0.92	1.38	1.4E-56
Rare Synonymous	Synonymous	--	(0.02)	0.16	0.98	0.80
rs41272114	Splice Donor	--	T (0.02)	-0.62	0.88	3.4E-7
Rare Loss of Function	Premature Stop/Splice site/Frameshift	--	(0.007)	-0.69	0.76	0.033

Fig. 4.2 Genetic variants in *LPA* are associated with increased and decreased risk of coronary heart disease based on logistic regression adjusted for potential confounders (CHD, coronary heart disease, Lp(a), lipoprotein(a)). (Reprinted from Connor et al. [20], with permission from Elsevier)

reduction as a 38.67 mg/dL reduction in LDL-C [25]. In a similar analysis, Lamina and colleagues used Mendelian randomization to predict the amount of Lp(a) lowering required to confer a reduction in CHD risk similar to a 38.67 mg/dL reduction in LDL-C and reached a different conclusion. Their model used 27 *LPA* SNPs and measured Lp(a) with outcomes data from existing large databases. They used Lp(a) measurements from 14,000 people, all performed in the same laboratory and suggest that a reduction of Lp(a) by 65.7 mg/dL would provide the same degree of CHD risk reduction as a reduction in LDL-C by 38.67 mg/dL [26]. Lp(a) is currently a target of ASO therapy (AKCEA-APO(a)-LRx, completed phase 2: NCT03070782; now called TQJ230 with planned phase 3 cardiovascular outcomes trial: NCT04023552) and siRNA therapy (AMG890, phase 1: NCT03626662). The results of these studies are highly anticipated and will test the findings from such Mendelian randomization studies in large, prospective, randomized controlled trials.

Bempedoic Acid

Cardiovascular genomics have also been used in an attempt to predict the effects of a small molecule called bempedoic acid in terms of its potential for LDL lowering and CVD risk reduction. A recent Mendelian randomization study involving 654,783 participants modeled the potential effects of inhibiting ATP citrate lyase using bempedoic acid by examining the effects of inherited variants in the gene for ATP citrate lyase (*ACLY*). The investigators compared these effects with those occurring with inherited variations in HMG-CoA-reductase (*HMGCR*) as a model for statin therapy. For every 10 mg/dL reduction in LDL-C in those with either variations in *ACLY* or *HMGCR*, the reduction in cardiovascular events was similar (OR for *ACLY* score was 0.823 (95% CI 0.78–0.87; $p = 4.0 \times 10^{-14}$, and OR for *HMGCR* score was 0.836 (95% CI, 0.81–0.87; $p = 3.9 \times 10^{-19}$). While this model cannot predict the outcome of a shorter term outcomes trial with bempedoic acid (which is currently underway, NCT02993406) or account for potential off target effects of the drug, it suggests that inhibition of ACLY with bempedoic might produce similar reductions in cardiovascular risk for a given reduction in LDL-C compared with statin therapy [27].

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibitors

PCSK9 inhibitors (monoclonal antibodies: alirocumab and evolocumab) are used for lipid-lowering and reduction of CVD risk in certain patients [28, 29], and their effectiveness has also been modeled and predicted using genomic methods. For example, a recent study examined the influence of variations in *PCSK9* on degree of LDL lowering, risk of cardiovascular death, and all-cause mortality. The presence of a greater number of weighted *PCSK9* alleles was associated with lower LDL, which was causally associated with reduced cardiovascular mortality but not a reduction in all-cause mortality. These findings are largely in-line with randomized controlled drug trials using PCSK9 inhibitors [28, 29]. Importantly, this study highlights differences which can emerge when studying the effects of genetic variants versus the effects of related pharmacologic interventions. For example, the authors observed that *PCSK9* variants did not affect Lp(a), while drug trials of monoclonal antibody inhibitors of PCSK9 have shown the potential to reduce Lp(a) [30].

Angiopoietin-like 3 Protein (ANGPTL3) and Angiopoietin-like 4 Protein (ANGPTL4)

Another example of translating the findings from cardiovascular genomics into drug development is in angiopoietin-like 3 protein (ANGPTL3; encoded by *ANGPTL3)* and angiopoietin-like 4 protein (ANGPTL4; encoded by *ANGPTL4*). These proteins are involved in lipid metabolism as inhibitors of lipoprotein lipase (LPL). From the Dallas Heart Study, mutations leading to loss

of function in *ANGPTL3* and *ANGPTL4* were associated with increased activity of LPL and lower triglyceride levels [31]. Investigators used exome sequencing in two members of a family with combined hypolipidemia (but without causative mutations in *APOB*) to search for mutations which might explain the hypolipidemia phenotype of low LDL, high-density lipoprotein (HDL), and triglycerides in this family. Their analysis suggested that nonsense mutations in *ANGPTL3* were responsible for hypolipidemia in this family [32]. Investigators have also linked mutations in *ANGPTL4* (resulting in reduced function) to a lower risk of CVD. This study simultaneously confirmed links between mutations in *LPA* (lipoprotein(a)) and *LPL* (lipoprotein lipase; loss-of-function) and an increased risk of CVD, and between mutations in *PCSK9* (PCSK9) and *LPL* (gain-of-function) and reduced CVD risk [33]. The link between loss-of-function mutations in *ANGPTL3* and reduced triglycerides, LDL, and HDL has prompted the development of drugs designed to inhibit ANGPTL3. A recent trial found that loss-of-function mutations in *ANGPTL3* are associated with a reduced risk of CVD, and that a monoclonal antibody (evinacumab) directed at ANGPTL3 reduces atherosclerosis in mice, and reduces triglycerides (up to 76%) and LDL (up to 23%) in humans [34]. Evinacumab is now in phase 2 clinical trials (NCT03175367) (and phase 3 for homozygous FH (HoFH): NCT03409744), and an ASO directed at ANGPTL3 (AKCEA ANGPTL3-LRx) is also in phase 2 trials (NCT03360747, NCT03514420, NCT03371355).

Conclusions

The field of cardiovascular genomics has rapidly advanced over the past 15–20 years, driven by the development of NGS technologies and the ambition of investigators to leverage these technologies through GWAS and Mendelian randomization. Cardiovascular genomics has contributed to our understanding of the genetic basis of CVD and holds great promise for use in personalized and precision medicine. As the predictive ability of polygenic risk scores continues to be refined, such tools are likely to be made available to clinicians and patients for use in routine care. The combination of a personalized, polygenic risk score with traditional risk factors is likely to help us refine and personalize CVD risk estimates and better identify patients who may benefit most from lifestyle change and pharmacotherapy to prevent CVD. Cardiovascular genomics has also rapidly accelerated the identification of potential targets for drug therapy. There are now many examples of the process by which GWAS has been used to identify potentially important genetic variants, hypotheses regarding the importance of genetic variants for CVD risk have been tested in Mendelian randomization, and findings have led to the development of a drug to reduce CVD risk. We are in a very exciting era and can expect the landscape of therapeutic lipidology to change dramatically in coming years. The process of translating findings from cardiovascular genomics to drug development is occurring rapidly and cardiovascular outcomes trials with novel agents developed through this process are currently underway and the results are highly anticipated.

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5 Epidemiology of Atherosclerotic Cardiovascular Disease

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Atherosclerotic Cardiovascular Disease in the USA

Atherosclerotic cardiovascular disease (ASCVD) encompasses a range of conditions resulting from atherosclerotic plaques in arterial beds, including those in the heart (coronary heart disease [CHD]), legs (peripheral arterial disease), aorta, carotid, cerebral, and renal arteries. In the United States, estimated lifetime risk for total cardiovascular disease (CVD) (fatal and nonfatal CHD, atherosclerotic and hemorrhagic stroke, congestive heart failure, and other CVD death) is >50% [1]. Data from 5 population-based cohorts included in The Cardiovascular Disease Lifetime Risk Pooling Project indicated that lifetime risk for total CVD among men and women free of CVD at 55 years of age is 60.2% and 56.3%, respectively [1]. According to a recent report by the American Heart Association (AHA), 121.5 million American adults had some form of CVD (CHD, stroke, heart failure, and hypertension) between 2013 and 2015, and over one million adults in the USA were expected to experience coronary events in 2019 [2]. In addition, approximately 795,000 Americans suffer a new or recurrent stroke annually. The incidence of stroke increases with advancing age in both men and women and is a leading cause of serious long-term disability; 3% of men and 2% of women in the USA report disability due to stroke [2, 3].

Direct and indirect costs associated with ASCVD represent a significant economic burden in the USA. Between 2014 and 2015, CVD and stroke accounted for 14% of health-related expenditures with an estimated total cost of $351.2 billion ($213.8 billion in direct costs and $137.4 billion in lost productivity/mortality) [2]. According to a 2016 report [4], total direct medical costs of CVD are projected to increase to $749 billion by 2035. Although the death rate from CVD in the USA decreased over the last decade, it remains the leading cause of death among adults and accounted for 840,768 (approximately 1 in 3) deaths in 2016 [3]. Of deaths attributable to CVD, CHD accounted for 43.2% and stroke 16.9%. Globally, CVD is also the leading cause of death and, according to the World Health Organization, accounted for more than 17.6 million deaths in 2016.

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_5

Atherothrombotic Process

The current understanding of the pathophysiology of atherothrombotic disease derives from the descriptive pathology of human autopsies and experimental studies in animal models. A detailed description of the atherothrombotic process is beyond the scope of this chapter but will be briefly described for context and depicted in Fig. 5.1. The process begins when cholesterol-rich, apolipoprotein (apo)B-containing lipoproteins penetrate and accumulate in lesion-prone areas of the arterial wall, where they are modified (e.g., oxidized, acetylated); this triggers unregulated uptake by macrophages and an inflammatory cascade. Low-density lipoprotein (LDL) cholesterol is the primary driver of this process, though recent evidence suggests that most apoB-containing lipoproteins (up to ~70 nm in diameter) are capable of promoting plaque formation, i.e., all but the largest very-low-density lipoprotein (VLDL) and chylomicron particles [5]. The accumulation of modified apoB lipoprotein particles in the arterial wall leads to an immune-inflammatory response characterized by increased secretion of adhesion molecules and

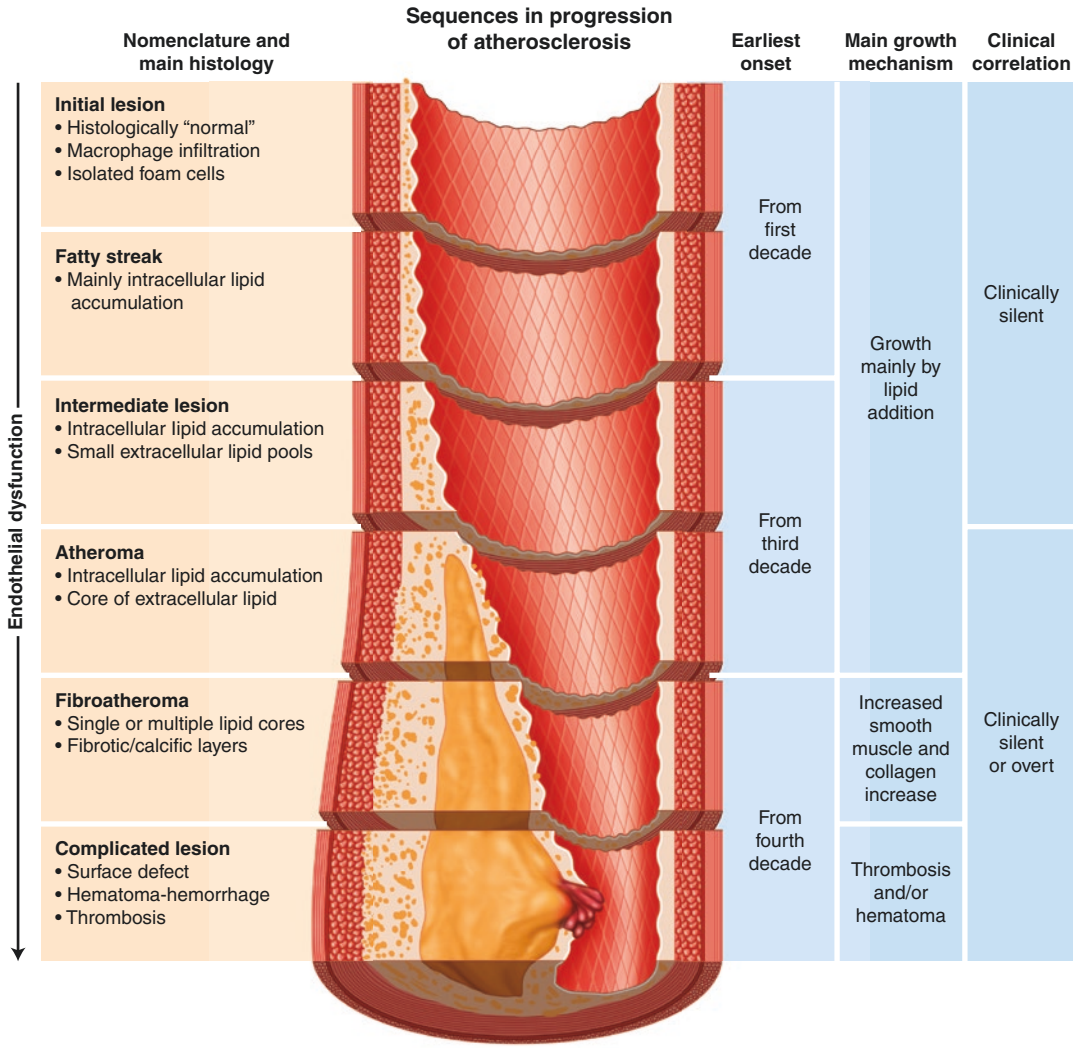

Fig. 5.1 Progression of the atherosclerotic lesion. (Anonymous, Stages of Endothelial Dysfunction in Atherosclerosis, CC BY-SA 3.0)

the recruitment of monocytes and leukocytes to the arterial lesion. In this inflammatory state, monocytes penetrate the arterial wall and differentiate into phagocytic macrophages that take up the oxidized apoB particles, forming lipid-rich foam cells that coalesce to form a fatty streak. Arterial smooth muscle cells simultaneously secrete extracellular matrix proteoglycans, collagen, and elastin fibers that form a fibrous cap over the growing lesion. Over time, the death of endothelial, smooth muscle, and foam cells leads to the formation of a soft and destabilizing lipid-rich core under the fibrous cap of the growing plaque. In the later stages of atherosclerosis, plaque progression and thickening of the arterial wall can lead to a significant narrowing of the lumen of the artery, limiting blood flow to the affected organ.

In the presence of abundant circulating atherogenic lipoprotein particles, the ongoing cycle of lipoprotein oxidation, foam cell formation, and cell death perpetuate the inflammatory state. Inflammatory processes are important in the development of plaque instability because inflammation can produce thinning of the fibrous cap, enhancing the probability of fissure formation, or frank rupture. Over time, hemodynamic stresses and degradation of the cellular matrix also increase the risk of plaque rupture. A ruptured plaque may result in the formation of a thrombus on the plaque's surface, or a piece of the plaque (or thrombus) may break off and become lodged in a different part of the artery. Either scenario can result in a partial or complete blockage of the arterial lumen, leading to downstream tissue ischemia. The main acute clinical complications of atherothrombotic disease are myocardial infarction (MI) and ischemic stroke, caused by a blockage in an artery of the heart or brain, respectively, from a ruptured plaque [6, 7].

Cardiovascular Epidemiology

Epidemiology is the study of the distribution and determinants of disease frequency in human populations and the application of that knowledge to evaluate interventions intended to reduce morbidity and mortality. Epidemiologists systematically investigate the associations between endogenous (e.g., cholesterol level) and exogenous (e.g., cigarette smoking) exposures and disease outcomes in populations, or subgroups of individuals within populations, to generate hypotheses about potential causal factors that increase or reduce the risk for human disease. Causal hypotheses generated through observational research may subsequently undergo evaluation in clinical intervention trials, which provide the strongest evidence in favor of an exposure being causally related to disease incidence. Historically, epidemiological methods were employed primarily in the study of infectious disease outbreaks or "epidemics." As the twentieth century progressed, there was a marked shift in the USA and other developed countries from infectious diseases to chronic diseases (characterized by latency periods of 10–20 years or more) as the major causes of mortality. As a result of these changes in disease distribution, the term "epidemic" was broadened to include any disease, infectious or chronic, occurring at an increased frequency in a population [8].

The fundamental measures of disease frequency in epidemiology are incidence and prevalence. Incidence quantifies the number of new occurrences of a disease in a population of at-risk individuals within a specified period of time. Two commonly used incidence measures are cumulative incidence (CInc) and incidence rate (IR).

CInc is defined as the proportion of individuals who become diseased during a specified period of time and is calculated as follows: CInc = number of individuals who develop a disease during a given time period/total number of individuals in the population at risk. It provides an estimate of the probability, or risk, that an individual will develop a disease during this time period. However, CInc assumes that the entire population is at risk for the duration of the specified time period and does not account for circumstances such as loss to follow-up or death from causes other than the disease of interest (competing risk). IR, on the other hand, accounts for the actual amount of follow-up time contributed by all individuals in the study population

(person-time units of observation). IR can be thought of as an instantaneous rate of disease development in a population and is calculated as follows: IR = number of individuals who develop a disease during a given time period/total observation time for all individuals followed. In contrast, prevalence measures the proportion of individuals in a population who have a disease at a given point in time. Using the formula prevalence = number of existing cases of a disease/total population, prevalence can be used to estimate the probability that an individual will have the disease at a specific point in time [8].

Observational Study Designs

Unlike clinical intervention studies, where individuals are allocated to the exposure of interest by study investigators, observational investigations examine the relationships between exposures occurring within a free-living population and disease outcomes. Observational studies have important limitations, such as the potential for residual confounding, and can be subject to biases that must be considered in the study design, analysis, and interpretation. Rigorously conducted observational studies, however, are capable of providing valuable insights that can be used to estimate disease risk, predict disease occurrence, and provide insights into potential causes of disease.

The two main types of observational study designs are the case-control study and the cohort study. A case-control study enrolls participants on the basis of whether they do or do not have the disease under study, defined as cases and controls, respectively. The proportion of individuals within each group with a history of exposure or characteristic of interest is then compared and used to assess the association between the exposure and disease. Case-control studies are particularly useful for studying rare diseases and diseases with very long latency periods, as well as for studying multiple potential etiologic exposures that might be associated with a specific disease, such as in the early investigation of a disease. A major advantage of case-control studies is that they can be conducted more quickly and less-expensively than studies requiring extended follow-up periods. A notable limitation of case-control studies, however, is the fact that both the exposure and the disease have already occurred at the time subjects are enrolled, making them susceptible to bias from participant selection and recall bias, and limiting inference regarding the temporal association between the exposure and disease.

In cohort studies, participants at risk, but (usually) free of the disease under study, are classified on the basis of exposure status, such as the presence or absence (exposed vs. unexposed) of a factor hypothesized to be related to a disease, or into categories of exposure such as quartiles, and followed over time to assess the relationship between exposure and disease incidence. An important advantage of the prospective cohort study design is the ability to more clearly establish a temporal relationship between exposure and disease. Since eligible participants are typically free from the disease or condition under investigation at the time exposure status is defined, direct calculation of IRs of the outcome in the exposed and non-exposed groups is also possible. Cohort studies are optimal for studying the effects of rare exposures since participants are selected based on their exposure status to ensure an adequate sample size for statistical analysis. In addition, cohort studies can be used to examine multiple health effects of a single exposure, providing a more comprehensive understanding of the range of potential health outcomes related to an exposure of interest. Limitations of the cohort study design include the time, personnel, and financial burdens associated with large sample sizes and often long duration of follow-up, and the potential impact that losses to follow-up of participants can have on the validity of the results.

Bias and Confounding in Observational Studies

The findings from all observational studies are susceptible to the influence of biases and residual confounding that, at times, no amount of statisti-

cal analysis can fully address. Bias can be broadly defined as any systematic error that results in an inaccurate estimate of the association between exposure and disease. Two main classes of bias found in epidemiological studies are selection bias and information bias. Selection bias occurs when the sample of individuals chosen for inclusion into a study differs from the target population it is intended to represent. This can occur as a result of procedures used to select participants, as well as from factors that influence study participation. Information bias arises when data on the exposure or outcome are obtained differently from different study groups. A common type of information bias present in observational studies is recall bias, defined as the tendency for individuals with a particular adverse health outcome to remember and report previous exposures differently from those who are not affected, or when those who have been exposed to a potential hazard report subsequent events with a different degree of completeness and accuracy than those who were not exposed. One particularly problematic type of information bias is misclassification, which occurs when individuals are incorrectly categorized with respect to exposure or disease status. The most effective way to minimize bias in observational studies is through thoughtful and meticulous study design. Analytical methods can also be used to evaluate and address some sources of bias from observational study results.

In addition to bias, confounding may also impact the validity of statistical associations from observational studies. Confounding is a mixing of effects, where the observed association between the exposure and disease outcome is fully or partially due to the effect of a third (confounding) factor. A confounding factor must be associated with the exposure and with the disease, but not lie on the causal pathway from exposure to disease. Imbalance of a known or unknown confounding factor between exposure groups can lead to an over- or underestimation of the true association between exposure and disease. In observational studies, confounding can be controlled through study design and/or statistical analysis. Two approaches to control confounding through study design are restriction, such as including only individuals within prespecified categories of a confounder, and matching, i.e., selecting subjects in a way that distributes potential confounding factors equally among exposure groups. Stratification is an analytical approach to control for confounding that evaluates the association between the exposure and disease within homogenous categories or strata of the confounding variable. For example, if sex is a confounding factor, estimates of the association should be calculated separately for men and women. Confounding is also addressed statistically through multivariable regression by including known and measured factors thought to be potential confounders in regression models.

Because some confounders may be unknown, or if known, be only crudely measured, residual confounding can occur. For example, numerous observational studies showed a strong, consistent and statistically significant association between vitamin E intake (including from dietary supplements) and risk for CHD [9–13]. However, randomized controlled trials (RCTs) of vitamin E supplements failed to demonstrate a significant protective effect of vitamin E supplementation against CHD risk, suggesting that the associations reported in observational studies were attributable to residual confounding. Thus, other behaviors associated with vitamin E supplement use, such as higher diet quality and physical activity, which may have been measured with low precision, likely confounded the association between vitamin E supplement use and CHD risk, despite attempts to adjust statistically for these factors [14].

RCTs

RCTs avoid many of the methodological challenges faced by observational studies and are therefore widely accepted as the gold standard for supporting causal relationships between exposures and health outcomes. In an RCT, study investigators randomly assign participants to separate groups to compare exposures, typically therapeutic or preventive interventions. Therefore,

each study participant has a pre-defined chance of being assigned to each treatment group, and thus, the groups should have similar prognoses. Importantly, not only will all known confounding variables, theoretically, be randomly distributed between or among groups, but all unmeasured and unknown confounders will also be balanced. Randomization also ensures that the results of the study are not biased by the way participants are assigned to an exposure status, further ensuring that the observed effects of an exposure are not due to other factors. RCTs, however, are not without limitations. Compared with observational studies, RCTs are often more difficult to design and conduct and present challenges related to ethics, feasibility, and costs [15].

Mendelian Randomization

An observational research method that has provided substantial contributions to the field of cardiovascular epidemiology is Mendelian randomization, which uses genetic variation as a proxy to investigate the relationship between potentially modifiable risk factors and disease outcomes. Mendelian randomization is defined as the random assortment of genes inherited by offspring from parents during meiosis. In Mendelian randomization studies, genetic variants, such as single-nucleotide polymorphisms, are used as "instrumental variables" for modifiable risk factors hypothesized to affect disease outcomes. An instrumental variable is one that is associated with the risk factor (exposure) of interest, not related to confounders, and has no potential effects on the disease under study except through the risk factor modified by the genetic variant. Though observational in nature, Mendelian randomization studies are less likely to be affected by bias, confounding, and reverse causation than traditional observational studies, because exposure-associated genetic variants are randomly allocated to individuals prior to any exposure or disease outcome [16–18]. An important contribution of the Mendelian randomization approach was the finding that individuals with mutations in proprotein convertase subtilisin kexin type 9 (PCSK9) and Niemann-Pick C1-Like 1, both of which result in lower levels of LDL cholesterol throughout life, were associated with lower ASCVD risk [19, 20]. Genetic variants in lipoprotein lipase, apoC3, and apoA5 that result in decreased triglyceride (TG) and TG-rich lipoprotein cholesterol levels have also been shown to be associated with reduced ASCVD risk [21].

Association vs. Causation

The results of individual observational studies provide evidence for associations between risk factors and health outcomes, but insight on causal relationships must be inferred from the totality of the evidence. In the mid-1960s, Sir Austin Bradford Hill proposed nine criteria that provide a framework for evaluating when there is sufficient evidence to establish causality [22]. The most relevant of these criteria for the investigation of a potentially causal factor in ASCVD include the *strength* and *consistency* of the relationship across studies and populations, *dose-response* (progressively greater exposure associated with progressively higher or lower disease risk), a *biologically plausible mechanism* to explain why the exposure might be causally related to the development of the disease, appropriateness of the *temporal relationship* between the risk factor and the disease (i.e., the risk factor precedes the disease), and the availability of *confirmatory evidence* from laboratory and clinical intervention studies. Epidemiological research has established associations between lifestyle factors and physiological changes that inform testable hypotheses regarding causal pathways that have helped to identify targets for intervention. For example, evidence from population studies showing a strong association between elevated blood cholesterol and ASCVD event and mortality rates, along with studies in animals indicating that experimental elevation in blood cholesterol produced atherosclerosis, laid the foundation for clinical trials that have since demonstrated that lowering elevated atherosclerotic lipoprotein cholesterol levels reduces ASCVD event risk [23, 24].

Framingham Heart Study

The Framingham Heart Study (FHS) was the first large-scale, prospective population-based investigation of CVD in the USA. In 1948, the FHS was initiated with the goal of identifying common factors or characteristics that contribute to CVD. At the time, little was known about the general causes of heart disease and stroke; the prevailing view was that the hardening of arteries was an unavoidable consequence of aging. The investigators measured characteristics of a group of approximately 5200 men and women between the ages of 30 and 62 from the town of Framingham, MA, and followed them (and eventually 2 generations of offspring) over decades to determine what characteristics were associated with CVD later in life. The FHS provided clear evidence that risk factors, many of which were identifiable years or even decades before clinical events, could predict CVD risk. These findings also suggested that risk factor modification might be helpful for disease prevention. The FHS identified 4 major modifiable risk factors for CVD: high cholesterol, high blood pressure, cigarette smoking, and diabetes mellitus. Building upon the success of the FHS, additional studies of CVD epidemiology both in the USA and internationally have confirmed and expanded these findings.

ASCVD Risk Factors

The FHS study paved the way for future studies of cardiovascular risk factors, and today, the major established ASCVD risk factors include elevation in cholesterol carried by atherogenic lipoproteins, a premature family history of CHD (defined as CHD in a male first-degree relative <55 years of age or CHD in a female first-degree relative <65 years of age), low high-density lipoprotein (HDL) cholesterol (<40 mg/dL for men and <50 mg/dL for women), age ≥45 years for men and ≥55 years for women, current cigarette smoking, hypertension (systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥80 mm Hg or use of antihypertensive medication for lowering blood pressure), and diabetes mellitus [25, 26].

In 2013, the American College of Cardiology (ACC) and AHA jointly released the race- (Black/White) and sex-specific (male/female) Pooled Cohort Equations to predict 10-year risk of a first 'hard' ASCVD event (nonfatal MI, fatal CHD, nonfatal or fatal stroke) and guide clinicians in primary prevention. The equations, which have also been used for risk stratification in the 2018 AHA/ACC/Multisociety Cholesterol Guideline [27] estimate 10-year risk based on an individual's age, total cholesterol, HDL cholesterol, systolic blood pressure (including treated or untreated status), diabetes mellitus, and current smoking status [28]. In response to the expanding evidence base of ASCVD epidemiology, current practice guidelines now recommend that clinicians estimate risk by assessing a number of established risk-enhancing factors in addition to the 2013 Pooled Cohort Equations. Collectively, these factors represent comorbid conditions or biomarkers that may not be a part of routine screening, but are effective for refining risk stratification to inform preventive treatment plans [29]. The risk-enhancing factors identified in the 2018 AHA/ACC/Multisociety Cholesterol Guideline are presented in Table 5.1.

Subclinical measures of ASCVD can also be used in addition to traditional risk factors to refine risk stratification and guide treatment. For example, coronary artery calcium (CAC) scoring is a robust marker of the presence and degree of subclinical atherosclerosis that integrates both measured and unmeasured risk factors and is recommended for use as a decision aid for initiating statin therapy when risk status is unclear [29]. Results from the Multi-Ethnic Study of Atherosclerosis which included over 6000 men and women, demonstrated that significant ASCVD risk heterogeneity exists among individuals eligible for statin therapy based on current guidelines [30]. This analysis showed that when considering CAC in risk stratification, a CAC score of zero reclassifies approximately half of primary prevention patients at borderline and intermediate risk, based on the Pooled Cohort Equation estimate, as being at low risk (<5% 10-year risk for an ASCVD event). Conversely, those with a CAC score ≥100 Agatston units consistently have a 10-year

Table 5.1 Risk-enhancing factors in ASCVD risk assessment[a]

Family history of premature ASCVD (males, age <55 years; female, age <65 years)
Primary hypercholesterolemia (LDL cholesterol, 160–189 mg/dL [4.1–4.8 mmol/L]; non-HDL cholesterol, 190–219 mg/dL [4.9–5.6 mmol/L])
Metabolic syndrome (increased waist circumference [by ethnically appropriate cut points], elevated TG [>150 mg/dL, non-fasting], elevated blood pressure, elevated glucose, and low HDL cholesterol [<40 mg/dL in men; <50 mg/dL in women]; a minimum of 3 factors denotes a diagnosis)
Chronic kidney disease (eGFR 15–59 mL/min/1.73 m^2 with or without albuminuria; not treated with dialysis or kidney transplantation) **Chronic inflammatory conditions**, such as psoriasis, rheumatoid arthritis, lupus, or HIV/AIDS
History of premature menopause (before age 40 y) and history of pregnancy-associated conditions such as preeclampsia
High-risk race/ethnicity (e.g., South Asian ancestry)
Lipids/biomarkers associated with increased ASCVD risk Persistently elevated primary hypertriglyceridemia (≥175 mg/dL, non-fasting) If measured: **Elevated high-sensitivity C-reactive protein** (≥2.0 mg/L) **Elevated Lp(a)** (≥50 mg/dL or ≥125 mmol/L): indication for measurement is a family history of premature ASCVD **Elevated apoB** (≥130 mg/dL, corresponds to an LDL cholesterol ≥160 mg/dL): indication for measurement is TG ≥200 mg/dL **Ankle-brachial index** (<0.9)

AIDS acquired immunodeficiency syndrome, *apoB* apolipoprotein B, *ASCVD* atherosclerotic cardiovascular disease, *eGFR* estimated glomerular filtration rate, *HDLC* high-density lipoprotein cholesterol, *HIV* human immunodeficiency virus, *LDLC* low-density lipoprotein cholesterol, *Lp(a)* lipoprotein (a), *TG* triglyceride.
[a]Adapted from Grundy et al. [27]

ASCVD event risk ≥7.5%, favoring the use of statin therapy. Such findings have important implications for identifying which patients are likely to have meaningful benefits, or not, of statin therapy and ensuring healthcare resources are allocated accordingly.

Additionally, lifestyle factors such as stress, lack of social support, poor diet quality, and physical inactivity are associated with an increased risk of CVD but do not factor into the current risk stratification guidelines, in part because they operate through effects on other risk factors that are included in the risk stratification process. Nonetheless, these are important for clinicians to assess and address as part of the treatment plan. The focus of the remainder of this chapter will be specifically on lipoprotein-related risk factors.

Lipoprotein-related Risk Factors

Hypercholesterolemia was one of the first well-established major risk factors for ASCVD. Since the critical role of elevated circulating cholesterol in the formation of atherosclerotic plaques was first identified, the understanding of the relationship between different types of lipoprotein cholesterol and ASCVD risk has evolved enormously. Cholesterol and TGs are not water-soluble and must be transported in the blood in lipoprotein particles. In the fasting state, the three main classes of circulating lipoproteins are LDL cholesterol, TG-rich lipoprotein cholesterol (VLDL cholesterol and a small number of chylomicron remnants), and HDL cholesterol. The main functions of LDL and VLDL are the transport of cholesterol and TGs, respectively, from the liver to peripheral tissues, whereas HDL is primarily involved in the return of cholesterol from peripheral tissues to the liver for excretion. Evidence from epidemiological, genetic, and experimental animal model studies, as well as RCTs, has established the central and causal role of apoB-containing lipoproteins (LDL, VLDL, chylomicron remnants) in the pathogenesis of ASCVD.

HDL Cholesterol Is an ASCVD Risk Factor But Not a Target of Therapy

Although low levels of HDL cholesterol and its main structural protein, apoA1, are strongly associated with an increased risk of ASCVD in observational studies, this relationship has proven to be complicated. Mendelian randomization studies of genetic variants that alter HDL cholesterol levels have not shown significant associations with ASCVD risk, unlike those associated with changes in LDL- and TG-rich lipoprotein cholesterol, as reviewed later in this chapter [31, 32]. While some evidence suggests that therapies that increase HDL cholesterol or apoA1 levels are associated with a reduction in ASCVD risk [33], clinical trials investigating the use of such therapies, such as niacin and cholesteryl ester transfer protein inhibitors, have not demonstrated ASCVD risk reduction. HDL cholesterol levels can be raised through a variety of mechanisms, and it is unclear whether all would produce benefits for CVD risk. Consequently, HDL cholesterol is not currently considered a target of ASCVD risk reduction therapy, although it is used in ASCVD risk assessment and stratification [25].

Evidence for a Causal Relationship for ApoB-containing Lipoproteins and ASCVD Risk

Genetic variants that alter apoB-containing lipoproteins, and the cholesterol carried by those lipoproteins, provide strong evidence supporting a causal relationship to ASCVD risk. It is well documented that genetically inherited forms of severely elevated LDL cholesterol, such as familial hypercholesterolemia (homozygous and heterozygous), familial defective apoB-100, and polygenic hypercholesterolemia, are associated with a substantially increased risk of premature CHD [34]. Also, prolonged exposure to low LDL cholesterol levels beginning early in life as a result of genetic polymorphisms is associated with a reduction in the risk of ASCVD that is larger than would be anticipated based on results from studies of lowering LDL cholesterol levels later in life with pharmacologic interventions [35]. For example, Cohen et al. examined the effect of DNA-sequence variations in PCSK9 associated with reduced levels of plasma LDL cholesterol throughout the lifespan on incident CHD (MI, fatal CHD, or coronary revascularization) over 15 years in black and white men and women in the Atherosclerosis Risk in Communities prospective cohort study [19]. Loss-of-function mutations in PCSK9 were associated with a 28% reduction in mean LDL cholesterol and an 89% reduction in the risk of ischemic CHD (hazard ratio 0.11, 95% confidence interval [CI] 0.02–0.81; $p = 0.03$).

Mendelian randomization studies also support the causal effects of elevations in plasma TGs and TG-rich lipoproteins for ASCVD risk, independent of LDL cholesterol [31, 32, 36, 37]. The degree of risk reduction associated with each mmol/L (39 mg/dL) lower level of cholesterol carried by LDL or TG-rich lipoprotein particles (VLDL and chylomicron remnants) produced by genetic variants is similar, and roughly twofold greater than would be predicted on the basis of results from RCTs of cholesterol-lowering therapies, which have had an average duration of ~5 years [38].

Non-HDL cholesterol is composed of cholesterol carried by all potentially atherogenic (apoB-containing) particles, including LDL, intermediate-density lipoproteins, Lp(a), VLDL, chylomicron particles, and their remnants. Both components of non-HDL cholesterol (LDL cholesterol and TG-rich lipoprotein cholesterol) independently predict atheroma progression in statin-treated patients with coronary artery disease [39]. A recent meta-regression analysis of data from clinical intervention trials by Marston et al. [40] showed that pharmacologic reduction in non-HDL cholesterol is strongly associated with a lower risk of major cardiovascular events, regardless of the class of lipid-lowering drug employed. For each 1 mmol/L (39 mg/dL) reduction in non-HDL cholesterol, the effect of statin therapy (relative risk 0.80, 95% CI 0.77–0.82), which mainly lowers LDL cholesterol, was similar to that of fibrate therapy (relative

risk 0.79, 95% CI 0.71–0.88), which mainly lowers VLDL cholesterol. There is no apparent threshold in the relationship between non-HDL cholesterol level and ASCVD risk and the available data suggest a continuous relationship down to very low levels [41].

A pooled analysis by the Cholesterol Treatment Trialists' Collaboration showed that each 1 mmol/L (39 mg/dL) reduction in LDL cholesterol produced by statin therapy was associated with a reduction of 23% (95% CI 20–26%) in risk for a major CHD event. Thus, each 10 mg/dL reduction in LDL cholesterol induced by statin therapy would be expected to lower CHD event risk by 6.5% [$1 - 0.77^{(10/38.7)} = 0.0653$ or 6.5%]. Ference et al. showed that each 10 mg/dL reduction in LDL cholesterol produced by genetic variants that affect LDL cholesterol was associated with a reduction of 13.8% (95% CI 12.5–15.1%) in CHD event risk. Each 10 mg/dL reduction in TG-rich lipoprotein cholesterol (estimated as TG/5) was associated with a similar risk reduction of 12.4% (95% CI 9.8 to 15.9%). Both estimates were from a model that contained the other lipid variable, indicating that the associations of LDL cholesterol and TG-rich lipoprotein cholesterol (the two components of non-HDL cholesterol) were independent of one another. Notably, the estimate for LDL cholesterol of 13.8% is more than twice the 6.5% value from the Cholesterol Treatment Trialists' analysis, suggesting that the full benefit of LDL cholesterol-lowering therapy may not be evident over a period of ~5 years. The findings summarized above support the views that both components of non-HDL cholesterol contribute to risk and that "lower for longer is better" with regard to non-HDL cholesterol and ASCVD risk.

Results of a risk-evaluation and modeling study by Brunner et al. [42] that included approximately 400,000 individuals from 19 countries across Europe, Australia, and North America provide strong evidence for the association of non-HDL cholesterol with ASCVD. Based on their findings, the authors developed a tool specific for age, sex, and cardiovascular risk factors to estimate the long-term probability of a cardiovascular event related to non-HDL cholesterol by age 75. With this tool, they also modeled risk reduction through lipid-lowering therapy, with results providing further support for the potential benefit of beginning lipid-lowering therapy early in life. For example, the tool predicted that a woman <45 years of age with a non-HDL cholesterol concentration of 145–185 mg/dL (3.7 to <4.8 mmol/L) and ≥2 additional risk factors had a 15.6% probability of having a major cardiovascular event by age 75; with a 50% reduction in non-HDL cholesterol levels, this probability could be reduced to 3.6%.

ApoB Concentration Is an Indicator of Atherogenic Particle Burden

For several decades, the custom in the USA has been to use measurements of lipoprotein cholesterol and TG to assess lipoprotein-related ASCVD risk and responses to interventions. The concentration of apoB reflects the total number of circulating lipoprotein particles with atherogenic potential because each VLDL, LDL, and chylomicron particle contains one molecule of apoB [note that intermediate-density lipoprotein and Lp(a) are typically in the LDL density range and thus included in LDL]. Unless an individual has a very high TG level, nearly all of the apoB is carried by VLDL and LDL particles in the fasting state, and <1% is carried by chylomicron remnants of intestinal origin that contain a truncated 48-amino acid form of apoB rather than the 100-amino acid form of hepatic origin. Using a Mendelian randomization study design, Ference et al. [43] demonstrated that both LDL cholesterol and TG level (a proxy for TG-rich lipoprotein cholesterol) lost statistical significance as predictors of CHD risk after adjustment for the concentration of apoB, suggesting that the clinical benefit of lowering LDL and TG-rich lipoprotein cholesterol levels may be a reflection of the degree of reduction in apoB-containing lipoprotein particles.

There is an ongoing debate about the merits of non-HDL cholesterol versus apoB for predicting ASCVD risk and assessing response to therapy in the clinical setting. Results from

observational studies and RCTs suggest that apoB level is modestly superior to non-HDL cholesterol concentration for these purposes [44–47]. However, the 2018 AHA/ACC/Multisociety Cholesterol Guideline favors the use of non-HDL cholesterol because it is universally available and requires no additional expense to measure compared with a standard lipid profile [25]. In some cases, however, an individual's apoB concentration may remain elevated despite having low levels of non-HDL and LDL cholesterol. For these individuals with discordantly elevated apoB, the circulating atherogenic lipoprotein particle burden is higher than would be predicted based on cholesterol measurements, and there is theoretical residual risk from this that could potentially be modified through efforts to further lower the circulating particle concentration, although this hypothesis has not been tested in prospective RCTs [25]. The National Lipid Association has recommended that consideration be given to measuring apoB (or the LDL particle concentration as an alternative) once desired levels of non-HDL and LDL cholesterol have been achieved, to identify such discordant individuals [25].

TG Elevation as a Marker for Metabolic Disturbances

When considering lipid-lowering approaches for ASCVD risk reduction, it is important to consider that TG elevation is often just one component of a group of metabolic disturbances and, therefore, some of the risk associated with increased TG levels may be due to non-lipid mechanisms. TG elevation is a component of the metabolic syndrome and is frequently associated with other metabolic disturbances that are not components of the syndrome, such as insulin resistance [48], chronic inflammation [49], and oxidative stress [50]. Thus, TG elevation may be useful for identification of individuals with strong potential to benefit from lifestyle intervention, as well as other interventions such as omega-3 fatty acid concentrates [51–53].

The results of the Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) suggest that at least some of the effects of icosapent ethyl (ethyl esters of the omega-3 fatty acid eicosapentaenoic acid) on CVD risk may be explained by mechanisms other than a reduction of TG levels [54]. REDUCE-IT compared the effect of 4 g/day icosapent ethyl versus placebo on a composite of cardiovascular death, nonfatal MI, nonfatal stroke, coronary revascularization, or unstable angina in patients with established CVD or with diabetes and other risk factors, who were receiving statin therapy and had a TG level of 135–499 mg/dL (1.52–5.63 mmol/L). Compared with placebo, patients treated with icosapent ethyl had a significantly lower risk of major cardiovascular events regardless of baseline TG levels and TG levels attained after the first year of the trial (≥150 or <150 mg/dL). The 25% relative risk reduction observed with icosapent ethyl far exceeded the ~9% risk reduction that would have been predicted from the 0.41 mmol/L (16 mg/dL) lowering of non-HDL cholesterol [40].

Lp(a) and ASCVD Risk

The biological plausibility of Lp(a) as a causal factor in ASCVD risk is two-fold. First, Lp(a) particles contain a large glycoprotein and an apo(a) protein bound to apoB by a disulfide bridge, making them structurally similar to plasminogen. As a result, Lp(a) competes with plasminogen for binding, impairing plasmin activation and hindering fibrinolysis. Second, the binding of Lp(a) to macrophages promotes the formation of foam cells and the deposition of cholesterol in atherosclerotic plaques. Meta-analyses of prospective observational studies have consistently shown that higher plasma concentrations of Lp(a) are associated with dose-dependent increases in the risk of CHD and stroke [55].

Results of a Mendelian randomization study that combined data from both the Copenhagen City Heart Study and Copenhagen General Population Study to include over 77,000 partici-

pants demonstrated a stepwise increase in MI risk with increasing levels of Lp(a) [56] and confirmed that elevated Lp(a) levels were associated with increased ASCVD risk in the general population, with levels >90 mg/dL predicting a threefold increase in risk [57]. Although Mendelian randomization studies collectively indicate that plasma Lp(a) is causally associated with CHD risk, RCTs of therapies that specifically target Lp(a) reduction are not yet available. Burgess et al. [58] conducted a Mendelian randomization analysis to estimate the magnitude of change in plasma Lp(a) levels that would be needed to produce a reduction in CHD risk similar to a ~39 mg/dL (1 mmol/L) decrease in LDL cholesterol levels, the amount shown in clinical trials to produce a clinically meaningful 20–23% reduction in the risk of cardiovascular events. Their results suggested that Lp(a) would need to be lowered by ~100 mg/dL to achieve the same CHD risk benefit attained by lowering LDL cholesterol levels by ~39 mg/dL. The practical implications of these findings are complicated by the fact the distribution of individual plasma Lp(a) concentrations are highly skewed, varying by up to 1000-fold among individuals in a given population [56]. The National Lipid Association has issued a Scientific Statement concluding that Lp(a) is an independent predictor of ASCVD risk that is additive to other risk factors including LDL and non-HDL cholesterol concentrations [25]. Current guidelines recommend that an Lp(a) concentration ≥50 mg/dL [or 125 nmol/L for Lp(a) particle concentration] be considered as a risk-enhancing factor (see Table 5.1) when considering pharmacotherapy for ASCVD risk management.

Statin therapy lowers LDL cholesterol and particle concentrations but has little effect on Lp(a) concentration. At the time of this writing, an antisense oligonucleotide agent is in development that will target Lp(a) reduction, but RCT data on cardiovascular outcomes are not available [59]. PCSK9 inhibitor therapy lowers the Lp(a) concentration by ~25%. Post hoc analyses from two secondary prevention trials with PCSK9 inhibitor therapy have provided suggestive evidence that the benefit of therapy may be greater in patients with higher baseline levels of Lp(a), consistent with the possibility that Lp(a) lowering contributes to ASCVD risk reduction [60, 61].

LDL Particle Size and ASCVD Risk

A large body of observational evidence has established an association of the small, dense LDL phenotype (known as LDL pattern B) with increased ASCVD risk [62, 63]. The biologic plausibility of this association is supported by atherogenic characteristics of small, dense LDL particles, such as extended time in circulation, enhanced susceptibility to oxidation, arterial proteoglycan binding, and ease of permeability through the endothelial barrier [64]. However, the pattern B phenotype is often associated with other high-risk characteristics such as elevated TGs; low HDL cholesterol and particle concentration; increased LDL particle and apoB concentrations; insulin resistance; diabetes; obesity; and metabolic syndrome [63]. Moreover, the association of the small, dense LDL particle or cholesterol concentration with ASCVD event risk typically loses statistical significance after adjustment for the number of circulating LDL particles or the apoB concentration [65]. Therefore, current guidelines do not recommend the use of LDL particle size or the LDL pattern B phenotype in ASCVD risk assessment.

Conclusions

The application of epidemiological methods of investigation has contributed immensely to the understanding of ASCVD etiology and led to the identification and testing of numerous therapeutic measures. Since the FHS first identified major risk factors associated with CVD, the understanding of ASCVD risk has expanded tremendously. The expanded knowledge of lipid-related risk factors, in particular, has contributed to major advances in the treatment of ASCVD. As a key driver of the atherothrombotic process, apoB-containing lipoprotein levels are used for risk

stratification and represent important therapeutic targets. Over the years, a large body of evidence has also demonstrated the important role of apoB particle number and Lp(a) in ASCVD risk. The relationship between some ASCVD risk factors, such as HDL cholesterol and TGs, has proven to be more complex and further research on how these factors should be addressed in the current treatment paradigm is warranted. In light of the growing epidemic of CVD worldwide, population and genetic studies continue to play an important role in advancing the field of cardiology. Additional investigation of lipid-related risk factors and interactions between risk factors will provide more effective means through which ASCVD can be effectively treated and ultimately prevented.

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6 Comparison of 2018 AHA-ACC Multi-Society Cholesterol Guidelines with 2013 ACC-AHA Cholesterol Guidelines

Carl E. Orringer and Neil J. Stone

The 2018 AHA–ACC Multi-Society Cholesterol Guideline (2018 GL) provides an update of the 2013 ACC–AHA Cholesterol Guideline (2013 GL). It incorporates new information published since the previous Guideline and presents a broadened perspective by including recommendations on lipid management in special populations. This chapter focuses on the key messages of the 2018 GL and accentuates the areas of similarities and differences between the two documents.

We begin with the Top 10 Take-Home Messages to Reduce Risk of Atherosclerotic Cardiovascular Disease Through Cholesterol Management from 2018 GL: [1]

1. Emphasize a heart-healthy lifestyle across the life course.
2. For patients with clinical ASCVD, reduce low-density lipoprotein cholesterol (LDL-C) with high-intensity statin therapy or maximally tolerated statin therapy with a goal of lowering LDL-C by ≥50%.
3. For patients with very high-risk ASCVD, use an LDL-C threshold of 70 mg/dL (1.8 mmol/L) to consider addition of non-statins to statin therapy.
4. For patients with severe primary hypercholesterolemia (LDL-C level ≥190 mg/dL [≥4.9 mmol/L]), begin high-intensity statin therapy without calculating 10-year ASCVD risk. If the LDL-C level remains ≥100 mg/dL (≥2.6 mmol/L), the addition ezetimibe is reasonable, and the further addition of a PCSK9 inhibitor may be considered for those with LDL-C persistently ≥100 mg/dL.
5. For patients 40–75 years of age with diabetes mellitus and LDL-C ≥70 mg/dL (≥1.8 mmol/L), start moderate-intensity statin therapy without calculating 10-year ASCVD risk. For those who are 50–75 years of age or have multiple additional major risk factors, use of a high-intensity statin is reasonable.
6. For adults 40–75 years of age without a history of ASCVD, a clinician–patient risk discussion should precede a discussion to start statin therapy.

Both authors indicate that they have no relevant relationships to report.
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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_6

7. For adults 40–75 years of age without diabetes mellitus and with LDL-C levels 70–189 mg/dL (≥1.8–4.1 mmol/L), and a 10-year ASCVD risk of ≥7.5%, initiation of a moderate-intensity statin is recommended.
8. For adults 40–75 years of age without diabetes mellitus and a 10-year risk of 7.5–19.9%, the presence of risk-enhancing factors favors the initiation of statin therapy.
9. For adults 40–75 years of age without diabetes mellitus and LDL-C 70–189 mg/dL (1.8–4.1 mmol/L) and a 10-year risk of 7.5–19.9% in whom the decision to initiate a statin is uncertain, coronary calcium scoring is reasonable to aid in decision-making.
10. Adherence to lifestyle and lipid lowering drug therapy should be assessed by measuring percentage reduction from baseline in LDL-C 4 to 12 weeks after statin initiation or dose adjustment, and this measurement should be repeated every 3–12 months as needed.

Secondary Prevention of ASCVD

The 2013 GL [2] provided the foundation for the 2018GL's [1] expanded recommendations for secondary prevention of ASCVD. The key points of comparison between the two guideline documents are provided in Table 6.1. Both advocate concomitant initiation of lifestyle therapy, and for those who are 75 years of age or younger, high-intensity, or the maximally tolerated statin intensity. When high-intensity statins are used, the objective is to achieve a ≥50% reduction from baseline LDL-C levels. For those not able to tolerate a high-intensity statin, a moderate-intensity statin should be initiated with a goal of achieving a 30–49% reduction.

Table 6.1 2013 versus 2018 cholesterol guideline: clinical ASCVD

Variable	2013	2018
Risk level categorization	All considered high risk	High vs. very-high risk
Statin treatment	High-intensity to achieve ≥50% LDL-C ↓ or use maximal intensity tolerated	High-intensity to achieve ≥50% LDL-C ↓ or use maximal intensity tolerated
Non-statins	No RCT support	RCT support for ezetimibe and PCSK9 inhibitors
LDL-C monitoring	Done to assess adherence and to identify those with less than anticipated LDL-C lowering response for possible consideration of non-statin	Done to assess adherence and to determine whether lipoprotein thresholds have been met to consider adding non-statin
Lipoproteins used in clinical decision-making	LDL-C	LDL-C, except may use non-HDLC when considering PCSK9 inhibitor addition
Statin intensity for adults ≥ age 75 years	Moderate	Moderate or high may be initiated or continued
Statin use in heart failure	No recommendation	Moderate intensity reasonable in selected individuals with ischemic etiology

Because of the proven efficacy of statins in ASCVD risk reduction in individuals with ASCVD, the initial clinician–patient discussion should emphasize the anticipated benefits versus potential adverse effects of statin therapy and that benefits far outweigh the risks. The value of maintaining long-term statin adherence to reduce recurrent myocardial infarction (MI) and coronary heart disease events was supported by a Medicare database study of 105,329 post-MI patients who were followed over a median of 1.9 to 2.3 years. The multivariate-adjusted hazard ratios (HR) comparing beneficiaries with low versus high statin adherence showed an increased odds of recurrent MI of 1.50 (95% CI: 1.30 to 1.73) and of percutaneous coronary intervention

or recurrent MI (CHD events) of 1.51 (95% CI: 1.34 to1.70) but did not meet criteria for a reduction in all-cause mortality of 0.96 (95% CI: 0.87 to 1.06) [3].

While the 2013 GL does not risk-stratify individuals with clinical ASCVD, the 2018 GL separates individuals with ASCVD into very-high versus high-risk categories. Very high-risk individuals have a history of two major ASCVD events or one major ASCVD event and two or more high-risk conditions. Major ASCVD events and high-risk conditions are identified in Table 6.2. Those patients with clinical ASCVD who do not meet the criteria for very high risk are classified as being "high risk."

Regarding the use of non-statins, the 2013 GL, in the absence of randomized controlled trials supporting their net ASCVD risk reduction benefit at that time, advised that the use of non-statins may be considered in those patients with ASCVD who have a less-than-anticipated LDL-C lowering response to maximally tolerated statin therapy or are unable to tolerate a less-than-recommended intensity of a statin or are completely statin-intolerant. Based upon the subsequent publication of randomized controlled trials supporting the net ASCVD risk reduction benefit of adding ezetimibe [4] and the PCSK9 inhibitors evolocumab [5] and alirocumab [6] to evidence-based statin therapy, the 2018 GL authors provide recommendations supporting the use of these agents in selected individuals 75 years of age or younger taking maximally tolerated statins. Ezetimibe treatment is reasonable in very-high risk patients who have an LDL-C ≥70 mg/dL, and may be reasonable in high-risk patients with a similar LDL-C level. Those very-high risk patients being considered for treatment with a PCSK9 inhibitor should first be treated with ezetimibe. For those with an LDL-C ≥70 mg/dL or non-HDL-C ≥100 mg/dL despite maximally tolerated statin and ezetimibe, it is reasonable to add a PCSK9 inhibitor following a clinician–patient discussion about net-benefit, safety, and cost.

Based on an estimated cost-value of >$150,000 per quality-adjusted life year at mid-2018 retail prices, PCSK9 inhibitors were felt to be low economic value therapeutic agents [7]. The impact on cost-effectiveness of retail price reductions of reported since publication of the 2018 GL can be estimated from the figure in the Cost-Effectiveness Section of the Guideline (Fig. 6.1).

The 2013 GL assessed the use of moderate-intensity statin therapy as being reasonable in patients older than 75 years of age with clinical ASCVD and to continue statin therapy in patients >75 with ASCVD who are tolerating statin therapy. In contrast, the 2018 GL advises that initiation of moderate or *high-intensity statins*, as well as continuation of high-intensity therapy is reasonable in such patients after evaluation of the potential for ASCVD risk reduction, adverse effects, drug–drug interactions, patient frailty, and patient preferences.

The 2013 GL made no recommendation on the use of statin therapy in patients with New York Heart Association class II to IV ischemic systolic heart failure. The 2018 GL also analyzed data from two randomized controlled

Table 6.2 Very high risk ASCVD: 2 or more major events or 1 major event and ≥2 high risk

Major ASCVD events	High risk conditions
Recent ACS (within the past 12 months)	Age ≥65 years
H/o MI (other than recent ACS event listed above)	Heterozygous familial hypercholesterolemia
H/o ischemic stroke	History of prior CABG or PCI outside of major ASCVD event(s)
Symptomatic peripheral arterial disease (history of claudication with ABI <0.85 or previous revascularization or amputation)	Diabetes mellitus
	Hypertension
	CKD (eGFR 15–59 mL/min/1.73 m^2)
	Current smoking
	LDL-C ≥ 100 despite maximally tolerated statin + ezetimibe
	H/o heart failure

H/o history of, *ABI* ankle brachial index, *CABG* Coronary artery bypass surgery, *PCI* Percutaneous coronary intervention, *CKD* Chronic kidney disease

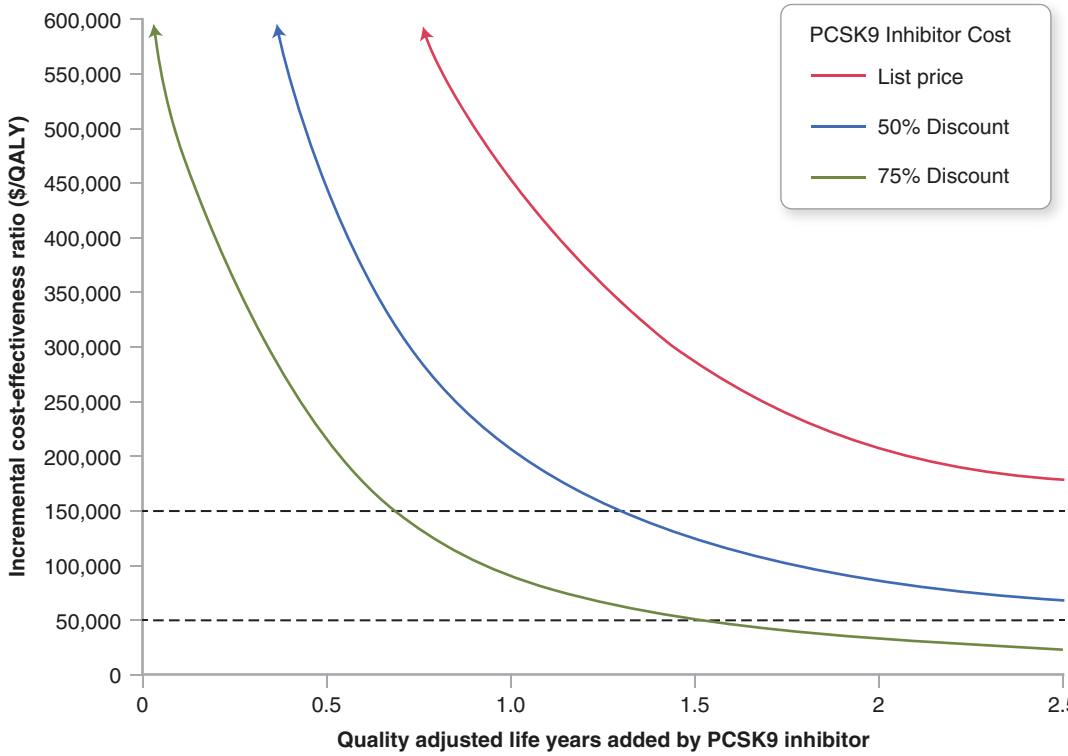

Fig. 6.1 PCSK9 inhibitor cost-effectiveness analysis. (Adapted from Hlatky and Kazi [7]. With permission from Elsevier)

trials that evaluated the efficacy of rosuvastatin 10 mg daily versus placebo to reduce ASCVD events in heart failure patients [8, 9]. Neither trial met its primary outcome. However, a subsequent analysis that accounted for repeat heart failure hospitalizations showed a significant reduction in heart failure hospitalizations [10], and a post-hoc analysis from one of these trials showed that patients with less advanced heart failure randomized to rosuvastatin had a significant reduction in the primary outcome [11]. Most importantly, a patient-level analysis pooling data from both of the above trials and accounting for competing causes of death showed a statistically significant relative risk reduction in the risk of MI in those with ischemic heart failure receiving rosuvastatin [12]. A patient management algorithm summarizing the secondary prevention recommendations of the 2018 GL is provided in Fig.6.2.

Primary Severe Hypercholesterolemia in Adults (LDL-C ≥ 190 mg/dL)

Primary severe hypercholesterolemia is defined in adults as an LDL-C ≥190 mg/dL in the absence of secondary causes. Whether the etiology is monogenic, as is the case in familial hypercholesterolemia, or polygenic and exacerbated by atherogenic lifestyle habits, these patients are at high or very-high risk for clinical ASCVD [13–15].The risk level depends upon the LDL-C concentration, the duration of exposure to hypercholesterolemia, and the presence of concomitant atherogenic risk factors [16]. In the absence of atherosclerotic cardiovascular disease outcomes, studies done exclusively in patients with LDL-C ≥190 mg/dL, the treatment focus in these patient is the use of pharmacotherapy that provides LDL-C lowering efficacy and has demonstrated safety. The 2018

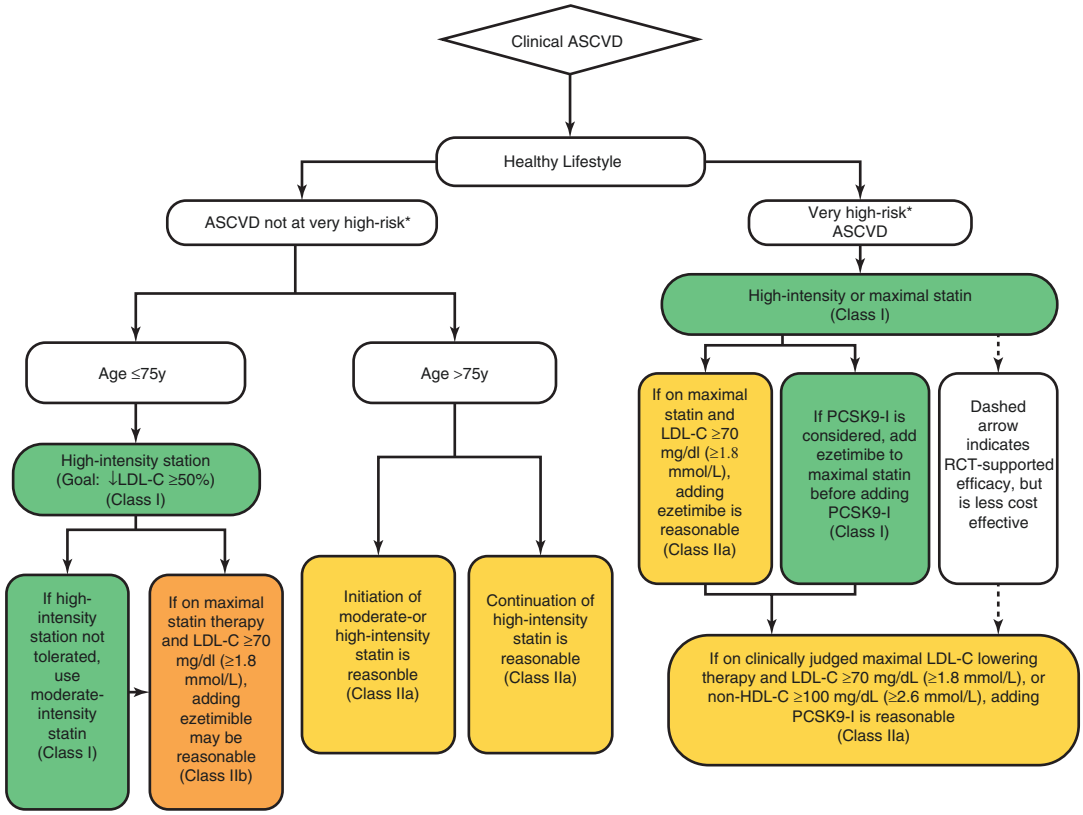

Fig. 6.2 Secondary prevention: 2018 AHA-ACC guideline. (Adapted from Grundy et al. [1]. With permission from AHA)

Table 6.3 Primary severe hypercholesterolemia (LDL-C ≥ 190 mg/dL)

	2013 Guideline	2018 Guideline
Age-based recommendations for statin therapy	≥21 years: high-intensity or maximally tolerated	20–75 years: high-intensity or maximally tolerated
LDL-C reduction objective	≥50%	≥50% and LDL-C <100 mg/dL
Adding non-statins	May be considered for those with <50% LDL-C ↓ (llb: limited evidence or expert opinion)	Selective, evidence-based use of ezetimibe, PCSK9 inhibitors, or bile acid sequestrants

GL, as compared to the 2013 GL (Table 6.3), was able to provide updated recommendations based on additional clinical trial data that were available following the publication of the 2013 GL.

A large placebo-controlled ASCVD outcomes RCT of both primary and secondary patients with a mean baseline LDL-C level of 192 ± 17 mg/dL [17] and a *post-hoc* analysis of exclusively primary prevention patients from this study [18] demonstrated a reduced incidence of MI and cardiovascular death in those treated with pravastatin 40 mg daily versus placebo. Retrospective cohort studies have supported the position that statin therapy reduces the incidence of MI, coronary heart disease [19], and all-cause death [20] in patients with phenotypic or genetically confirmed familial hypercholesterolemia. As statin therapy reduces ASCVD risk both in primary and secondary prevention trials and as high-intensity therapy results in greater risk reduction than moderate-intensity [21], adults 20–75 years of age with primary severe hypercholesterolemia should be treated with maximally tolerated statin therapy.

Both the 2013 and 2018 GL indicate that for patients with severe hypercholesterolemia,

ASCVD risk reduction from statins is deemed optimal and evidence-based when the patient achieves a ≥50% reduction from the baseline LDL-C level. The 2018 GL advises that when less-than-anticipated LDL-C lowering is encountered, and particularly when LDL-C remains >100 mg/dL, a level at which an increased odds of clinical ASCVD is encountered in patients with familial hypercholesterolemia [16], the addition of a second LDL-C lowering drug is reasonable. A randomized controlled trial of 720 familial hypercholesterolemia patients treated with moderate-intensity statin plus ezetimibe vs. moderate-intensity statin plus placebo demonstrated greater LDL-C lowering and good tolerability of ezetimibe therapy [22]. Adults who have suffered a recent acute coronary syndrome and were treated with a moderate-intensity statin plus ezetimibe, compared to statin monotherapy, have been shown to demonstrate improved ASCVD outcomes [4]. Thus, the addition of ezetimibe, an evidence-based, generic, well-tolerated drug, is reasonable in those with severe hypercholesterolemia whose LDL-C remains ≥100 mg/dL despite maximally tolerated statin therapy.

Bile acid sequestrants may be considered as an additional LDL-C lowering option for selected patients with severe hypercholesterolemia taking maximally tolerated statin therapy and ezetimi be and having an LDL-C ≥100 mg/dL. Colesevelam 3.75 grams daily was shown in a 12-week randomized placebo-controlled trial study of 86 patients who met clinical criteria for familial hypercholesterolemia and had an LDL-C ≥100 mg/dL, while on a statin plus ezetimibe, to provide an additional 18.5% reduction in LDL-C with good tolerability [23]. The use of bile acid sequestrants is limited by inconvenient dosing forms, the absence of well-tolerated generic formulations, and drug–drug interactions. Because they may raise triglycerides, their use should be avoided in patients with fasting triglycerides ≥300 mg/dL.

Some patients with severe hypercholesterolemia are unable to achieve a ≥50% LDL-C reduction and LDL-C <100 mg/dL with the use of diet, maximally tolerated statins, and ezetimibe. Two placebo-controlled randomized trials examining the efficacy and safety of PCSK9 inhibitors in patients with heterozygous familial hypercholesterolemia showed that treatment with these agents results in an additional ≥50% LDL-C reduction and is well tolerated [24, 25]. As the incidence of ASCVD events is higher in heterozygous familial hypercholesterolemia patients >30 years of age as compared to those who are younger [16] and as there are insufficient data to guide treatment in those older than 75 years of age, the addition of either evolocumab or alirocumab to the medical regimen of heterozygous familial hypercholesterolemia patients, 30–75 years of age, taking maximally tolerated statin and ezetimibe with an LDL-C ≥100 mg/dL may be reasonable.

Most patients with LDL-C ≥190 mg/dL do not have monogenic familial hypercholesterolemia [13]. However, these patients still have a considerably higher risk of clinical ASCVD than those with LDL-C <130 mg/dL [14]. Consequently, those patients who have an LDL-C ≥220 mg/dL, but do not meet clinical criteria or have genetic confirmation of the diagnosis of familial hypercholesterolemia and have an LDL-C ≥100 mg/dL while on maximally tolerated statin and ezetimibe, may be considered for treatment with a PCSK9 inhibitor. The economic value of PCSK9 inhibitors at mid-2018 retail prices for patients with primary severe hypercholesterolemia was deemed uncertain by the 2018 GL. The impact on cost-effectiveness of retail price reductions reported since publication of the 2018 GL can be estimated from the figure in the Cost-Effectiveness Section of the guideline (Fig. 6.1).

Statin Safety and Statin-associated Side Effects

Because statin therapy provides ASCVD risk reduction in both primary and secondary prevention and because lack of adherence is associated with less favorable outcomes [3, 26], clinicians need to understand statin safety and the diagnosis and management of statin-associated side effects. The decision about whether to initiate a statin

should be based on a clinician–patient discussion that weighs the potential for ASCVD risk reduction against the potential for statin-associated side effects and statin drug–drug interactions. Patients should be reassured that side effects occurring during the course of statin therapy can be effectively addressed. Prior to the initiation of a statin, patients should be evaluated for predisposing factors to statin-associated side effects, including pre-existing musculoskeletal symptoms and/or insulin-resistant states that predispose to new-onset diabetes. Routine measurement of creatine kinase is felt to be of no clinical utility in statin treated patients but is of value in those who are symptomatic.

The most commonly encountered side effects are statin-associated muscle symptoms (SAMS), which must be differentiated from other causes of muscle symptoms such as muscular exercise, polymyalgia rheumatica, or primary muscle disorders. SAMS are most often bilateral, proximal, occur within the first few weeks or months after initiation of therapy, and resolve within several weeks after discontinuation of the medication. Objective muscle weakness (myopathy) and associated CK elevation (myositis) are far less common side effects and mandate statin discontinuation and exploration for exacerbating factors. Rhabdomyolysis, defined as CK elevation >10 times the upper limit of normal, associated with renal injury, is a very rare side effect that occurs primarily in those with predisposing factors and/or drug–drug interactions. When the diagnosis is made, the statin and any other potentially interacting drug should be promptly discontinued, any predisposing conditions addressed and, generally, intravenous hydration provided until renal function improves and stabilizes.

When the clinician encounters probable SAMS, the 2018 GL supports a strategy, in those with less-than-severe symptoms, of re-challenging the patient using a lower dose of the same agent, or a different statin; or using alternative dosing strategies (low intensity or less-than-daily, preferably long acting statins); or adding to these alternative statin-based regimens randomized controlled trial-proven non-statins. In those who are completely statin-intolerant, non-statins alone or in combination may be considered. The use of co-enzyme Q-10 to ameliorate or prevent SAMS was shown in a high-quality double blind trial that tested CoQ10 in those proven to have statin myalgia to be of no benefit as compared to placebo [27]. A rare disorder, statin-associated autoimmune myopathy, is diagnosed in patients with persistent muscle weakness following statin discontinuation, persistent CK elevation, and the presence of HMG CoA reductase antibodies [28] Such patients may benefit from neurology consultation and consideration of immunosuppressive therapy for symptom control [29].

Statin therapy is associated with a mildly increased risk for incident diabetes mellitus in those with predisposing risk factors for diabetes, or the metabolic syndrome, or receiving higher-intensity statins. A 2015 meta-analysis of 129,170 participants of 20 statin trials with a mean follow-up of 4.2 years showed an odds ratio of 1.12 for incident diabetes (95% CI 1.06-1.18) in those taking statins versus placebo [30]. For those at increased diabetes risk, the clinician should place increased emphasis on maintaining heart-healthy dietary patterns, regular aerobic exercise and weight loss, particularly for those who are overweight or obese. The atherosclerotic cardiovascular disease risk reduction benefits accrued in those who take statins significantly outweigh the potential to develop diabetes. Thus, the potential development of diabetes should not be viewed by patients or clinicians as a reason to avoid the initiation of therapy. In addition, those who do develop diabetes have a well-established indication for statin therapy.

Although patients treated with statins may occasionally develop transaminase elevations, elevation of transaminases to greater than three times the upper limit of normal occurs infrequently and usually resolves with dose reduction or the use of an alternate statin. Clinically significant hepatotoxicity related to statins is rare, and its incidence is not predicted by routine hepatic function testing. Consequently, routine measurement of transaminase levels is deemed to be of no benefit in patients prescribed statins. In addition, statins may be safely administered to patients with chronic stable liver disease who have the established indications for therapy.

Primary Prevention of ASCVD

As heart disease and stroke are the first and fifth leading causes of death in the United States [31], respectively, a key patient care priority is the identification of those primary prevention patients in whom evidence-based lifestyle, and drug therapy reduces the risk of clinical ASCVD events. The detailed evidence review of the 2013 GL identified two primary prevention groups whose increased risk of ASCVD was shown to be reduced by statin therapy. These include individuals 40–75 years of age with diabetes mellitus, no clinical ASCVD, and LDL-C 70–189 mg/dL; and individuals without diabetes, with LDL-C 70–189 mg/dL and 10-year ASCVD risk ≥7.5% using the Pooled Cohort Equations. Management of patients in these two groups and considerations unique to lipid management in special populations will be addressed in this section.

The 2013 ACC/AHA Blood Cholesterol Guideline (2013 GL) used three exclusively primary prevention randomized controlled trials (RCTs) as the foundation for their evidence-based recommendations for primary prevention [2]. The 2018 GL [1]used data from another exclusively primary prevention trial (Heart Outcomes Prevention Evaluation or HOPE-3) published after the 2013 GL to further inform their recommendations [32].

The Importance of a Healthy Lifestyle

Both the 2013 and 2018 guidelines emphasized heart-healthy lifestyle, atherosclerotic cardiovascular disease (ASCVD) risk assessment, clinician–patient risk discussion before statin treatment in primary prevention, and reclassification to resolve uncertainty regarding treatment based on further risk assessment evaluation.

A healthy lifestyle is foundational therapy for those individuals who wish to reduce their risk of heart attack and stroke. This perspective was emphasized in both Guideline documents and amplified in companion documents. The 2013 GL was published simultaneously with the 2013 Lifestyle Guideline [33] and the 2018 GL was published 4 months prior to the 2019 ACC-AHA Prevention Guideline [34] Importantly, advice on adherence to healthy lifestyle is the first of the Top Ten Take Home Messages.

A healthy lifestyle is mentioned frequently in the 2018 GL, not only as a required first step in ASCVD risk reduction over the life course but also as front-line therapy for those with the metabolic syndrome (MS), in which obesity and insulin resistance are the core determinants. Individuals with three or more of the following components are diagnosed as having the MS. [35] These include increased waist circumference measured at the iliac crest; elevated triglycerides; low HDL-Cholesterol; elevated blood glucose and elevated blood pressure. Lifestyle therapy is especially powerful because adherence to recommended diet and exercise, and often modest amounts of weight loss, can improve all of the MS risk factors. Finally, a lifestyle characterized by a sedentary existence with a diet rich in calories, refined sugars, fatty meats, increased alcohol, and LDL-cholesterol raising foods, such as butter, deep fried foods, and egg yolks is a known secondary cause of both elevated cholesterol and triglycerides.

Lipid Management for ASCVD Risk Reduction in Patients with Diabetes

The importance of employing evidence-based lipid management for ASCVD risk reduction in diabetic patients has been consistently emphasized. In both the 2013 and 2018 GL, Type 1 and type 2 DM are considered together. Although there is much less information on ASCVD risk reduction using statin therapy in Type I as compared to Type II DM, ASCVD remains an important cause of death in both conditions [36].

A key point stated in the Top 10 Messages is that in patients 40–75 years of age with DM and LDL-C ≥70 mg/dL ~~(≥1.8 mmol/L)~~, clinicians should initiate therapy with a moderate intensity statin to reduce ASCVD risk. The high lifetime risk in DM makes calculation of 10-year risk unnecessary. Both the 2013 and 2018 GL endorse

high-intensity stain therapy in those with DM at increased risk, such as those with multiple risk factors or those 50–75 years of age. The goal in such patients is to lower LDL-C by ≥50% to provide optimal reduction in absolute ASCVD risk.

Analysis of primary-prevention trials in large cohorts with DM demonstrates that moderate-intensity statin therapy provides clinically significant benefit that exceeds the risk of therapy [37, 38]. Moreover, a large primary prevention RCT in men ≥50 years of age and women ≥60 years of age, with high sensitivity C-reactive protein ≥2.0 mg and a high prevalence of MS (approximately 41%) showed benefit with statin therapy that lowered LDL-C on average 50% from baseline [39]. Given the long period of increased ASCVD risk of those with Type 1 and Type 2 DM and the high risk at the onset of clinical ASCVD in those with diabetes, both 2013 and 2018 GL recognize that high-intensity statin therapy maximizes reduction of both LDL-C and risk of clinical ASCVD.

On the other hand, both guidelines acknowledge the wide range of risk among those with diabetes. The 2013 GL suggested that high-intensity statin therapy be considered if 10-year ASCVD risk was at least 7.5% (usually the case over age 50) in the context of a risk discussion. In the 2018 GL, clinicians are advised to consider advancing age, concomitant major ASCVD risk factors, and DM-specific risk enhancers in decision-making about statin intensity, including (1) long duration (≥10 years for type 2 diabetes mellitus or ≥20 years for type 1 DM); (2) albuminuria ≥30 mcg of albumin/mg creatinine; (3) eGFR <60 mL/min/1.73 m^2; (4) retinopathy; (5) neuropathy; and (6) ankle brachial index <0.9.

Both the 2013 and 2018 GL provide management recommendations for those with diabetes in an age group where there is inadequate evidence for Class I recommendations. For those <40 years of age or >75 years of age, or whose LDL-C is <70 mg/dL, the 2013 GL suggested that statin therapy should be individualized on the basis of considerations of ASCVD risk-reduction benefits, the potential for adverse effects, drug–drug interactions, and patient preferences. The 2018 GL expands these recommendations by providing diabetes-specific risk enhancers factors as noted above. As many patients with type 1 DM are first diagnosed during their teenage years, the risk enhancer of long duration (≥20 years) of Type 1 DM helps to guide patient care for those under 40 years of age who may benefit from statin therapy. Similarly, adults >75 years of age with DM are at high ASCVD risk and generally benefit from statin therapy, although benefit has to be evaluated in terms of competing risks that reduce longevity [40].

Lipid Management for ASCVD Risk Reduction in Primary Prevention Adults

For those adults 40–75 years of age with LDL-C 70–189 mg/dL and no diabetes, the 2013 GL recommended ASCVD risk assessment using the Pooled Cohort Equations. A clinician–patient risk discussion was advised to provide an explanation of the significance of the calculated risk and respect patient preferences, as shared decision-making enhances the likelihood of long-term adherence to therapeutic recommendations. The 2018 GL builds on this approach in the following ways:

1. *10-year ASCVD risk assessments used to define 4 levels of risk*: <5% low risk; 5–7.4% borderline risk; 7.5–19.9% intermediate risk; and ≥20% high risk. In each of these categories, a healthy lifestyle is the underpinning of therapy. In those with borderline, intermediate or high risk, the benefits versus risks of statin therapy must be clearly defined by the clinician and understood by the patient as an essential part of the process of shared decision-making.
2. *The clinician–patient risk discussion now utilizes "risk-enhancing" factors to personalize the ASCVD risk*. Both the 2013 and the 2018 GL recommend moderate-intensity statins for those with a 10-year ASCVD risk of ≥7.5%. The low cost of these widely available and cost-effective drugs [41] that most patients take without adverse effects have the potential

to prevent 475,000 future cardiovascular events [42]. On the other hand, this increased sensitivity is associated with decreased specificity if statins are assigned only based on the patient's ASCVD 10-year risk score ≥7.5%. Statin assignment was not meant to be automatic by the 2013 Guideline, but was recommended to occur after a clinician–patient risk discussion that described the potential for benefit, adverse effects, drug–drug interactions, and respected patient preferences. Various factors that served to reclassify risk including family history of premature ASCVD, LDL-C ≥160 mg/dL, hs-CRP ≥2.0 mg/L; ankle brachial index <0.9 or a coronary artery calcium (CAC) score ≥300 Agatston units were suggested if a quantitative risk decision was uncertain.

The 2018 Guideline extends this approach by providing "risk enhancing factors" as a way to personalize ASCVD risk. They include a family history of premature ASCVD (before 55 years of age in male and 65 years of age in female first degree relatives); lipid parameters such as LDL-C ≥160 mg/dl or a persistent triglyceride elevation ≥175 mg/dL; high risk ethnicity such as South Asian ancestry; high risk conditions such as metabolic syndrome; chronic kidney disease with or without microalbuminuria (but not receiving hemodialysis); chronic inflammatory diseases such as HIV, rheumatoid arthritis, or psoriasis; conditions specific to women such as a history of preeclampsia or a premature menopause before age 40; and, if measured, apolipoprotein B ≥130 mg/dL and Lp(a) ≥50 mg/dL or ≥125 nmol/L; hs-CRP ≥2.0 g/L; and ankle-brachial index <0.9. These are stable factors that help patients to understand the dimensions of their ASCVD risk in greater detail. They include genetic and acquired characteristics other than the established risk factors that may be available in selected but not all patients. The presence of one or more of these factors favors the initiation of statin therapy.

3. *Coronary artery calcium (CAC) scoring is now the preferred way to reclassify and make therapeutic decisions, if a risk decision remains uncertain*. In the intermediate risk, and occasionally, in the borderline risk range, there are individuals in whom the need for a "tie-breaker" regarding a statin therapy decision is needed. Since 2013, it has become clear that the best test to reclassify ASCVD risk is the CAC score. High-quality prospective data from both the MESA [43] and BioImage cohorts [44], in which subjects had their 10-year ASCVD risk assessed, examined the clinical impact of a CAC of zero. This score in those presumed to be at "intermediate risk" reclassifies risk usually to less than the threshold for statin therapy. In these patients, in the context of shared decision-making, it could be prudent to withhold or delay initiation of statin therapy. A score of zero may also have increased utility in those patients 60–75 years of age, in whom age plays an increasingly strong role in determining 10-year ASCVD risk. The 2018 GL, however, points out that a CAC score of zero does not exclude the presence of non-calcified plaque and should not be used to support deferral of statin therapy in active cigarette smokers, diabetics, or those with a family history of premature ASCVD. The GL states that it is reasonable, based upon the increased risk observed in observational studies, to recommend statin therapy in those with CAC scores of ≥100 Agatston units (or ≥75th percentile for the patient's age, sex, and race) and in those with scores of 1–99 ≥55 years of age. A patient management algorithm summarizing the primary prevention recommendations of the 2018 GL is provided in Fig. 6.3.

Lipid Management for ASCVD Risk Reduction in Special Populations

The 2018 GL, unlike the 2013 GL, has a section on children and adolescents. It emphasizes the importance of intensifying lifestyle therapy in children with lipid disorders related to obesity and indicates that lifestyle counseling may be beneficial for lowering LDL-C. Children and adolescents 10 years of age and older with an

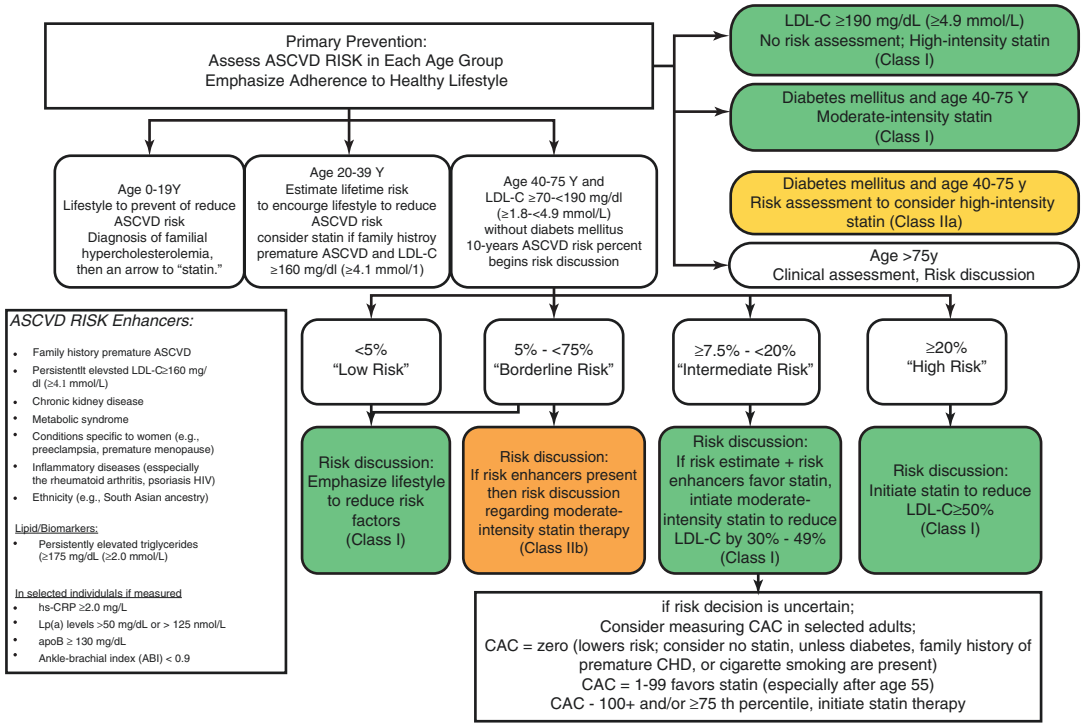

Fig. 6.3 Primary prevention: 2018 cholesterol guideline. (Adapted from Grundy et al. [1]. With permission from AHA)

LDL-C ≥190 mg/dL or ≥160 mg/dL and a clinical presentation consistent with familial hypercholesterolemia (FH); and failure to respond adequately to 3–6 months of lifestyle therapy are reasonable candidates for statin therapy. The section on young adults 20–39 years of age highlights the importance of recognizing those with unrecognized moderate or severe elevations of LDL-C, for whom statin therapy may be considered or those with multiple risk factors of the metabolic syndrome, for whom lifestyle therapy is the initial approach.

The management of older adults was addressed in both the 2013 and 2018 GL. The 2013 GL found a paucity of evidence to support a recommendation for statin therapy for those over 75 years of age. In view of a weaker database to guide therapeutic decision-making for these individuals, the clinician–patient discussion must focus on the potential for benefit versus adverse effects from statin therapy, clinical characteristics of the patient, and patient preferences. The 2018 GL gives a class IIb recommendation for the initiation of a moderate intensity statin in those 75 and older, supported by the combined subgroup analysis of the JUPITER and HOPE-3 participants [45]. The evidence for benefit, however, is not strong and only extends to age 80. This was paired with another class IIb recommendation that it may be reasonable to stop statin therapy when physical or cognitive decline, multi-morbidity, frailty, or reduced life-expectancy limits the potential benefits of statin therapy. Taken together the two recommendations indicate the strong need for a thoughtful clinician–patient discussion before either decision is made. Finally, the 2018 GL provides a class IIb recommendation that it may be reasonable in selected patients, 76–80 years of age and with a paucity of ASCVD risk factors, to measure CAC to reclassify risk. These lower strength recommendations should serve to encourage further research are to be used only in specific instances and do not support widespread treatment of older adults for primary prevention of ASCVD.

The 2018 GL also addresses several issues related to lipid testing. Non-fasting lipid panels are now deemed acceptable for children and adults for risk assessment in primary prevention and for assessment of baseline LDL-C levels before the initiation of a statin in primary and secondary prevention. For situations in which increased precision is required (as in hypertriglyceridemia and suspicion of genetic hyperlipidemia), fasting lipids can be measured. This approach will lessen the morning scheduling burden of outpatient laboratories and will be more convenient for patients who have afternoon appointments.

Second, the 2018 GL recognizes the unreliability of the Friedewald-calculated LDL-C levels both when LDL-C levels are <70 mg/dL and/or triglycerides are ≥150 mg/dL. It indicates that it is reasonable in such patients to use either a directly measured LDL-C value or a newer validated approach (Martin-Hopkins method) to estimate LDL-C. This method estimates LDL-C using an adjustable factor for the triglyceride/very low density lipoprotein-cholesterol ratio and does not entail additional cost to the patient, as does direct LDL-C measurement [46].

Third, the 2018 GL provides recommendations on when and how often lipid panels should be drawn. A fasting or non-fasting lipid panel may be drawn as early as 2 years of age in children with a family history of early CVD, significant hypercholesterolemia (LDL-C ≥190 mg/dL or non-HDL-C ≥220 mg/dL) or known primary hypercholesterolemia and advocates reverse cascade screening of family members when moderate or severe hypercholesterolemia is found. It also advises, as did the 2013 GL, that follow-up lipids should be drawn to assess adherence and percentage response to LDL-C lowering medications and lifestyle therapy. Lipid measurements should be done 4–12 weeks after statin initiation or dose adjustment and be repeated every 3–12 months as needed. Follow-up lipid testing is especially important because there is considerable individual variation in the LDL-C lowering response to all doses of statins [47]. In a large-scale primary prevention ASCVD outcomes RCT that compared assignment of 20 mg/day of rosuvastatin to placebo, the variability in percentage LDL-C reduction was wide and the magnitude of this percentage reduction in LDL-C directly related to statin efficacy in reducing ASCVD outcomes [48]. The authors felt that their data supported the of use percentage reduction in LDL-C in Guideline recommendations.

In summary, the 2018 GL extends and improves the recommendations for primary prevention. This Guideline is informed by a major RCT in a diverse, multi-ethnic, multi-national cohort (HOPE-3) and uses "risk-enhancing factors" to personalize risk assessment in the borderline and intermediate risk ranges. It identifies CAC scoring as the most useful test for reclassifying ASCVD risk, especially in patients in the 5–19.9% 10-year ASCVD risk range. The inclusion of class IIb recommendations is designed to increase awareness of important lipid management issues for which there are limited available data and to highlight important gaps in our knowledge base that require additional investigation. Those who wish to understand the major thrust of the 2018 GL should study the Top Ten Take-Home Messages. They concisely identify recommendations of highest priority to facilitate optimal lipid management for ASCVD risk reduction.

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7 The Low-Density Lipoprotein Cholesterol Hypothesis: An Update

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Low-Density Lipoprotein-Cholesterol

Cholesterol is an important part of the human body as it constitutes a structural component of cell membranes, is a precursor for bile acids and steroid hormones, and takes part in vitamin D synthesis [1]. As discussed in prior chapters, serum cholesterol is produced in the liver as well as obtained exogenously through the gut, where lipids are absorbed via apolipoprotein B-48 (apoB48), which is produced exclusively in the intestine through a unique RNA editing mechanism by the apobec-1 enzyme complex [2]. The lipids are then packaged into chylomicrons and released into the bloodstream. Lipoprotein lipase, found in the capillary endothelium, hydrolyzes these chylomicrons. Chylomicron remnants and degradation products of this process can be taken up into the liver where they are synthesized into very-low-density lipoprotein (VLDL). Further catabolism of VLDL by lipoprotein lipase results in higher density particles including high-density lipoprotein (HDL), intermediate-density protein (IDL), and low-density lipoprotein (LDL) [3].

Each LDL is about 200 Å in diameter and contains a single apolipoprotein B-100 (ApoB100) protein. ApoB100, mainly produced by the liver where it is required for the synthesis and secretion of VLDL, acts as the ligand for hepatic LDL-receptor (LDL-R) clearance of not only LDL but also IDL and VLDL [2]. The core of the LDL particle is made of esterified cholesterol and triacylglycerol covered by a surface containing a phospholipid monolayer overlying unesterified cholesterol [4].

Cholesterol is primarily transported in the blood by LDL, and to a lesser degree by HDL and lipoprotein(a) [Lp(a)]. The main mechanism of clearing LDL particles is through a hepatic transmembrane LDL-R to which an LDL-cholesterol (LDL-C) particle binds followed by internalization via endocytosis. The LDL-C and LDL-R split, allowing for the recycling of the receptor and LDL-C is then degraded in lysosomes where cholesterol content can be released [5]. Measurement of cholesterol in the LDL pool represents the steady state of production of VLDL, its metabolism to LDL, and the receptor-mediated clearance of LDL [2].

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_7

The Link Between Cholesterol and Atherosclerosis –Illustrative Trials

The first mention of a link between cholesterol and the formation of atherosclerotic disease was shown in 1913 by Anitschkow in rabbits who were fed oil [6]. In 1933, Muller described families with inherited high cholesterol and increased risk for cardiovascular disease [7]. Starting in the 1970s, the Framingham Heart Study revealed an association between total cholesterol and coronary heart disease [8]. Brown and Goldstein even won the Nobel Prize in 1985 for their work in identifying the lack of LDL-receptor function in hereditary familial hypercholesterolemia. Such individuals had high levels of LDL-C and suffered from increased burden of atherosclerotic disease. With this background, as well as powerful lipid lowering medications (statins) introduced into the market, it was in the 1990s when numerous clinical outcome studies involving the lowering of LDL-C began to be published.

One of the first seminal trials was the Scandinavian Simvastatin Survival Study (4S) in 1994, which enrolled 4444 patients with angina or prior myocardial infarction (MI). Simvastatin reduced LDL-C by a mean of 25% over a mean follow-up of 5.4 years, yielding a relative risk of 0.7 for total mortality, suggesting that statins, or perhaps a decrease in LDL-C, led to improved survival in such patients [9]. The 1995 West of Scotland Coronary Prevention Study (WOSCOPS) utilized 6595 men and showed that pravastatin decreased LDL-C by approximately 26%. Over an average follow-up of 4.9 years, there was a significant reduction (31%) in fatal or non-fatal MI in patients with elevated cholesterol levels without history of MI [10]. In 1996, the Cholesterol and Recurrent Events (CARE) Trial was published, involving 4159 patients with "average" LDL-C levels (115–174 mg/dL) and a history of MI. The pravastatin arm lowered LDL-C and achieved a 24% reduction in risk for the primary endpoint (fatal and non-fatal coronary events), as well as a reduction in frequency of stroke by 31% [11]. Similar results were achieved in the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) trial in 1998 which included 9014 patients with history of MI or hospitalization for unstable angina. Pravastatin showed improved outcomes with a 22% overall mortality reduction [12].

The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) from 1996 was unique in that it aimed to address whether reduction of LDL-C was beneficial for patients without cardiovascular disease and average serum cholesterol levels (mean LDL-C 150 mg/dL). This work also included women and the elderly, populations neglected in prior studies. Lovastatin along with a low-saturated fat and low-cholesterol diet showed decreased relative risk of first major coronary event (RR 0.63), MI (RR 0.60), and cardiovascular events (RR 0.75) in this study of over 6500 participants [13]. The Heart Protection Study (HPS) included over 20,000 individuals with coronary disease, other occlusive arterial disease, or diabetes and showed simvastatin lowered LDL-C and improved outcomes. Most notable was a 24% reduction in first occurrence of a major vascular event which was significant across all subcategories of patients including those without coronary disease but had cerebrovascular disease or peripheral disease, diabetics, men, and women, those over and under age 70, as well as those with initial LDL-C below 116 mg/dL [14]. The PROspective study of pravastatin in the elderly at risk (PROSPER) in 2003 focused on the elderly (5804 patients) with history of or risk factors for vascular disease. Pravastatin reduced LDL-C by 34% and showed significant reduction in coronary disease [15].

By this point, it was evident that lowering levels of cholesterol improved outcomes. Trials then shifted focus to determine if the degree of reduction in cholesterol changed outcomes. This was largely achieved by comparing two different strengths of the same antihyperlipidemic drug class (statins). In 2004, the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT–TIMI 22) study was published. In over 4162 patients recently hospitalized for acute coronary syndrome (ACS), a moderate-intensity

statin (pravastatin) lowered LDL-C to a mean of 95 mg/dL over 2 years compared to a high-intensity statin (atorvastatin) which lowered LDL-C to a mean of 62 mg/dL. Those randomized to the higher intensity statin group showed a 16% relative risk reduction of the composite primary end point (all-cause mortality, MI, unstable angina, revascularization, and stroke). This study suggested that lowering LDL-C levels below current target levels may have benefit [16]. The following year, the Treating to New Targets (TNT) trial was published with 10,001 patients with coronary heart disease (CHD) and LDL-C levels already below 130 mg/dL. Low dose atorvastatin (10 mg/day) decreased LDL-C to a mean of 101 mg/dL in a 4.9 year follow-up compared to high dose atorvastatin (80 mg/day), which lowered LDL-C to a mean of 77 mg/dL. Again, the group with lower LDL-C revealed a 22% relative risk reduction in occurrence of first major cardiovascular event compared to those wih higher residual LDL-C. However, it is important to note there was no difference in mortality in this study [17]. The Incremental Decrease in Events through Aggressive Lipid Lowering (IDEAL) study, also released in 2005, had slightly different findings. With 8888 patients with prior MI and a mean follow-up of 4.8 years, simvastatin lowered LDL to 104 mg/dL, while atorvastatin lowered LDL to 81 mg/dL. While this study did not show a significant reduction in major coronary events, it did result in reduction of non-fatal MI [18].

In a slight throwback to the trials of the 1990s, the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial in 2008 compared rosuvastatin against a placebo. It included over 17,000 healthy men and women with an LDL-C less than 130 mg/dL with a C-reactive protein (CRP) greater than 2.0 mg per liter. Rosuvastatin lowered LDL-C by approximately 50% and lowered CRP significantly. The trial was stopped early after a median follow-up of 1.9 years due to remarkable reduction in major cardiovascular events (HR 0.56), findings which were consistent across all subgroups [19].

While these outcomes were being published, lipid research also progressed in a different direction. Other trials aimed to assess whether lipid lowering by statins would directly reduce atherosclerotic burden via serial imaging of atherosclerosis and plaque. In the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial in 2004, over 500 patients with initial mean LDL-C of 150 mg/dL were placed on pravastatin, which lowered LDL-C to a mean of 110 mg/dL after 18 months versus atorvastatin, which lowered LDL-C to a mean of 79 mg/dL. Outcomes revealed halting of atheroma progression seen on intravascular ultrasound (IVUS) at 18 months in the high-intensity statin group compared to progression of atherosclerosis seen in the pravastatin group [20]. Two years later, A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden (ASTEROID) took this one step further and attempted to evaluate whether intensive statin therapy could actually reverse atherosclerosis as determined by IVUS. Around 350 patents with mean baseline LDL-C of 130 mg/dL were given rosuvastatin and evaluated with serial IVUS. In 2 years' time, the mean LDL-C was reduced to 60.3 mg/dL and there was regression in atherosclerosis across 3 different IVUS measures, suggesting that treating LDL-C to levels below current guidelines may decrease atherosclerotic burden. There was no control group, and clinical outcomes were not evaluated [21].

In 2007, a study Measuring Effects on Intima-Media Thickness: An Evaluation of Rosuvastatin (METEOR) aimed to address whether statin therapy could slow progression of atherosclerosis in those with low Framingham risk score and mild-to-moderate subclinical atherosclerosis. Nearly 1000 individuals with mean LDL-C 155 mg/dL received either rosuvastatin 40 mg daily or placebo and outcomes were measured by assessing rate of change in maximum carotid intima-media thickness (CIMT) over 2 years at 12 extracranial carotid artery sites. Rosuvastatin lowered LDL to a mean of 78 mg/dL and showed significant reduction in rate of progression of max CIMT; however, plaque regression was not seen [22]. The Study of Coronary Atheroma by Travascular Ultrasound: Effect of Rosuvastatin Versus AtorvastatiN Trial (SATURN) from 2011 showed

significant regression of coronary atherosclerosis with both arms (rosuvastatin 40 mg daily vs atorvastatin 80 mg daily) as LDL-C was reduced to mean of 62.6 mg/dL and 70.2 mg/dL, respectively, after 104 weeks of therapy [23].

A large meta-analysis by the Cholesterol Treatment Trialists' (CTT) Collaboration in 2005 included data from 14 trials and over 90,000 patients followed over 5 years. Results revealed a 12% reduction in all-cause mortality per 1 mmol/L (38.7 mg/dL) of LDL-C reduction, largely irrespective of initial lipid profile and other presenting characteristics [24]. In 2010, the CTT published another meta-analysis which included five trials that compared low to high intensity statin use and 21 trials comparing statins to control. Nearly 170,000 individuals were analyzed. For each type of trial, the average risk reduction, as well as the average risk reduction per 1.0 mmol/L LDL-C, was calculated after 1 year of randomization. Across all 26 trials combined, all-cause mortality was reduced by 10% per 1.0 mmol/L (38.7 mg/dL) LDL-C reduction and the risk of occlusive vascular events was decreased by 20% irrespective of baseline cholesterol. There was also no evidence of any threshold to this effect within the range studied [25]. Even when LDL-C levels were below 2.0 mmol/L, additional reduction in LDL-C with more intensive statin therapy reduced the incidence of major vascular events. The authors suggested that the primary goal for patients at high risk of occlusive vascular events should be to achieve the largest LDL-C reduction possible [25].

Measurement of Low-Density Lipoprotein

A small note must be made about the measurement of LDL-C. Currently, the most commonly used method of reporting LDL-C on routine laboratory tests is through the Friedewald equation, where LDL-C = [total cholesterol (TC)] – [HDL-C] – [triglycerides (TG)/5], which becomes inaccurate if TG are greater than 400 mg/dL. Even at levels of TG less than this, it is established that LDL-C underestimates risk of atherosclerotic cardiovascular disease (ASCVD) in the setting of hypertriglyceridemia [2].To convert LDL-C from mmol/L to mg/dL, divide by 0.0259 [26].

The Friedewald formula underestimates true LDL-C at high levels of TG and low levels of LDL-C and overestimates it at higher LDL-C levels [27]. In an attempt to rectify the weakness of the Friedewald equation (which assumes a fixed ratio of TG to VLDL of 5), a few other equations have been proposed. One, which has been adopted by many laboratories around the United States was proposed by Martin, where the ratio is adjustable rather than fixed at five. This results in a more accurate risk classification [28]. External validation when compared to β-quantification, however, suggested that this method still falls short when TG are above 400 mg/dL. Although the Martin equation does provide higher (more accurate) estimates for LDL-C compared to the Friedewald equation, particularly when LDL-C levels are lower [29]. Due to such shortcomings of the Freidewald equation as well as other formulas, some suggest repeating LDL-C measurement by direct assay, particularly when TG are greater than 200 mg/dL or when LDL-C is less than 70 mg/dL or greater than 130 mg/dL [27].

We will also briefly address how lipoprotein (a) [Lp(a)] can affect calculated LDL-C levels. Lp(a) will be discussed in detail in another chapter, but is a class of lipoproteins which consists of a cholesterol-laden LDL-like particle bound to apolipoprotein (a). The Friedewald formula does not distinguish between cholesterol derived from LDL and Lp(a) and actually represents their sum. As Lp(a) contains a significant percentage of cholesterol, high levels will cause LDL-C to be overestimated. To "correct" for this, we can adjust the Friedewald formula as the following: LDL-C = HDL-C – TG/5 – 0.3Lp(a), with Lp(a) measured as a mass in mg/dL. Several studies have validated that this correction factor is significant when Lp(a) is moderately elevated (over 30 mg/dL). This has consequences because medications such as statins lower LDL, but not Lp(a), making Lp(a)-C comprise a larger fraction of routinely measured "LDL levels" in those on therapy [30, 31].

While the terms LDL and LDL-C are often used interchangeably, there is an important dis-

tinction. LDL is a collection of heterogeneous particles which vary in terms of size and cholesterol content. Work by Kraus revealed two major patterns of LDL sub-populations. Those with fractions showing large and buoyant LDL are considered to have pattern A while those with smaller, denser LDL have pattern B [32–34]. Small and dense LDL correlate negatively with HDL and triglycerides and are associated with increased risk of CVD and diabetes mellitus [34]. Increased susceptibility of oxidization and glycation may contribute to the pro-atherogenic properties of small-dense LDL, although some propose that this is due to the increased LDL particle number (LDL-P) [2]. NMR spectroscopy is one way to measure LDL-P concentrations more directly. The Multi-Ethnic Study of Atherosclerosis (MESA) demonstrated that when LDL-C and LDL-P are discordant, LDL-P predicts cardiovascular events better than LDL-C [2]. There is still debate on whether to measure LDL size routinely, with some arguing that measuring subfractions of LDL-C does not have sufficient incremental value to merit routine adoption [33, 35].

As we will see in the ODYSSEY OUTCOMES trial, non-HDL and apoB100 measurements have also been utilized more frequently. Since most of apoB100 is found in LDL particles, studies have shown that apoB100 levels correlate with LDL-P and may be superior markers of ASCVD risk compared to LDL-C. Various studies have also looked at the measurement of non-HDL cholesterol as a marker for ASCVD showing slight superiority over routine LDL-C measurements. Although one meta-analysis revealed apoB100 levels as the most superior marker for ASCVD [36], another suggested LDL-C, Non-HDL, and apoB were equivalent markers of cardiovascular events [2, 37]. Some suggest that this difference was due to the population under study. When LDL particles have normal cholesterol content, LDL-C, non-HDL, and apoB are relatively equal markers of cardiovascular disease risk [2]. A relatively more frequent scenario in which LDL-C appears to have a discordant risk compared to non-HDL-C and apoB occurs when TG are greater than 200 mg/dL. The American Association of Clinical Endocrinologists (AACE) and The European Society of Cardiology and European Atherosclerosis Society (ESC/EAS) guidelines take this into account and offer specific target levels for these values as well [38].

The Low-Density Lipoprotein Hypothesis

As we can see from the previously discussed trials, there appears to be a correlation between lowering LDL-C and improved outcomes. These trials, along with animal experiments on atherosclerosis and early-atherosclerotic disease in genetic conditions with high cholesterol levels led to the development of the LDL hypothesis, considered by some to be "one of the best-supported hypotheses in modern medicine." [1] The LDL hypothesis is the concept that excess LDL is a causal factor for the development of ASCVD and that lowering LDL-C levels correlates with a decrease in cardiovascular events, regardless of the method of LDL reduction [39].

However, not everyone was in agreement with the LDL hypothesis. Some argued that if LDL-C was the major cause of ASCVD, then there should be an exposure-response in trials; the more LDL-C is lowered, the better outcomes should be. While various trials have seemingly shown this, some argued that it was impossible to know if the effect was truly from lowering LDL-C levels or due to the pleiotropic effects of statins [26]. In order to understand what this means, and realize why statins were considered a possible confounding variable in all these trials, we will briefly discuss statins and their mechanisms of action.

Nearly two-thirds of the body's cholesterol is synthesized in the liver, with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase acting as the rate limiting enzyme in this biochemical pathway. HMG-CoA reductase inhibitors or "statins," initially isolated in 1976, work by reversibly inhibiting HMG-CoA reductase. Not only does reduction in cholesterol synthesis lower LDL-C levels; this decrease in LDL-C concentration is accompanied by an

increase in LDL-receptor activity, causing LDL-C decrease via two mechanisms [40]. However, these medications also have pleiotropic effects, and some argue that the protective effects shown in trials were actually in large part due to the other pathways of statin action, rather than due to decreased LDL-C levels. This idea is referred to as "the statin hypothesis." [39]

One common mechanism by which statins exert other effects is through the inhibition of Rho and Ras (small GTP-binding proteins) isoprenylation leading to downstream effects on the vascular wall. Decreasing smooth muscle proliferation, inhibiting platelet aggregation, increasing circulating endothelial progenitor cells, mediating vascular relaxation through increased eNOS, and antioxidant properties, and reducing vascular inflammation are all LDL-C independent mechanisms by which statins are thought to promote and account for cardiovascular benefits [39–41].

Proving the Hypothesis

With a few negative trials showing no significant incremental benefit in adding a non-statin lipid lowering drug to statin therapy, and data from the JUPITER trial showing that rosuvastatin reduced CRP levels (supporting the statin hypothesis), the 2013 ACC/AHA emphasized the use of statin medications to reduce risk rather than aiming for specific LDL-C targets.

With this background, with The IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) published in 2015 was the first clinical trial to show benefit of adding a non-statin lipid-modifying agent to statin therapy. Over 18,144 patients with ACS were randomized to simvastatin plus ezetimibe or simvastatin plus placebo. The primary end point was a composite of cardiovascular death, major coronary event (nonfatal myocardial infarction, unstable angina, or coronary revascularization), or nonfatal stroke. Within 1 year, mean LDL-C levels differed and were 53 mg/dL and 70 mg/dL, respectively. With a follow-up of 7 years, the rate of primary endpoint was significantly lower by 2% in the group with lower LDL-C levels, suggesting that lowering LDL-C in a statin-independent manner did indeed correlate with improved outcomes [39].

It was not until the proprotein convertase subtilsin-kexin type 9 (PCSK9) inhibitor trials were published that the evidence supporting the LDL hypothesis became indisputable. By 2014, results of phase 3 trials involving PCSK9 inhibitors were well underway and the Durable Effect of PCSK9 Antibody Compared with Placebo Study (DESCARTES) was published. Patients were started on background lipid-lowering therapy in five different ways. Diet alone, diet plus low-dose atorvastatin, high-dose atorvastatin, or high-dose atorvastatin plus ezetimibe. Each group was then randomized to either adding evolocumab or a placebo. Evolocumab decreased LDL-C by an additional 56 mg/dL, 62 mg/dL, 57 mg/dL, and 48 mg/dL, respectively, suggesting that this medication significantly lowers LDL-C levels. Similar LDL-C reductions were seen in other trials, such as the ODYSSEY FH I and II, RUTHERFORD-2, and GAUSS-2. LDL-C levels were now being lowered to previously unattainable levels in a statin-independent manner [42, 43].

The following year, The Open-Label Study of Long-Term Evaluation against LDL Cholesterol (OSLER-1) and (OSLER-2) results were published. They followed patients who had completed various phase 2 and 3 trials of evolocumab with the aim of collecting long-term data. While these were open-label trials, they included 4465 patients who were randomized to receive evolocumab plus standard therapy vs standard therapy alone. With approximately 1 year follow-up, evolocumab showed a mean LDL-C reduction by 61% from 120 mg/dL to 48 mg/mL and most importantly, also showed a reduced incidence of cardiovascular events [43]. With similar results to the DESCARTES trial in terms of LDL-C reduction, The Long-term Safety and Tolerability of Alirocumab in High Cardiovascular Risk Patients with Hypercholesterolemia Not Adequately Controlled with Their Lipid Modifying Therapy (ODYSSEY LONG-TERM) study, published in 2015, showed that alirocumab

significantly lowered LDL-C levels in those already on maximum-tolerated statin therapy in high risk patients with LDL-C levels greater than 70 mg/dL. Furthermore, a post-hoc analysis which included 78 weeks total follow-up suggested a significant reduction in major cardiovascular events as well [44].

In 2016, reminiscent of prior plaque regression studies from a decade earlier, the results of the GLobal Assessment of plaque reGression with a PCSK9 antibOdy as measured by intraVascular ultrasound (GLAGOV) trial, which included 968 patients with coronary disease was published. Patients were randomized to the addition of evolocumab versus placebo to their current therapy. The PCSK9 inhibitor arm lowered LDL-C levels to a mean of 36.6 mg/dL compared to 93.0 mg/dL in the control group. Serial IVUS over 78 weeks showed a significant decrease in plaque atheroma value by 0.95% with evolocumab compared to a slight increase (0.05%) with the control group. Secondary outcomes included a significant increase in the percentage of patients showing plaque regression as well as a decrease in normalized total atheroma volume [45].

The same year, a large meta-analysis by Silverman with data from over 300,000 participants was published aiming to evaluate the association between lowering LDL-C and relative cardiovascular risk across statin and non-statin therapies. Baseline mean LDL-C was 122.3 mg/dL. Relative risk for major vascular events (a composite of cardiovascular death, acute MI or other acute coronary syndrome, coronary revascularization, or stroke) per 1 mmol/L (38.7 mg/dL) reduction in LDL-C was 0.77 for statins and 0.75 for established non-statin interventions which work primarily via up-regulation of LDL-R expression (diet, bile acid sequestrants, ileal bypass, and ezetimibe) giving further evidence for the LDL hypothesis [26]. Overall, combining data from 33 trials showed that there was a 23% relative risk reduction of major vascular events per 1 mmol/L (38.7 mg/dL) reduction in LDL-C, results that are very similar to the findings of the Cholesterol Treatment Trialists' Collaboration almost a decade prior [22].

One of the most pivotal studies involving PCSK9 inhibitors with respect to LDL was published in 2017, the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial. 27,564 patients with ASCVD and LDL-C >70 mg/dL (median 92 mg/dL) already on statin therapy were randomized to evolocumab or placebo. Evolocumab lowered LDL-C by 59% to a mean of 30 mg/dL. With a median follow-up of 2.2 years, the primary endpoint (composite of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization) was reduced significantly from 11.3% to 9.8%. This suggested that patients with atherosclerotic disease benefit from lowering LDL-C levels beyond current targets [46]. The result of this trial showed unequivocal evidence that lowering LDL-C levels is associated with reduced atherosclerotic risk in a statin-independent manner.

Toward the end of 2018, the ODYSSEY OUTCOMES trial was published. 18,924 patients with recent ACS and LDL-C of at least 70 mg/dL, non-HDL of at least 100 mg/dL, or apoB level of at least 80 mg/dL on high-intensity statin or the maximally tolerated dose were randomized to alirocumab with a dose adjusted to target an LDL-C of 25 mg/dL to 50 mg/dL against placebo. A median follow-up of 2.8 years showed a decrease in the primary endpoint (composite of death from coronary heart disease, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, or unstable angina requiring hospitalization) from 11.1% to 9.5%. Notably, the absolute risk reduction in cardiovascular events was greatest in those with a baseline LDL-C of 100 mg/dL or more, adding support to the theory of the pathologic nature of LDL [47].

A 2018 meta-analysis by Sabatine incorporated data from the Cholesterol Treatment Trialists' Collaboration and newer trials where non-statin medications were added to statin therapy. An important distinction is that the mean starting LDL-C had to be 70 mg/dL or less in the population included in their study. They found consistent and significant relative risk reduction in major vascular events in groups with starting LDL-C as low

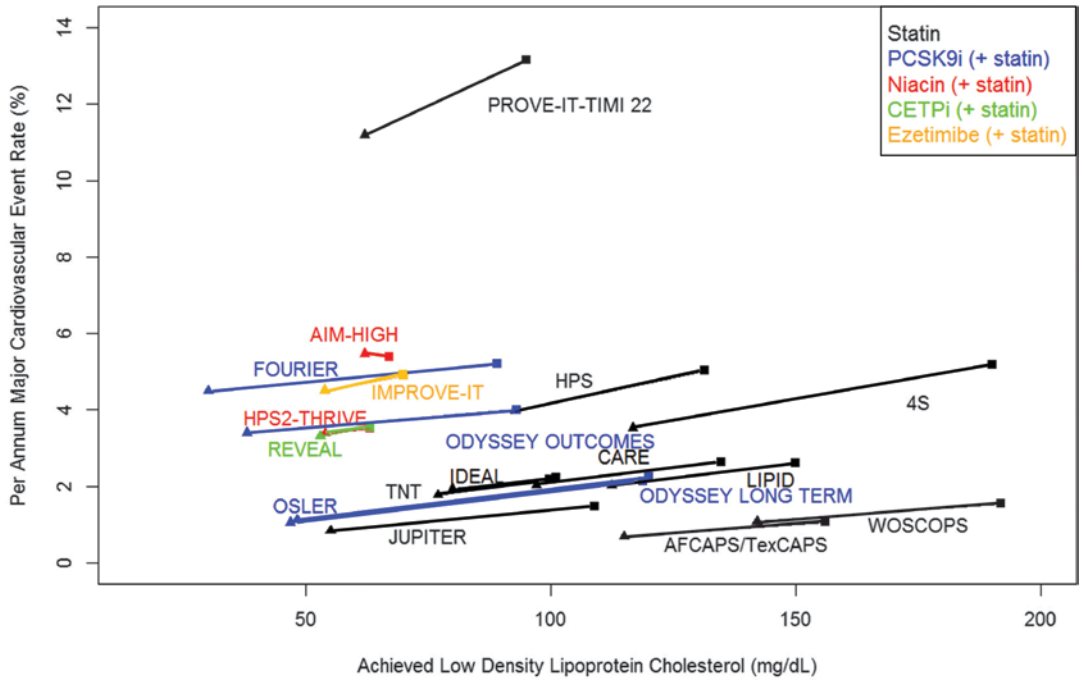

Fig. 7.1 The relation between achieved low-density lipoprotein cholesterol of control (square) and treatment (triangle) groups with per annum major cardiovascular event rate in select clinical trials where the annual event rate could be calculated. The baseline values for the control groups of LIPID, AFCAPS/TexCAPS, and HPS2-THRIVE were taken as achieved levels. Note that the definition of an event was not the same across trials

as a median of 63 mg/dL and while achieving levels as low as 21 mg/dL with the addition of non-statin agents to statin therapy. In fact, the relative risk of vascular events with a 1 mmol/L (38.7 mg/dL) reduction in LDL-C was 0.79 with both non-statin therapy and where non-statins were added to statins, further suggesting that reduction in LDL-C levels are associated with reduction in disease risk in a statin independent manner [48].

Using data from the major statin and non-statin trials included in this chapter where major cardiovascular event rate per annum could be calculated, we illustrate the relation between achieved LDL-C in control and treatment groups and the rate of cardiovascular events (Fig. 7.1). In nearly every trial, as LDL-C is decreased, we see a corresponding reduction in annual cardiovascular event rate. It is important to note, however, that even at extremely low LDL-C levels, the cardiovascular event rate would not actually hit zero. In addition, focusing on the landmark cardiovascular outcome trials discussed in this chapter with an achieved LDL-C less than 100 md/dL, we highlight the relation between achieved low-density lipoprotein cholesterol for the treatment group and the relative risk reduction of the composite endpoint in relationship to a control or placebo group (Fig. 7.2). Again, almost every major trial reveals a significant relative risk reduction with LDL-C reduction.

Mechanisms of Low Density Lipoprotein Pathogenicity

The "response to retention hypothesis" states that retention of lipoproteins in the artery wall is an important initiating event in the development of atherosclerosis. It was based on decades of work showing retention of apoB containing lipoproteins, such as LDL, in arterial walls through interactions with proteoglycans. It is important to note that native LDL does not induce macrophage foam cell formation or the pro-inflammatory milieu in vitro, leading

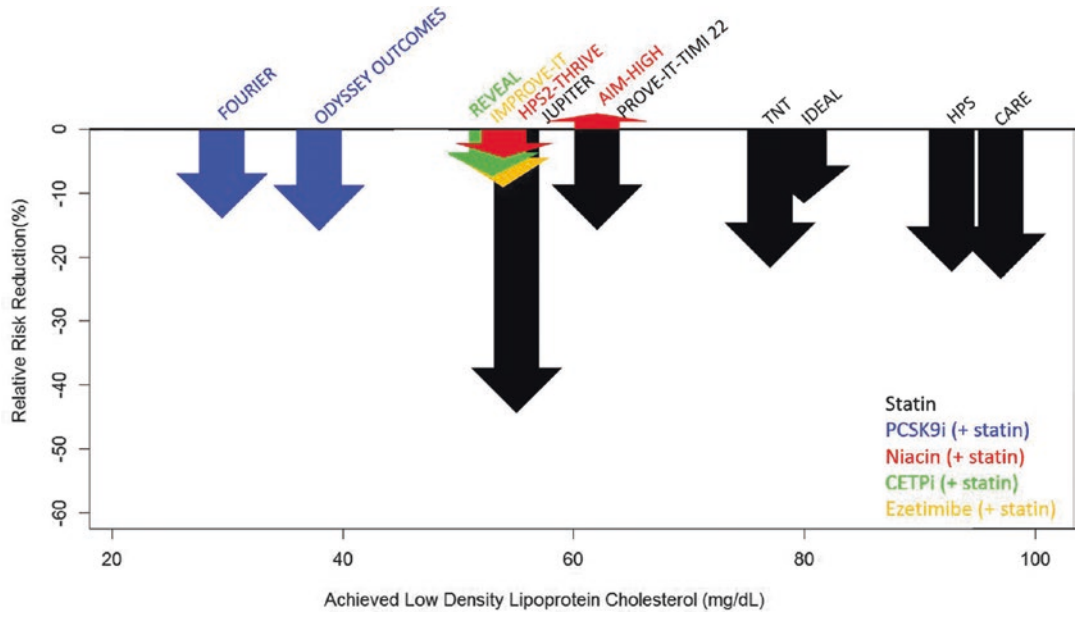

Fig. 7.2 The relation between achieved low-density lipoprotein cholesterol for the treatment group and the relative risk reduction of the composite endpoint in relationship to a control or placebo group for dedicated cardiovascular outcome trials. PCSK9i = proprotein convertase subtilsinkexin type 9 inhibitor, CETPi = cholesteryl ester transfer protein inhibitor

to the hypothesis that LDL needs to be modified prior to its effects in vivo [2]. Oxidization of LDL in the endothelial wall is an early event in the formation of atherosclerosis according to the oxidative hypothesis. LDL enters the arterial wall from the vasculature through transcytosis [49]. Basic amino acids in apoB100 bind to negatively charged sulfate groups of proteoglycans in the extracellular matrix causing structural changes which impact the configuration of apoB100 and make LDL more readily oxidized [2]. Oxidized LDL (OxLDL) contributes to atherosclerosis through four mechanisms: triggering endothelial dysfunction, increasing foam cell formation, promoting vascular smooth-muscle cell (VSMC) migration and proliferation, and stimulating the induction of platelet adhesion and aggregation [49].

In the initiation and fatty streak phase of atherosclerosis, endothelial dysfunction leads to increased accumulation of apoB containing lipoproteins which are then modified through oxidization [2]. OxLDL activates endothelial cells which then induce activation of rolling and adhesion of monocytes and T cells which then migrate into the tunica media. Specifically, OxLDL causes the up-regulation of endothelial adhesion molecules as ICAM-1 and VCAM-1 [in a lectin-like receptor (LOX-1) dependent manner]. In addition, nitric oxide (NO) production from endothelial cells is inhibited by OxLDL. This is important, as NO normally has vasodilator properties and inhibits adhesion, attachment, and transmigration [49].

Once inside the arterial wall, monocytes differentiate into macrophages. Normally, macrophages, which contain LDL receptors, endocytose LDL particles. LDL is then degraded in lysosomes into free cholesterol. Excess free cholesterol is taken to the endoplasmic reticulum and esterified by acyl CoA: cholesterol acyltransferase (ACAT). The resulting choleresteryl ester is packaged in cytoplasmic lipid droplets, which is characteristic of foam cells. It is important to note that normally, this process of LDL internalization occurs at a very slow rate and free cholesterol actually down-regulates the LDL receptor, thus maintaining cholesterol homeostasis and preventing foam cell formation [2, 49].

However, oxidization enhances phenotypic change in macrophages and other cells leading to increased expression of scavenger receptors such as CD36, scavenger receptor A (SRA), lectin-like receptors (LOX), and toll-like receptors (TLR4), none of which are down-regulated by free cholesterol [2]. In the presence of OxLDL, there is intracellular lipid accumulation resulting in a cycle of macrophage trapping and foam cell formation [49]. Macrophages' inflammatory pathways are also activated by OxLDL, leading to increased inflammation and oxidative stress, perpetuating the cycle of LDL oxidation, endothelial cell activation, monocyte recruitment, and foam cell formation. Other immune cells are attracted by OxLDL including dendritic cells, mast cells, T cells, and B cells; all of which contribute to the development of atherosclerotic plaque formation [2].

The next step in atherosclerosis progression involves chemo-attractants from the pro-inflammatory macrophages promoting migration and proliferation of VSMCs from the tunica media into the tunica intima. These VSMCs produce an extracellular matrix made up of collagen, proteoglycans, and elastin to form a fibrous cap over a core of foam cells. This thick cap on a stable plaque limits thrombus rupture and protects against exposure of the prothrombotic factors to blood [2]. OxLDL enhances the expression and secretion of various growth factors by macrophages and endothelial cells promoting VSMC migration. OxLDL also directly induces changes in the smooth-muscle cell's phenotype causing them to produce larger amounts of extracellular matrix, thereby expanding the atherosclerotic lesion size [49].

Eventually though, the lesion, a non-resolving inflammatory condition, becomes vulnerable through the formation of a necrotic core and thinning of the fibrous cap. Increased macrophage apoptosis and defective efferocytosis of apoptotic cells result in cell death. This causes an enlargement of the necrotic core composed of inflammatory and oxidative components which in turn causes smooth muscle cell death. This decreases the production of the extracellular matrix that the fibrous cap is composed of [2]. OxLDL also induces LOX-1 expression in smooth-muscle cells, which causes increased reactive oxygen species (ROS) generation further contributing to cell death and directly increasing the risk of rupture [49]. If the fibrous cap ruptures, there can be clinically significant cardiovascular events [2].

Platelets also play an important role in this process, especially after plaque rupture, where they promote thrombus formation. Decreased NO production from endothelial cells (due to OxLDL) is associated with an increase in prostaglandin secretion which leads to increased platelet aggregation. Through various pathways (including CD36, Src kinases, and Rho kinases), OxLDL promotes platelet activation. LOX-1 is also expressed in activated platelets and may mediate platelet adhesion to endothelial cells [49]. Overall, nearly every step of atherosclerotic disease progression is affected by LDL for the worse.

Normal Physiological Levels of Low-Density Lipoprotein

As we have touched upon the uses of cholesterol in the body, as well as the pathways of creation and degradation of the LDL particle, we need to address the question of determining what levels of cholesterol are required to maintain normal biological functions. While this may at first seem simple, decades of research have led to ever increasing and changing knowledge that cause a continually changing paradigm. The major flaw in simply using the mean or median values of LDL-C in our population as the norm is presented here.

While diet and lifestyle have changed considerably over the last few generations, the human genome as a whole has remained relatively stable in the last 10,000 years. With such discordance between what our body is designed for and the nutritional, cultural, and activity patterns in modern populations, the assumption that current average levels of LDL-C in the population is "normal" is inaccurate [50]. In fact, certain hunter-gatherer populations (living indigenous lifestyles) have estimated LDL-C levels of around 50 mg/dL to

75 mg/dL. As a comparison, healthy adult primates in the wild (who do not develop atherosclerotic disease) have LDL-C levels from 40 mg/dL to 80 mg/dL. When including hunter-gatherer populations, primates, and other mammals, the average levels of LDL-C are actually as low as 35 mg/dL to 70 mg/dL, further providing evidence that our ideal LDL levels should be lower than currently proposed [1].

Cholesterol is extremely important in the brain, where it plays a role in synaptic transmission. In fact, links between cholesterol metabolism defects and neurodegenerative disorders have been shown [51]. This raised alarm over lowering levels of LDL-C well below the traditional norm, especially after a few small statin trials suggested the possibility of an increase in cognitive deficits, such as memory loss or confusion. The Evaluating PCSK9 Binding antiBody Influence oNcoGnitiveHeAlth in High cardiovascUlar Risk Subjects (EBBINGHAUS) substudy of the FOURIER trial put this concern to rest. 1204 patients were followed for a median of 19 months with cognitive function assessed objectively using the Cambridge Neuropsychological Test Automated Battery (CANTAB). Findings suggested no difference in the primary endpoint (spatial working memory strategy index of executive function) even when stratified by LDL-C levels, confirming the lack of cognitive harm of low LDL-C levels [52]. Physiologically, this makes sense as well, since the blood–brain barrier prevents uptake of cholesterol from the bloodstream. Most cholesterol in the central nervous system (at least 95%) comes from in situ synthesis [51]. Therefore, fluctuations in peripheral cholesterol concentrations should have minimal impact on levels in the brain.

In terms of a lower limit of normal and safety with very low levels of LDL, we can also look to patients with heterozygous hypobetalipoproteinemia. Through a genetic mutation, these patients have LDL-C levels as low as 30 mg/dL and show no adverse effects; this suggests that only very low cholesterol levels are needed to maintain normal biological functions. In fact, such patients with very low LDL-C levels often exhibit longevity [1].

Low-Density Lipoprotein-Cholesterol Goals

As newer trials help elucidate more information on LDL and its pathogenic nature, guidelines have also been regularly updated to aid the clinician and patient. We will briefly mention major recommendations as they apply to LDL-C. The 2013 American College of Cardiology and the American Heart Association ACC/AHA guidelines took the approach of risk stratification based on various factors including the presence of ASCVD, initial LDL-C level, age, and the presence of diabetes. In addition, calculation of 10-year ASCVD risk using the Pooled Cohort Equations was used to determine whether patients should be on a high or moderate intensity statin. Unlike the previous Adult Treatment Panel III (ATP III) guidelines, there was no specific LDL-C target goal [53]. With newer data published since 2013, including from the PCSK9 inhibitor trials, the ACC/AHA reintroduced LDL-C goals for certain high-risk groups in their latest 2018 update [2, 54].

The American Association of Clinical Endocrinologists (AACE) 2017 guidelines utilize five risk categories ranging from low risk (goal LDL-C less than 130 mg/dL) to extreme risk (LDL-C goal less than 55 mg/dL). The risk category a patient falls into is based on risk factors including the 10-year ASCVD risk based on Framingham Global Risk calculation, the presence of cardiovascular disease, diabetes, kidney disease, and lipid disorders [55]. The European Society of Cardiology and European Atherosclerosis Society (ESC/EAS) Task Force's 2016 guidelines use four risk categories ranging from low risk to very-high risk. Again, factors including renal function, comorbidities such as diabetes mellitus and cardiovascular disease, and even blood pressure affect risk stratification; however, an important calculation is the use of the SCORE chart (which includes smoking, age, gender, SBP, and cholesterol) to estimate 10-year risk of fatal CVD. Goals for the lowest risk groups are to keep LDL-C below 190 mg/dL while the very-high risk patients should aim for an LDL-C goal of less than 70 mg/dL [56].

An important point was also illustrated in a 2018 meta-analysis; Navarese looked at intensive and less intensive therapy and how baseline LDL-C levels were related to outcomes. With data from over 270,000 participants from 34 trials, all cause and cardiovascular mortality were clearly lower with intensive therapy. However, those stratified into groups with higher LDL-C at baseline had greater risk reduction, suggesting that the greatest benefit of LDL-C lowering therapy is in those with a higher LDL-C baseline [57].

We have reviewed several trials and have touched upon the mechanisms by which LDL confer pathogenicity. In addition, we have seen that perhaps our current average levels of LDL-C in the population are not historically or physiologically normal. With this being the case, many argue for even more stringent control of LDL-C in an attempt to further decrease or perhaps even eliminate ASCVD.

We know that atherosclerosis begins early in life. In fact, a study from the 1990s looked at 111 casualties of the Korean War who died from noncardiac trauma. 78.3% of these relatively healthy individuals with a mean age of 26 years showed coronary atherosclerosis. Over one-fifth had greater than 50% narrowing and just as many had left main or significant two and three-vessel involvement suggesting that ASCVD begins much earlier in life than usually presumed [58]. In addition, The Bogalusa Heart Study found that serum LDL-C is significantly related to the extent of atherosclerotic lesions in people below the age of 40 years [59].

A related concept to take into account is that the effect of LDL-C on atherosclerosis is not only causal, but cumulative over time. There is evidence of this based on Mendelian randomization studies which have shown that long-term exposure to lower LDL-C levels is associated with three times greater proportional risk reduction in cardiovascular disease compared to shorter term treatment after atherosclerosis has already developed [60, 61]. In one of their consensus statements, the European Heart Journal best illustrates this concept after integrating Mendelian randomization studies in addition to randomized controlled trials. They suggest that each mmol/L reduction in LDL-C reduces the relative risk of ASCVD events by 10%. However, this is for the first year. By 3 years of treatment, this relative risk reduction increases to 20%, with an additional 1.5% proportional decrease in events each year after. In 40 years, this would theoretically reduce ASCVD events by 50–55% per mmol/L decreased [60].

In an older paper by O'Keefe from 2003, a regression based on data from randomized placebo controlled trials, including many we have discussed, suggest that atherosclerosis does not progress when LDL-C is below 67 mg/dL [1]. Again, aggregating data from randomized controlled trials suggest LDL-C cutoffs of 57 mg/dL or 30 mg/dL, below which the cardiovascular event rate approaches zero for primary prevention secondary prevention, respectively [1]. The Progression of Early Subclinical Atherosclerosis (PESA) study, published in 2017, included only healthy patients without any cardiovascular risk factors. LDL-C remained the strongest modifiable risk factor for the progression of atherosclerosis, suggesting a central role of LDL-C in early human atherogenesis. This study found that atherosclerosis develops above an LDL-C threshold of 50 mg/dL to 60 mg/dL. All of these findings raise implications for primordial prevention and lower LDL-C goals [62].

Therapeutic Options

Statins inhibit intrahepatic activity of the enzyme HMG-CoA, reducing cholesterol synthesis, leading to reduction in LDL-C levels. In addition, they exhibit pleitropic effects we have discussed previously. The most common side effects based on randomized controlled trials involving over 160,000 patients include myopathy and rhabdomyolysis, as well as marginally increased rates of new-onset diabetes. Other perceived side effects seen observationally such as cognitive impairment, cataract formation, or erectile dysfunction have not been confirmed, even with extensive databases [35]. Depending on the exact statin and dosage of the medication,

they can lower LDL-C by 21–55% and have a beneficial effect on TG and HDL-C as well. Liver function needs to be checked prior to and during therapy with myalgia and weakness being commonly described side effects [55]. Statins remain the first line of treatment for hyperlipidemia in those without contraindications.

Ezetimibe works by blocking the transport protein NPC1L1 in the brush border of enterocytes, preventing uptake of dietary sterols. It also inhibits NPC1L1 in the plasma membrane of macrophages, lowering the uptake of oxidized LDL. In addition, there may be additional anti-inflammatory and immune-modulatory effects [63].While ezetimibe can be used as monotherapy for reducing LDL-C and apoB, especially in statin-intolerant individuals, it is usually used as an adjunct with statin in those who have failed to achieve LDL-C goals. Ezetimibe may decrease LDL by 10–18% in monotherapy and by an additional 25% when added to statin therapy (for a total 34–61% reduction in LDL-C) [63]. Myopathy and rhabdomyolysis, while rare, are more likely when adding ezetimibe to a statin or fibrate [55].

Bile acid sequestrants are also used to reduce LDL-C and apoB levels, especially in statin-intolerant individuals. They reduce LDL-C by a little over 10% and may also have favorable effects on glycemic markers [64].These medications have various drug interactions and can reduce the absorption of folic acid and fat-soluble vitamins. Common side effects include constipation and bloating [55]. Again, they are not traditionally used as monotherapy and usually are considered an add-on to statin therapy.

Fibrates (PPAR agonists) work by inducing the transcription of genes involved in peroxisomal beta-oxidation which is mediated by factors called peroxisomal proliferator activated receptors (PPARs). While effects of fibrates, such as decreasing triglycerides and increasing HDL-C, are discussed elsewhere, with regard to LDL-C, they increase the formation of LDL with a higher affinity for the LDL-R, which is catabolized more rapidly. As the cholesterol ester content also is increased, fibrates end up decreasing the small-dense LDL fraction, increasing the buoyant LDL fraction which may be less susceptible to oxidation and therefore less atherogenic [32]. In some cases, a modest decrease in LDL-C of 20–25% may occur; however, in others, they may actually cause a reciprocal rise in LDL-C levels by 10–15%, although it is felt to be a less atherogenic form of LDL [32, 55]. Fibrates are also traditionally used in combination with statins; those with elevated TG and low HDL-C baselines obtaining the most benefit [32]. Omega-3 acids are primarily used in the treatment of hypertriglyceridemia. While one omega-3, icosapent, may lower LDL-C by 5%, omega-3 acid ethyl esters can increase LDL-C by 45% [55].

Niacin, or nicotinic acid, reduces TG, LDL-C, Lp(a) and increases HDL-C. Its effects on LDL occur by inhibiting hepatic diacylglycerol acyltransferase–2 (DGAT2), resulting in decreased TG synthesis leading to increased intra-hepatic apoB degradation. This decreases VLDL and in turn LDL. In addition, through the same mechanism, less large TG-rich VLDL is produced, shifting away from the production of small-dense LDL to a more favorable distribution of LDL [65]. Overall, they may reduce LDL-C by 1% to 25% [66]. Unfortunately, trials have shown little to no outcome benefit. The AIM-HIGH trial showed that extended release niacin lowered LDL-C to 62 mg/dL from an already low mean of 74 mg/dL, without benefit [67]. The HPS2-THRIVE study, with over 25,000 participants, added extended-release niacin and laropiprant versus placebo to a background of statins. LDL-C was reduced by around 10 mg/dL, but there were no differences in cardiovascular events and there was an increase in select non-cardiovascular complications [68, 69]. However, some older studies (CLAS, FATS, HATS, and AFREGS) used niacin either alone or in addition to other medications and all showed reduction of LDL-C of 26–43% with regression of atherosclerosis, leading some to argue that niacin may have a role in select populations [70]. Most common side effects include flushing and increased insulin resistance [66]. New nicotinic-acid related compounds are being developed currently [66].

While CETP inhibitors are traditionally used to increase HDL-C levels, more potent CETP inhibi-

tors also decrease LDL-C, up to 35% with evacetrapib, for example. Even with combination therapy with statin, evacetrapib reduced LDL-C by an additional 11–14% in one study [71]. However in major trials, when CETP inhibitors were added to statin therapy, torcetrapib increased cardiovascular disease (likely due to off-target effects) and the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events Outcomes (ILLUMINATE) trial was stopped early. The dal-OUTCOMES trial and Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition with Evacetrapib in Patients with a High Risk for Vascular Outcomes (ACCELERATE) trial were also stopped early due to lack of efficacy [72, 73]. The Randomized Evaluation of the Effects of Anacetrapib through Lipid modification (REVEAL) trial with 30,449 adults on atorvastatin who were given anacetrapib did show improved outcomes, but whether this was attributed to further decrease in LDL-C (non-HDL cholesterol measurements were used in this study) or increase in HDL-C was not determined [74].

Proprotein convertase subtilsin-kexin type 9 (PCSK9) was identified in 2003 and degrades hepatic LDL receptors, leading to increased LDL-C concentration in the circulation. Interestingly, statins actually tend to increase serum PCSK9 levels. Knowing these two facts led to the development of a class of medications that inhibit this protease, which in turn leads to increased LDL receptors. PCSK9 inhibitors lower LDL-C by 48–71% and also may have a synergistic effect with statins. The current recommended group of patients whom this class may be ideal for include those who have high risk of side effects with statins, those who fail to meet target LDL-C on current therapy, as well as those with heterozygous familial hypercholesterolemia [55, 75]. Currently available PCSK9 inhibitors are all monoclonal antibodies, so local site reactions can occur as a side effect. Additionally, the antibodies do not cross the blood–brain barrier, leaving cholesterol metabolism of the central nervous system untouched [76]. There is ongoing research into gene silencing approaches to PCSK9 inhibition with RNA interference and antisense oligonucleotides and even peptide-based vaccines against PCSK9 in development [66].

In addition to current therapies, numerous medications are being researched as future therapeutic options. Two drugs currently approved for homozygous familial hypercholesterolemia that are being evaluated for use in routine ASCVD include lomitapide and mipomerson which work in similar, but slightly varied mechanisms to decrease the formation of apoB-containing lipoproteins, and thus also lower LDL-C levels. Acetyl Coenzyme A Carboxylase Inhibitors and ANGPTL3 Inhibitors are also being researched; however, they have a long way to go before possible use in clinical practice [66].

Phytosterols (sitosterol, campesterol, and stigmaterol) occur naturally in vegetable oils and in small amounts in vegetables, fruits, legumes, and grains. They compete with intestinal absorption of cholesterol, lowering levels. The consumption of 2 g daily can lower TC and LDL-C by 7–10%. No specific studies have been performed to evaluate cardiovascular benefits though. Saturated fatty acids have the greatest impact on LDL-C levels. Every additional 1% increase in energy from saturated fatty acids increases LDL-C by 0.8 mg/dL to 1.6 mg/dL. Partially hydrogenated fatty acids (trans-fatty acids) not only increase LDL-C, but also decrease HDL-C. Dietary carbohydrates are neutral on LDL-C; however, refined carbohydrates are not recommended as they can elevate TG and lower HDL-C. Dietary fibers (especially soluble fibers) have a hypocholesterolemic effect [56].

A healthy lifestyle and diet should be the foundation of any therapy. In terms of pharmacologic agents, most guidelines favor statins as first-line therapy. The AACE even claims that the risk of mild increase in new onset type-II diabetes mellitus does not outweigh their benefit [49]. Once statins are at the highest tolerated dose, a second agent, ezetimibe or a bile acid sequestrant, is added (ezetimibe is preferred). PCSK9 inhibitors currently are added to therapy only if LDL-C goals are not met in high-risk individuals or if patients are statin intolerant, although this may change in the future [56].

Conclusion

In this chapter, we have comprehensively reviewed aspects of LDL, starting from its chemical structure to the biochemical pathways of its creation and degradation. We discussed pertinent historical, as well as the latest clinical trials, which highlight the association between LDL and ASCVD. We provided evidence to unequivocally prove the LDL hypothesis, showing that LDL is a causal factor for the development of ASCVD. We discussed mechanisms by which LDL confers such harm. We provided information about the measurement and normal range of LDL, as well as updates on the latest LDL guidelines and practical therapeutic management of LDL. All statements are backed by evidence-based data referenced throughout the chapter allowing readers to easily find information on topics discussed within. We hope that this chapter has aided the clinician in broadening their knowledge and will serve well in the care of their patients.

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8 Triglyceride-rich Lipoprotein Cholesterol (Remnant Cholesterol) as a Therapeutic Target for Cardiovascular Disease Risk

Børge G. Nordestgaard and Anette Varbo

Introduction

In preventive cardiology, vascular medicine, and clinical lipidology, the focus over the past many years has been on reduction in atherogenic LDL cholesterol to reduce the burden of atherosclerotic cardiovascular disease, that is, ischemic heart disease and ischemic stroke. This evidence-based medical practice is founded on huge scientific evidence for disease causality [1] coupled with cardiovascular benefit from safe LDL cholesterol reduction [2, 3].

However, we now understand that even after maximal LDL cholesterol reduction, substantial residual cardiovascular disease risk remains. In a very large fraction of high-risk patients, this is due to elevated remnant cholesterol, i.e., the cholesterol content of triglyceride-rich lipoproteins. This chapter focuses on the main scientific and clinical evidence for why this is so.

Historical Development

In the 1970s and 1980s, most clinical lipidologists understood that patients at high risk of cardiovascular disease were either those with familial hypercholesterolemia and elevated LDL cholesterol or those with combined hyperlipidemia and elevated remnants and triglyceride-rich lipoproteins. Together with the rare genetic form of the chylomicronemia syndrome, these were the typical two types of patients followed in lipid clinics.

Then focus centered on LDL cholesterol, due to understanding of the genetic cause of familial hypercholesterolemia as mutations in the LDL receptor – leading to the Nobel Prize in 1985 given to Brown and Goldstein for their pioneering work [4]. The focus on LDL cholesterol was further supported by statin trials showing reduced cardiovascular morbidity, cardiovascular mortality, and all-cause mortality [3, 2]. Because statins work via the upregulation of the LDL receptor on hepatocytes and LDL cholesterol in consequence is lowered, for easy communication, statins were marketed as LDL-cholesterol-lowering drug; however, the fact that statins also reduce triglyceride-rich lipoproteins [5] and remnants was ignored by most drug companies, and therefore, this facet went unrecognized by the common clinician prescribing statins.

Next came the focus on high-density lipoprotein (HDL) cholesterol starting in the 1990s due to very strong epidemiological evidence that low HDL cholesterol is associated with high risk of cardiovascular disease. Many believed that increasing HDL cholesterol would be beneficial just like lowering of LDL cholesterol, and numer-

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_8

ous drug companies developed HDL cholesterol increasing drugs. However, starting around the year 2000, genetic evidence could not confirm a causal relationship between low HDL cholesterol and cardiovascular disease [6–10]. The hypothesis of HDL cholesterol as cardioprotective finally fell into neglect when none of the HDL-cholesterol-raising trials led to cardiovascular benefit trough HDL cholesterol increases [11–16], and one trial even increased cardiovascular morbidity and mortality [11]. Unfortunately, scientists and drug companies alike were misled by confounding fact that individuals with low HDL cholesterol often have high levels of remnant cholesterol and triglyceride-rich lipoproteins – the real cause of high cardiovascular risk in those with low HDL cholesterol [17].

Renewed interest in triglycerides, remnants, and triglyceride-rich lipoproteins surfaced in 2007, with special focus on the nonfasting state to better capture the risk associated with these lipoproteins [18–20]. This was followed by numerous genetic Mendelian randomization studies documenting causality between elevated remnant cholesterol/triglyceride-rich lipoproteins and cardiovascular disease and all-cause mortality [21–32]. Finally, three large-scale triglyceride-lowering trials in patients with elevated triglyceride-rich lipoproteins despite statin therapy were initiated [33–35].

Calculation and Direct Measurement

A standard lipid profile includes reporting of total, LDL, and HDL cholesterol together with plasma triglycerides [36]. Attempting to change the focus away from triglycerides per se, in 2007, we introduced the term "remnant cholesterol" to direct focus toward the cholesterol content in triglyceride-rich lipoproteins [18]. As cholesterol, and not triglycerides, accumulates in the atherosclerotic plaque, our simple aim was to help scientists, clinicians, patients, and drug companies to understand that the cholesterol in these particles likely is equally important to LDL cholesterol in leading to atherosclerosis and cardiovascular disease.

Remnant cholesterol can be calculated from a standard lipid profile as total cholesterol minus HDL cholesterol minus LDL cholesterol. Either nonfasting or fasting blood samples can be used, where nonfasting values best capture the average levels seen during a 24-hour cycle [37, 38, 36]. Remnant cholesterol levels are typically 8 mg/dL (0.2 mmol/L) higher 3–4 hours after a normal meal compared with those in the fasting state; for plasma triglycerides, the corresponding value is 26 mg/dL (0.3 mmol/L) higher levels.

The calculated version of remnant cholesterol comes at no additional cost; however, remnant cholesterol (triglyceride-rich lipoprotein cholesterol) can also be measured using a newly developed direct homogenous assay for auto-analyzers from Denka Seiken[39], ultracentrifugation, or nuclear magnetic resonance (NMR) technology[40]. It is possible that directly measured versus calculated remnant cholesterol will better capture the true risk associated with elevated levels, as levels of the two entities do not agree perfectly on a person-to-person basis (Fig. 8.1) [39].

Metabolism

Triglycerides and cholesterol in the diet are absorbed in the small intestine to be incorporated in very large chylomicrons (Fig. 8.2). Chylomicrons are transferred to the bloodstream via lymph, where triglyceride degradation starts immediately by the lipoprotein lipase enzyme, mainly in fat and muscle tissue. The hereby produced smaller, cholesterol-enriched chylomicron remnants are rapidly taken up by liver cells.

Triglycerides and cholesterol in the liver are packed into medium-sized very-low-density lipoprotein (VLDL) particles and secreted into the bloodstream (Fig. 8.2). Triglycerides in VLDL are then degraded slowly in fat and muscle tissue by lipoprotein lipase, and the smaller, cholesterol-rich intermediate-density lipoprotein (IDL) particle is formed. Some IDL particles are cleared directly by liver cells while others are converted to LDL particles through the action of the triglyceride-degrading enzyme hepatic lipase. LDL particles are taken up via the LDL receptor in the liver and other tissues.

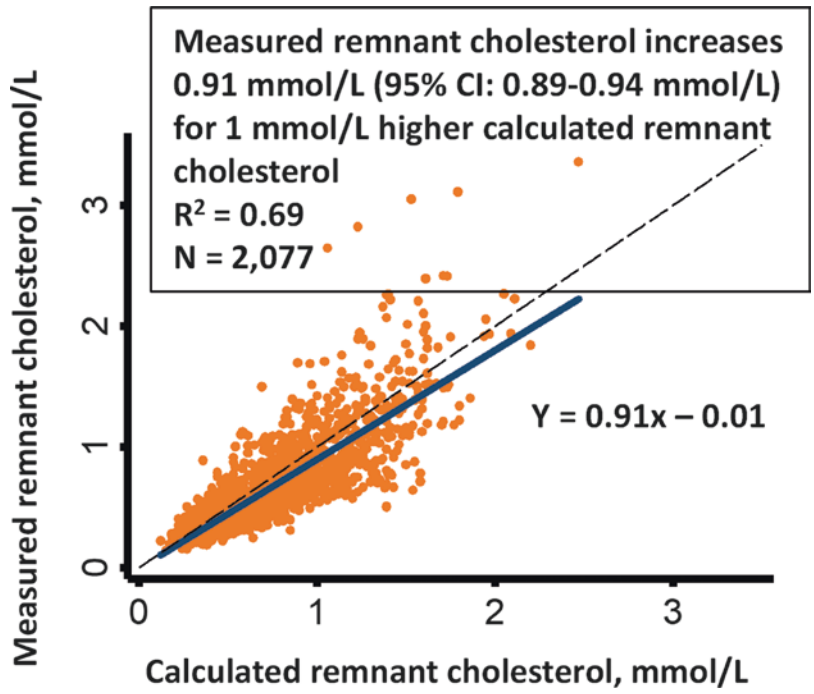

Fig. 8.1 Association between calculated and directly measured remnant cholesterol, using a newly developed direct homogenous assay for auto-analyzers from Denka Seiken. Based on 2077 individuals from the Copenhagen General Population Study. To convert cholesterol levels in mmol/L to mg/dL, multiply by 38.6. (Adapted from Varbo et al. [39])

In the fasting state, triglyceride-rich lipoproteins include VLDL and IDL, while in the nonfasting state, chylomicron remnants are also present as a minor fraction of all triglyceride-rich lipoproteins. Except for in the very rare situation of complete lipoprotein lipase deficiency, lipoprotein lipase-mediated triglyceride hydrolysis in chylomicrons and nascent VLDL particles are started immediately after these particles appear in the blood. Therefore, all triglyceride-rich lipoproteins in plasma represent some form of remnants. This is the reason why we in 2007 started using the term "remnant cholesterol" [18], which is easier to communicate to patients and the common medical doctor compared with the parallel term "triglyceride-rich lipoprotein cholesterol." However, some specialists prefer the latter term, and different specialists often have their personal idea of what should be called a remnant and what should not.

As lipid and cardiovascular guidelines worldwide now increasingly advise that lipid profiles can be measured in blood drawn in the nonfasting state [38, 41–48, 36], remnant cholesterol typically includes the cholesterol present in IDL, VLDL, and chylomicron remnants (Fig. 8.2) – all particles that are small enough to enter the arterial intima and cause the development of atherosclerosis. In other words, remnant cholesterol includes all cholesterol not found in HDL and LDL. The proteins apolipoprotein AV and glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) promotes the action of lipoprotein lipase and thus lower remnant cholesterol levels through faster removal of triglyceride-rich lipoproteins from plasma, while the lipoprotein lipase inhibitors apolipoprotein C3, angiopoietin-like 3 (ANGPTL3), and ANGPTL4 increase remnant cholesterol levels (Fig. 8.2).

Population Distribution

Like for plasma triglycerides, the population distribution of remnant cholesterol is skewed with a tail toward the extremely high levels in both women and men (Fig. 8.3). In a typical affluent country like Denmark, 26.5% of women and 45.0% of men have triglycerides of 150–500 mg/dL (1.7–5.7 mmol/), while 0.5% of women and 1.9% of men have triglycerides above 500 mg/dL (5.7 mmol/L). Correspondingly, 26.0% of women and 44.5% of men have remnant cholesterol of

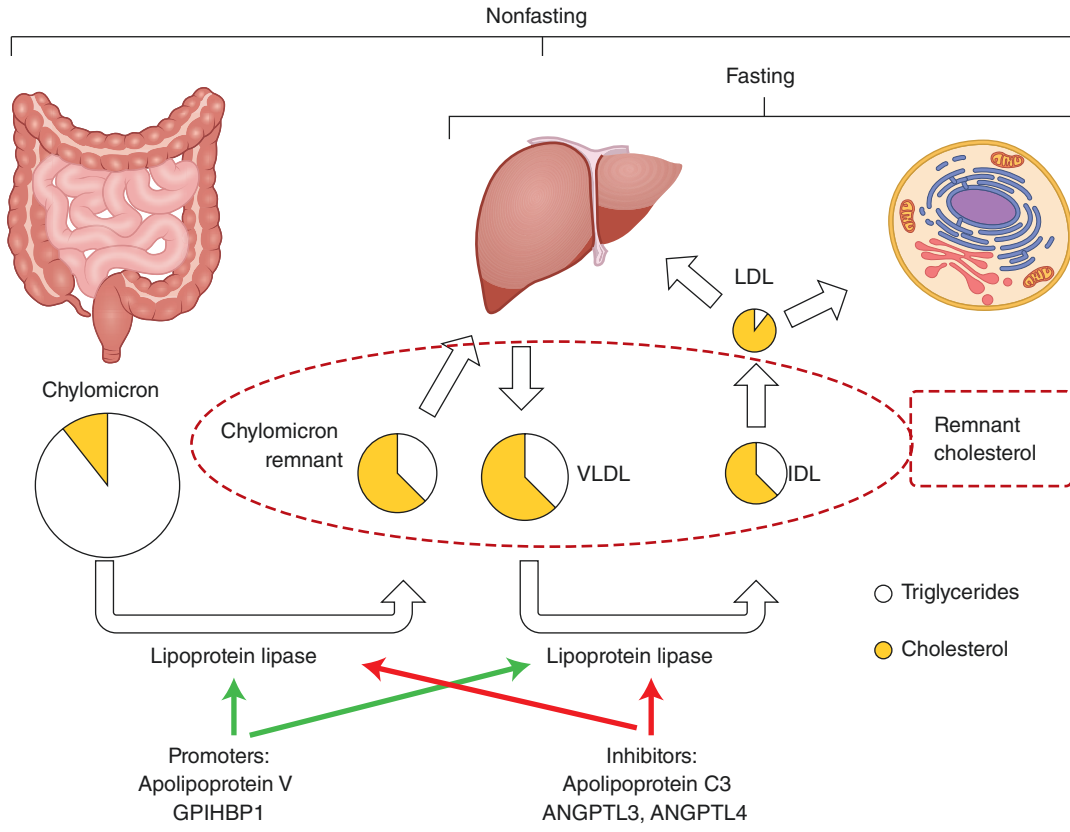

Fig. 8.2 Chylomicrons are secreted to the bloodstream from the intestine and very-low-density lipoprotein (VLDL) from the liver. IDL intermediate-density lipoprotein. LDL low-density lipoprotein. GHIHBP1 glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1. ANGPTL 3 & 4 angiopoietin-like 3 & 4. (Courtesy of Børge G. Nordestgaard, MD, and Anette Varbo, MD. Adapted.)

30–100 mg/dL (0.8–2.6 mmol/), while 0.2% of women and 0.7% of men have even higher levels.

Newborns typically have triglyceride levels of 20 mg/dL (0.3 mmol/L), while levels in children and adolescents typically are double those values (Fig. 8.4), unless the child is overweight or obese when triglyceride levels may be even higher. In men including those with overweight or obesity, levels of both triglycerides and remnant cholesterol increase from age 20 and onward, while a similar increase is only observed in women after age 40.

Elevated Levels in Obesity

The most common causes of elevated remnant cholesterol are overweight and obesity. Indeed, body mass index explains 12% of all variation in remnant cholesterol in individuals in the population at large (Fig. 8.5) [39]. At a body mass index (BMI) of 18.5 kg/m^2, remnant cholesterol levels are on average 20 mg/dL (0.5 mmol/L). At higher and higher BMI values, remnant cholesterol increases until the BMI of 35 kg/m^2 to a value of 39 mg/dl (1 mmol/L) (Fig. 8.5). At even higher BMI values, remnant cholesterol does not appear to increase further in the average person.

In individuals in the Danish general population, the average remnant cholesterol levels were 15 mg/dL (0.4 mmol/L) in underweight individuals, 19 mg/dL (0.5 mmol/L) in normal weight individuals, 27 mg/dL (0.7 mmol/L) in overweight individuals, 32 mg/dL (0.8 mmol/L) in obese individuals, 39 mg/dL (1.0 mmol/L) in severe obese individuals, and 39 mg/dL (1.0 mmol/L) in extreme obese individuals

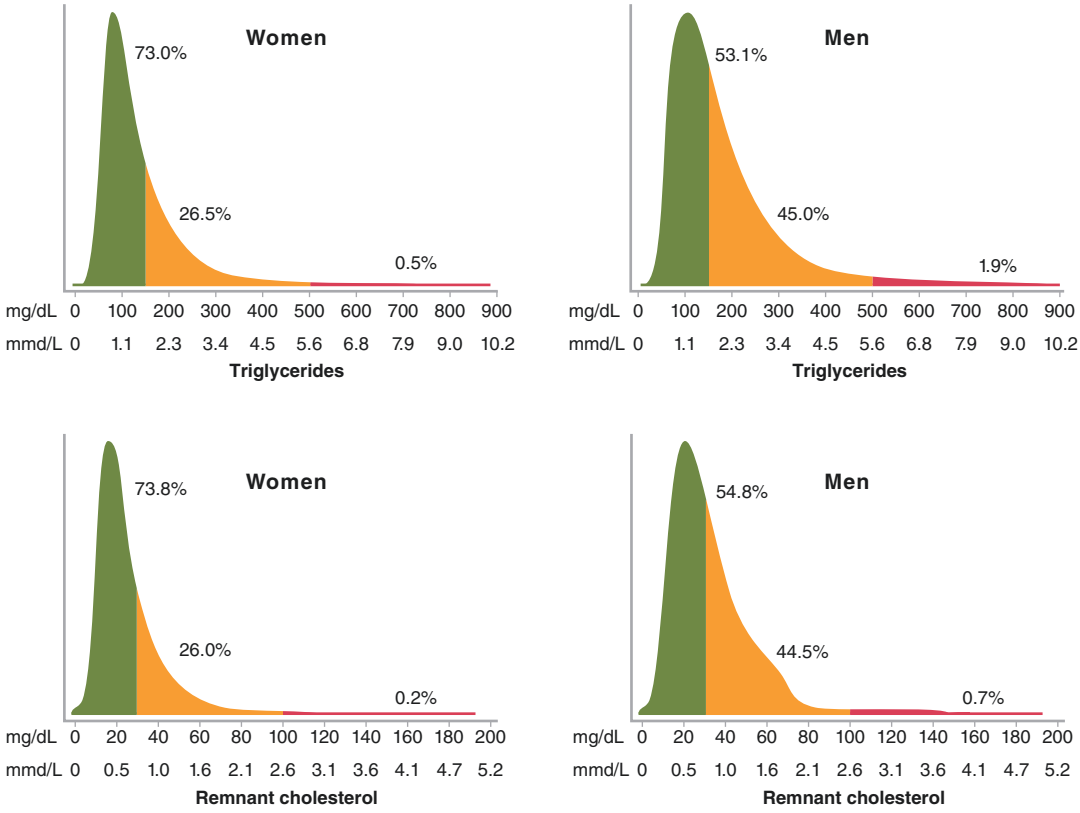

Fig. 8.3 Distributions of nonfasting triglycerides and calculated remnant cholesterol in 58,558 women and 47,811 men aged 20–100 from the Copenhagen General Population Study. Green indicates relatively low levels, orange moderately elevated levels, and red high levels. (Courtesy of Børge G. Nordestgaard, MD, and Anette Varbo, MD. Adapted.)

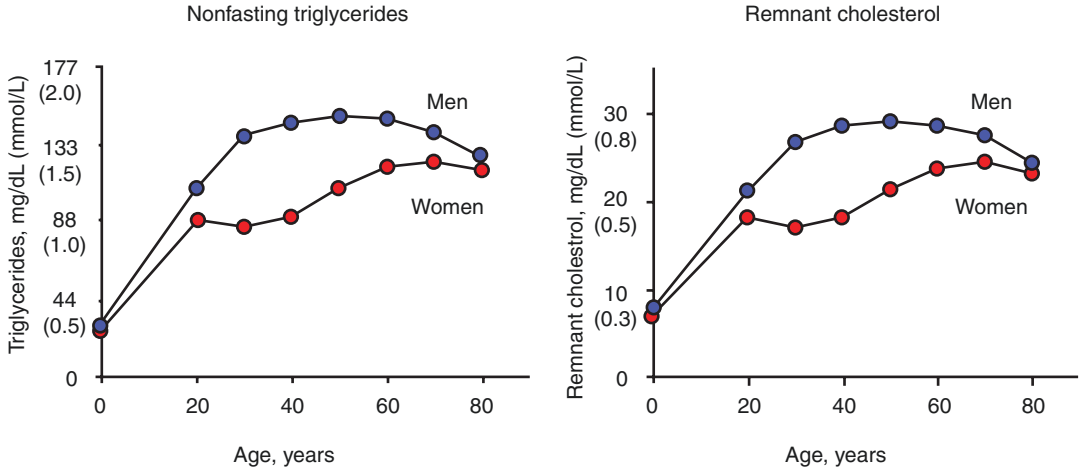

Fig. 8.4 Median levels of nonfasting triglycerides and calculated remnant cholesterol in men and women from the general population by age. Based on 58,558 women and 47,811 men from the Copenhagen General Population Study while levels in newborns are shown for comparison. (Courtesy of Børge G. Nordestgaard, MD, and Anette Varbo, MD. Adapted.)

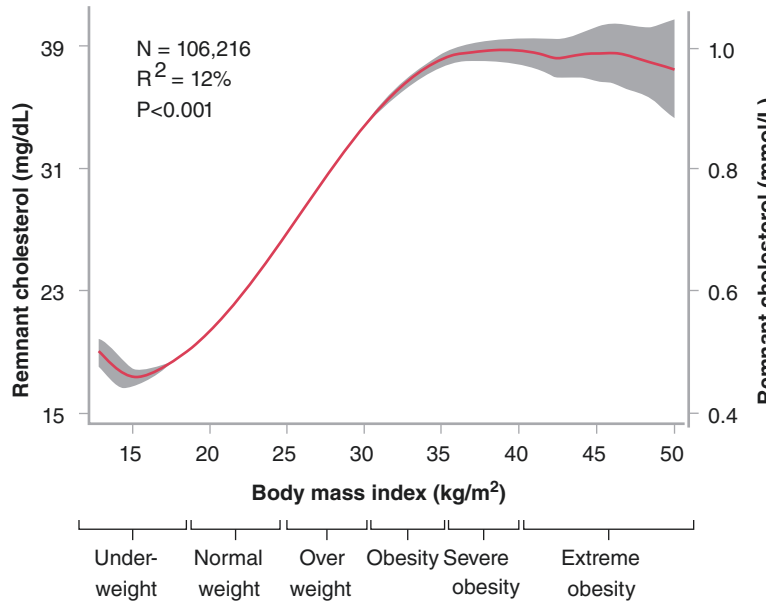

Fig. 8.5 Association between remnant cholesterol and body mass index in individuals from the general population. (Adapted from Varbo et al. [39].}

Remnant-Cholesterol median(IQR)

mg/dL	15(10)	19(13)	27(20)	32(23)	34(23)	34(22)
mmol/L	0.4(0.2)	0.5(0.3)	0.7(0.5)	0.8(0.6)	0.9(0.6)	0.9(0.6)

(Fig. 8.5). Corresponding values were 30 mg/dL (0.8 mmol/L) and 24 mg/dL (0.6 mmol/L) in those with and without diabetes mellitus, partly explained by the higher BMI in those with versus without diabetes.

Other factors like genetics, fat intake, carbohydrate intake, and exercise naturally will also influence remnant cholesterol and triglyceride levels. Thus, within each BMI category, there will be a remnant cholesterol distribution like that shown in Fig. 8.3, such that some individuals have lower and some individuals have higher levels than the average person [39]. That said, it appears that BMI is the strongest determinant of remnant cholesterol levels. To put it simple, when energy intake in the form of fat, carbohydrates, and alcohol far surpasses that used for basal metabolism and exercise, triglycerides are deposited in fat and liver tissue which is balanced with higher and higher levels of triglyceride-rich lipoproteins and thus remnant cholesterol in plasma.

Atherosclerotic Cardiovascular Disease

Like LDL particles, remnants/triglyceride-rich lipoproteins can penetrate from blood into the arterial intima [49–51], driven mainly by the blood pressure gradient from the arterial lumen through the entire arterial wall ending with no pressure in the adventitia. Entrance into the arterial intima also depends on lipoprotein size: the larger the lipoprotein, the slower the penetration [50, 36]. However, only the very large chylomicrons with diameters above 70 nm will not be able to inter the intima [52, 53], that is, the dominant lipoprotein type when plasma triglycerides are above 4000–5000 mg/dL (45–57 mmol/L). Therefore, at triglyceride levels of 150–4000 mg/dL (1.7–45 mmol/L), most if not all lipoproteins are small enough to enter the intima.

Because remnants/triglyceride-rich lipoproteins are slightly larger than LDL, these lipoproteins will more easily be trapped within the

intima compared with LDL [54–56]. Neither remnants nor LDL can penetrate the elastic laminas in the media and therefore can only leave the arterial intima through the endothelial layer, that is, against a blood pressure gradient.

Upon entrapment in the arterial intima, remnants are taken up directly without modification by macrophages [57, 51] (Fig. 8.6, bottom part). This leads to triglyceride hydrolysis and degradation of the protein part of the lipoproteins. However, as cholesterol cannot be degraded by human cells, cholesterol will accumulate within macrophages to produce the hallmark cell of the atherosclerotic plaque, the cholesterol-filled foam cell [58]. These pathological processes are like those for LDL, except that LDL needs to be modified before such particles are taken up by macrophages in the intima [59].

Unlike for LDL, however, triglyceride-rich lipoproteins in the intima will also lead to triglyceride hydrolysis locally, liberating tissue-toxic free fatty acids that will cause local inflammation [60–64](Fig. 8.6, top part). This process likely is like how triglyceride hydrolysis of chylomicron in the vicinity of the pancreas can lead to acute pancreatitis involving severe local inflammation [65, 66]. Therefore, the combination of intimal foam cells, atherosclerosis, and inflammation likely is the cocktail that explains the high risk of myocardial infarction and ischemic stroke observed in individuals with elevated remnant cholesterol/triglyceride-rich lipoproteins.

The higher the triglycerides, the higher the risk of acute pancreatitis, starting already at triglyceride levels above 150 mg/dL (1.7 mmol/L) [67, 68] (Fig. 8.7). Risk of myocardial infarction, ischemic stroke, other atherosclerotic cardiovascular diseases, and all-cause mortality increase already at triglyceride levels above 88 mg/dL (1 mmol/L) [18, 20, 69]; however, at some not well-defined high levels of triglycerides, perhaps around 2000–3000 mg/dl (23–34 mmol/L), most lipoproteins become too large to enter the intima, and thus, development of atherosclerosis and risk of atherosclerotic cardiovascular disease decrease with even higher triglyceride levels (Fig. 8.7).

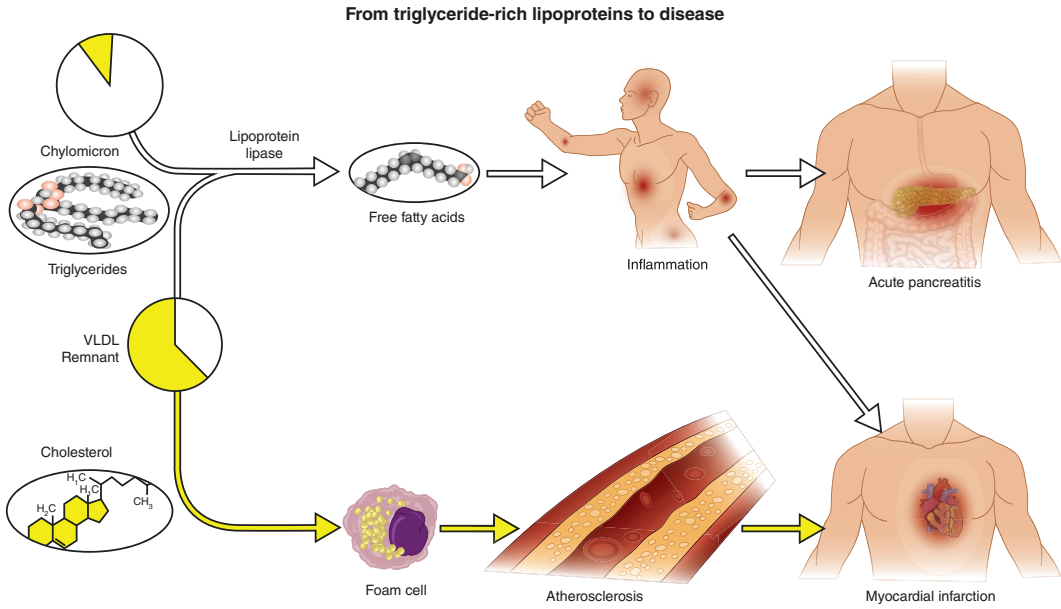

Fig. 8.6 Proposed pathway by which remnants and their cholesterol and triglyceride content cause inflammation, acute pancreatitis, atherosclerosis, and myocardial infarction. VLDL very-low-density lipoprotein. (Courtesy of Børge G. Nordestgaard, MD, and Anette Varbo, MD. Adapted.)

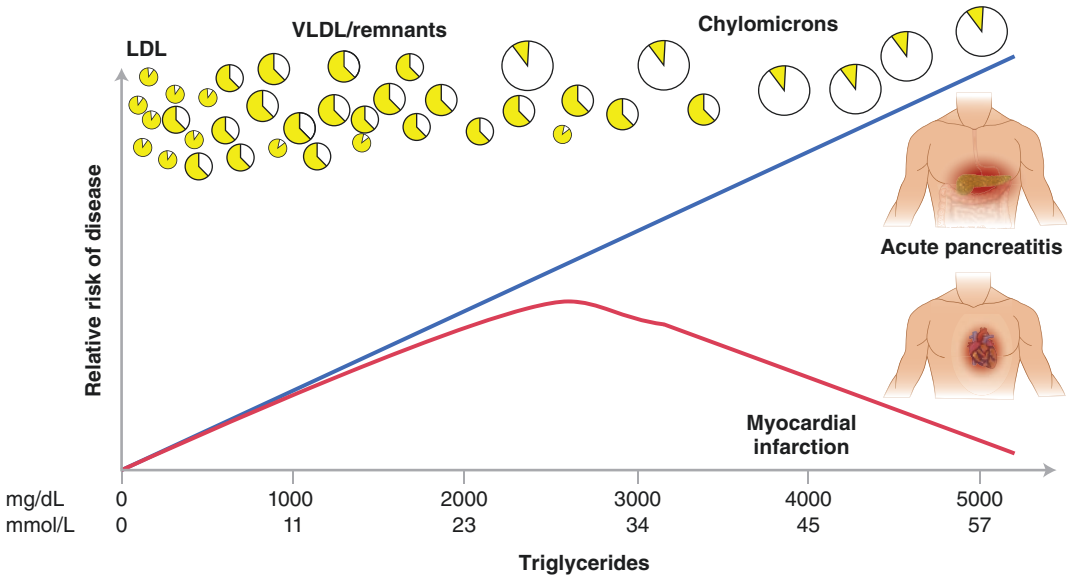

Fig. 8.7 Relative risk of acute pancreatitis and myocardial infarction as a function of triglyceride levels and size of lipoproteins. LDL low-density lipoprotein. VLDL very-low-density lipoprotein. (Courtesy of Børge G. Nordestgaard, MD, and Anette Varbo, MD. Adapted.)

Observational Studies

There is extensive evidence from observational epidemiology showing associations between high levels of triglycerides and increased cardiovascular disease risk [69]. For example, in 2007 to 2008, publications from the Women's Health Study with ~26,500 women [19, 70] and from the Copenhagen City Heart Study [18, 20] with 7587 women and 6394 men found increased risk of cardiovascular disease in individuals with high nonfasting triglyceride levels. Also, combined evidence from ~96,000 individuals from the two prospective cohort studies, the Copenhagen City Heart Study and the Copenhagen General Population Study, found five times higher risk of myocardial infarction, three times higher risk of ischemic stroke and ischemic heart disease, and 2.5 times higher risk of all-cause mortality in individuals with nonfasting triglycerides above 5 mmol/L (440 mg/dL), when compared to individuals with nonfasting triglycerides below 1 mmol/L (89 mg/dL) [69] (Fig. 8.8).

The emerging risk factors collaboration [71] including 302,430 individuals from 68 studies found similar hazard ratios; however, like many other observational lipid studies, they did not look at individuals with extremely high triglyceride levels separately, and thereby, they may have missed the very high risk in individuals with the very highest levels, that is, the individuals most needing intervention to lower triglycerides and remnant cholesterol. In that study, the authors found that the risk of coronary heart disease and ischemic stroke for high triglycerides was not significant after adjustment for HDL cholesterol and non-HDL cholesterol and concluded from this that triglycerides can be disregarded for risk assessment in vascular disease. The problem with this conclusion is, however, that it is only expected that hazard ratios for atherosclerotic cardiovascular disease at high triglycerides will diminish when adjusted for HDL cholesterol and non-HDL cholesterol, for two reasons. First, triglyceride and HDL cholesterol levels are inversely correlated because of the lipoprotein metabolism where triglycerides and cholesterol esters are exchanged between remnants and HDL through the action cholesteryl ester transfer protein (CETP); thus, low HDL cholesterol can be viewed as a monitor of long-term average increased triglycerides just

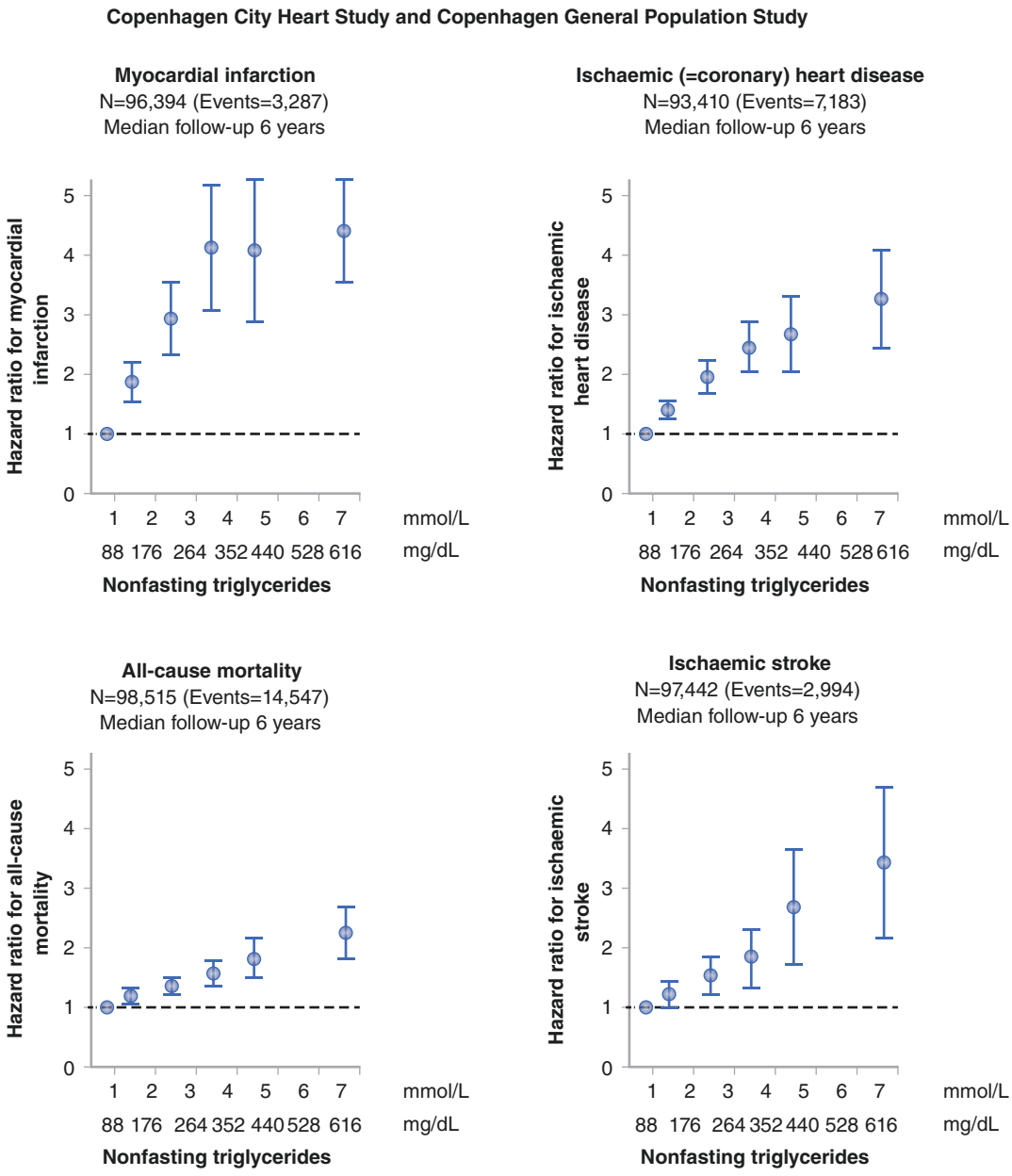

Fig. 8.8 Observational association between nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, all-cause mortality, and ischemic stroke in individuals from the Copenhagen City Heart Study and the Copenhagen General Population Study combined. Hazard ratios were estimated by Cox proportional hazard regression models, adjusted for age (timescale), sex, and trial group. (Adapted from Nordestgaard and Varbo [69].)

as high hemoglobin A1c is a monitor of long-term average increased glucose levels [17]. Second, non-HDL cholesterol is remnant cholesterol plus LDL cholesterol where the former is highly correlated with triglyceride levels. Hazard ratios for atherosclerotic cardiovascular disease therefore naturally diminish after such overadjustment, but this does not mean that the cocktail of remnant cholesterol and triglycerides is not atherogenic at elevated levels.

For elevated remnant cholesterol on the risk of atherosclerotic cardiovascular disease, there is also extensive evidence from observational studies, and more is emerging continuously. Evidence should however be interpreted similarly for elevated triglycerides and elevated remnant cholesterol as they both are constituents of the same triglyceride- and cholesterol-rich remnant particles and as the two levels are highly correlated. Most observational evidence for remnant cholesterol, the way that we define it as the cholesterol content of all lipoproteins that are not HDL or LDL, is from the Copenhagen City Heart Study and the Copenhagen General population Study [21–23, 72–74, 39, 75]; however, international interest is emerging, and other research groups have recently reported on the increased risk of atherosclerosis with inflammation and atherosclerotic plaque progression for high levels of remnant cholesterol [76, 77].

Causal, Genetic Studies

In order to avoid confounding and reverse causation that biases observational epidemiology, genetics can be used to further explore whether a risk factor is merely associated with disease risk or is actually a causal factor, that is, causes the disease directly [78, 79]. For triglycerides and remnant cholesterol, several studies have found associations between common genetic variants giving lifelong higher levels and risk of atherosclerotic cardiovascular disease. One of the first to examine the causality of remnant cholesterol on the risk of ischemic heart disease was our own study in 2013, where we found that a 1 mmol/L (39 mg/dL) genetically higher remnant cholesterol was associated with a 2.8-fold higher risk of ischemic heart disease [21]. That finding was supported by evidence from a large meta-analysis from the same year of genome-wide association studies (GWAS) using the Metabochip [24]. Several other genetic studies have come to essentially the same conclusion: elevated triglyceride-rich lipoproteins or remnant cholesterol are causal risk factors for atherosclerotic cardiovascular disease and even all-cause mortality, independent of LDL and HDL cholesterol levels [22, 23, 32, 29].

In individuals in the general population, the risk of myocardial infarction for a genetically caused 1 mmol/L (39 mg/dL) higher level of remnant cholesterol is similar in effect size to the other causal types of lipoproteins carrying cholesterol, that is, LDL and lipoprotein(a) [80] (Fig. 8.9). For the corresponding observational analyses with much narrower 95% confidence interval, a 1 mmol/L (39 mg/dL) higher level had higher risk estimates for remnant cholesterol compared to for LDL cholesterol.

Several studies have examined associations between rare mutations in key steps of the lipoprotein metabolism causing lifelong higher or lower triglycerides (and remnant cholesterol) and risk of atherosclerotic cardiovascular disease. First, heterozygosity for loss-of-function mutations in lipoprotein lipase, the key enzyme degrading triglycerides in plasma (Fig.8.2), leads to lifelong increased triglycerides and increased risk of atherosclerotic cardiovascular disease [81, 31]. Second, two important papers on loss-of-function mutations in APOC3 leading to higher lipoprotein activity were published in 2014 [25, 26]. Both came to the same conclusion that mutations giving lifelong lower triglycerides through APOC3 mutations were associated with lower atherosclerotic cardiovascular disease risk. Similar results have been found for loss-of-function mutations in ANGPTL3 and ANGPTL4 that, like apoC3, both inhibit lipoprotein lipase function (Fig.8.2). Mutations in these genes likewise lead to lifelong lower triglycerides and lower risk of atherosclerotic cardiovascular disease [82, 28, 30, 27].

Randomized, Controlled Trials

Evidence showing that reduction of triglycerides (and thus remnant cholesterol) in those with elevated levels leads to reduced cardiovascular disease is emerging. Most fibrate- and statin trials pretending to examine whether triglyceride reduc-

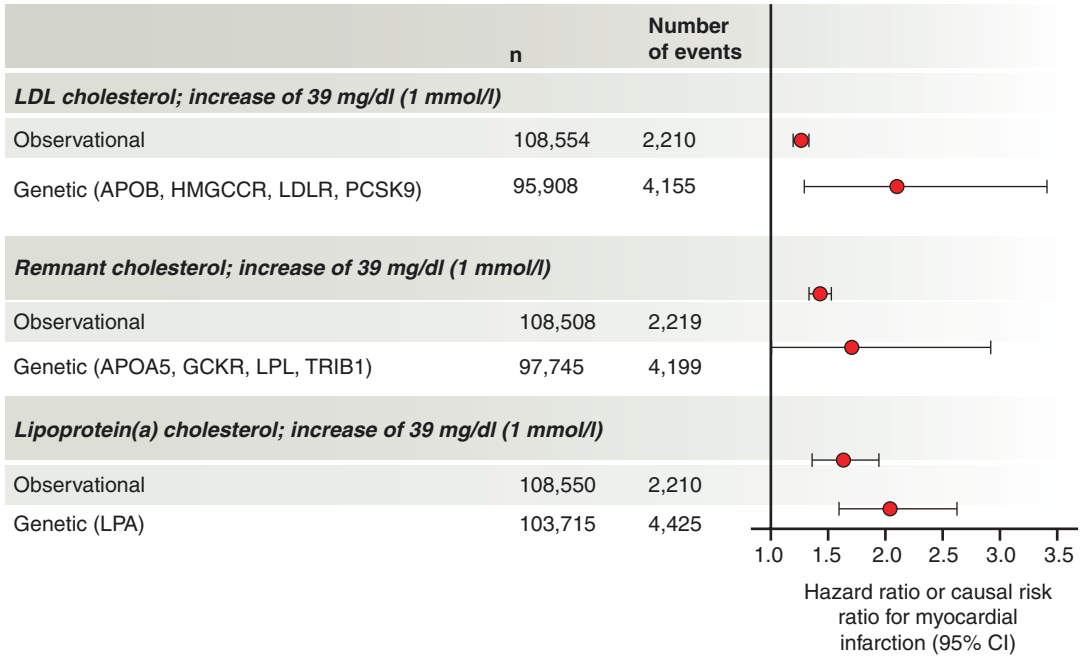

Fig. 8.9 Observational and genetic associations for 1 mmol/L (39 mg/dL) increase in LDL cholesterol, remnant cholesterol, and lipoprotein(a) cholesterol with the risk of myocardial infarction in the Copenhagen General Population Study. (Adapted from Nordestgaard et al. [80].)

tion leads to lower atherosclerotic cardiovascular disease did however not include participants based on triglyceride levels, and most participants in those studies therefore had relatively low levels. This led to disappointing results showing that triglyceride reduction did not appear to lower cardiovascular risk, and to the misperception that triglyceride reduction was without cardiovascular benefit. However, post hoc analyses of fibrate trials including only individuals with high triglycerides at baseline found reduction in atherosclerotic cardiovascular disease risk in a dose-dependent manner, so that for higher triglyceride reduction, the atherosclerotic cardiovascular disease risk reduction was also higher [69, 83–88] (Fig. 8.10).

Recent evidence from the REDUCE-IT trial also showed the cardiovascular benefit of triglyceride-lowering in individuals with elevated levels [33]. Bhatt et al. found a 25% (95% confidence interval 17–32%; P-value = 0.00000001) lower risk of a composite atherosclerotic cardiovascular disease endpoint for the group receiving 4 g/day of icosapent ethyl (omega-3 free fatty acids with 100% EPA), compared to placebo. With a 5-year event rate of 23.0% in the icosapent ethyl arm and 28.3% in the placebo arm, the 25% relative risk reduction translates into a 4.8% absolute risk reduction and a number needed to treat for 5 years to prevent one event of only 21 (95% confidence interval 15–33). Individuals included in that study had established cardiovascular disease, had diabetes, or were at high risk because of other risk factors. EPA was given on top of statins and on average reduced triglyceride levels by 20% and C-reactive protein by 40%, compared to placebo. Interestingly, the largest benefit was observed in those with the highest triglycerides at study entry [89].

Two further triglyceride-lowering trials in high-risk, statin-treated patients are ongoing, the STRENGTH [34] and PROMINENT [35] trials, and results from these will be very interesting to see in light of the positive findings from the REDUCE-IT trial. STRENGTH will examine whether Epanova 4 g daily (omega-3 free fatty acids with 75% EPA

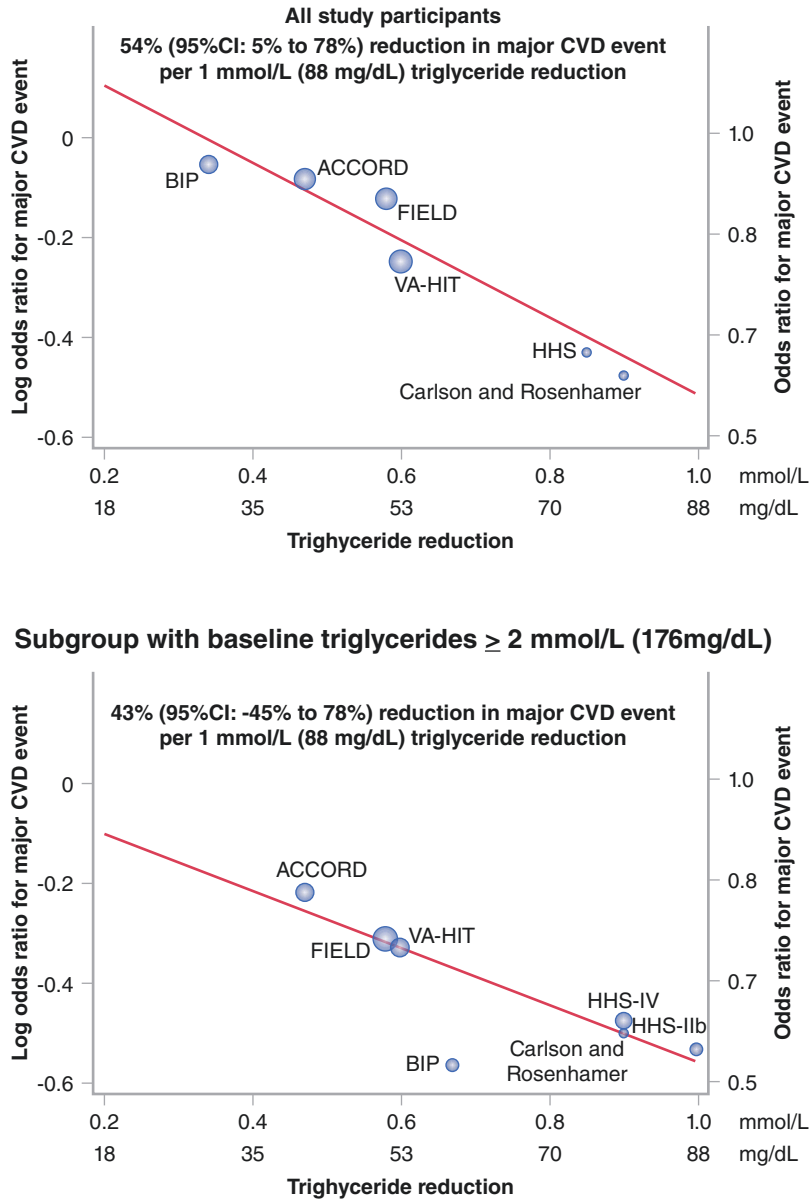

Fig. 8.10 Meta-regression estimating association between the extent of triglyceride lowering and reduction in risk of major cardiovascular events in large controlled trials with fibrates. CVD, cardiovascular disease. Carlson and Rosenhamer [83]. HHS-IIb Helsinki Heart Study subgroup of participants with Fredrickson's type IIb hyperlipidemia[84]. HHS-IV Helsinki Heart Study subgroup of participants with Fredrickson's type IV hyperlipidemia [84]. VA-HIT The Veterans Affairs High-Density Lipoprotein Intervention Trial [85]. BIP Bezafibrate Infarction Prevention study [86]. FIELD Fenofibrate Intervention and Event Lowering in Diabetes trial [87]. ACCORD Action to Control Cardiovascular Risk in Diabetes Lipid Trial [88]. (Adapted from Nordestgaard and Varbo [69].)

and 25% DHA) reduces the rate of cardiovascular events in high-risk, statin-treated patients with hypertriglyceridemia and low levels of HDL-C. PROMINENT will examine whether the selective peroxisome proliferator-activated receptor alpha modulator (SPPARM-α), pemafibrate at 0.2 mg twice daily, will reduce the rate of atherosclerotic cardiovascular disease events in high-risk, statin-treated patients with diabetes, hypertriglyceridemia, and low levels of HDL-C.

Residual Risk After Statin Therapy

Before the REDUCE-IT trial [89], many other studies of patients on statins have documented that part of residual atherosclerotic cardiovascular disease and all-cause mortality risk is due to elevated triglyceride-rich lipoproteins and remnant cholesterol [90–94, 74, 95–97]. Some studies examined triglycerides, while others examined remnant cholesterol (triglyceride-rich lipoprotein cholesterol)

or very-low-density lipoproteins as explanations for residual risk. With these differences in study design, all studies however came to the same conclusion: triglyceride-rich remnants explain cardiovascular morbidity and mortality residual risk beyond statin therapy, independent of LDL and HDL cholesterol levels.

Lack of Knowledge

Most importantly, we await the publication of results from the two ongoing randomized controlled trials of reduction in triglyceride-rich lipoproteins in statin-treated, high-risk patients, the STRENGTH [34] and PROMINENT [35] trials. Likewise important is the insight into whether the 25% relative reduction in cardiovascular endpoints in the REDUCE-IT trial [89, 33] can be explained mainly due to the reduction in triglyceride-rich lipoprotein coupled with the corresponding reduction in low-grade inflammation, the key to understanding these important findings; as elevated triglycerides and remnant cholesterol (unlike LDL cholesterol) are causally related to increased low-grade inflammation (measured as elevated plasma high-sensitivity C-reactive protein) [22, 68, 98], the reduction in C-reactive protein observed in the REDUCE-IT trial may be part of explaining the reduced risk observed.

Also, it would be valuable to better understand whether it is the cholesterol and/or triglyceride content of triglyceride-rich remnants that explains the development of atherosclerosis, and even more important the transition from an atherosclerotic plaque into clinical manifest atherosclerotic disease including myocardial infarction and ischemic stroke. Further, a better understanding of the cardiovascular risk in the relatively few individuals with very high triglycerides and remnant cholesterol is urgently needed. Finally, yet more randomized controlled trials of lowering of triglyceride-ride rich lipoproteins are needed, in individuals with triglyceride of 150–500 mg/dL (1.7–5.7 mmol/L) as well as in those with even higher levels.

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9 Medical Nutrition Therapy for Lipid and Lipoprotein Disorders

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By the end of this chapter, the reader will:

- Understand the role of medical nutrition therapy as a cornerstone in the treatment of dyslipidemia
- Recognize evidence-based recommendations for the dietary management of the following:
  - High LDL-C and non-HDL-C
  - High triglycerides
  - Low HDL-C
- Understand the benefits of referring patients to a registered dietitian nutritionist (RDN) for the management of dyslipidemia and the role that RDNs can play in the nutritional management of lipid and lipoprotein disorders
- Be aware of nutrition-related education resources for health care providers and patients for the management of lipid and lipoprotein disorders

Introduction

Management of dyslipidemia is a cornerstone of atherosclerotic cardiovascular disease (ASCVD) risk reduction [1]. The most prevalent lipid disorders are elevated apolipoprotein B (apo B)-containing particles, i.e., LDL-cholesterol (LDL-C), non-HDL-cholesterol (non-HDL-C), and triglyceride (TG)-carrying lipoproteins, as well as low HDL-cholesterol (HDL-C) [2]. Dietary intervention is recommended as first-line therapy in guideline-based treatment of dyslipidemias (e.g., elevated LDL-C and non-HDL-C, high TG, and low HDL-C) [1, 3]. For all lipid/lipoprotein disorders, treatment should be multifaceted and include a team-based care approach. This chapter summarizes evidence-based nutrition recommendations for the treatment and management of dyslipidemias that can be implemented in medical practice. For comprehensive medical nutrition therapy (MNT), patients should be referred to a registered dietitian nutritionist (RDN).

Medical Nutrition Therapy for High LDL-C (and Non-HDL-C)

LDL-C and non-HDL-C are primary treatment targets the management of ASCVD risk. Interventions that lower LDL-C will also lower non-HDL-C. LDL-C lowering of ~39 mg/dL is

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_9

associated with a 23% relative risk reduction for major vascular events [4]. For all patients with an elevated LDL-C, including those on pharmacologic therapy, a healthy lifestyle including a healthy diet should be the foundation for therapy [5]. Key dietary recommendations to lower LDL-C (and non-HDL-C) include the following :

1. *Reducing saturated fat (SFA) intake to < 7% of total energy* [6, 7] with a target of 5–6% of total energy recommended by AHA/ACC [3]. Lowering SFA intake to meet these targets is expected to lower LDL-C by 8–10% [8]. Currently, in the United States, the average consumption of SFA is 11%, and the predominant contributors to SFA intake are pizza, mixed fast-food chicken dishes, grain-based desserts, cheese, and red and processed meat [9, 10]. To achieve the SFA targets, it is recommended that dietary sources of SFA be replaced with food sources of mono- and polyunsaturated fats (MUFA and PUFA). Replacing 5% of energy from SFA with MUFA, PUFA, or whole grains will reduce LDL-C by 9.0, 6.5, and 6.0 mg/dL, respectively [3]. Choosing nonfat or low-fat dairy products (milk, cheese, and yogurt), liquid vegetable oils (nontropical), and spreads instead of butter and choosing lean cuts of meat and avoiding processed meat are recommended to reduce SFA intake. Furthermore, choosing nuts as a snack instead of processed snacks including potato chips and crackers may also result in reduction of SFA intake.
2. *Increasing intake of plant sterol/stanols or phytosterols to ~ 2 g/day* will lower LDL-C by ≈6–15% [7]. A 2014 meta-analysis of 124 clinical trials with over 9600 individuals reported LDL-C reductions of 6–12% with phytosterol intakes averaging 2.1 g/day [11]. The reduction was 6–12% when intake was 0.6–3.3 g/day with the greatest reductions at 3.3 g/day [11]. A recent meta-analysis of studies conducted from 2002–2016 reported a reduction in LDL-C of 12–15 mg/dL with plant sterol intake in the range of 1.5 to 3 g/day [12]. Plant sterol/stanol intake in the average American diet does not approach 1 g/day, with the average intake being 200–500 mg/day; the higher end of the range is consumed by vegans and vegetarians [13–15]. Therefore, the addition of phytosterol-fortified spreads and foods/beverages is necessary to achieve an LDL-C lowering of 6–12%.
3. *Increasing intake of viscous fiber to 5–10 g/day,* which is expected to lower LDL-C by 3–5% [7]. Two meta-analyses, including 94 randomized controlled trials, concluded that 5–10 g/day of viscous fiber, in the form of beta-glucan, psyllium, guar gum, and pectin, lowered LDL-C by 5.5–11.0 mg/dL [7, 16, 17]. A 2000 kcal/day diet should include 31 g of fiber based on the American Heart Association (AHA) eating pattern recommendations [18]. At least 5–10 g of the 31 g should come from viscous fiber for LDL-C lowering [7]. Viscous fiber is only found in plant foods, and consuming a diet rich in fruits, vegetables, nuts, legumes, and whole grains will help achieve the recommended daily viscous fiber intake. Rich sources of viscous fiber are beans, legumes, oats, broccoli, and sweet potatoes.
4. *Reducing dietary cholesterol to < 200 mg/day* [7]. Limiting dietary cholesterol to <200 mg/day is recommended by the National Lipid Association (NLA). A recent meta-regression analysis including 55 randomized, controlled dietary intervention studies found a dose-response relationship between dietary cholesterol and LDL-C, after controlling for intakes of SFA, MUFA, and PUFA. Vincent et al. [19] reported that for each 100 mg/d increase in dietary cholesterol, LDL-C would be expected to increase by 1.90 mg/dL to 4.58 mg/dL (depending on the model used) [19]. SFA more potently increases LDL-C than dietary cholesterol; however, many dietary sources of SFA are also sources of dietary cholesterol, and thus, recommendations to limit SFA will also lower dietary cholesterol. Meat (including poultry, mixed dishes, red meat, processed meat, shrimp, and other shellfish), eggs, baked goods, and full-fat dairy products are the major sources of dietary cholesterol in the US diet and should be limited to lower cholesterol intake [7].

5. *Weight loss of 3–5% of body weight* in individuals with overweight or obesity typically contributes to LDL-C lowering, with greater weight loss expected to result in additional LDL-C lowering [20]. In a systematic review of 13 studies that evaluated the effects of weight loss on lipids and lipoproteins, Poobalan et al. reported that weight loss of 10 kg lowered total cholesterol by 8.9 mg/dL (approximately 5%) and LDL-C by approximately 7.7 mg/dL [21]. The magnitude of LDL-C lowering with weight loss will depend on the SFA content of the weight loss diet, and therefore, to achieve maximum LDL-C lowering, a hypocaloric diet low in SFA is recommended.
6. *Referral to an RDN.* RDN-delivered MNT lowers LDL-C by 7–14% [22]. A Cochrane review of 12 randomized controlled trials concluded that greater reductions in total cholesterol were achieved with advice from dietitians compared with doctors (−9.7 mg/dL (95% CI −14.3, −4.6 mg/dL); LDL-C was only measured in one study included in this review. In addition, a systematic review and meta-analysis conducted by Sikand et al. reported that in a pooled analysis of 10 studies MNT, compared to control/usual care, decreased LDL-C by 10.3 mg/dL (95% CI, −13.9 to −6.7 mg/dL) [23]. Based on the totality of the evidence, the NLA includes a Grade A (strong) recommendation and cites moderate evidence to support nutrition counseling and follow-up from an RDN for the management of dyslipidemia [7].

In summary, decreasing SFA intake by replacing sources with unsaturated fats will confer clinically significant LDL-C lowering. Other recommendations to lower LDL-C include increasing intake of soluble viscous fiber, plant sterols, and stanols and lowering dietary cholesterol. Weight loss, if indicated, may decrease LDL-C further. Referral to an RDN is recommended for individualized comprehensive dietary management to help acheive optimal LDL-C reduction (Table 9.1).

Table 9.1 Summary of lifestyle recommendation to lower LDL-C [3, 7, 21, 22]

Dietary component	Dietary change	Estimated LDL reduction
Saturated fat	< 7% of calories (target 5–6% or lower) achieved by replacing sources of saturated fat with PUFA, MUFA, or whole grains	8–10% PUFA (replace 5% kcal): −9.0 mg/dL MUFA (replace 5% kcal): −6.5 mg/dL Whole grains (replace 5% kcal): −6.0 mg/dL
Viscous fiber	5–10 g/day	3–5%
Plant sterol/ stanols	2 g/day	6–15%
Dietary cholesterol	< 200 mg/day	3–5%
Weight reduction	3–5%	7.7 mg/dL[a]
RDN referral	Medical nutrition therapy	7–14%

[a]Estimated reduction varies based on total weight lost and composition of the diet (e.g., saturated fat content)

Medical Nutrition Therapy for Hypertriglyceridemia

Hypertriglyceridemia is a target for ASCVD risk reduction [24]. Elevated concentrations of TG-rich lipoproteins are causally related to CVD, as well as all-cause mortality [25, 26]. Dietary interventions for hypertriglyceridemia can lower TG by 20–50% [7]. Specific recommendations for the management of patients with elevated TG are as follows:

1. *Reduce alcohol intake (moderate to less intake if TG 150–500 mg/dL) or eliminate (TG > 500 mg/dL)* [1, 6]. TG are increased by 0.2 mg/dL per gram of alcohol consumed per day; consuming two standard drinks per day would be expected to increase TG by ~6 mg/dL [27]. Moderate consumption of alcohol is defined as ≤7 standard drinks/day for women

and ≤ 14 standard drinks/week for men (one standard drink is equivalent to 12 oz. beer, 5 oz. wine, and 1.5 oz. liquor) [28]. In individuals with obesity and in those with severely elevated TG, complete abstinence from alcohol will decrease the risk of pancreatitis [3].

2. *Reduce intake of refined grains (< 50% of total carbohydrates) and added sugar (<10% of kcal for TG 150–499; <5% of kcal for TG 500–999; near 0% of kcal for TG > 1000 mg/dL)* [6]**.** Lowering intake of refined grains and added sugars is expected to lower TG levels by ~10–25% [29–31]. Refined grains (such as white breads, pasta, rice, chips, crackers, processed breakfast cereals) should be replaced with whole grains (whole wheat breads, pastas, crackers, brown rice, oats) or with lean protein foods and unsaturated fatty acids. Furthermore, sources of added sugar should be limited. In the United States (US), added sugar contributes ~13% of kcal (range ~ 11–20%) [32]. The greatest contributors to added sugar intake in the US diet are sweetened beverages (soda, fruit drinks, tea products, sports drinks, energy drinks), sugar added to tea and coffee, sweet bakery products (cakes, cookies, pies), desserts (pudding and ice cream), and candy [33].
3. *Increase consumption of omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) (TG 150–199 mg/dL: 0.25–1 g/day; TG 200–499 mg/dL: 1–2 g/day; TG 500–999 mg/dL: 2–4 g/day; TG > 1000 mg/dL: 3–4 g/day),* which would be expected to lower TG by 3–45% depending on baseline TG levels [6]. Significant dietary sources of omega-3 fatty acids include salmon, mackerel, albacore tuna, trout, and sardines. The average American diet is low in EPA and DHA, with estimates showing that intake is less than 100 mg/day [34], which is much lower than current recommendations of 250 mg/day or about 8 oz. of fish (with emphasis on fatty fish) per week [35]. Fish oil is a rich source of EPA and DHA and is available as an over-the-counter omega-3 fatty acid supplement and as prescription capsule formulation, which may be necessary for patients who need higher doses. Prescription capsule formulations provide higher doses. A recent clinical trial showed that in high-CVD-risk patients with elevated triglycerides (135 to 499 mg/dL) on a statin drug, 2 g of icosapent ethyl (prescription EPA) twice daily for 4.9 years lowered TG by 14% and reduced the primary composite endpoint by 25% (cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization or unstable angina) [36]. This aligns with the results from the Japan EPA Lipid Intervention Study, which reported that in patients with hypercholesterolemia who were randomly assigned to receive either low-dose statin therapy plus 1.8 g of EPA ethyl ester daily or statin therapy alone, there was a 19% decrease in major coronary events in the group that received EPA plus statin therapy [37].
4. *Increase aerobic exercise with the goal of 150–300 minutes of moderate aerobic physical activity per week [38].* Aerobic exercise lowers TG by about 5% [31]; long-term lowering of TG from exercise is likely due to weight loss and a reduction in body fat [39].
5. *Weight loss of 5–10% of body weight* if individuals present with overweight or obesity [6]. Weight management remains a mainstay of TG treatment [24]. Weight loss of >8% of total body weight is expected to reduce TG by >20% [40]. See weight loss section for specific guidance on weight management.
6. *Referral to an RDN* [6]. In individuals with excessively high TG (over 1000 mg/dL), a very low-fat diet (10–15% of total energy from fat) is required along with a high dose of omega-3 fatty acids (either OTC or by prescription) and other pharmacotherapy to prevent acute pancreatitis [1]. Referral to an RDN is recommended for the management of TG >1000 mg/dL. An RDN has the requisite expertise to counsel patients on an very low-fat diet, which is challenging to follow without education and follow-up. Moreover, the systematic review and meta-analysis con-

Table 9.2 Summary of lifestyle recommendations to lower TG [7, 31, 40–42]

Dietary component	Dietary change	Estimated TG reduction
Alcohol	Reduction (TG 150–500 mg/dL) Elimination (TG >500 mg/dL)	20 mg/dL
Refined carbohydrates and added sugar	Refined carbohydrates <50% of total energy intake Added sugars **(<10% of kcal for TG 150–499, <5% of kcal for TG 500–999, near 0% of kcal for TG > 1000 mg/dL)**	10–25%
Omega-3 fatty acids	**0.25–1 g/day (TG 150–199 mg/dL)** **1–2 g/day (TG 200–499 mg/dL)** **2-4 g/day (TG 500–999 mg/dL)** **3–4 g/day (TG > 1000 mg/dL)**	3–45%
Exercise	150–300 min/wk	5%
Weight reduction	> 8% Reduction in body weight	>20%
RDN referral	Medical nutrition therapy (multiple visits over 6–12 weeks)	11–31%

ducted by Sikand et al. [23] reported that in a pooled analysis of 10 studies MNT, versus control/usual care decreased TG levels 15.9 mg/dl compared to the control/usual care group.

In summary, reducing or eliminating alcohol intake and reducing intake of refined grains and added sugars will elicit clinically significant reductions in TG. Other treatment strategies to lower TG include increasing intake of omega-3 fatty acids and increasing aerobic exercise. Weight loss, if indicated in combination with the recommended dietary changes, will decrease TG further. Referral to an RDN is recommended for individualized comprehensive dietary management to achieve a greater TG reduction (Table 9.2).

Medical Nutrition Therapy for Mixed Dyslipidemia

Lifestyle recommendations for individuals with mixed dyslipidemia (i.e., elevated LDL-C/non-HDL-C, and TG) are consistent with the recommendations for lowering LDL-C/non-HDL-C and TG; these patients will benefit from the specific lifestyle changes for each of these lipid/lipoprotein disorders.

Low HDL-C levels are typically associated with increased levels of triglyceride-rich lipoproteins [43]. Interventions to increase HDL-C should be patient-specific and require the assessment of the complete lipid profile and overall health. Some dietary interventions aimed at reducing LDL-C, and overall ASCVD risk could reduce HDL-C, but physical activity can attenuate HDL-C reductions and even raise HDL-C [44, 45]. Physical activity is especially important for patients with low HDL-C. The Second Edition of the Physical Activity Guidelines for Americans (2018) provides science-based guidance to help individuals improve their health by participating in regular physical activity (see Table 9.3 for the physical activity recommendations for adults). Although physical activity may not improve HDL-C in all individuals, it still confers many other health benefits.

Diet Therapy for Rare Lipid Disorders

Dietary intervention, along with pharmacotherapy, is necessary for patients with rare lipid disorders [46]. Familial hypercholesterolemia (FH)

Table 9.3 Physical activity recommendations for adults [38]

Physical activity Guidelines for Americans	150–300 minutes of moderate-intensity aerobic activity weekly *Or* 75–150 minutes of vigorous-intensity aerobic activity weekly *And* muscle-strengthening exercises ≥2 days/week

affects nearly one million people in the United States and increases the risk of coronary heart disease by fivefold [47]. The International FH Foundation recommends that all adults with FH receive advice on lifestyle modification [42]; this recommendation is endorsed by the NLA [6]. The dietary recommendations for FH are consistent with those outlined in the LDL-C management section. Patients with FH must work with a lipid specialist for the management of FH [48].

Familial chylomicronemia syndrome affects approximately 1 in 1–2 million people and increases the risk of developing acute and chronic pancreatitis without medication and diet therapy [49]. In these patients, TG levels are usually >750 mg/dL but may be in the thousands. A very low-fat diet is recommended (< 15 to 20 g of fat per day or < 10 to <15% of calories); medium-chain TG oil may be used as a dietary fat and calorie source because it does not increase chylomicron production [6, 50]. Abstinence from alcohol is also recommended. The recommendations outlined in the TG management section are appropriate for these patients. Patients should work with a lipid specialist and RDN to assure that essential fatty acid needs are met (i.e., 2–4% of daily calorie intake of alpha-linolenic acid and linoleic acid) [50].

The Integral Role of Weight Management in ASCVD Risk Reduction

In patients with overweight or obesity, weight loss is indicated for ASCVD risk reduction and may assist with the management of dyslipidemias. The ABCDEF framework provides a guide for approaching weight management with patients (Table 9.4) [51]. In addition, dietary recommendations for patient counseling follow.

1. *Adjust energy intake to avoid weight gain or, in patients with overweight or obesity, to promote weight loss.* A 500 kcal/day deficit is expected to result in weight loss of 1 lb./wk. Current evidence does not define one diet or a "best diet" for weight loss [20, 52]. Thus, to increase patient motivation, a strategy that aligns with patients' interests, values, and preferences and is reasonably appealing and convenient for them is recommended [51]. With a prescribed energy deficit, a variety of

Table 9.4: ABCDEF framework [51] steps

Implementation		
A	Ask permission	Determine patient's readiness to discuss weight status
B	Be systematic in the clinical examination	Inquire about weight history; determine physiological, pharmacological, and behavioral factors known to affect weight status; consider social determinants and barriers
C	Counseling	Provide recommendations consistent with current weight-loss guidelines (AHA/ACC/TOS) Emphasize healthy dietary patterns for weight management Recommend reputable, useful online tools and resources (i.e., DGA, diabetes prevention program) Discuss current physical activity level and set small goals
D	Determine current health status	Evaluate the patient for the presence of weight-related comorbidities; consider the quality of life and physical abilities
E	Escalate treatment when indicated	Discuss medical/surgical interventions if weight-related comorbidities are present: Pharmaceutical therapy (BMI ≥ 27) Bariatric surgery (BMI ≥ 35)
F	Follow up frequently	Check on patient's progress at future appointments; offer continued support Use a team-based approach (RDN, health coaches, physicians, etc.) Recommend community or commercial weight loss programs that may be accessible to the patient

Adapted from Kahan and Manson [51]

approaches can result in weight loss, including a Mediterranean-style diet, macronutrient-targeted diets (carbohydrate, fat, protein), and vegetarian or vegan diets [20]. Weight loss of as little as 3–5% of body weight can result in clinically meaningful improvements in health, and this can be motivating to patients [20].

While improvements in lipids and lipoproteins can result from weight loss on restrictive diets, it is imperative that patients do not sacrifice a healthy dietary pattern. These restrictive diets may produce rapid weight loss, but research has shown it is typically not maintained and may be nutritionally incomplete [53, 54]. Individuals who successfully maintain long term weight loss report being more physically active, have a lower calorie intake, and have increased dietary restraint (conscious effort to decrease/reduce caloric intake) compared to those who regain weight [55]. Patients with significant weight to lose should see an RDN for long-term counseling and may be referred for medical or surgical management of weight.

2. *Increase physical activity with the goal of ≥150 minutes/week of aerobic activity.* Higher levels of physical activity ~200–300 minutes/week are recommended to maintain weight loss and prevent weight regain [20]. Moving more and sitting less are the key recommendations from the current Physical Activity Guidelines [38]. In the current Physical Activity Guidelines, the requirement for exercise to occur in bouts of at least 10 minutes has been removed; therefore, any exercise counts toward daily goals. Step counters (pedometers) and other wearable activity monitors, combined with other behavioral strategies, may increase physical activity by providing feedback to the user. Discussing daily step goals with patients may assist in increasing physical activity [38, 56].
3. *Use of technology for self-monitoring.* Patients may benefit from keeping a food log or using a mobile app to keep track of their intake, activity, and goals. Use of apps is associated with greater weight loss and more physical activity in some studies [57, 58]. Electronically delivered interventions that include tracking are associated with increased weight loss beyond usual care or simple educational interventions, and this approach is recommended by the 2013AHA/ACC/TOS Guideline for the Management of Overweight and Obesity in Adults [20].
4. *Referral to an RDN or behavioral weight loss intervention*. In the AHA/ACC/TOS Guideline, Grade A (strong) Class 1 evidence is cited for referral to a comprehensive lifestyle program that assists patients in reducing caloric intake and increasing physical activity through behavioral strategies for sustained weight loss. This may include onsite, high-intensity (i.e., ≥14 sessions in 6 months) individual or group sessions. This type of program would be expected to lower body weight by 5–10%.

In summary, weight management interventions necessitate a team-based approach that includes dietary changes, physical activity, and behavior therapy. The ABCDEF framework may be used in medical practice to approach weight management with patients. There is no single dietary recommendation for weight loss; however, the approach used must include a caloric deficit to elicit sustained weight loss. The use of technology to self-monitor diet and physical activity may assist patients to achieve meaningful weight loss.

Dietary Patterns for Prevention of CVD

The evidence-based dietary and physical activity recommendations for modifying lipids and lipoproteins discussed herein should be implemented in the context of a healthy dietary pattern, a core foundation of ASCVD risk reduction. While there are many healthy dietary patterns, they all share common principles and emphasize the intake of vegetables, fruits, legumes, nuts, whole grains, vegetable or lean animal protein, and fish/seafood. Furthermore, it is recommended that the intake of trans fats, sodium, processed meats, added sugars, and sweetened

beverages be limited [3, 7]. Small improvements in diet with the goal of greater adherence to a healthy dietary pattern may reduce ASCVD risk by >10% [59–61].

Since there are a number of healthy dietary patterns for ASCVD prevention and management, patient's personal and dietary preferences should be taken into consideration when choosing a healthy dietary pattern. The USDA 2015 Dietary Guidelines for Americans (DGA) recommends three healthy eating patterns for overall population health [70]; however, these are also suitable for ASCVD prevention and management with minor adjustments for additional saturated fat and sodium lowering (see Table 9.5) [3]. The DASH dietary pattern is similar to the USDA patterns and is recommended for the prevention *and* treatment of ASCVD (see Table 9.5) [1, 3, 62]. These dietary patterns are consistently rated among the top diets by US News and World Report, which rates diets for safety, ease, and effectiveness [63].

Case Study: Using the ABCDs of Lifestyle Counseling

Mr. Young, a 35-year-old male, presents to your practice for a yearly physical and health screening. The patient is overweight (BMI: 28.5 kg/m^2) and reports feeling run-down. He is an investment banker and admits he has little time for physical activity or cooking/food preparation. You complete his history and physical and send him for bloodwork (CBC and Chem-24). When you receive the results, they show an abnormal lipid panel consistent with dyslipidemia (see Table 9.6).

Table 9.5 DASH diet and DGA healthy eating patterns at a 2000 kcal level [64, 65, 70]

Food group	Healthy Mediterranean-style eating pattern	Healthy vegetarian eating pattern	DGA healthy American diet	DASH diet
Vegetables	2 ½ c-eq/day	2 ½ c-eq/day	2 ½ c-eq/day	2–5 c/day
Dark green	1 ½ c-eq/week	1 ½ c-eq/week	1 ½ c-eq/week	–
Red and orange	5 ½ c-eq/week	5 ½ c-eq/week	5 ½ c-eq/week	–
Legumes (beans and peas)	1 ½ c-eq/week	3 c-eq/week	1 ½ c-eq/week	2–2 ½ c/week
Starchy	5 c-eq/week	5 c-eq/week	5 c-eq/week	–
Other	4 c-eq/week	4 c-eq/week	4 c-eq/week	–
Fruits	2 ½ c-eq/day	2 c-eq/day	2 c-eq/day	2–2 ½ c/day
Grains	6 oz-eq/day	6 ½ oz-eq/day	6 oz-eq/day	6–8 oz./day
Whole grains	≥ 3 oz-eq/day	≥ 3 ½ oz-eq/day	≥ 3 oz-eq/day	–
Refined grains	≤ 3 oz-eq/day	≤ 3 oz-eq/day	≤ 3 oz-eq/day	–
Dairy	2 c-eq/day	3 c-eq/day	3 c-eq/day	2–3 c/day
Protein foods	6 ½ oz-eq/day	3 ½ oz-eq/day	5 ½ oz-eq/day	≤ 6 oz./day
Seafood	15 oz-eq/week	Not applicable	8 oz-eq/week	–
Meat, poultry, eggs	26 oz-eq/week	3 oz-eq/wk. (eggs)	26 oz-eq/week	–
Nuts, seeds, soy	5 oz-eq/week	15 oz-eq/wk. (7 oz. nuts/seed, 8 oz. soy)	5 oz-eq/week	6–7 ½ oz./week
Oils	27 g/day	27 g/day	27 g/day	8–12 g/day
Limit calories from other uses (% of calories)	260 kcal/day (13%)	290 kcal/day (15%)	270 kcal/day (14%)	–
Saturated fat	<8% or 18 g/day[a]	<8% or 18 g/day[a]	<8% or 18 g/day[a]	<7% [b]
Added sugars	30 g/day	30 g/day	30 g/day	~35 g/day
Sodium	≤ 2300 mg/day	≤ 2300 mg/day	≤ 2300 mg/day	≤ 2300 mg/day

[a]Saturated fat estimate derived from ACC/AHA recommendations [3]
[b]Saturated fat estimate derived from DASH feeding study [66]

Table 9.6 Mr. Young's lipid profile

Lipid/lipoprotein	Value
Total cholesterol	270 mg/dL
LDL-cholesterol	195 mg/dL
Triglycerides	185 mg/dL
HDL-cholesterol	38 mg/dL

Assessment of readiness to change. Mr. Young is scheduled for a follow-up visit. You ask questions about his habitual diet and lifestyle to assess his perception of his lifestyle and motivation to change. He says most of his meals are deli sandwiches or burgers and fries at lunch and microwaved meals or takeout for dinner followed by a few beers. He expresses concern that these are not healthy options but admits he does not know how to make better choices. He reports that he is willing to pack lunches and cook for himself.

Barriers to successful change. During your conversation, Mr. Young admits that he has difficulty preparing his meals at home because he is unaware of what and how much he should be eating. He also states that he lacks the time and skill for cooking and meal preparation. Additionally, he reports that physical activity used to be a part of his life before his job became more demanding. However, he is planning to get back to the gym and ride his bike again.

Commit to measurable goals. You start by explaining that despite his age, Mr. Young is at risk for CVD-related complications and that small changes can help to reduce his risk factors. Using evidence-based guidelines for the management of dyslipidemia, you explain the steps Mr. Young can take to improve his dyslipidemia and reduce his ASCVD risk. You challenge Mr. Young to commit to specific, measurable, and attainable health behavior goals along with steps that may help him to achieve his goals. You schedule a follow-up visit in 1 month.

You prescribe the following changes for Mr. Young (he agrees that he will try to take the steps outlined below):

1. You encourage Mr. Young to replace his burger with a lean protein (i.e., grilled chicken breast) and replace his French fries with a side salad or vegetable option when dining out.
2. You recommend that Mr. Young purchase microwavable meals that follow these guidelines (< 600 kcal, <600 mg sodium, <10% saturated fat) and add a side of vegetables (steamed or raw).
3. You encourage Mr. Young to consume no more than two alcoholic drinks per day.
4. You refer Mr. Young for four MNT visits with an RDN that are covered by his health insurance plan. You ask that he set up a meeting with an RDN before your next follow-up meeting in 1 month.
5. You recommend that Mr. Young start walking for a minimum of 10 minutes per day with a goal of 7000 or 7500 steps daily.

Demonstrate progress toward goals. You encourage Mr. Young to keep a food log of his daily intake for 1 week so he can share this information with the RDN, and they can discuss the changes he made at his follow-up visit. You also encourage him to purchase a pedometer so he can track his steps and record his progress. He is agreeable to the health behavior goals you prescribed and will bring the requested progress reports to his scheduled follow-up visit.

Conclusion

Poor diet is responsible for greater than 45% of preventable cardiometabolic deaths in the United States [67], but few physician visits (<25%) include any nutrition counseling with patients [68]. This chapter summarizes the evidence-based nutrition recommendations for the management of dyslipidemias that can be used by physicians/health care providers with their patients (see Table 9.7 for a summary). The ABCDEF framework offers an organized approach for physicians to begin planning lifestyle interventions with patients. A team-based approach is recommended, and referral to an RDN for further counseling will assist with the management of dyslipidemias. Importantly, when physicians recommend that patients make diet and lifestyle changes, including seeing an RDN for MNT, patients are more likely to make sustained changes with improved

Table 9.7 Summary of dietary recommendations for elevated LDL-C and TG: specific food-based examples

Dyslipidemia	Recommendation	Specific example
Elevated LDL-C	↓ SFA	Replace high-fat and processed meats with white meat chicken (without skin), fish/seafood, and plant protein foods, e.g., nuts/nut butters and tofu When selecting red meat, choose lean cuts lean ground beef <90% fat and remove visible fat from meat Replace full-fat dairy products with skim or fat-free dairy products Cook with nontropical liquid vegetable oil instead of solid fats such as butter, lard, beef tallow, ghee, coconut, and palm oil
	↑ Plant sterols	Replace butter with soft margarines containing plant stanols/sterols
	↑ Viscous fiber	Choose whole grains such as oats and replace breads and pastas made with white flour with beans, legumes, nuts, and vegetables
	↓ Dietary cholesterol	Replace some animal protein with plant proteins (inclusion of legumes, beans, tofu in salads instead of meats; veggie burgers instead of beef burgers made with high-fat hamburger meat) Avoid mixed dishes containing full-fat dairy products and processed meats
Elevated TG	↓ Alcohol	Reduce alcohol intake to a maximum of 14/week men (<2 drinks/day) and 7/week for women (1 drink/day)
	↓ Added sugars and Refined grains	Decrease consumption of sugar sweetened beverages. Replace refined grains (white bread, crackers, pretzels, white pasta, and rice) with whole grains (whole wheat breads, wheat crackers, brown rice, whole wheat pasta); avoid commercial baked goods
	↑ Omega-3 fatty acids	Replace cheese or croutons on a salad with a tbsp. of walnuts or add flax or chia seeds to oatmeal or yogurt Eat at least 8 ounces of fatty fish per week

outcomes [69]. The lifestyle recommendations discussed in this chapter are expected to improve dyslipidemias by 3–45%, depending on the intervention. Thus, diet and physical activity interventions are integral components in the prevention and management of dyslipidemia.

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Nutraceuticals and Lipid Management

10

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Introduction

All industrialized countries have observed an important rise in life expectancy over the past few decades. Consequently, even moderately high levels of cardiovascular (CV) risk factors are now more likely to give rise to acute CV events given the longer duration of exposure. However, the opportunity for preventive treatment has also changed within this context; appropriate monitoring and control of risk factors, carried out in a timely and continuous manner, can play an even greater role in prevention.

Coronary heart disease (CHD) remains the leading cause of mortality worldwide, both in men and women, whereas stroke is also an important cause of mortality and a major cause of disability. The common link is atherosclerosis and reference is made to the "total burden" of atherosclerotic cardiovascular disease (CVD). In 2008, the economic cost of CVD in the European Union was reported to amount to approx. 192 billion Euros in terms of direct and indirect healthcare costs [1, 2]. Thus, it is very important to establish prevention programs in order to correct modifiable risk factors, such as dyslipidemias, but also hypertension and smoking. These programs should include both subjects with established CVD (secondary prevention) and those at high risk of experiencing a first CV event based on risk factor scores (primary prevention). It is noteworthy to say that atherosclerosis develops very slowly, starting at an early age, and making a healthy lifestyle in youth is most likely a very effective method to address the worldwide CVD epidemic.

Considerable scientific evidence supports the effectiveness of the reduction of total cholesterol (TC) as well as low-density lipoprotein cholesterol (LDL-C) in preventing CVD events [1, 2]. It

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_10

is generally accepted that the most effective lifestyle changes designed to reduce TC and LDL-C levels, increase high density lipoprotein cholesterol (HDL-C) levels and reduce triglyceride (TG) levels (other two strong and independent predictors of CVD), refer to the reduction in dietary saturated fat, trans fat and mono-/disaccharides, use of functional foods enriched with phytosterols, reduction in excessive body weight, a moderate alcohol intake and increase in habitual physical activity [1]. Current evidence confirms that such lipid management either reduces the likelihood of CVD or slows down its progression [3].Consequently, it is crucial that healthcare professionals make appropriate use of all the available intervention strategies to control these modifiable risk factors as appropriate: from dietary improvement and adequate physical activity (lifestyle changes) to the use of nutraceuticals, functional foods, food supplements, and/or their combination with drugs.

Nutraceuticals that Improve Plasma LDL-C Levels

Until recently (a decade ago), diet and cholesterol-lowering agents have been the main interventions prescribed to subjects at CV risk. However, adherence to a healthy and "cardioprotective" diet (low in salt and without saturated fat) is quite low and the change of dietary habits is not easy to implement in the long term. Current guidelines suggest the use of innovative nutritional strategies in lipid management (especially high LDL-C and TC) based on the consumption of specifically targeted "health" functional foods and/or dietary supplements—the so-called nutraceuticals—which can be used, together with dietary measures, either as alternatives or in addition to lipid-lowering drugs [3]. Research into nutraceuticals and functional foods is progressing rapidly, and several effective products can be found in the market without mandatory prescription or medical advice. Moreover, it is widely accepted that "natural equals safe", but sometimes there are potential risks associated with the inappropriate use of these products. Nutraceutical (a portmanteau of the words "nutrient" and "pharmaceutical") is a nutritional product that provides health and medical benefits, including the prevention and treatment of disease [4]; the nutraceuticals are defined as the phytocomplex (derivatives of vegetal origin) and as the pool of secondary metabolites (derivatives of animal origin), concentrated and administered in a suitable pharmaceutical form. On the other hand, functional foods are conventional, and daily foods that are consumed as part of a standard diet but composed of naturally occurring components, which were absent or in low concentrations in the conventional ones [5]. It improves well-being and life quality by reducing the risk of disease or beneficially affects target functions beyond its basic nutritional functions.

This chapter summarizes currently available evidence about the effect of the most frequently occurring lipid-lowering substances in nutraceuticals, functional foods, or in supplements. Also, we highlight the differences between nutraceutical, functional food, and supplements, giving a brief summary on the available international regulations. The impact of nutraceutical agents on the lipid profile and a summary of their mechanisms of action are detailed in Table 10.1. In addition, we discuss the use of nutraceuticals in special patient populations and the role of physicians in the control of lipid levels by such approaches.

Red Yeast Rice

Monascuspurpureus is a mold that ferments rice to produce Red Yeast Rice (RYR), which is red and contains molecules that inhibit hepatic cholesterol biosynthesis. Between 70 and 83% of these molecules are monacolin K, both as a lactone(K) and open-ring acid (Ka) mixture. It is very interesting that monacolin K is identical to the statin lovastatin and inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis. For unknown reasons, RYR monacolins are more bioavailable than pure lovastatin [6].It has been shown that monacolin K is able to

Table 10.1 Summary of nutraceuticals that impact serum lipids with recommended dosing and expected effects on lipid profile

Active ingredient	Daily dose	Effects on lipid profile	(Possible) mechanisms
Red yeast rice (RYR) [7]	3–10 mg (titrated in Monacolin K)	↓ LDL-C ↓ TG (especially in subjects with high TG) Minor effects on HDL-C	inhibits HMG-CoA reductase
Sterols and plant stanols [12]	1.5–2.0 g	↓ LDL-C ↓ TG (in subjects with T2DM)	inhibit cholesterol absorption via the formation of mixed micelles, subsequently internalized by the NPC1L1 protein
Beta-Glucan [16]	3.5 g	↓ LDL-C ↓ non-HDL-C ↓ apoB	not yet clearly understood; seems to ↑ fecal excretion of cholesterol, bile acids, and/or dietary fat
Glucomannan [19, 20]	1.24–15.1 g	↓ LDL-C ↓ TG ↓ non-HDL-C	↓ the absorption of cholesterol in the jejunum and the absorption of bile acids in the ileum
Psyllium [21, 22]	3–20 g	↓ LDL-C	↑ excretion of bile acids, ↓ absorption of intestinal cholesterol, ↓ hepatic cholesterol synthesis
Chitosan [23]	1–6 g	↓ LDL-C	inhibits cholesterol absorption in the bowel
Berberine [24]	500–1500 mg	↓ LDL-C ↓TG ↑ HDL-C	yet to be defined; Most probably ↓ PCSK9 mRNA in addition to direct effects on LDL receptors, stabilizing their encoding mRNA ↑ *Akkermansia*
Omega 3 fatty acids [29–34]	2–4 g	↓ LDL-C ↓ TG ↑ HDL-C ↑apo-AI ↑ large LDL and HDL particles ↓ VLDL-C	activation of PPARs → increase expression of genes encoding proteins that participate in fatty acid oxidation, inhibition of fatty acid incorporation into TG, and ↓ VLDL
Garlic [46]	5–6 g	↓ TC ↓ TG	antioxidant activity, antiplatelet action and favorable hemostatic effects
Soy [48]	25–100 g	↓ TC ↓ LDL-C ↓ Non-HDL-C ↓ apoB	promotes the expression of LDL receptors
(Poly)phenols [51, 52] Bergamot	500–1000 mg	↓ TC ↓ TG ↑ HDL-C ↓ LDL-C ↑ larger and more buoyant LDL particles ↓ small, dense LDL-3, −4, and 5 particles	inhibits HMG-CoA reductase

(continued)

Table 10.1 (continued)

Active ingredient	Daily dose	Effects on lipid profile	(Possible) mechanisms
Probiotics [55, 56]	Strain dependent	↓ TC ↓ LDL-C	Unclear; it is possible to interact with the intestinal cholesterol; contain some enzymes (cholesteroldehydrogenase/isomerase) able to catalyze the transformation of cholesterol into cholest-4-en-3-one;↓ the enterohepatic circulation of bile salts:↑ bowel pH;the formation of micelles;the transport pathways of cholesterol and/or lipoprotein (such as NPC1L1 gene expression), and cholesteryl esters
Vitamin D [57]		↓ TC ↓ LDL-C ↓ TG	↓ SREBP-2 and inhibits HMG-CoA reductase expression
Astaxanthin [64]	4-20 mg	–	inhibits LDL peroxidation → the inhibition of lipoproteins from being converted into pro-atherogenic particles
CoQ10 [69, 70]	combination of CoQ10 with astaxanthin, RYR, berberina, policosanol, and folic acid	↓ TC ↓ LDL-C ↓ TG ↑ HDL-C ↓ Lp(a)	Inhibition of LDL oxidation
Turmeric and curcumin [73, 74]	1–3 g	↓ TC ↓ LDL-C ↓ TG ↓ Non-HDL-C ↓ Lp(a)	antioxidant and anti-inflammatory effects unclear inhibits the expression of the NPC1L1 transporter via the SREBP2 transcription factor;↑ the efflux of cholesterol via expression of ABCA1
Green tea [79, 80]	25–100 g	↓ TC ↓ LDL-C ↓ TG ↓ apo B ↑ HDL-C ↑ apo A ↑ VHDL ↑ lipoprotein lipase	inhibits lipid synthesis via SREBPs inhibition, ↑ sirtuin proteins and ↑ AMP kinase, and ↓ the PI3K/AKT/mTOR pathway
Resveratrol [84, 85]	100 mg	↓ TC ↓ triacylglycerol	antioxidant anti-inflammatory cardioprotective effects
Artichoke [87, 88]	1–3 g	↓ TC ↓ LDL-C ↓ TG	the interaction of luteolin with HMG-CoA reductase and the pathways regulating hepatic SREBPs and ACAT

ABCA1 ATP-binding cassette transporter, *ACAT* the acyl-CoA:cholesterol O-acyltransferase enzyme, *apoB* apolipoprotein B, *HDL-C* high density lipoprotein cholesterol, *HMG-CoA* 3-hydroxy-3- methylglutaryl-coenzyme A, *Lp(a)* lipoprotein(a), *LDL-C* low-density lipoprotein cholesterol, *mTOR* rapamycin, *NPC1L1* the trans-membrane transport protein Niemann-Pick C1-Like 1 protein, *PCSK9* Proprotein Convertase Subtilisin/Kexin Type 9, *PI3K* the phosphatidylinositol-3-kinase, *PPARs* peroxisome proliferator-activated receptors, *SREBP-2* sterol regulatory-element binding protein-2, *TC* total cholesterol, *TG* triglycerides, *T2DM* type 2 diabetes mellitus, *VLDL-C* very low-density lipoprotein cholesterol
↑ increase; ↓ decrease; → result in

reduce LDL-C concentrations up to 20–25%, at doses of 3–10 mg/day [7], its effects on HDL-C are usually of minor importance, while TG is reduced especially in subjects with hypertriglyceridemia (Table 10.1).

The first randomized controlled trial (RCT) was performed in China including 5000 subjects with previous coronary events, including myocardial infarction (China Coronary Secondary Prevention Study), where RYR extracts (xuezhikang) with

2.5–3.2 mg of monacolin provided an average reduction in LDL-C levels of about 20%. Noteworthy, this cholesterol-lowering effect was associated with significant reductions in fatal and non-fatal coronary events, stroke, and all-cause mortality (−31%, −44%, and −32%, respectively) [8]. RYR supplements are often considered as a feasible alternative to statins with a low incidence of adverse effects [9]. However, the issue about safety on RYR use is still not conclusive and the dose of 10 mg/day has been suggested with medical supervision [3].

Monacolin K is metabolized by cytochrome P450 (more specifically by isoenzyme 3A4 involved in the metabolism of almost 30% of all drugs). Therefore, the co-administration with medicines containing itraconazole, ketoconazole, erythromycin, clarithromycin, telithromycin, HIVprotease inhibitors, cyclosporine, nefazodone, in addition to grapefruit juice (≥0.2 L/day), is contraindicated, while use in combination with statins should be avoided [3].

Phytosterols and Stanols

The second most widely known class of nutraceuticals are the plant sterols (known as phytosterols) and stanols, which are structurally similar to cholesterol (they are also comprised of a substituted steroid nucleus) and can be found to different extents in all plant-based products [10]. In the intestine, phytosterols compete with cholesterol and inhibit its absorption via the formation of mixed micelles, subsequently internalized by the *trans*-membrane transport protein Niemann-Pick C1-Like 1 (NPC1L1) protein [10]. Phytosterols are then re-secreted into the intestinal lumen, mainly through the ATP binding membrane cassette transport proteins (ABC) ABC-G5 and ABC-G8 [10].

Phytosterols inhibit both the dietary and biliary intestinal absorption of cholesterol in a dose-dependent way. There is a significant cholesterol-lowering effect after intakes of more than 1.5 g of phytosterols per day. There is no effect on plasma HDL-C and TG, but they reduce LDL-C by about 9–10% [11], when taken as functional foods (beverages or margarines) at doses of 1.5–2.0 g/day. A study in subjects with type 2 diabetes mellitus (T2DM) reported a decrease in both LDL-C and TG levels [12]. Moreover, phytosterols may improve vascular endothelial function and exert anti-inflammatory actions [13, 14]. It is usually recommended that phytosterol-based functional foods or nutraceuticals be taken during main meals, because cholesterol is more abundant in the gut lumen, following the stimulation of biliary secretions and the presence of cholesterol derived from dietary sources [13].The disadvantage of phytosterol therapy is the decreased absorption of carotenoids and fat-soluble vitamins; therefore, patients treated with phytosterols should consume higher amounts of fruits and vegetables containing such nutrients [13]. Their use is recommended in subjects with dyslipidemia at low CVD risk or in addition to statins in those at high CVD risk (or other drugs in case of statin intolerance), as well as those with familial hypercholesterolemia including children over 6 years of age [15].

Soluble Fiber: The Case for Beta-Glucan

Non-digestible carbohydrates play an important role in controlling plasma LDL-C levels, but their exact mechanisms of action are not yet clearly understood. The more probable mechanism seems to be an increased fecal excretion of cholesterol, bile acids, and/or dietary fat; especially seen for viscous soluble fiber which absorbs water and forms water-based gels in the intestine [3].

Beta-glucan (a class of non-starch polysaccharides: (1 → 3) (1 → 4)-β-d-glucan) can be found in small amounts in grains and cereals and certain mushrooms and, in larger quantities, in barley and oats as well as in some supplements and functional foods [3]. One meta-analysis reported that beta-glucan (at a daily dose of 3.5 g) reduced LDL-C, non-HDL-C, and apolipoprotein (apo) B [16]. Moreover, glucomannan, plantago/psyllium, and chitosan are also effective in lipid management [17].Glucomannan seems to reduce

the absorption of cholesterol in the jejunum and the absorption of bile acids in the ileum, leading to improvements in apo B and plasma LDL-C levels [18]. A meta-analysis [19]including 14 RCTs (n = 531 subjects) showed that glucomannan supplementation (at doses ranging between 1.24 and 15.1 g/d) significantly reduced LDL-C and TG (by −0.41 mmol/L and − 0.13 mmol/L, respectively, $p < 0.05$ for both) compared with placebo. These findings have been also confirmed by recent meta-analysis [20] reporting that the intake of 3 g glucomannan/day resulted in the reductions in LDL- and non-HDL-C cholesterol of 10% and 7%, respectively.

Psyllium is a source of concentrated fibers derived from the husks of blonde psyllium seed with the mechanisms of action similar to those of other fibers: increased excretion of bile acids and reduced absorption of intestinal cholesterol and hepatic cholesterol synthesis [21]. An average intake of psyllium of 10 g/day results in an average LDL-C reduction of 7% [22]. Doses up to 20 g/day are safe.

Chitosan is a non-fiber lipid-lowering agent isolated from shellfish and sea crustaceans that inhibits cholesterol absorption in the bowel. A recent meta-analysis [23] including 14 RCTs provided evidence that chitosan supplementation (mean dosage 2 g/day) leads to significant effects on weight loss (−1.01 kg, 95% CI: −1.67 to −0.34), improves plasma lipid profile (LDL-C -0.83 mmol/L, 95% CI, −1.64 to −0.01; TG −1.06 mmol/L (95% CI, −1.67 to −0.45)) and CV outcomes (the most significant improvement was observed in systolic and diastolic blood pressure (SBP and DBP): −2.68 mm Hg (95% CI: −4.19 to −1.18) and − 2.14 mm Hg (95% CI: −4.14 to −0.14), respectively) compared with placebo.

Berberine

Berberine is an alkaloid found in and extracted from the root of *Berberis aristata* and other congeners. It has been shown to have LDL-C lowering effects (in the range 10–20%) [24]. It also decreases plasma TG (the standardized mean difference (SMD) = − 0.50 mmol/L; 95% confidence interval (CI) – 0.69 to – 0.31) and increases HDL-C concentrations (SMD = 0.05 mmol/L; 95% CI 0.02–0.09). Berberine has low (2–3%) bioavailability and the exact mechanisms of action are yet to be defined. Most probably berberine reduces the levels of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) mRNA in addition to direct effects on LDL receptors, stabilizing their encoding mRNA. Berberine also decreases TG levels inhibiting MAP and enhancing AMP kinase [25] Safe limit for daily intake for berberine is about 500–1500 mg [26], however, it should be emphasized that it has mainly been studied in Asian populations, and in the Western world is under investigation (often found in products containing also RYR) [13]. In addition to the lipid-lowering effects, its effects are associated with an increased growth of *Akkermansia* in the gut and this is probably the reason for its possible anti-atherosclerotic effect, as observed in animal models [3, 27].Such specific bacterial taxa (an increase in the abundance of *Akkermansia* and consequently modulation of gut microbiota) may contribute to the anti-atherosclerotic and metabolic protective effects of berberine which is poorly absorbed orally, but significantly accumulated in the intestines. A recent randomized, double-blind, placebo-controlled, single-center pilot study [28], showed that supplementation with *A. muciniphila* bacteria improves several metabolic parameters, including TC (−8.68 ± 2.38%, $p = 0.02$).

Marine-Derived Omega 3 Fatty Acids

Fish oil is rich in the omega-3 polyunsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with a recommended daily dose 2–4 g. Several meta-analyses have shown a consistent TG-lowering effect of fish oil supplements, especially in patients with hypertriglyceridemia [29–32], with simultaneous, although small, reductions in levels of LDL-C, with the exception of krill oil [33]. Krill oil has a greater ratio of EPA to DHA than fish oil, and most of them are in the form of phospholipids,

which have higher bioavailability (the reason why lower doses of krill oil have similar TG-lowering effects compared to higher doses of fish oil). Another interesting finding from the above-mentioned meta-analyses is that although DHA is more efficient in lowering TG levels than EPA, DHA decreases LDL-C levels to a greater extent than EPA. DHA also increases HDL-C and apo-AI concentrations, increases the number of large LDL and HDL particles, and decreases very low-density lipoprotein cholesterol (VLDL-C) [30, 34].

The main TG-lowering action mechanism includes activation of peroxisome proliferator-activated receptors (PPARs) to increase expression of genes encoding proteins that participate in fatty acid oxidation, inhibition of fatty acid incorporation into TG, and reduction of hepatic VLDL synthesis and secretion. EPA and DHA also increase lipoprotein lipase activity, thus enhancing TG clearance [31]. Given the fact that hypertriglyceridemia is often combined with low HDL-C levels, a concomitant increase in levels of HDL-C with fish oil supplementation might be expected; however, there are no exact and definite data. One meta-analysis has demonstrated that monotherapy with DHA increased HDL-C levels considerably more than placebo [31], whereas a RCT found that krill oil, but not fish oil supplements, significantly increased levels of HDL-C [35]. EPA monotherapy is a cornerstone for the treatment of high TG and can complement fibrate treatment [36]. Combination of DHA/EPA preparations may reduce TG, but they do not reduce risk for CV events. Cardioprotective effects were suggested after the consumption of n-3 polyunsaturated fatty acids (PUFA) from fish oil, including both DHA and EPA [37, 38]; however, recent findings did not show positive effects on CVD risk reduction [39, 40]. Only the REDUCE-IT trial [41] has shown the reduction of CVD events and CVD death in subjects with CVD and hypertriglyceridemia after treatment with 4.0 g daily of Icosapent ethyl—a highly purified EPA.

Apart from their direct effects on plasma lipids, fish oils have also improved other CV risk factors such as body weight (by 5.6 +/− 0.8 kg [42]) and blood pressure (DHA reduced SBP −5.8 ± 2.1 mm Hg, p = 0.022 and DBP −3.3 ± 1.3 mm Hg, p = 0.029 [43]), although a large meta-analysis did not demonstrate a statistically significant reduction in total mortality, cardiac mortality, or cardiac events after fish oil supplement consumption [32]. However, a significant protective effect on heart failure was observed (in terms of mortality and admission to hospital for CV reasons) [44], and one analysis found a significantly reduced risk of cardiac death and all-cause mortality in high-risk groups consuming fish oil supplements (a recommended daily dose 2–4 g) [45].

Garlic

Garlic (*Allium sativum,* its main compound *Allicin*, formed from the conversion of alliin by the enzyme allinase) has been shown to have possible cardioprotective actions, including lipid lowering action, antioxidant activity, antiplatelet action, and favorable hemostatic effects [46]. It has been shown that garlic, both in powder and non-powder form, can significantly reduce plasma lipid levels over a 1–3 months period: 8% reduction in serum cholesterol with dried powder and 15% with non-powder preparations, as well a significant reduction in plasma TG level, while HDL-C remained stable without any significant increase [46]. These effects seemed to be similar in a daily dose range of 600–900 mg, concerning the garlic powder preparations, while there is not an exact equivalence with fresh garlic because of the variation in the concentration of *allicin* [46]. The main adverse and undesirable effect for the public is odor, which appears to be uncommon with the standardized preparations, especially in lower doses. The available recent data from meta-analysis [47] also suggest that garlic is superior to placebo in reducing total cholesterol levels (the weighted mean difference was −0.41 mmol/L). It should be mentioned that garlic is not yet an established as a licensed supplement and more research in its use in clinical practice is needed.

Other Cholesterol-Lowering Agents

Due to the great public interest in functional foods and nutraceuticals, there are today many other substances which are under investigation to evaluate their effects on lipids. Soy, lupin and its products, contain isoflavones, lecithin, and proteins that promote the expression of LDL receptors. The plasmatotal- and LDL-C reduction following a consumption of high amounts of soy protein (25 g/day) is not very high (4–6%) and is mostly applicable to patients with high basal cholesterol concentrations [3]. Interestingly, reduced LDL-C, non-HDL-C and apoB levels were found when animal proteins were replaced with soybeans [48]. However, a meta-analysis of the 10 RCTs revealed no significant effect of soy isoflavones on plasma lipoprotein(a) (Lp(a)) concentrations [49]. On the other hand, another meta-analysis showed an inverse association between soy protein intake and CHD and ischemic stroke risk in case control studies [50].

(Poly)phenols, namely flavonoids such as quercetin and those contained in bergamot extracts (that might competitively inhibit HMG-CoA reductase), have been tested in patients with both primary dyslipidemic and the metabolic syndrome (MetS) [51], showing beneficial effects on the lipid profile (TC decreased form 6.6 +/− 0.4 to 5.8 +/− 1.1 mmol/l, $p < 0.0001$; LDL-C from 4.6 +/− 0.2 to 3.7 +/− 1.0 mmol/l, $p < 0.0001$ and TG from 1.8 +/− 0.6 to 1.5 +/− 0.9 mmol/l, $p = 0.002$, while HDL-C increased from 1.3 +/− 0.2 to 1.4 +/− 0.4 mmol/l, $p < 0.0007$), including favorable changes in LDL-C quality, a shift toward larger and more buoyant LDL particles (LDL-1 particles increased from 41.2 +/− 0.2 to 49.6 +/− 0.2%, $p < 0.0001$, while small, dense LDL-3, −4, and 5 particles decreased from 14.5 +/− 0.1 to 9.0 +/− 0.1% $p < 0.0001$; 3.2 +/− 0.1 to 1.5 +/− 0.1% $p = 0.0053$; 0.3 +/− 0.0% to 0.1 +/− 0.0% $p = 0.0133$, respectively) [52].

Policosanol has been proven ineffective [53], and the use of probiotics is yet quite limited as cholesterol-lowering agents [54]. Results from several meta-analyses indicate that probiotics can significantly reduce serum TC (SMD = −13.27, 95% CI (−16.74 to 9.80), $p < 0.05$) [55] as well as both TC and LDL-C (by −0.25 mmol/L (95% CI: −0.39, −0.12) and − 0.17 mmol/L (95% CI: −0.25, −0.09, respectively) [56], and it seems that the subjects with higher TC levels at baseline and longer intervention time may have more benefit. Of note, more studies are necessary in order to better define their efficacy.

A large meta-analysis of 41 RCTs including 3434 participants evaluated the effect of vitamin D supplementation on lipids plasma levels [57], showing a beneficial effect on serum TC (SMD = −0.17 (−0.28 to −0.06)), LDL-C(SMD = −0.12 (−0.23 to −0.01)); and TG (SMD = −0.12 (−0.25 to 0.01)) but not on HDL-C. The main mechanism through which vitamin D affects circulating cholesterol levels may be through the action on the transcription activity of vitamin receptor and insulin-induced gene-2 (Insig-2) expression which further downregulates sterol regulatory-element binding protein-2 (SREBP-2) activation and inhibits HMG-CoA reductase expression. In addition, there is growing evidence that vitamin D deficiency significantly increases the risk of a CV event, while a sufficient or optimum vitamin D status is protective [58]. Furthermore, it should be mentioned that changes in vitamin D status may confound some statin studies finding CV risk reduction [59].

Nutraceutical Combinations

Given the natural effect of the nutraceuticals and functional foods, many different combinations were tested in order to increase their lipid-lowering effect. The more representative example could be the association of berberine and monacolin (which increases the expression of PCSK9) [60] or berberine with silymarin [61], then the concomitant use of monacolin and phytosterols. It should be noted that before such combinations are prescribed, more of high-quality data must arise in order to clarify the exact effects of these combinations and the possible adverse events [3, 13]. Furthermore, after a short-term supplementation with a combination

of RYR (10 mg), phytosterols (800 mg), and L-tyrosol (5 mg) [62], a reduction in the estimated CVD has been reported.

It should be mentioned that combinations of lipid-lowering nutraceuticals could further improve their safety (reducing the dosages of the single components), but the data from RTC about their efficacy are rare, and some of the investigated combinations contained under-dosed components [17]. On the other hand, some combinations such as the combination of RYR, berberine, and policosanol confirmed their efficacy in the long-term with a positive impact on LDL-C but also other CVD risk factors (endothelial function, pulse wave velocity).

Antioxidants

Antioxidants are another alternative in the treatment of dyslipidemias. The mechanism of their action seems to be very clear, since after entering into the subendothelial space of the intima, LDL is oxidized by reactive oxygen species (ROS) and transformed into oxidized proatherogenic particles. In this step, increased antioxidant stores through the administration of antioxidants, such as astaxanthin and coenzyme Q10, might reduce LDL oxidation and peroxidation, thereby slowing atherogenesis, though work in this area is by no means definitive [63].

Astaxanthin

Astaxanthin is a red–orange carotenoid obtained from the microalgae *Haematococcuspluvialis*, which is one of the most potent naturally occurring antioxidants (100–500 times more potent than vitamin E). Astaxanthin, at doses of 1.8, 3.6, 14.4, and 21.6 mg for 14 days, inhibits LDL peroxidation (LDL lag time was longer (5.0%, 26.2%, 42.3%, and 30.7%, respectively) compared with day 0 after consuming astaxanthin at the above mentioned doses) [64], resulting in the inhibition of lipoproteins from being converted into pro-atherogenic particles [63]. However, a random-effect meta-analysis from 7 RCTs (10 treatment arms) did not show any significant effect of astaxanthin supplementation (dose from 4 mg to 20 mg/day) on lipids [65], indicating a need for further, well-designed trials.

Coenzyme Q10

It is well known that CoQ10 is the only lipid-soluble antioxidant that slows lipid peroxidation in the circulation, plays a crucial role in oxidative phosphorylation (i.e. ATP biosynthesis), and stabilizes Ca-dependent channels, cell signaling, and cell growth by regulating levels of cytosolic redox intermediates (nicotinamide adenine dinucleotide phosphate (NADPH)) [66]. CoQ10 deficiency may play an etiologic role in the development and progression of heart failure [67].

The bioavailability of CoQ10 is variable (in terms of the mode of administration) and the release method, formulation, dosage, particle size, and ubiquinol (the reduced form) seems to be the most available form of CoQ10. However, absorption of dietary CoQ10 is limited and slow (it takes about 6 h to reach the maximum concentration) due to its hydrophobicity and large molecular weight, while the elimination half-life is about 33 h. In healthy adults, the range for plasma CoQ10 is from 0.40 to 1.91 μmol/l. Recently, soft-gel capsules containing ubiquinone or ubiquinol were reported to be the best absorbable formulations among seven different formulations of CoQ10 [68]. However, there is not yet enough evidence supporting its use in clinical practice, emphasizing the gap between the pathophysiological process and the clinical evidence [63].

Supplementation with CoQ10 may impact the treatment of hyperlipidemia. A combination of CoQ10 with astaxanthin, RYR, berberina, policosanol, and folic acid decreased TC (−26.15 mg/dL, $p < 0.001$), LDL-C (−23.85 mg/dL, $p < 0.001$), TG (−13.83 mg/dL, $p < 0.001$), increased HDL-C (2.53 mg/dL, $p < 0.001$) levels [69]. CoQ10 has some efficacy for reducing plasma Lp(a) (an inverse association was observed between administered CoQ10 dose and

Lp(a) lowering; slope: 0.04; 95% CI: 0.01, 0.07; $p = 0.004$) [70]. In addition, CoQ10 inhibits lipid peroxidation of LDL particles [66, 71].

Turmeric and Curcumin

Turmeric (*Curcuma longa*) is a yellow pigment that is used worldwide in cooking, cosmetics, dyes, and medicines, used frequently as food additive in Southeast Asia, improving color and flavor of food preparations [72]. Curcumin (chemical name: diferuloylmethane) is an active component of turmeric and has the capacity to interact with hundreds of molecular targets. A large meta-analysis demonstrated that subjects who received turmeric and curcumin experienced a cardioprotective effect with lowering of serum LDL-C (SMD = −0.340, 95% CI: −0.530 to −0.150, $p < 0.0001$) and TG (SMD = -0.214, 95% CI: −0.369 to −0.059, $p = 0.007$) levels, compared to subjects who did not receive this supplement [73]. The efficacy of turmeric and curcumin on serum TC levels (−0.934, 95% CI: −1.289 to −0.579, $p < 0.0001$) has been confirmed also in subjects with the MetS and higher CVD risk [73] and such effect in TC lowering may be greater by turmeric extract (SMD = −0.584, 95% CI: −0.980 to −0.188, $p = 0.004$). It is premature to recommend the use of these compounds in clinical practice due to uncertainties related to formulation, dose, and medication frequency. Supplementation with curcuminoids (1000 mg/day) plus piperine as an absorption enhancer (10 mg/day) or placebo, showed a reduction in atherogenic lipid indices including non-HDL-C (−23.42+/−25.13 versus −16.84+/−41.42, respectively; $p = 0.014$) and Lp(a)(−1.50+/−1.61 versus −0.34+/−1.73, respectively; $p = 0.001$) in T2DM subjects [74]. Results from pre-clinical and clinical studies indicate the curcumin impacts CVD through its anti-hypercholesterolemic and anti-atherosclerotic effects as well as its protective properties against cardiac ischemia and reperfusion [75]. One limitation to curcumin therapy is its low bioavailability; new curcumin nanomedicine formulations are being developed in order to solve this problem. In addition, a natural supplement (Kepar) containing curcuma longa, silymarin, guggul, chlorogenic acid, and inulin, at a dose of 2 pills/day for 4 months, significantly reduced TC (from 4.8 ± 1.4 to 4.5 ± 1.0 mmol/l, $p = 0.03$) and improved anthropometric parameters in subjects with the MetS [76].

Green Tea

Green tea is one of the most popular beverages worldwide, and its major components are polyphenols, including catechins (about 30% of its dry weight), alkaloids, and polysaccharides [77, 78]. Supplementation with green tea extract leads to significant reductions in TC (from 5.4 ± 1.0 mmol to 5.0 ± 0.9 mmol; $p = 0.009$), LDL-C (from 3.5 ± 1.0 mmol to 3.1 ± 0.9 mmol; $p = 0.011$) and TG (from 1.4 ± 0.6 mmol to 1.1 ± 0.5 mmol; $p = 0.004$), while HDL-C increases (from 1.2 ± 0.2 mmol to 1.4 ± 0.3 mmol; $p < 0.001$) [79]. A recent animal study [80] showed that a basal diet plus 10 g/kg green tea powder for 12 weeks resulted in an increase in HDL, apo A1, and very high-density lipoprotein (VHDL) ($p < 0.01$, respectively), while apo B, TG, TC ($p < 0.01$, respectively), and LDL ($p < 0.05$) decreased after 8 weeks feeding. Lipoprotein lipase expression in the liver was increased after 8 to 12 weeks feeding when compared to the control group ($p < 0.05$) [80]. Increased HDL and apo A was observed in Portuguese adults who were given 1 L of green tea per day for 4 weeks [81].

Resveratrol

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) is a natural polyphenolic compound in grapes, nuts, fruits, and vegetables [82], which has been approved as a dietary supplement by the Food and Drug Administration (FDA) because of its multiple functions and low cytotoxicity. According to the current evidence, resveratrol has health-promoting functions such as anti-inflammatory, antioxidant, and anti-tumor activities, as well as cardioprotective effects [83].

One recent meta-analysis [84] demonstrated that resveratrol supplementation (up to 3000 mg/day) in subjects with the MetS significantly reduced TC, without effects on TG, LDL-, and HDL-C. In subjects with dyslipidemia, resveratrol supplementation (100 mg/day) significantly reduces TC (201.4 +/− 34.4 versus 220.6 +/− 37.4, $p = 0.04$) and triacylglycerol (133.4 +/− 55.3 versus 166.7 +/− 68.5, $p = 0.04$) compared with placebo [85]. Furthermore, resveratrol alters lipid metabolism in cancer via various mechanisms [86]: inhibits lipid synthesis via SREBP inhibition, activates sirtu in proteins concomitantly with the activation of AMP kinase, and downregulates the phosphatidylinositol-3-kinase (PI3K)/AKT/ rapamycin (mTOR) pathway, resulting in cancer cell apoptosis.

Artichoke (*Cynara scolymus, Cynara cardunculus*)

Artichoke leaf extract (ALE) has potential hypolipidemic as well as hepatoprotective effects that can be attributed to its antioxidant action; the primary constituent substances are mono- and dicaffeoylquinic acid (chlorogenic acid and cynarin), caffeic acid (1%), volatile sesquiterpene, and flavonoids (1% which include the glycosides luteolin-7-beta-rutinoside (scolymoside), luteolin-7- beta-D-glucoside, and luteolin-4-beta-D-glucoside) [17]. A recent 6-month randomized, double-blind, placebo-controlled study [87], using a natural supplement containing chlorogenic acid and luteolin, reported a statistically significant improvement in lipid profile (TC −19.59 mg/dl (95% CI −23.71, −15.47), TG −35.14 mg/dl (95% CI −44.83, −25.45) and LDL-C −24.79 mg/dl (−95% CI 31.43, −18,16), $p < 0.001$ for all) as well as other investigated cardio-metabolic parameters. These findings are consistent with the most recent meta-analysis of 9 RCTs including 702 participants [88]. A hepatoprotective effect observed after ALE supplementation suggests possible usefulness in statin-intolerant patients with elevated alanine transaminase levels. However, long-term safety studies are needed.

Suitable Candidates for the Use of Nutraceuticals and Functional Foods

There is evidence that highlights the link between diet and human health potentiating food products with added value beyond hunger satisfaction and nutrient supply. Nutraceuticals, functional foods, and food supplements are at the interface between pharma and nutrition and represent therapeutic alternatives for the prevention and the management of chronic diseases, such as dyslipidemia, in combination with prescribed medication. There are remarkable differences between these concepts which are often confused and they are used interchangeably by consumers. Statutory definitions differ among international regulations in Europe, United States, and Japan (as described in details in [89]). However, in all regions, these functional products meet the following three requirements: (1) promote health; (2) lead to better wellbeing, life quality, and function of the whole organism, and (3) reduce risk and prevent diseases. However, specific harmonized regulation for these products is still needed, while further research will show which combinations are most favorable and suitable for each metabolic disorder. Nutrition–pharma combinations could reduce the total doses of prescribed medicines without interfering with their pharmaceutical effect as well as the side effects, but there are several issues that must be considered with particular attention: efficacy, food–medicine interactions, indirect stimulation of self-medication, and long-term effects of the combination. Lipid-lowering agents are of particular interest (together with blood pressure-lowering agents) since they are able to reduce the risk of CVD or slow down its progression [89].

Healthcare professionals should make appropriate use of all the available strategies in order to control risk factors: from positive lifestyle changes, dietary improvement to the use of nutraceuticals, functional foods and food supplements, in combination with drugs. It is evident that currently available functional foods and supplements can effectively reduce plasma LDL-C levels (from 5% to 25%), either alone or in combination [13].

Mainly individuals at low absolute CV risk at a young age or according to classic algorithms are more suitable candidates for these products. The effects of the most frequently occurring cholesterol-lowering substances in functional foods or in supplements, such as RYR, plant sterols and stanols, beta-glucans and berberine, were described above. On the other hand, it should be mentioned that some evidence supports the fact that a healthy diet and lifestyle can reduce CV risk through mechanisms independent of LDL-C reduction and such strategies are recommended even in the absence of clinically significant hypercholesterolemia. However, if LDL-C is high (above the target values by 10% or more), it appears reasonable to complement diet and lifestyle with other interventions, focused on LDL control, from the very beginning of treatment [13]. More evidence-based evaluation is need in this context.

Studies in Special Patient Populations

Patients with Chronic Kidney Disease (CKD)

The use of cholesterol-reducing nutraceuticals in patients with chronic kidney disease should be further investigated, although current evidence indicates that lipid-lowering nutraceuticals may represent potential therapeutic options [90]. Those with concomitant CVD and CKD should be treated early and intensively in order to minimize their very high risk and possibly, progression of CKD.

An international lipid expert panel statement [91] supports the evidence that nutraceuticals may be used to achieve LDL-C target, especially in cases of statin intolerance. Some concerns have been raised about potential toxic side effects of RYR supplementation (related to musculoskeletal disorders), leading to safety warnings from the US Food and Drug Administration and the European Food Safety Authority (EFSA) panel on Food Additives and Nutrient Sources, although later meta-analysis including 53 RCT with more than 8000 subjects did not confirm such effect and a similar high tolerability of RYR has been shown by further subgroup analyses considering different daily RYR doses and type of nutraceuticals (RYR alone or combined with other nutraceutical), co-administration with statin and type of control (statin or placebo) [90].

Although a little evidence is showing the beneficial effects of nutraceuticals on vascular health in subjects with CKD, such effects are supported by improved vascular-related outcomes in animal models, healthy human, and aging populations [92]. Three emerging nutraceutical strategies show promising roles in preventing or reversing vascular dysregulation in CKD: polyphenols, dietary nitrates, and selective mitochondria therapies, although limited direct evidence exists [93]. Omega 3 fatty acids represent an attractive and safe therapeutic option in all CKD stages, including end-stage renal disease (ESRD). According to the ESC/EAS guidelines [94], 2–3 g/day of omega 3 fatty acids can decrease TG levels up to 30%. It is recommended to initiate with omega 3 fatty acids supplementation if TG concentrations remain >200 mg/dl despite statin ± fibrate therapy [94]. In terms of CV benefits, omega 3 fatty acids have been shown to decrease lipids TG levels, oxidative stress, inflammation, and platelet activity in CKD patients [95]. One meta-analysis reported that supplementation with omega 3 fatty acids significantly reduced CVD death in dialysis patients and prevented ESRD in CKD patients [96]. In addition, an inverse association was found between serum omega 3 fatty acids levels and sudden cardiac death in dialysis patients [97], while a long-term (40 months) supplementation of omega 3 fatty acids resulted in renoprotective in subjects (n = 2344) with a history of myocardial infarction [98].

Patients Who Cannot Tolerate Statins

Nutraceuticals with lipid-lowering properties (such as bergamot, berberine, artichoke, and their combinations), may be considered for further LDL-C lowering in those subjects with statin-related muscle symptoms or statin-intolerant

patients being close to the target [99]. Finally, it should be mentioned that it is reasonable to consider all the possible options to reduce LDL-C with nutraceuticals, especially in populations where there is a higher risk of statin intolerance, such as athletes and individuals on regular intense exercise programs [100]. However, large, well-designed trials are still required.

The supplementation aimed to control LDL-C levels can be initiated in parallel with diet and lifestyle interventions in patient who: (1) are aged below 40 years, for whom a risk algorithm-like SCORE cannot be used; (2) have a global CVD risk ≤1% at 10 years; (3) have the MetS or complex metabolic disorders with a low absolute CVD risk, both according to the SCORE algorithm; and (4) clinically require lipid-lowering pharmacological treatment but refuse to take drugs (statins) based on beliefs or preferences [13]. Finally, potential differences in lipid-lowering efficacy in subjects with specific genetic patterns should also be explored as Mendelian randomization studies showed that cholesterol-lowering polymorphisms, inducing low or moderate plasma LDL reduction, reduce CVD risk as effectively as high intensity but shorter interventions [101].

The Physician's Role in the Use of Nutraceuticals and Functional Foods in Lipid Management

It is important to emphasize that lipid-lowering therapy cannot be replaced by nutraceuticals, but their use might help to optimize it (reducing CV residual risk) as adjunctive therapy. Based on the current evidence of their usefulness in terms of lipid lowering, it seems that such a therapeutic approach might be especially important for subjects with mixed dyslipidemia, atherogenic dyslipidemia in patients with T2DM and the MetS, patients with mild-to-moderate hypercholesterolemia not at target, including those who are statin-intolerant or those who cannot be treated with statins/suitable doses of statins and are at higher CV risk [17]. However, it remains to be addressed by future studies which lipid-lowering effects of nutraceuticals are clinically relevant, which maintain their efficacy in the long term and which might also be associated with an improvement in CVD risk.

Of note, it has been suggested that the way of preparation of RYR can vary substantially and such differences could cause safety issues [90]. As some nutraceuticals have been shown to improve the efficacy of standard pharmacological treatments, an evidence-based approach to their use could improve the quality of the treatment, including achievement of the LDL-C goal in clinical practice, but also increasing therapy adherence [17]. Physicians and other healthcare professionals engaged in the diagnosis and management of subjects with lipid disorders (especially in the primary care setting) are encouraged to consider the position [17] in their clinical practice in making the strategies for the prevention, diagnosis, and treatment of dyslipidemia. It should be highlighted that, despite being freely available for purchase, nutraceuticals and functional foods should be used for the control of lipids level following shared agreement between the physician and the patient. Obviously, the focus should be on the strategies for which there are data from studies of at-risk human populations as well as other disease states, and/or results of translational investigations [93]. It should be emphasized that the management of subjects with hypercholesterolemia should take into consideration an assessment of global CV risk, including family history for CVD, abdominal obesity, asymptomatic organ damage, as well as the use of the risk assessment algorithms [13]. Patients should be counseled about the role and importance of each supplement in their management so as to enhance long-term treatment adherence.

Conclusions

Overall, nutraceuticals may prove useful in terms of lipid lowering, including in some special populations such as statin-intolerant patients. However, there is limited evidence for the effects of nutraceuticals on vascular function and in

patients with CKD. Clinical efficacy on the lipid profile has to be investigated in large, placebo-controlled, double-blind studies, using the product (or combining active ingredients) at commercially available doses for an adequate time, including subjects with or without lipid alterations at baseline. In the future, there will be new safe and evidence-based lipid-lowering options by nutraceuticals, including more details about the mechanism of action of active ingredients which will also shed light on their potential effects on intermediate endpoints such as endothelial function, systemic microinflammation, and overall CV risk.

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Pharmacology of Statins

11

Kenneth Kellick and Joseph J. Saseen

Historical Perspective

Statins have been extensively studied over the past three decades and have demonstrable significant clinical benefits [1, 2]. However, the statin chronology starts with the recognition that aortic atherosclerotic plaques contain significant concentrations of cholesterol compared to normal aortas. Clinical research had already proven that feeding pure cholesterol to rabbits resulted in severe aortic atherosclerosis [1]. The microbiologists known as Brown and Goldstein won a Nobel Prize for the discovery that the condition known as familial hypercholesterolemia was associated with impaired HMG-CoA reductase activity [2, 3].

The Japanese scientist Akira Endo worked for Sankyo Japan microbiology group and was given the opportunity to work on a project of his own choosing. There was fascination that various fungi produced compounds that could inhibit HMG Co-A reductase. Experimentation with the blue-green mold, *Penicillium citrinum*, produced several metabolites, one of them known as ML-236B or compactin. The latter showed potent activity in vivo and in vitro to inhibit cholesterol synthesis. Over the years, after a number of failed animal experiments, including rats, it was found that compactin significantly lowered the cholesterol in hens, dogs, and monkeys. After about 4 years of continued human and animal trials with compactin, thsse clinical development was suspended in 1980. There were reports of transaminase elevations in humans as well as the development of lymphoma in dogs.

In the late 1970s, Merck research laboratories developed a new compound from *Aspergillus terreus* and named it mevinolin which later became known as lovastatin [4]. Anecdotally, they later found a compound from cultures of *Monascus ruber* and labeled it monacolin K. It was later found that monacolin K (marketed as red yeast rice) and mevinolin were the same compound.

Lovastatin, the first statin approved by the US Food and Drug Administration (FDA) in 1987 had some clinical limitations that resulted in some commercial obstacles. While being the first statin to market, its ability to lower LDL-C was not deemed optimal. This coupled with the lack of patent protection in many countries led to the development of additional statin agents. Adding a methyl group at the 2′ position led to the development of simvastatin. The latter had more lipophilicity than its predecessor as well as greater LDL-C lowering potency. One controversy with these first

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_11

two moieties was the closed lactone ring structure. Both lovastatin and simvastatin are prodrugs that require hydrolysis in the liver and some in the gut to the open acid formulation, which acts as the inhibitor of HMG CoA reductase.

During the time that Merck was developing simvastatin, Sankyo in Japan was developing pravastatin. Unlike lovastatin and simvastatin, pravastatin had the first open-ring statin structure. Pravastatin is produced by chemical modification of lovastatin in a two-step fermentation reaction performed by the bacterium *Nocardia autotrophica.* Bristol Myers Squibb acquired US marketing rights and began to develop the drug for marketing and use in the United States. During the development of pravastatin, the concepts of the hydrophilicity and the open lactone ring fostered speculation that might lead to better tissue selectivity than the first two statins. While this appeared to be a desired characteristic, there was never any substantial proof of this being a clinical benefit [5]. In the PRIMO study, pravastatin 40 mg was shown to have only 10.9% over all muscle symptoms than atorvastatin 40–80 mg daily (14.9%) or simvastatin 40–80 mg (18.2%). The 40 mg daily dose of fluvastatin XL in the PRIMO study had an overall myalgia rate of 5.1%. The discussion of the safest statin with fewer statin-associated muscle symptoms still continues today [6].

The pharmacokinetic differences between statins have been well described in the literature [7, 8]. As the newer statins have longer serum half-lives (Table 11.1), leading to the concept of a longer half-life increased the time of the inhibition of HMG Co-A reductase and amplified the LDL-C lowering effect. Cerivastatin was another statin with a half-life of 2–4 hours, similar to simvastatin and pravastatin. However, it was administered using relatively low mg doses compared to other statins. Pharmacokinetic studies of cerivastatin showed that despite a shorter half-life, it had a higher enzyme affinity compared to other statins, fueling an argument that it may be advantageous [9, 10]. Unfortunately, cerivastatin was withdrawn from the US market in 2001 due to a documented increase in deaths and other permanent statin-associated side events, most of which

Table 11.1 Pharmacokinetic properties of statin medications

	Absorption		Distribution		Metabolism			Elimination	
Statin (year approved)	Bioavailability (%)	T_{max} (h)	protein binding (%)	Lipophilicity (log *P*)[a]	CYP hepatic enzyme	Pro-drug	Active metabolite	renal excretion (%)	$t_{1/2}$
Atorvastatin (1996)	14 (decreased with food)	1–2	≥98	4.1	3A4	No	Yes	<2	14
Fluvastatin (1993)	24 (decreased with food)	<1	98	3.2	2C9 (2C8, 3A4 minor)	No	No	5	3[b]
Lovastatin (1987)	<5 (increased with food)	2–4	>95	4.3	3A4	Yes	Yes	10	2–3[b]
Pitavastatin (2009)	43–51	1	99	1.5	2C9 (2C8 minor)	No	No	15	12
Pravastatin (1991)	17 (decreased with food)	1–1.5	50	−0.2	None	No	No	20	1.8[b]
Rosuvastatin (2003)	20	3–5	88	−0.3	2C9	No	Minimal	10	19
Simvastatin (1991)	<5	4	95	4.7	3A4	Yes	Yes	13	2[b]

Ref: web supplement and AHA statement

CYP indicates cytochrome P450, *h* hour, T_{max} time until maximum serum concentration achieved, $t_{1/2}$ drug half-life

[a]Log *P* values that are <1 indicate the statin is hydrophilic and values >1 indicate the statin is lipophilic

[b]Should be dosed in the evening to assure maximal response because of short $t_{1/2}$

were due to a major drug-drug interaction with gemfibrozil. Moreover, in premarketing surveillance, the 80 mg dose of rosuvastatin was abandoned by the manufacturer due to a significantly increased incidence of serious statin-associated muscle symptoms (clinical rhabdomyolysis) with increased creatinine kinase levels [11]. In 2011, the FDA limited the simvastatin maximum dose as a result of data from the SEARCH trial and capped the maximum dose at 40 mg daily and included several simvastatin dose restrictions to mitigate drug-drug interactions [12, 13].

Mechanism of Action

Statins reduce cholesterol biosynthesis and subsequently hepatic cholesterol through competitive inhibition of HMG CoA reductase (Fig. 11.1). Subsequent to the decrease in the production of cholesterol, there is an increased expression of LDL receptors in the liver. These increased receptors pull LDL particles into the hepatocyte, and this results in increased clearance of LDL-C from the blood. In certain patients, statins also can affect the production rate of apoB100 containing lipoproteins resulting in additional reductions in cholesterol and triglyceride levels. The reduction in both LDL-C and triglyceride levels is dose dependent, with greater reductions seen with higher statin doses. Moreover, the potency of statins in their ability to lower LDL-C varies, with atorvastatin and rosuvastatin resulting in the most robust LDL-C reductions.

Pharmacokinetics and Pharmacodynamics

The pharmacokinetics and pharmacodynamics of statins are summarized in Table 11.1. The absorption of most statins is relatively predictable, but food has a significant effect on increasing the bioavailability of lovastatin. Additionally, the absorption of lovastatin is enhanced by splitting the 40 or 80 mg/day dose into twice-daily dosing. Atorvastatin, pitavastatin, and rosuvastatin all have long half-lives and may be taken any time of the day, but other statins should be administered in the evening to maximize reduction in LDL-C due to the diurnal rhythm of HMG CoA reductase in vivo. The long half-life of rosuvastatin can also facilitate the use of nontraditional, off-label use of non-daily dosing (one to three times weekly) in patients who have statin-associated muscle symptoms with daily dosing of statins.

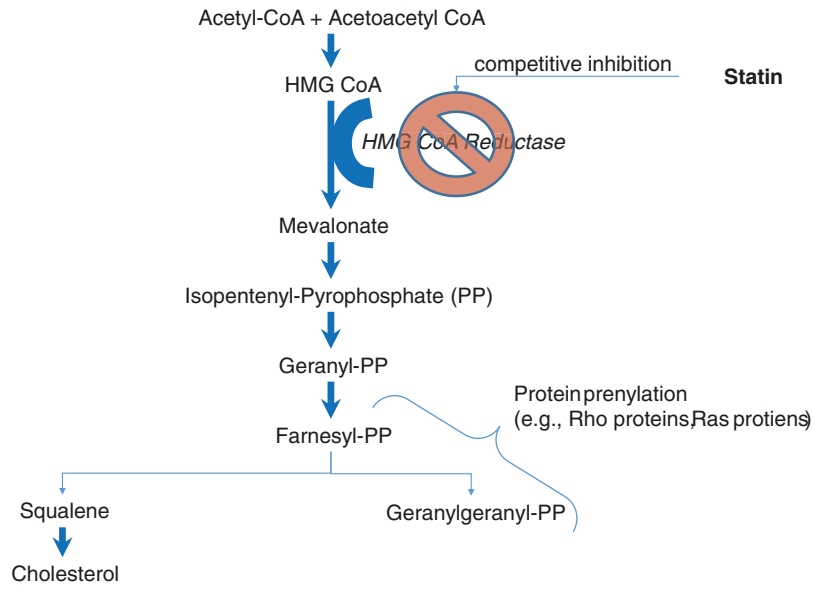

Fig. 11.1 Mechanism of action of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitors (aka statins)

In addition to requiring metabolism through common hepatic cytochrome metabolic enzymes, statins can be either substrates or inhibitors of other enzymes. The general regulation of organic transport proteins (OATP) appears to be multifactorial. Hepatic OATP1B1 and OATP1B3 may be controlled by the liver-enriched hepatocyte nuclear factor 1a (HNF1α). Both transcription (the synthesis of RNA from DNA) and translation (synthesis of a protein from mRNA) may be involved in regulating OATP activity. With respect to drug development, the complexity of OATP regulation requires in vitro measurements of more than one OATP substrates and metabolites. Table 11.2 displays the pertinent transporters involved with respect to statin metabolism.

Asians patients have higher serum levels of statin medications than Caucasians. The FDA has issued caution when treating Chinese patients with simvastatin doses exceeding 20 mg/day administered with niacin. This followed the observation in the Heart Protection Study 2 of the increased risk of myopathy in those taking simvastatin 40 mg administered with niacin-containing products (>1 g/day). The rosuvastatin product labeling notes that higher blood levels are seen in patients of Asian heritage (Filipino, Chinese, Japanese, Korean, Vietnamese, or Asian-Indian). A 5 mg rosuvastatin starting dose may be appropriate for this group. Pitavastatin was approved based on research in Japanese patients. Differences in Japanese and Caucasian pharmacokinetics with pitavastatin are still under investigation. No specific recommendations appear in the pitavastatin labeling. Atorvastatin and fluvastatin offer no current special population warning for Asian patients. Statin product labeling in Asian countries recommends lower doses compared to the US-approved product labeling. Initiation of therapy with low doses of all statins in Asian and Asian-American patients remains the most prudent approach [14].

Of important note, OATP is not the only transporter or enzyme involved in statin metabolism. Table 11.2 shows the full gamut of transporters with respect to the individual statin medication.

Table 11.2 Transporters and enzymes that are involved in statin metabolism and elimination

Statin	Transporters and enzymes involved
Simvastatin Lovastatin	CYP3A4 (intestinal and hepatic) OAT1B1 p-Glycoprotein MDR1 BCRP
Atorvastatin	BCRP (intestinal) CYP3A4 (intestinal and hepatic) OAT1B1 and OAT2B1 p-Glycoprotein
Rosuvastatin	BCRP (intestinal) CYP2C9 (minor) OAT1B1 andOAT1B3 NTCP OAT2B1
Pravastatin	BCRP (intestinal) OAT1B1 and OAT1B3 OAT2B1
Pitavastatin	BCRP (intestinal) MDR1 OAT1B1 andOAT1B3 OATP2B1 CYP2C9 (minor)

Ref: adapted from Harper et al. [55]
Abbreviations: *BCRP* breast cancer resistant protein, *CYP* cytochrome P450, *MDR1* multidrug resistant protein, *OAT* organic anion transporters, *OATP* organic anion-transporting polypeptides, *NTCP* sodium-taurocholate cotransporting polypeptide

Statin-Associated Side Effects

Hepatic

When first introduced to the US market, there were warnings to stop statins when liver function testing revealed serum hepatic transaminase elevations that were at or exceeded three times the upper limit of normal (ULN). Transient elevations in these enzymes were often seen following the initiation of therapy and were a common reason for discontinuance of the medications by primary care providers. In their recent surveillance summary, the FDA reviewed post-marketing data to evaluate the risk of clinically serious hepatotoxicity associated with statins. The FDA had conducted several post-marketing reviews of statins and hepatotoxicity between the years 2000 and 2009 by searching the Agency's Adverse Event Reporting System (AERS) database. Those reviews consistently noted that reporting of statin-associated serious liver injury

to the AERS database was extremely low (reporting rate of ≤2 per 1 million patient-years). The FDA's updated review focused on cases of severe liver injury, defined as a 4 (severe liver injury) or a 5 (death or liver transplant) using the Drug-Induced Liver Injury Network (DILIN) liver injury severity scale, which were reported to AERS from the marketing of each statin through 2009. Cases meeting criteria were identified. Seventy-five cases—27 cases with a severity score of 4 and 48 cases with a severity score of 5 (these included 37 deaths and 11 liver transplants)—were then assessed for causality. Of the 75 cases, 30 cases (14 deaths, 7 liver transplantations, and 9 severe liver injury) were determined as possibly or probably associated with statin therapy. No cases were determined to be highly likely or definitely associated with statin therapy. The FDA concluded that, despite a rising use of statins as a class since the late 1990s, there was not a detectable increase in the annual rates of fatal or severe liver injury cases possibly or probably causally associated with statin use.

The FDA also reviewed cases from the DILIN and Acute Liver Failure Study Group (ALFSG), organizations that have been submitting reports to the FDA of drug-associated liver injury in their liver injury outcome studies. As of January 1, 2011, DILIN had submitted 25 reports of statin-associated liver injury to the FDA, 12 of which gave hospitalization as an outcome. A 2010 article from ALFSG included 133 prospectively identified cases of idiopathic drug-induced liver injury resulting in acute liver failure. Of these 133 patients, 15 were taking statins, and in six of these 15 individuals, a statin was identified as the only potential drug to cause drug-induced liver injury. The most recent FDA and 2018 Cholesterol Guideline recommends baseline liver function testing, but ONLY periodically thereafter if symptoms suggest hepatotoxicity [15, 16]. Other causes of abnormal liver function tests include nonalcoholic fatty liver disease, chronic hepatitis, celiac disease, congestive heart failure, and excessive alcohol use as well as others. Importantly, in patients with most of these conditions, especially nonalcoholic fatty liver disease, statin therapy can still be safely used [16].

Muscle

Statin-associated muscle symptoms (SAMS) include myalgia and/or myositis and are often reasons for a patient to discontinue statin therapy. The definitions associated with muscle abnormalities are important to understand as too often providers discontinue statins labeling the SAMS as rhabdomyolysis when often it is mild to moderate myonecrosis. The National Lipid Association has clarified the definitions as follows [17]:

- Myalgia: often characterized by various complaints including "flu-like" syndrome, aches, soreness, stiffness, tenderness, and cramping with or without exercise.
- Myopathy: defined as muscle weakness, which is not always painful, may or may not be associated with an elevated creatinine kinase.
- Myositis: muscle inflammation can be associated with some of the aforementioned symptoms with varying severity.
- Myonecrosis: present when accompanied by muscle enzyme elevations, typically including hyperCKemia (elevated serum creatinine kinase). The degrees of myonecrosis are mild (3–10 times the ULN or baseline untreated CK level), moderate (10–49 times the ULN or baseline untreated CK level), and severe (≥50 times the ULN or baseline untreated CK level).
- Rhabdomyolysis: to make the critical diagnosis of rhabdomyolysis, there must be myonecrosis with concomitant myoglobinuria or acute renal failure defined as an increase in serum creatinine ≥0.5 mg/dL.

The mechanisms for SAMS are unclear and widely postulated. The possibility of mitochondrial damage that normalizes after statin discontinuation may explain the effect of statins on some patients. However, other data show that muscle structure in the face of elevated creatinine kinase levels remains normal [18, 19]. Statins decrease the production of ubiquinone (CoQ10). This decrease has been postulated as a mechanism of SAMS. Studies suggest that intramuscular concentrations of CoQ10 do not appear to be affected in patients with SAMS [20]. As shown in Fig. 11.1,

statins decrease the production of farnesyl pyrophosphate and geranylgeranyl pyrophosphate. This is hypothesized to result in an imbalance of RAS and Rho. These changes might possibly affect cell maintenance, growth, and apoptosis. It may be that the geranylgeranyl pyrophosphate changes cause these side effects [21]. Another plausible theory centers on impaired calcium signaling. Simvastatin has been shown to trigger mitochondrial depolarization and calcium efflux. The resulting change in mitochondrial function may lead to an elevated cytoplasmic calcium concentration and subsequent calcium waves. Changes in the velocity of calcium waves may affect smooth muscle activity and may contribute to the muscle symptoms associated with statin use [22].

There are other factors associated with SAMs: older age (>80 years old), small body frame and frailty, multiorgan pathologies, large quantities of grapefruit juice, trauma resulting from major surgery, and significant changes in physical activity as well as the use of alcohol [23]. Single-nucleotide genetic polymorphisms in any of the organic transport proteins or other carriers may also explain some of the predisposition to statin-induced muscle symptoms.

Diabetes Mellitus

The first reports of impaired glucose tolerance and increased risk of new-onset diabetes with statin therapy emerged with the publication of the JUPITER trial results [24]. This was followed by two meta-analyses demonstrating an increased risk of new-onset diabetes with statin therapy [25, 26]. The first meta-analysis demonstrated an increased risk when evaluating clinical trials comparing statin to placebo, but this was also seen in clinical trials comparing intensive-dose versus moderate-dose statin therapy. The FDA responded by revising the statin labeling. In the product labeling revision that removed the need for routine liver function monitoring, the FDA summarized the risk for increased glycosylated HbA1c and fasting plasma glucose associated with statins [15]. This FDA product labeling revision went on to note that high-dose atorvastatin was associated with the same clinical effect as rosuvastatin, and finally in a study of postmenopausal women, the clinical issue appeared to be a class effect [15]. This controverted an earlier meta-analysis, which suggested that hyperglycemia was not a class effect of statins [27]. However, the FDA clearly indicated in this labeling revision that the risk of new-onset diabetes is small, and does not outweigh the benefit of reducing CV events.

The mechanism by which statins predispose individuals to diabetes is unclear. The hypotheses revolve around impaired glucose secretion and decreased insulin sensitivity. The decrease in the production of farnesyl pyrophosphate, geranylgeranyl pyrophosphate, and ubiquinone may be the cause of the altered insulin action. Pravastatin is not reported to have the hyperglycemic effect of the other statins based on data from the WOSCOPS study [28]. This may be due to its effect on adiponectin. Adiponectin is a known sensitizing agent found in adipocytes. Pitavastatin also does not appear to have this metabolic adverse effect [29]. Rosuvastatin and simvastatin have been shown to decrease plasma adiponectin levels and insulin sensitivity while pravastatin increased both [30, 31]. In all the clinical trials where statins showed cardiovascular risk reduction, there was no evidence that the effect of statins on atherosclerotic vascular disease was blunted by the development of new-onset diabetes or by any increases in blood glucose levels.

Despite the small increase in the risk of new-onset diabetes, major organizations, including the 2018 Cholesterol Guideline and the American Diabetes Association, strongly recommend statin therapy in eligible patients [16, 32]. These recommendations are steadfast in concluding that benefit outweighs the small risk. To proactively identify this potential risk and to balance this against significant benefits, the 2018 Cholesterol Guideline recommends a patient-clinician discussion and shared decision-making to assure proper explanation of this rather complex patient conversation. Importantly, the excess cases of new-onset diabetes only seem to affect patients with at least one of the four major diabetes risk factors: A1C $\geq$ 6%, obesity, impaired fasting glucose, or metabolic syndrome [16, 33]. For patients who develop new-

onset diabetes after starting statin therapy, the recommendation in the 2018 Cholesterol Guideline is to continue statin therapy [16].

Memory

The topic of memory loss with statins takes on the image of a two-headed monster; do statins cause or do statins protect against memory loss? Single case reports of statin-induced memory loss have been reported to the FDA since the entry of statins to the market. The FDA has recognized this as a possible statin-associated side effect in the revised product labeling in 2011 [15]. However, in a meta-analysis from 2013 that included over 23,000 patients, three clinical trials found no association with statin use and memory loss, and five actually found a favorable effect resulting in a 29% reduction in incident dementia in statin-treated patients [34].

Due to anti-inflammatory effects seen with statin therapy, there has been investigation as to whether or not statins could protect from the development of Alzheimer's disease (AD) [35]. One review evaluated the treatment of AD with atorvastatin and simvastatin. Using standard AD testing, no significant benefit on cognition was seen. These trials did not include patients with hyperlipidemia where there would be a therapeutic indication for the statins. In several large cardiovascular outcome clinical trials, there was no noted evidence of dementia comparing the two treatment arms [36, 37]. The LEADe study was a randomized prospective study that evaluated the safety and efficacy of atorvastatin 80 mg daily versus placebo in 640 patients with mild to probable AD that were on donepezil. There was no improvement in cognition as measured by standard AD testing metrics [38].

In the National Lipid Association taskforce statements regarding statins and memory, the authors conclude that there is no evidence for baseline cognitive testing. The review concluded that there was a lack of substantive evidence to state that statins as a class had adverse effects on cognition. In the event of memory loss, cognitive testing could be performed to determine whether stopping the statin, lowering the dose, or switching from a lipophilic to a hydrophilic statin should be discussed with the patient [39].

Pleiotropic Effects

There has been much speculation about the pleiotropic benefits of statins that are beyond those related to lipid lowering [40]. It has long been postulated that statins may work to improve reduced vascular function. In one experiment on vascular reactivity, 23 patients with elevated cholesterol were randomly assigned to lovastatin 40 mg twice daily or placebo. They were studied 12 days and 6 months after randomization. After intracoronary infusions of acetylcholine, there was paradoxical vasoconstriction in the placebo group, whereas the lovastatin group had preserved or improved the vascular wall reaction to the acetylcholine infusion [41]. The mechanisms are not fully elucidated but have been postulated to involve increased nitric oxide (NO) synthase and subsequent release of NO. The result may be the inhibition of platelet aggregation and alleviation of transient ischemic symptoms [42].

Various uses of statins that capitalize on the anti-inflammatory effects are noted in the literature:

- Breast, colorectal, hepatocellular, and other cancers may benefit from these effects [43].
- High-dose statins are now the mainstay of therapy after a stroke with strong evidence that they can prevent future events especially in very-high-risk populations [44, 45]
- Ischemic bowel disease (IBD) has been of interest in expanding statin usage. It appears that in patients exposed to statin use, there may be a decreased incidence in IBD onset. Markers of inflammation may be reduced when patients are administered a statin. Additionally, glucocorticoid use may be reduced in these patients who are also given statins [46–48].
- There is increased interest in the use of statins to ameliorate symptoms in rheumatoid arthritis (RA). Biomarkers testing may show improved results when patients are given statins [49, 50].

- There may a role for statins to decrease the progression of symptoms in multiple sclerosis (MS). In secondary progressive MS, simvastatin was found to reduce atrophy by 43% [51]. Relapsing-remitting MS may not have consistent results when co-administered with other anti-inflammatory agents [52].

Over-the-Counter Statins

In the year 2000, the FDA convened a meeting to review applications from both Bristol Myers Squibb to market pravastatin 10 mg and Merck to market lovastatin 10 mg as over-the-counter (OTC) agents. Both manufacturers sought approval for treating patients with total cholesterol levels of 200–240 mg/dL and low-density lipoprotein (LDL) levels ≥130 mg/dL. The proposed OTC lovastatin indication was for men aged ≥40 years and postmenopausal women who did not have established cardiovascular disease or diabetes. The proposed pravastatin OTC indication would have been for individuals who did not have established cardiovascular disease or diabetes. This attempt and several more in the years to come were rejected by the FDA who cited the need for monitoring by a medical professional, despite analyses suggesting cost-effectiveness [53].

Drug-Drug Interactions

Various transporters can influence the drug-drug interaction profile of statins. As noted in Table 11.3, drugs can be either substrates or inhibitors of transporters involved with drug metabolism. The organic anion-transporting polypeptide (OATP) transporters are often at the same time inhibitors or inducers of CYP enzymes and other transporters. Isolation of specific OATP actions is therefore difficult.

Gemfibrozil and gemfibrozil-glucuronide are known inhibitors of OATP1B1 and OATP2B1. Co-administration of gemfibrozil with substrate drugs of OATP1B1 and OATP2B1 can decrease the hepatic clearance of statins and, specific to OATP2B1, can result in alterations of intestinal drug absorption. The combination of gemfibrozil with statin therapy (especially cerivastatin) has been shown to result in decreased metabolism and/or reduction of hepatic uptake of statin therapy, ultimately resulting in rhabdomyolysis and renal failure in some patients [54].

Rifampin is transported by OATP1B1 and OATP1B3. Rifampin induces metabolic enzymes and transporters via binding to the pregnane-x-receptor (PXR), which can activate CYP3A4. Statins such as atorvastatin and simvastatin when co-administered with rifampin can result in decreased concentration of the acid metabolite and decrease in area under the curve (AUC) of these statins. Due to the dual interaction mechanism of rifampin, simultaneous co-administration of atorvastatin with rifampin is recommended, as delayed administration of atorvastatin after administration of rifampin has been associated with a significant reduction in atorvastatin plasma concentrations.

Cyclosporine has a demonstrable effect when given with statins. Cyclosporine is both an inhibitor of OATP2B1 and OATP1B1, as well as a substrate for CYP3A4. This dual effect can result in either

Table 11.3 Summary of transporters for statins in the body

Transporter (gene)	Substrate	Inhibitor	Where located
OAT1B1 aka OATP-C or OATP2 (SLCO1B1)	Statins and other drugs	Cyclosporine, rifampicin, and some protease inhibitors	Hepatocytes (sinusoidal)
OAT1B3 aka OATP-8 (SLCO1B3)	Statins and other drugs	Same as above	Hepatocytes (sinusoidal)
OATP1A2 aka OATP-A (SLCO1A2)	Statins and other drugs	rifampicin and some protease inhibitors	Brain capillaries, endothelia, cholangiocytes, distal nephron

Abbreviations: *OAT* organic anion transporters, *OATP* organic anion-transporting polypeptides, *SLCO* solute carrier organic anion transporter family member

inhibition or increased exposure to CYP3A4 substrates. The third possible mechanism is increased hepatocellular accumulation. Cyclosporine also interacts with intestinal ABCB1 or ABCC2. Drugs co-administered with cyclosporine can show enhanced intestinal absorption and enhanced clinical effects.

Drug interactions between statins and antiretroviral medications are challenging.

When given with elvitegravir/cobicistat, atorvastatin has a clinically significant interaction in which the AUC for atorvastatin can increase 2.6-fold. Atorvastatin should not exceed 20 mg daily when given with this combination. Rosuvastatin has also been known to have a similar pharmacokinetic effect when co-administered with elvitegravir/cobicistat.

Protease inhibitors can significantly inhibit statin metabolism. When given with lopinavir/ritonavir, the AUC of rosuvastatin has been shown to increase up to twofold. Therefore, doses of rosuvastatin should be limited to 10 mg daily when given with protease inhibitors. Simvastatin (and lovastatin) is contraindicated with all protease inhibitors.

New drugs for human immunodeficiency virus (HIV) infection emerge on the market every year. Either OATP or interaction with CYP hepatic enzymes can often lead to clinically significant drug-drug interactions. The National Institutes of Health (NIH) publishes an up-to-date list of drug-drug interactions with antiretroviral drugs, and this resource should be consulted when selecting statin therapy in patients with HIV infection. https://aidsinfo.nih.gov/guidelines/search?q=drug[space]interaction&c=guidelines&htmldocId=1&startAt=0. Refer to Table 11.4 for a more comprehensive reference for statin drug interactions.

Table 11.4 Statin drug interactions

	Level 1 (severe)	Level 2 (major)	Level 3 (moderate)
	"Do not use"	"Use with caution"	"Less likely to cause severe drug interaction"
Simvastatin/Lovastatin	Protease inhibitors Amprenavir Atazanavir Boceprevir Clarithromycin Cobicistat Conivaptan Cyclosporine Danazol Delavirdine Darunavir Elvitegravir Emtricitabine Erythromycin Gemfibrozil Idelalisib Itraconazole Ketoconazole Mifepristone Nefazodone Nelfinavir Ombitasvir Paritaprevir Posaconazole Red yeast rice Ribociclib Telaprevir Telithromycin Tenofovir Tipranavir Voriconazole	Aliskiren Amiodarone Amlodipine Ceritinib Diltiazem Dronedarone Everolimus Fenofibrate Fenofibric acid Fluconazole Glecaprevir Grapefruit juice Imatinib Lanthanum Carb. Leflunomide Letermovir Lomitapide Pibrentasvir Ranolazine Sirolimus Tacrolimus Teriflunomide Ticagrelor Tolvaptan Troleandomycin Verapamil	Aprepitant Crizotinib Efavirenz Esomeprazole Fluvoxamine Fosnetupitant Fosphenytoin Lansoprazole Netupitant Niacin Niacinamide Omeprazole Pantoprazole Palonosetron Phenytoin Quinine Repaglinide Rifampin St. John's wort Warfarin

(continued)

Table 11.4 (continued)

	Level 1 (severe)	Level 2 (major)	Level 3 (moderate)
	"Do not use"	"Use with caution"	"Less likely to cause severe drug interaction"
Atorvastatin	Dasabuvir Ombitasvir Paritaprevir Ritonavir Posaconazole Red yeast rice Telithromycin Voriconazole	Atazanavir Boceprevir Ciprofloxacin Clarithromycin Cobicistat Conivaptan Cyclosporine Dasabuvir; Darunavir Delavirdine Digoxin Diltiazem Enzalutamide Erythromycin Fluconazole Fosamprenavir Gemfibrozil Glecaprevir Grapefruit juice Idelalisib Imatinib Indinavir Itraconazole Ketoconazole Leflunomide Letermovir Lopinavir/Ritonavir Mitotane Nefazodone Nelfinavir Other fibrates Ombitasvir Paritaprevir Pibrentasvir Ribociclib Saquinavir Telaprevir Telithromycin Teriflunomide Tipranavir Velpatasvir Verapamil Voriconazole	Aliskiren Amiodarone Antacids Apalutamide Aprepitant Fosaprepitant Atazanavir Bosentan Brigatinib Ceritinib Colchicine Colestipol Daclatasvir Dalfopristin/Quin. Danazol Daptomycin Dronedarone Efavirenz Elbasvir Eliglustat Eltrombopag Emtricitabine Etravirine Everolimus Esomeprazole Fosphenytoin Grazoprevir Hydantoins Isoniazid Isavuconazonium Ivacaftor Lansoprazole Ledipasvir Lumacaftor Mifepristone Niacin Niacinamide Nilotinib Omeprazole Oritavancin Oxcarbazeine Pazopanib Quinine Raltegravir Ranolazine Riluzole Rifampin Sarilumab Sapropterin Simeprevir Sirolimus St. John's Wort Talazoparib Telbivudine Tenofovir Temsirolimus Tocilizumab Vemurafenib Warfarin

Table 11.4 (continued)

	Level 1 (severe)	Level 2 (major)	Level 3 (moderate)
	"Do not use"	"Use with caution"	"Less likely to cause severe drug interaction"
Rosuvastatin	Red yeast rice	Antacids Atazanavir Cimetidine Clarithromycin Cobicistat Cyclosporine Darunavir Fenofibrate Fenofibric acid Fosamprenavir Glecaprevir Gemfibrozil Lanthanum Carbonate Ledipasvir Leflunomide Lopinavir/Ritonavir Nelfinavir Ombitasvir Pibrentasvir Ponatinib Paritaprevir Ritonavir Saquinavir Simeprevir Sofosbuvir Telithromycin Teriflunomide Tipranavir Tolvaptan Velpatasvir	Clofarabine Clopidogrel Colchicine Daclatasvir Daptomycin Darunavir Elagolix Elbasvir Eltrombopag Eluxadoline Elvitegravir Etravirine Fostamatinib Grazoprevir Indinavir Itraconazole Letermovir Niacin Niacinamide Obeticholic acid Oritavancin Osimertinib Ponatinib Raltegravir Regorafenib Rolapitant Safinamide Simeprevir Tacrolimus Tedizolid Telaprevir Telbivudine Warfarin
Pravastatin	Red yeast rice	Bile acid resins Cimetidine Clarithromycin Cobicistat Cyclosporine Darunavir Erythromycin Fenofibrate Fenofibric acid Gemfibrozil Glecaprevir IdelalisibLanthanum Carbonate Leflunomide Ombitasvir Paritaprevir Pibrentasvir Ritonavir Telithromycin Teriflunomide Tolvaptan	Atazanavir Azithromycin Boceprevir Clofarabine Colchicine Daclatasvir Daptomycin Eltrombopag Erythromycin Etravirine Everolimus Itraconazole Letermovir Niacin Niacinamide Rifampin Orlistat Simeprevir Sirolimus Tacrolimus Telbivudine Voxilaprevir Warfarin

(continued)

Table 11.4 (continued)

	Level 1 (severe)	Level 2 (major)	Level 3 (moderate)
	"Do not use"	"Use with caution"	"Less likely to cause severe drug interaction"
Fluvastatin	Red yeast rice	Cobicistat Ceritinib Cimetidine Cyclosporine Erythromycin Fenofibrate Fenofibric acid Gemfibrozil Glecaprevir Lanthanum Carbonate Leflunomide Fluconazole Mifepristone Pibrentasvir Telithromycin Teriflunomide Tolvaptan	Amiodarone Amprenavir Anti-retroviral protease inhibitors Atazanavir Capecitabine Cobicistat Cholestyramine Cimetidine Clarithromycin Colchicine Darunavir Daptomycin Deferasirox Delavirdine Diclofenac Digoxin Dronabinol Efavirenz Eltrombopag Elvitegravir Ethanol Etravirine Everolimus Fluoxetine Fluvoxamine Fosamprenavir Fosphenytoin Glimepiride Glyburide Grazoprevir Imatinib Indinavir Isoniazid Lesinurad Letermovir Niacin Niacinamide Nelfinivir Nilotinib Ombitasvir Omeprazole Oritavancin Paritaprevir Phenytoin Raltegrivir Ranitidine Rifampin Ritonavir Rucaparib Saqinavir Simeprevir Sirolumus Sulfinpyrazone Tacroliumus Telaprevir Telbivudine Terbinafine Tipranavir Vemurafenib Voxilaprevir Voriconazole Warfarin

Table 11.4 (continued)

	Level 1 (severe)	Level 2 (major)	Level 3 (moderate)
	"Do not use"	"Use with caution"	"Less likely to cause severe drug interaction"
Pitavastatin	Cyclosporine	Cobicistat Erythromycin Everolimus Fosamprenavir Gemfibrozil and other fibrates Glecaprevir Letermovir Lopinavir Ritonavir Rifampin Ritonavir Saquinavir Sirolimus Tacrolimus Telithromycin Tipranavir Tolvaptan	Abiraterone Capecitabine Clofarabine Clopidogrel Colchicine Eltrombopag Oritavancin Raltegravir Simeprevir Telaprevir

Source package labeling from https://www.accessdata.fda.gov/scripts/cder/daf/#labeli

Summary

Statins have an extensive evidence base that demonstrates reduced cardiovascular morbidity and mortality. While many statins have potential drug-drug interactions that can result in clinically significant changes in pharmacodynamics and pharmacokinetic, they are the foundation of pharmacologic therapy for most patients with hypercholesterolemia according to the 2018 Cholesterol Guidelines [16]. Given the generic availability of nearly all of the statins (only pitavastatin is a brand name), their clinical use is widespread with the hope that increased use and implementation of guideline-directed medical therapy will continue to reduce the incidence of atherosclerotic cardiovascular disease.

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Management of Statin Intolerance

12

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Abbreviations

ACL	ATP-citrate lyase
ACS	Acute coronary syndromes
ACSVL1	Very-long-chain acyl CoA synthetase-1
ASCVD	Atherosclerotic cardiovascular disease
CAD	Coronary artery disease
CHD	Coronary heart disease
CK	Creatine kinase
CKD	Chronic kidney disease
CVD	Cardiovascular disease
DM	Diabetes mellitus
EAS	European Atherosclerosis Society
FABP3	Fatty acid-binding protein 3
FOURIER	Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk
HDL-C	High-density lipoprotein cholesterol
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme A
ILEP	International Lipid Expert Panel
LDL-C	Low-density lipoprotein cholesterol
Lp(a)	Lipoprotein(a)
MI	Myocardial infarction
MLC1	Myosin light chain 1
NLA	National Lipid Association
NOD	New-onset diabetes
ODYSSEY Outcomes	Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab
PCSK9	Proprotein convertase subtilisin/kexin type 9
RCTs	Randomized controlled trials
SAMS	Statin-associated muscle symptoms
SMCI	Statin Myalgia Clinical Index
TG	Triglycerides
ULN	Upper limit of normal
USAGE	The Understanding Statin Use in America and Gaps in Patient Education

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_12

Introduction (Fig. 12.1)

Statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) are the first-line lipid-modifying drugs for the primary and secondary prevention of cardiovascular diseases (CVD) in patients with acute coronary syndromes (ACS), dyslipidemia, coronary artery disease (CAD), hypertension, diabetes mellitus (DM), stroke, and chronic kidney disease (CKD), irrespective of cholesterol levels [1]. Lowering low-density lipoprotein cholesterol (LDL-C) by 38.7 mg/dl (1.0 mmol/l) results in 21% decrease in CVD morbidity and mortality [1], and therapy is generally well tolerated [2]. Nevertheless, statin therapy is associated with adverse effects in some patients [3, 4]. Muscle-related side effects are the most frequently reported complication – even in 29% of patients on statins [5] – and are the most common reason for discontinuation statin therapy [6]. Some reports suggest that discontinuation rates can be very high, with up to 50% of patients with coronary heart disease (CHD) discontinuing after 1 year [6]. Cessation of statin therapy leads to an elevated risk for acute CV events, CHD, and recurrent myocardial infarction (MI) [7]. Thus, it is essential that statin intolerance can be accurately diagnosed, that its risk factors are understood, and that physicians know how to manage the condition. Many patients, even over 90%, who initially present with some symptoms of statin intolerance can, in fact, take a statin. This may be achieved by switching to a different statin or altering the dosing regimen. However, if patients cannot tolerate any statin or the maximally tolerated dose does not enable lipid-targets to be reached, non-statin drugs and nutraceuticals should be considered as monotherapy or as an add-on to statins [2, 8].

Fig. 12.1 Professor Banach is the founder and president of the International Lipid Expert Panel (ILEP), Dr. Bartlomiejczyk is the secretary, and Dr. Penson is a member: www.ilep.eu

Defining and Diagnosing Statin Intolerance

Definition of Statin Intolerance

Despite the numerous well-documented advantages of statin therapy, approximately 20% of patients are unable to take their prescribed daily dose of statin because of some degree of intolerance [9], and 40%–75% stop therapy within 2 years [7]. However, there is no universally accepted definition of statin intolerance, and the term has been used in several different ways and for different purposes. In many cases, clinical trials of non-statin lipid-lowering agents such as proprotein convertase subtilisin/kexin type 9 (PCSK9) recruit patients who are unable to take statins and thus have some definition if statin intolerance inherent in their inclusion criteria. Such approaches are summarized in Table 12.1. Sullivan et al. in the GAUSS trial with evolocumab defined statin intolerance based upon the inability to take 1 statin (at a dose specified for each statin) because of intolerable myalgia or myopathy, which resolved upon statin discontinuation [10]. The definition was simplified and refined in the GAUSS-2 [13] and GAUSS-3 trials [12]; a similar approach was taken in the ODYSSEY ALTERNATIVE trial of alirocumab [13] (Table 12.1). In the ongoing CLEAR-OUTCOMES trial with bempedoic acid, the definition of statin intolerance is even easier, as statin intolerance is defined as an inability to tolerate more than two statins at any dose or one statin at any dose and unwilling to attempt a second or advised by a physician not to attempt a second statin.

More formal approaches to the definition of statin intolerance are summarized in Table 12.2.

Table 12.1 Definition of statin intolerance used in clinical trial inclusion criteria

Research trial name (Ref #)	Proposed definition of statin intolerance
GAUSS trial with evolocumab [10]	"Inability to tolerate at least 1 statin at any dose or an increase in dose above weekly maximums of 35 mg rosuvastatin; 70 mg atorvastatin; 140 mg simvastatin; 140 mg pravastatin; 140 mg lovastatin; or 280 mg fluvastatin, because of intolerable myalgia (muscle pain, soreness, weakness, or cramps) or myopathy (myalgia plus elevated CK) and having symptom improvement or resolution with statin discontinuation"
GAUSS-2 trial [11]	"Inability to tolerate ≥2 statins at any dose or increase the dose above the smallest tablet strength because of intolerable muscle-related side effects, which resolved or improved significantly upon dose decrease or discontinuation"
GAUSS-3 [12]	"Inability to tolerate atorvastatin at an average dose of 10 mg/day and unable to tolerate any other statin at any dose due to skeletal muscle-related symptoms OR the inability to tolerate at least three statins – one statin at the lowest starting average daily dose and any other two statins at any dose due to skeletal muscle-related symptoms OR documented history of CK elevation >10 times the ULN accompanied by muscle symptoms while on statin therapy, which resolved upon discontinuation AND symptoms resolved or improved when statin dose was decreased or discontinued"
ODYSSEY Alternative [13]	"Inability to tolerate at least two different statins because of unexplained skeletal muscle-related symptoms, such as pain, aches, weakness, or cramping that began or increased during statin therapy and returned to baseline when statin therapy was discontinued. For each patient to meet this definition, one of the statins that was discontinued had to have been at the lowest approved daily starting dose (i.e., 5 mg rosuvastatin, 10 mg atorvastatin, 10 mg simvastatin, 20 mg lovastatin, 40 mg pravastatin, 40 mg fluvastatin, 2 mg pitavastatin); the other statin was at any dose"

Table 12.2 Formal definitions of statin intolerance

Society, year (Ref #)	Definition of statin intolerance
National Lipid Association (NLA), 2014 [14]	"Inability to tolerate at least two statins: one statin at the lowest starting daily dose and another statin at any daily dose, due to either objectionable symptoms (real or perceived) or abnormal laboratory determinations, which are temporally related to statin treatment and reversible upon statin discontinuation"
European Atherosclerosis Society (EAS), 2015 [15]	"The assessment of statin-associated muscle symptoms (SAMS) includes the nature of muscle symptoms, increased creatine kinase levels and their temporal association with initiation of therapy with statin, and statin therapy suspension and rechallenge"
International Lipid Expert Panel (ILEP) Unified Definition, 2015 [16, 17]	1. The inability to tolerate at least two different statins – one statin at the lowest starting average daily dose and the other statin at any dose 2. Intolerance associated with confirmed, intolerable statin-related adverse effect(s) or significant biomarker abnormalities 3. Symptom or biomarker changes resolution or significant improvement upon dose decrease or discontinuation 4. Symptoms or biomarker changes not attributable to established predispositions such as drug-drug interactions and recognized conditions increasing the risk of statin intolerance.
Canadian Consensus Working Group, 2016 [18]	"A clinical syndrome, not caused by drug interactions or risk factors for untreated intolerance and characterized by significant symptoms and/or biomarker abnormalities that prevent the long-term use and adherence to statins documented by challenge/dechallenge/rechallenge, where appropriate, using at least two statins, including atorvastatin and rosuvastatin, and that leads to failure of maintenance of therapeutic goals, as defined by national guidelines" Current Guidelines [15, 19, 20]

In June 2014, the National Lipid Association (NLA) Expert Panel on Statin Intolerance suggested the first formal definition. Later, the International Lipid Expert Panel (ILEP) developed a universal definition which defined statin intolerance as (a) the inability to tolerate at least two different statins – one statin at the lowest starting average daily dose and the other statin at any dose (the lowest starting daily doses are rosuvastatin (5 mg), simvastatin (10 mg), atorvastatin (10 mg), lovastatin (20 mg), fluvastatin (40 mg), pravastatin (40 mg), and pitavastatin 2 mg)), (b) intolerance associated with confirmed, intolerable statin-related adverse effect(s) or significant biomarker abnormalities, (c) symptom or biomarker changes resolution or significant improvement upon dose decrease or discontinuation, and (d) symptoms or biomarker changes not attributable to established predispositions such as drug-drug interactions and recognized conditions increasing the risk of statin intolerance (Table 12.2) [16, 17].

This approach seeks to distinguish between perceived effects, which may result from the drucebo effect [21], and genuine pharmacological effects of statins. By promoting the consideration of and, where appropriate, correction of factors predisposing patients to, the ILEP definition involved changes in biomarkers or resolution of symptoms which are not attributable to predispositions, increasing the risk of statin intolerance [16, 17]. Subsequently, the European Atherosclerosis Society (EAS) proposed its own definition (Table 12.2). Finally, in 2016, the Canadian Consensus Working Group proposes a pragmatic definition, which defines statin intolerance as occurring when adverse effects of statin therapy prevent lipid targets being met, according to current clinical guidelines (Table 12.2). This is important, because it recognizes the importance of partial intolerance, whereby patients can tolerate some statins at some doses [18, 22] but may not be able to receive optimal lipid-lowering therapy. Most patients who experience statin intolerance have partial intolerance, and so, this is an important group to consider. Only a relatively small number of individuals (3%–5%) have complete statin intolerance, whereby they cannot take any statin at all without severe adverse effects.

Symptoms of Statin Intolerance

The ILEP definition of statin intolerance encompasses both patient-reported and biochemically confirmed adverse effects [16, 17]. Most reports of statin intolerance symptoms are mostly related to patient-reported adverse effects, which can never be confirmed by blood tests [23]. The most specific adverse effects of statin treatment are statin-associated muscle symptoms (SAMS) including myalgias (3–20%), myopathy (0.1–1.5%), and rhabdomyolysis (0.01%). Statins have also been causally linked with new-onset diabetes (NOD) [23]. Risk of NOD is associated not only with patient predispositions (hyperglycemia, overweight, low high-density lipoprotein cholesterol (HDL-C), high triglycerides (TG), elevated blood pressure, carbohydrate metabolism disorders, insulin resistance) but also with time and intensity of statin treatment. However, the JUPITER study demonstrated that the benefits of statin therapy outweigh the risks, even if NOD develops and that therapy with rosuvastatin accelerated diagnosis of NOD by an average of only 5.4 weeks compared to placebo [24].

SAMS is classified by NLA as four distinct entities – myopathy, myalgia, myonecrosis, and myositis – and is one of the most common reasons for stopping statin therapy [19]. In 90% of cases, it appears within 6 months of the start of statin therapy (or change of dose), and in 75% of cases, it occurs within 10–12 weeks [25]. Myalgia manifests as muscle pain without changes in creatine kinase (CK) levels [19]. Normal or higher CK level and muscle weakness (without pain) are symptoms of myopathy [26]. Several groups of patients are predisposed to the occurrence of myopathy including patients over 75 years, in females, those with low body mass index, those with hepatic and renal dysfunction, and those with a genetic susceptibility. Risk is elevated in the perioperative period and by alcohol abuse, extreme-intensity exercise, hypothyroidism, and concomitant use of drugs which affect the metabolism of statins [27–29]. Myositis (muscle inflammation) is associated with CK elevation and leukocyte infiltration into muscle tissue. Extreme serum concentrations of CK associated

with muscle injury are symptoms of myonecrosis [26] where the most serious form is rhabdomyolysis (1.6 per 100,000 patients). It is manifested with very high levels of CK and myoglobin (as a result of muscle breakdown) causing acute renal failure and myoglobinuria [26].

Biomarkers of Statin Intolerance

There are currently no effective biomarkers of statin intolerance. Currently available biomarkers cannot be commonly used because of costs, complexities in methodology, and undefined specificity and sensitivity [5]. The most commonly used serum marker is CK [30]; however, it is not specific to myopathy and can be elevated by exercise, genetic variants, drug interaction, and deficiencies of vitamin D or coenzyme Q10 (CoQ10). Analysis of liver function is no longer recommended because of cost and low diagnostic yield. What's more, liver abnormalities caused by statins are rare, dose-related, and mild, and it is possible to return to baseline levels after 2–4 weeks. Finally, in most of the liver disease, statins should be not only used but also recommended, as they significantly reduce the risk of CVD events, mortality, and even primary liver cancer (the only contraindication are acute liver diseases) [31, 32].

Lactate dehydrogenase has been suggested as a potential early biomarker on the basis of *in vitro* experiments, but its usefulness has not been validated in myopathy induced by statins [33]. Other biomarkers of skeletal muscle toxicity under investigation include (myosin light chain 1 (MLC1) and fatty acid-binding protein 3 (FABP3)) [33, 34] and microRNA 133a/b and 499-5p [35, 36].

Diagnosis of Statin Intolerance

SAMS are the most common related to statin side effects. Rosenson et al. proposed a Statin Myalgia Clinical Index (SMCI) [37] that might be useful when identifying statin-associated muscle symptoms in patients suffering from statin intolerance. The SMCI is focused on the location of muscle pain, its symmetry (bilateral or unilateral), time of occurrence the symptoms (similar to the time the treatment started), time/degree of improvement after stopping statins, and return of symptoms after returning to therapy. The SMCI score provides an intuitive method to help diagnose whether muscle symptoms are related to statins and further large-scale evaluation of its usefulness is warranted [38]. Correct classification and diagnosis of the side effects of statin-treated patients are crucial for effective diagnosis of statin intolerance. Effective diagnosis requires recognition of the symptoms of statin intolerance and enables a rational approach to the management of the symptoms. Banach and Mikhailidis proposed a simplified four-step diagnosis scheme for statin intolerance [8] (Table 12.3).

Diagnosis of statin intolerance resulting from SAMS is challenging because fatigue, muscle pain, and nocturnal cramps are common among the population; thus, errors in diagnosis are not rare. Joint pain is often misattributed to statins [45–47]. Myopathy induced by statins is usually symmetrical and bilateral, affects large groups of muscles, and can be exacerbated by exercise (therefore, each patient that is adherent to lifestyle changes at the time of initiation or statin dose increase should be asked for regular exercises) [19]. Particular attention should also be paid to muscle symptoms if they appear weeks or months after increase in statin dose (or the initiation of therapy). Such symptoms are more likely to be caused by statin therapy than those which begin within days of commencing treatment or those which occur after a long duration of treatment [25, 48]. It can be useful to consider "Koch's postulates" of causality and leave off statin treatment for 2–4 weeks to investigate whether the symptoms are temporally related to drug exposure (drug dechallenge) [39]. Common errors in the diagnosis of statin intolerance result from incorrectly making a diagnosis based on false assumptions made by the patient. The Understanding Statin Use in America and Gaps in Patient Education (USAGE) recommends that patients are informed about the benefits and side effects of statin treatment to improve adherence and outcomes [49].

Table 12.3 Four-step diagnosis of patients with statin intolerance [8]

Step	Issue to investigate	Explanation	Reference [#]
1	Ask when statin treatment started or whether the dose has been increased over the past few weeks?	Over 75% of symptoms usually develop in the first 12 weeks and nearly 90% within first 6 months. Thus, patients taking statins for years are less likely to have statin intolerance. Only if a new external factor did not appear	[16, 17]
2	Ask about family history and check for conditions causing increased risk of statin intolerance	SAMS may be caused by (the most influential factors): 1. Hypothyroidism/hyperthyroidism 2. New intensive exercise (e.g., with the initiation of statin therapy when there is also a recommendation for lifestyle changes) 3. Vitamin D deficiency (especially in countries with limited sun access annually) 4. Concomitant therapy (e.g., antibiotics, calcium antagonists, some antifungal medications, HIV protease inhibitors, and/or medicines link: amiodarone, ranolazine, cyclosporine, danazol) 5. Family history (genetic predisposition).	[16, 17, 39–42]
3	Examine if muscle symptoms are caused by statins and eliminate nocebo effect	At the beginning, use the SAMS-Clinical Index to check whether muscle pain is statin-related. SAMS symptoms (e.g., large muscle symmetric aches, bilateral aches of the smaller distal or proximal musculature) are different then non-statin-related myalgia (e.g., groin pain, whole-body fatigue). Therefore, it is very important to perform a patient examination with emphasis on the character of muscle pain	[5, 16, 17, 21, 37, 41]
4	Ask about the acceptability of the symptoms and clearly highlight the benefits of statin therapy	Check for CK (so far the only confirmed and effective predictor which can be used in everyday clinical practice), and follow principal rules based on recent guidelines: 1. If muscle pain at CK higher or equal to 4 ULN occur, statin treatment should be suspended until the CK normalization and regression of pain (usually 4–6 weeks) 2. If muscle pain is tolerable and CK less than 4 ULN, statin treatment with reduced dose (and CK monitoring) should be continued, but if CK concentration is increased and/or muscle pain (or other clinical symptoms) is exacerbated, statin treatment should be stopped till the regression of pain and CK normalization (usually 4–6 weeks) 3. If muscle pain is intolerable at CK less than 4 ULN, statin treatment should be stopped till the regression of pain and CK normalization (usually 4–6 weeks)	[27, 42–44]

Managing Statin Intolerance

The initial approach to a patient with suspected statin intolerance should be to conduct a careful interview with the patient about the tolerability of their muscle symptoms. If the patient is able to tolerate their symptoms, the treatment should be continued, because the symptoms may well be temporary and disappear after 2–4 weeks [16, 17]. Simultaneously, the patient should be carefully observed in order to detect any worsening of symptoms or biochemical results. It is essential to make patients aware of the benefits of statin therapy and the fact that CV risk is elevated after stopping treatment or when treatment targets are not met [8]. This is important because patients are often well informed about the side effects of statin therapy from the mass media, but they are not aware of treatment benefits to the same extent. This makes patients susceptible to the drucebo effect. Drucebo (a combination of DRUg and plaCEBO or noCEBO) introduced by Banach and

Penson on behalf of ILEP relates to beneficial or (as in the case of statins) adverse effects of a drug, which result from expectation and are not pharmacologically caused by the drug. A recent systematic review of randomized controlled trials of statin therapy, which had a blinded phase and an open-label period of statin therapy, found that between 38% and 78% of statin adverse effects could be attributed to the drucebo effect [21]. This result is consistent with findings that, after implementing the four-step diagnosis for patients with statin intolerance and after applying different management methods (combination therapy, statin replacement, dose reduction, replacement therapy), more than 90% of patients initially reporting with intolerance can be still treated with statins. Therefore, when treatment has been interrupted owing to adverse effects, it is important to "rechallenge" patients with statins to identify which statins and which dose of those statins they can tolerate [8]. With this in mind, Banach and Mikhailidis outlined practical tips to optimize lipid-lowering therapy in patients with some degree of statin intolerance [8]. Strategies included employing statin therapy at a reduced dose or dosing on alternate days, changing to a different statin, and employing combination therapy with ezetimibe, PCSK9 inhibitors, other lipid-lowering drugs, or nutraceuticals.

Switching Between Statins

Mechanisms and symptoms of statin intolerance vary between individuals. In some cases, the intolerance may be a "class effect" (patient is intolerant to any statin at any dose). However, in many cases, the intolerance may be to a particular drug (or even a particular formulation of a drug). Rechallenging a patient with a different statin after a break in treatment may be sufficient to resolve the symptoms of intolerance. Changing from one statin to another may resolve symptoms, which result from individual variations in metabolism or drug-drug interactions or because of the physiochemical properties of particular drugs. Atorvastatin, simvastatin, fluvastatin, lovastatin, and pitavastatin are lipophilic, whereas pravastatin and rosuvastatin are hydrophilic (hydrophilic statins seem to be better tolerated, and, e.g., they are recommended in the elderly patients who are at the higher risk of statin intolerance). From an empirical point of view, it would seem rational to try a drug with different properties to that which caused symptoms [2, 15–17]. The patient may be reassured by the fact that they are receiving a different drug – thus lessening the likelihood of the drucebo effect [21].

Reduced Dose or Alternate Dose Strategies

Pharmacological and toxicological responses to statins appear to be dose-dependent; therefore, dose reduction is a logical approach to managing statin-induced adverse effects [50]. A practical approach to dose reduction is alternate-day dosing of statins. This approach appears to have merit. A meta-analysis of randomized controlled trials (RCTs) and quasi-RCTs was recently conducted to synthesize evidence about the efficacy and safety of alternate-day vs daily dosing of statins. A total of 13 studies and 1023 patients were included in the analysis. Pooled analysis revealed no statistically significant difference between alternate-day and daily regimens of atorvastatin and rosuvastatin in terms of change in LDL-C and TG ($p > 0.05$). Further work is required to investigate the long-term outcomes of this approach, and combination therapy with other lipid-lowering agents may be necessary when targets cannot be met with reduced-dose strategies.

Use of Other Lipid-Lowering Drugs

When lipid-lowering targets cannot be met by statins alone, other lipid-lowering drugs should be considered in order to reduce the patient's cardiovascular risk. In the case of partial statin intolerance, other drugs can be used in combination with statins. When complete statin intolerance occurs, other drugs can be used in monotherapy

(or in combinations which exclude statins). Nutraceuticals may also be useful in helping to reach lipid-lowering targets. Alternative approaches to lipid-lowering, which may be beneficial in statin intolerance are outlined below.

Ezetimibe

Ezetimibe reduces the intestinal absorption of cholesterol by blocking the Niemann-Pick C1-like 1 protein on epithelial cells. It causes a modest reduction in LDL-C (15–20%) in comparison with statins. In the IMPROVE-IT study, ezetimibe was demonstrated to reduce cardiovascular events in combination with statin therapy [51]. Thus, ezetimibe is an excellent choice as an add-on when treatment targets cannot be met with statin therapy. Ezetimibe may also be useful as monotherapy (for example, when a patient suffers complete statin intolerance). As monotherapy, ezetimibe effectively lowers LDL-C [52] and Lp(a) (despite some controversies on these data) [53], although outcome data are still lacking, and where they are available, PCSK9 inhibitors might be preferred in this situation, owing to their superior lipid-lowering properties.

PCSK9 Inhibition by Monoclonal Antibodies

PCSK9 is a protein which binds to LDL-receptors on the surface of hepatocytes and marks them for internalization. Inhibition of PCSK9 increases LDL-receptor density and results in improved clearance of LDL from plasma. Monoclonal antibodies against PCSK9 (alirocumab and evolocumab) markedly reduce LDL-C (even over 60%) and present an enormous advance in the treatment of dyslipidemias, particularly in individuals with statin intolerance [54–56]. Importantly, these drugs have been investigated in the setting of statin intolerance with very encouraging results. Patients with confirmed statin intolerance were recruited into the GAUSS-3 trial and were treated with evolocumab, which was associated with a 53% reduction in LDL-C at 24 weeks. Muscle symptoms were reported in only 21% of evolocumab-treated patients [12]. Similarly, in the ODYSSEY-Alternative trial, alirocumab reduced mean LDL-C by 45% at 24 weeks [57]. Although outcome data are limited in this setting, PCSK9 inhibition has been demonstrated to reduce CV events when assed to statin therapy in the FOURIER [58] and ODYSSEY-Outcomes [59] trials (15% relative risk reduction of the primary endpoint). Currently, the acquisition cost of PCSK9 inhibitors is high, and reimbursement is challenging in many jurisdictions, but these agents may play an increasingly important role in the management of statin intolerance. Therefore nowadays the most important issue would be to present the relatively easy and possible to common use definition of statin intolerance in order to establish the real number of patients with this condition what enable to apply for the PCSK9 inhibitors reimbursement for this group, besides existing in many countries reimbursement for familial hypercholesterolemia and extremely high risk patients after myocardial infarction.

PCSK9 Inhibition by siRNA

The ORION-1 clinical trial was performed in patients with high cardiovascular risk and elevated LDL-C level. This study demonstrated that inclisiran (a synthetic siRNA designed to target PCSK9 mRNA) is able to significantly lower PCSK9 and LDL-C levels [60]. Over the 180 days, 501 participants took 200, 300, or 500 mg inclisiran in single doses or two doses, at the first and 90th days, of 100, 200, or 300 mg [60]. On the last day, biochemical tests (compared to placebo) showed significantly ($p < 0.001$) reduced LDL-C levels from 27.9% to 41.9% and 35.5% to 52.6% in patients taking single or two doses, respectively. The largest reduction in LDL levels was obtained in patients treated with two 300 mg doses of inclisiran (48% of patients had LDL-C < 1.3 mmol/l (50 mg/dl) at day 180) [60]. These results suggest that inclisiran might be very useful for the management of statin-intolerant patients [60]. The fact that this drug only needs to be administered every 6 months may be a particular benefit in individuals who have experienced problems with medicines in the past.

Bempedoic Acid

Bempedoic acid is a small-molecule prodrug, which is metabolized in the liver to produce bempedoic acid-CoA, an inhibitor of ATP-Citrate Lyase (ACL). ACL is an enzyme necessary for the hepatic production of cholesterol. In the liver, inhibition of ACL (which lies upstream of HMG-CoA reductase) results in reduced synthesis of cholesterol and of fatty acids; however, the enzyme (very-long-chain acyl CoA synthetase-1 (ACSVL1)) needed to produce the active drug from the prodrug is specific to the liver. This reduces the chance of bempedoic acid resulting in off-target adverse effects [61]. The CLEAR-Harmony randomized controlled trial demonstrated that over 53 weeks' treatment, bempedoic acid resulted in a 16.5% reduction in LDL-C from baseline, with a similar adverse effect profile to placebo [62]. In recently published CLEAR-Serenity trial, 345 patients with hypercholesterolemia and a history of intolerance to at least two statins (one at the lowest available dose) were randomized to 180 mg bempedoic acid or placebo once daily for 24 weeks [63]. Bempedoic acid treatment significantly reduced LDL-C by 21.4% ($p < 0.001$); significant reductions were also observed in non-HDL-C (−17.9%), total cholesterol (−14.8%), apolipoprotein B (−15.0%), and high-sensitivity C-reactive protein (−24.3%; $p < 0.001$ for all comparisons). Bempedoic acid was safe and well tolerated [63]. Although outcome data are not yet available, bempedoic acid represents a very exciting development in the management of statin intolerance (ongoing CLEAR-Outcomes study) [64].

Nutraceuticals

Several nutraceuticals have been demonstrated to have beneficial effects on plasma lipids and profiles, and as such may be useful in optimizing lipid-lowering therapy, especially when targets are not met by conventional therapy. Mechanisms and evidence supporting various nutraceuticals have been reviewed elsewhere [43], and the International Lipid Expert Panel has produced extensive guidance on these subjects with statin intolerance [65].

Conclusions

Statins are a key preventive medicine for ASCVD, and their most common side effect is associated with muscles. Adverse effects leading to decrease in a patient's quality of life may result in a patient taking a suboptimal dose of statin therapy or even discontinuing therapy altogether. Various definitions have been proposed for this phenomenon of "statin intolerance." Most definitions require some form of "rechallenge" after cessation of statin therapy, thus defining statin intolerance is the inability to tolerate at least two different statins. However, it is universally agreed that statin intolerance leads to poor cardiovascular clinical outcomes. Thus, effective diagnosis and distinction of true statin-related side effects from unrelated conditions are necessary to avoid the unnecessary withdrawal of these very effective drugs.

The four-step approach to the diagnosis of statin intolerance is a practical approach to effective diagnosis, which enables optimal management. Management strategies include altering the dose, switching between statins, or supplementing or replacing statins with other drugs. The International Lipid Expert Panel is currently producing a step-by-step practical guide to the diagnosis and management of statin intolerance. It is hoped that this position paper, which will be published in 2020, will assist physicians in the accurate diagnosis and effective management of this challenging condition.

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13 Intestinally Active Therapies for Hypercholesterolemia: Ezetimibe, Bile Acid Resins, and Plant Sterols

Thomas Dayspring and Gregory S. Pokrywka

Abbreviations

ABC	ATP-binding cassette transporter
ApoB	Apolipoprotein B
BA	Bile acid
BAS	Bile acid sequestrants
CE	Cholesteryl ester
CHD	Coronary heart disease
CVD	Cardiovascular disease
FA	Fatty acid
FC	Free or unesterified cholesterol
HDL-C	High density lipoprotein cholesterol
LDL-C	Low density lipoprotein cholesterol
LXR	Liver X receptor
LysoPL	Lysophospholipids
Non-HDL-C	Non-high-density lipoprotein cholesterol
NPC1L1	Niemann-Pick C1-Like 1 protein
PL	Phospholipids
PS	Phytosterols
RXR	Retinoid X receptor
SREBP	Sterol regulatory element binding protein
TC	Total cholesterol
TG	Triglyceride
TICE	Transintestinal cholesterol efflux
VLDL-C	Very low-density lipoprotein cholesterol

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Introduction

The hepatobiliary system affects sterol homeostasis [1]. A subsection of the emerging field called lipidomics is "cholesterolomics" which deals with the identification and quantification of cholesterol, its presqualene aliphatic and post squalene aromatic precursors. Cholesterol is a 27-carbon molecule which is essential to human health, serving in cell membrane structure and function and as a substrate from which various steroid hornones and bile acids are produced. Yet, since excess cellular cholesterol has an ability to crystalize it can also be toxic to cells [2, 3]. The intestine plays a major role in cholesterol homeostasis through enterocytic synthesis, absorption and excretion, lipoprotein production and delipidation, as well as handling, reabsorption, and excretion of bile acids [4].

Unesterified or free cholesterol (FC) is a member of the sterol family of molecules (Fig. 13.1), which are a group of sterane-derived alcohols hav-

M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_13

ing an aromatic center of 4 rings with an aliphatic hydrocarbon side chain of 8–10 carbons which may or may not contain double bonds attached at the 17-beta (β) position of the fourth ring and a hydroxyl group (-OH) at the 3-β position of the first ring. The bond (Δ) between carbons 5 and 6 of the second ring is unsaturated. Because of the relative hydrophilicity of the hydroxyl end and hydrophobicity at the hydrocarbon side chain, amphiphilic sterols can be incorporated into phospholipid (PL) bilayers of the cytoplasmic membrane or into single layers on the surface of a lipoprotein [5]. FC may also become part of sophisticated lipid rafts serving in areas of sphingolipid-rich complex membrane domains regulating cell membrane transport and cellular signal transduction [6].

FC is a zoosterol, as it is predominantly synthesized by members of the animal kingdom including humans and is infrequently found in plants [7], whereas the structurally similar phytosterols (PS) are produced solely by the plant kingdom including fungi and yeasts. Both FC and PS are part of the human diet and subject to intestinal absorption. Sterols other than cholesterol, which include aromatic sterol cholesterol and hormone precursors and PS, have long been referred to as "non-cholesterol" sterols or xenosterols (xeno, meaning "other"). The PS group is large consisting of sitosterol, campesterol, stigmasterol, and numerous others, which have a very similar structure to cholesterol, in that they all are 4-desmethylsterols (no methyl group at C4) but vary in the makeup of additional methyl or ethyl groups in the aliphatic tail attached at C-17 and/or in the location of some double bonds [8]. All sterols can be individually measured in plasma using gas or liquid chromatography with mass spectrometry (GC-MS or LC-MS) [8], but clinicians are unaware that routine cholesterol assays are in fact collective measures of all sterols and stanols, e.g., total cholesterol assays identify all of the lipoprotein-trafficked cholesterol and xenosterols and stanols per unit of plasma volume [9]. The same applies to lipoprotein subfraction cholesterol concentrations such as low-density lipoprotein-cholesterol (LDL-C) which is actually LDL-cholesterol + LDL-sitosterol + LDL-campesterol + LDL-stigmasterol + LDL-any sterol + LDL-any stanol. The same applies to high-density lipoprotein-cholesterol (HDL-C).

PS are present in Western Diets in amounts equal to cholesterol (150–350 mg daily) from sources such as nuts, seeds, fruits, vegetables and their oils, and thus are available for absorption by the intestinal epithelium [10]. Vegetarians consume larger amounts (500–1000 mg daily) and there are also many commercially available food

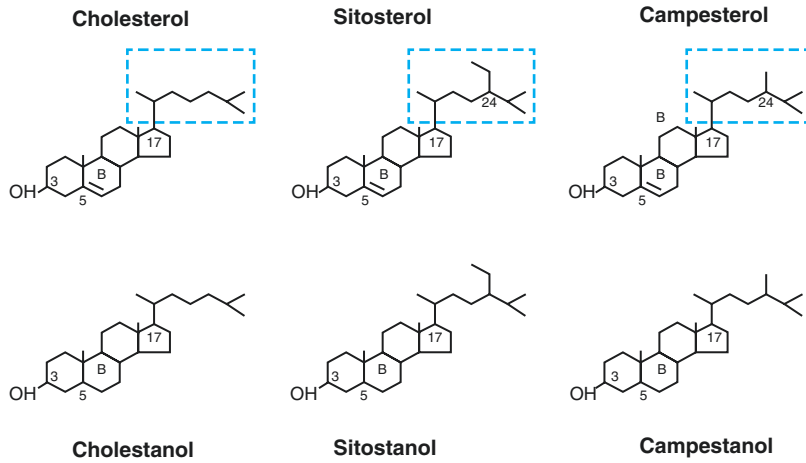

Fig. 13.1 Sterols and stanols. Structures of FC with its aliphatic tail at C17, sitosterol (with an ethyl group at C24 of its tail), campesterol (with methyl group at C24 at its tail), as well as their respective stanol counterparts which lack the Δ5 double bond of the B ring. All have a hydroxyl group at C3. Replacing the C3-hydroxyl group with an acyl group converts the molecule to a sterol-ester, e.g., cholesterol becomes cholesteryl ester (CE)

additives enriched in PS and phytostanols which carry any number of medical claim benefits. Such products contain very large doses (>2 g) of PS, which is far in excess of what a typical vegetarian consumes [11]. Stanols are simply sterols that have a saturated (hydrogenated) Δ5 bond in the B-ring but that subtle structural difference has profound effects on its molecular handling. Stanols in the diet are mainly from plant sources with an intake of about 50 mg daily. With saturation of Δ5 bond of the B-ring, cholesterol, sitosterol, campesterol, etc., respectively become cholestanol (or its β-isomer coprostanol), sitostanol, and campestanol [12, 13]. Sitostanol esters are a commercially available stanol product used to reduce LDL-C.

Atherosclerosis is a disease caused by arterial wall accumulation of sterol-laden foams cells, but that sterol does not have to be exclusively cholesterol. In 1974, two patients were described relating tendon xanthomas to a new "lipid storage disease" characterized by elevated plasma sitosterol concentrations [14]. Subsequently, numerous other such patients were described with "sitosterolemia" associated with premature atherosclerosis and other pathologies. With the recognition of increased concentrations of multiple other PS in these patients, the disease sitosterolemia is more accurately classified as phytosterolemia or xenosterolemia. Much is now known about cholesterol homeostasis and how sterols are absorbed, synthesized, trafficked, and excreted, and such knowledge helps us better understand atherogenesis and impacts ways of preventing or treating patients.

Cholesterol

FC is acquired primarily through endogenous cellular synthesis (800–1200 mg daily) and to a far lesser degree also from exogenous dietary sources (300–500 mg daily) and from shredded intestinal epithelia (300 mg daily). FC is synthesized in every cell of the body including the enterocytes, via a multistep process from acetate or acetyl coenzyme A, into lengthening aliphatic molecules and isoprene units (squalene) before aromatizing into a structure of 4 rings, the first of which is lanosterol. Subsequent steps of choelsterol synthesis occurs through many steps utilyzing at least two distinct pathways, cholesterol is formed with the penultimate sterols such as lathosterol (product of Kandutsch–Russel path) or desmosterol (Bloch path) (Fig. 13.2). When a fatty acid (acyl group) esterifies to the hydroxyl group at position 3 of the first ring, the amphiphilic FC molecule converts to a larger (higher molecular weight) hydrophobic cholesteryl ester (CE) molecule, which can be stored in cells or transported to the cores of lipoproteins [15]. CE can be de-esterified to FC via the action of esterolases.

FC is synthesized in all cells of the body including the enterocytes, via a multistep process from acetate or acetyl coenzyme A, into lengthening aliphatic molecules and isoprene units (squalene) before aromatizing into a series of 4 rings, the first of which is lanosterol [16, 17]. After several more steps utilyzing at least two distinct pathways, cholesterol is formed with the penultimate sterols being lathosterol (product of Kandutsch–Russel path) or desmosterol (product of Bloch path). Both of those sterols serve as readily available clinical biomarkers of cholesterol synthesis [18]. When a fatty acid esterifies a hydroxyl group at position 3 of the first ring, the potentially active cholesterol becomes amphipathic cholesteryl ester (CE) which is stored in cells or transported in the cores of lipoproteins [14, 16].

The liver is responsible for about 15% of cholesterol synthesis and the remainder is synthesized by extrahepatic peripheral cells [19]. Brain cholesterol synthesis and regulation are totally independent of that in the liver and other peripheral tissues. Cholesterol synthesis is a four-step process starting from its precursor acetate: CH_3-COO^-. In the first step, acetyl-CoA condenses with acetoacetyl-CoA upon the action of cytosolic HMG synthase and becomes hydroxymethylglutaryl-CoA (HMG-CoA). The catalytic action (rate limiting state) of HMG-CoA reductase, an integral part of the smooth endoplasmic reticulum, forms mevalonate (a six-carbon intermediate) with NADPH serving as the reductant [8, 16]. SREBP-2, a membrane-

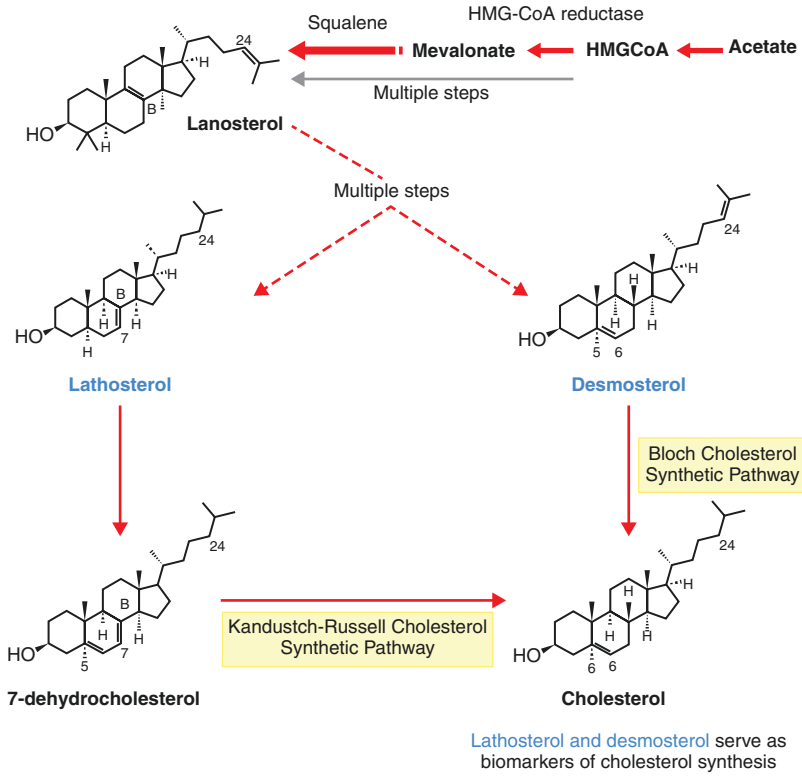

Fig. 13.2 Cholesterol synthesis pathway. Truncated pathways of cholesterol synthesis from acetate and acetylCoA, aromatization of linear squalene to lanosterol and the Kandutsch–Russell (thru lathosterol) and Block (thru desmosterol) paths. Note the transformation of the double bonds in specific carbon atoms in the ringed structures. Failure to saturate C7 of lathosterol or C24 of desmosterol results, respectively, into the rare sterol disorders lathosterolosis or desmosterolosis. Cholesterol precursors are present in plasma at concentrations roughly 1:1000 of that of cholesterol

bound transcription factor [4], tightly regulates production of the rate-limiting HMG-CoA reductase whose activity can be partially inhibited by statin therapy. Sterol homeostasis is also regulated by additional sterol/bile acid sensing nuclear transcription factors such as liver X receptors (LXR) and farnesoid X receptors (FXR) [20].

In the second step, mevalonate is phosphorylated from ATP to isoprene units or isoprenoids, namely isopentyl pyrophosphate which can isomerize or inter-convert to dimethylallyl pyrophosphate. In the third step, isoprenoids react with each other to form geranyl pyrophosphate and condensation with another isopentyl-PP yields farnesyl pyrophosphate. Squalene synthase catalyzes the condensation of two molecules of farnesyl-PP with reduction by NADPH to make squalene. The fourth step involves conversion of the linear squalene molecule via the enzymes squalene epoxidase and oxidocyclase to the four-ringed sterol molecule called lanosterol. Conversion to FC takes about 19–20 reactions via enzymes (cytochrome P_{450}) in the mitochondria and endoplasmic reticulum that include migration and removal of methyl groups.

There are several intermediary, cholesterol precursor sterols which evolve into one of two penultimate sterols, specifically lathosterol and desmosterol, whose concentrations have clinical use as biomarkers of cholesterol synthesis [16, 18]. Intermediary products of the cholesterol synthesis pathway such as farnesyl-PP are precursors for other isoprenoids used in the synthesis of other compounds such as dolichol or ubiquinone or prenylation of cellular proteins, many of which are involved in cell signaling. FC can be converted into reproductive or adrenocortical hormones in steroidogenic tissue or into bile acids in the liver. In one of the bile acid production pathways, saturation of Δ5 carbon of cholesterol forms cholestanol in an intermediary step. Defects in the enzymes of that path lead to significant excess cholestanol causing the disease cerebrotendinous xanthomatosis [20, 21]. In the gut lumen, free cholesterol upon the action of microbes can be saturated forming the α-isoform

cholestanol or β-isomer coprostanol which usually are very poorly absorbed and thus excreted in stool. Since cholestanol is not readily absorbed, it also serves as a plasma biomarker of cholesterol absorption [22].

Xenosterols

As noted, the term xenosterols technically includes every sterol that is not cholesterol but includes PS, shellfish sterols like fucosterol, and others as well as sterol intermediaries in the cholesterol and some hormonal synthesis pathways. Plants, fungi, and yeasts cannot convert squalene to cholesterol but to other structurally similar sterols: stigmasterol, sitosterol, campesterol, stigmasterol, ergosterol, brassicosterol, avenosterol, etc. which are present in Western Diets in amounts equal to cholesterol (150–350 mg daily) and thus available for absorption by the intestinal epithelium [23]. The predominant PS and stanols in human diets are sitosterol (66%), campesterol (22%), stigmasterol (8%), and sitostanol plus campestanol (4%). Because of their different aliphatic tails at carbon 17 and different degrees of saturation in the aromatic rings, PS compared to FC are far less likely to be recognized and internalized by enterocyte membrane sterol influx proteins and their plasma measurement serves as useful biomarkers of cholesterol absorption. Plant sterols are found in and trafficked within all lipoproteins with the highest concentrations in low- and high-density lipoproteins (LDL and HDL) and high-density lipoproteins (HDL). Because human cells cannot synthesize PS, their presence in plasma is reflective of intestinal sterol (including cholesterol) absorption [24]. Physiologic absorption of plant sterols and stanols is much lower than that of PS and stanols varying between 0.5 and 1.9%. Absolute plasma concentrations are as follows: cholesterol: 5.5 mmol/L, phytosterols: 7–24 μmol/L (0.3–1.0 mg/dl), and phytostanols: 0.05–0.3 μmol/L (0.002–0.012 mg/dl) [25] (Fig. 13.3).

Bile Acids

Hepatobiliary circulation of bile acids (BA) is an important part of cholesterol homeostasis.

The primary bile acids scholic and chenodeoxycholic acids are amphipathic molecules synthesized in the liver from cholesterol utilizing the enzyme 7α-hydroxylase in the neutral path or sterol 27-hydroxylase acidic path. BA are secreted from hepatocytes into bile via the ATP binding cassette transporter (ABC) B11 (ABCB11). Along with FC and PL, BA are major constituents of bile

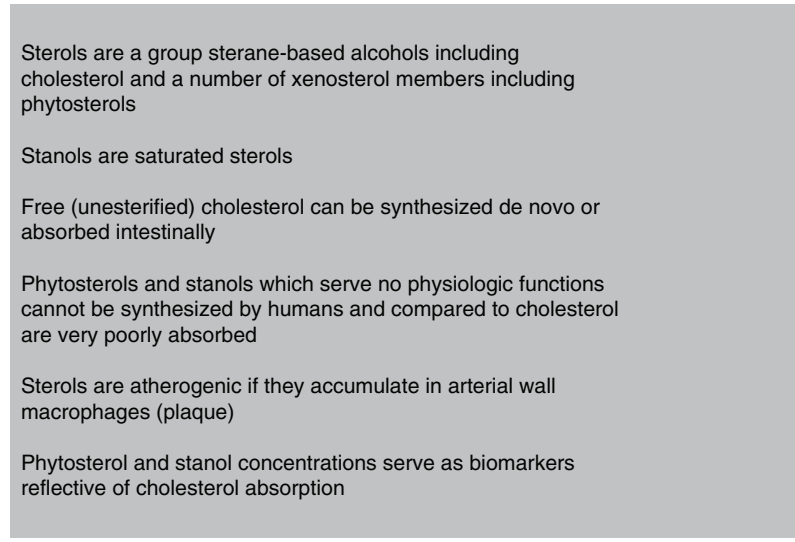

Fig. 13.3 Sterol/stanol key points

which is transiently stored in the gall bladder and typically released after eating by the gall bladder contraction influenced by hormone cholecystokinin [26]. Gut microbes can transform the primary bile acids into secondary bile acids deoxycholic and lithocholic acids [27]. Ultimately, almost all BA are internalized into ileal enterocytes via the apical sodium-dependent BA transporter (ASBAT) and then secreted into portal plasma via the basolateral BA transporter and organic solute transporter-α where they bind to albumin and return to the liver via the portal circulation where they are internalized by taurocholate cotransporting protein (NTCP, SLC10A1) [27, 28]. Approximately 5% of BA is excreted in the stool [29]. The major way the human body can rid itself of *excess*, unneeded, potentially toxic FC is to convert it to a BA which can exit the body in feces. BA indirectly influence many cardiometabolic functions by being potent signaling molecules via Farnesoid and Liver X receptors (FXR and LXR), the G protein-coupled bile acid receptor 1 (TGR5/GPBAR1), pregnane X receptor (PXR/NR1I2), and vitamin D receptor [30, 31].

Intestinal Absorption of Sterols

Sterol entry into the plasma is tightly regulated in enterocytes and hepatocytes through the action of a variety of genes which regulate the expression and function of specific membrane transporters which large part control influx from gut lumen to enterocytes or from the bile to the liver and efflux from enterocyte to gut lumen and hepatocytes to bile. Ultimately, trafficking of sterols in plasma is accomplished by binding to proteins such as lipoproteins or albumin or by incorporation into circulating erythrocyte membranes [32]. Incorporation and packaging of sterols into lipoproteins in enterocytes and hepatocytes are equally complex [33].

There is a subtle but crucial terminology distinction between cholesterol entry from the gut lumen into enterocytes and cholesterol absorption which refers to systemic presence of cholesterol within lymphatic vessels and plasma. Not all sterols that are internalized into enterocytes from the gut lumen find their way into the systemic circulation for distribution to tissues. A multitude of complex forces regulate the quantity of FC and free xenosterols in the gut lumen that pass into enterocytes and then either gain systemic entry into plasma or secreted back to the gut lumen. Sterol/stanol absorption is an individualized, multiple-step process involving (a) unesterified sterol entry from the gut lumen into intestinal epithelial cells (enterocytes), (b) subsequent esterification with fatty acids, (c) incorporation into apoB48-containing lipoproteins, (d) efflux to HDL particles, and finally ultimate delivery to tissues including the liver. However, unesterified sterols/stanols that are denied systemic entry are subject to enterocyte excretion into the intestinal lumen in a process called transintestinal cholesterol efflux (TICE). Gut sterol uptake and absorption, most of which occurs in the duodenum or proximal jejunum, is regulated by multiple genes, nuclear transcription factors, gut microbiota, and other forces. The mean value of FC absorption in humans is 56% with individual rates being highly reproducible. The process is incomplete with variations due to apoE genotype, gender, and age [34, 35].

From food sources, after a meal, the intestine contains a pool of fatty acids and monoacylglycerols and lysophospholipids formed from triglycerides or phospholipids hydrolyzed by pancreatic lipases. Ingested sterols consist of PS, CE and some FC. Since only free sterols can be interanlized by enterocytes, esterified sterols require hydrolysis by pancreatic cholesteryl esterolase. It is important to recognize that in most humans the majority of the FC eligible for enterocyte absorption is from a biliary not a food origin but in contradistinction the vast majority of PS availabe for absorption is from food. FC and fatty acids after being emulsified by biliary phospholipids and then enwrapped by amphipathic bile acids form mixed biliary micelles [26]. The nonpolar end of the bile acid surrounds the lipids and the polar end bulges outward making the micellar collection of lipids soluble in aqueous intestinal fluids. The micelles transport or "ferry" the lipids through a diffusion barrier consisting of unstirred water and mucous coat layer [36, 37] to the brush border (microvilli) of the intestinal epithelium

where their delipidation will occur. Fatty acids enter into the enterocyte by both passive diffusion and transportation by the fatty acid transporter protein glycoprotein CD36 [38].

Aside from gut lumen absorption enterocytes have other sources of cholesterol including (1) endogenous synthesis and (2) direct delivery of FC or CE via TICE pathways. Also available to enterocytes via absorption, packaged within mixed biliary micelles are free PS, fatty acids, monoacylglycerols, partially de-esterified phospholipids called lysophospholipids (LysoPL) and BA [41] (Fig. 13.4).

Delipidation of sterols from mixed micelles and sterol influx occurs at the microvilli of the brush border of the small intestine via the action of enterocyte membraneinflux transporters or "permeases," the most important of which is the Niemann-Pick C1-like 1 protein (NPC1L1) [42] which was reported in 2004 as the human sterol absorption protein. NPC1L1 is expressed at both the enterocyte/gut lumen (apical) and the hepatobiliary (canalicular) interface. NPC1L1 has a sterol-sensing domain (SSD) which is a region consisting of around 180 amino acids that form five predicted membrane-spanning helices with short intervening loops [43]. At enterocyte and hepatocyte membrane borders NPC1L1 protein interacts with the adaptor protein-2 (AP2) complex consisting of four proteins forming a cholesterol carrying core and appendage domains which bind to clathrin which has a triskelion shape (three interlocked spirals) composed of three heavy chains and three light chains aligned to form small vesicles capable of internalizing cholesterol and xenosterols. This vesicular complex which, with the help of myosin, translocates along cytosolic microfilaments to a storage endosome called the endocytic recycling compartment (ERC). When intracellular cholesterol becomes low, the NPC1L1 recycles back to the cell membrane [24, 44]. The sterol-sensing domain of NPC1L1 has the highest affinity for cholesterol, far less for phytosterols, and least for stanols [23]. The *NPC1L1* gene may be influenced (downregulated) by PPAR-δ [29] as well as LXR and dietary cholesterol ingestion [45]. Various SNPs associated with loss of function of NPC1L1 are associated with reductions in LDL-cholesterol and reduced risk form atherosclerotic

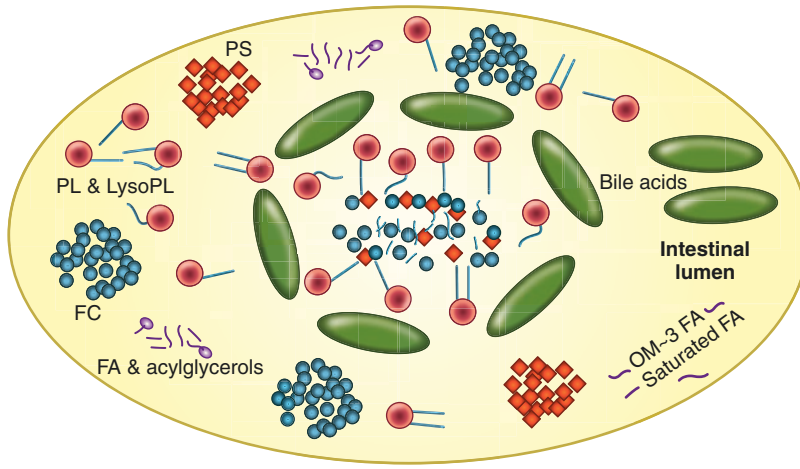

Fig. 13.4 Mixed micelle formation. Bile acids are amphipathic molecules with polar and nonpolar ends. Mixed micelles contain FC, PS, BA, FA including omega-3 FA, and monoacylglycerols and lysophospholipids (LysoPL) derived from TG or PL. The mixed micelles traffic the lipids to the brush border (microvilli) of enterocytes where lipid absorption occurs

events [46] which support the concept that reducing sterol absorption has cardiovascular benefits.

Other mechanisms for enterocyte sterol entry have also been described including a membrane-bound ectoenzyme aminopeptidase N (alanyl)-aminopeptidase (APN), which facilitates the endocytosis of cholesterol-rich membrane microdomains [47, 48], and Caveolin-1 (CAV1), a protein which binds with cholesterol and helps shape caveolae [49]. CAV1 can also complex with annexin-2 (ANX2), cyclophilin A, and cyclophilin 40 to traffic cholesterol from caveolae to the endoplasmic reticulum where esterification will occur [50–52]. Factors such as scavenger receptor-B type 1 (SR-B1) may also be involved with cholesteryl ester movement but is not obligatory [53].

Once within enterocytes, sterol homeostasis is complex. FC can be transformed into hydrophobic-CE by acyl-cholesterol acyl-transferase (ACAT) enabling incorporation into the core of apoB48-containing chylomicrons or FC can be effluxed by membrane ABCA1 transporters to free apoA-I, apoE, or partially phospholipidated preβ-HDLs. FC can also be effluxed back to the gut lumen by ABCG5 and ABCG8 transporters (originally named sterolin 1 and 2). FC because of its higher affinity for ACAT proceeds down to esterification pathways while noncholesterol sterols are directed to sterol efflux transporters. ACAT2 displays the greatest capacity to differentiate cholesterol from sitosterol [54]. In mice combined ACAT2 and LDL receptor (LDLr), deficiency leads to the redirecting of FC to intestinal ABCA1 and efflux to apoA-I [55]. Most of the absorbed FC in preparation for entry into plasma is esterified to CE and with the aid of microsomal triglyceride transfer factor (MTF) at the endoplasmic reticulum joins apolipoprotein B48, which consists of the N-terminal-2152 amino acids of hepatic produced apoB100 in the formation of chylomicrons [56, 57]. ApoB48 is synthesized in the endoplasmic reticulum and then transported to the Golgi-apparatus by a small GTP-binding protein called ADP ribosylation factor 1 [58] acquiring phospholipids in the process [59]. Also influencing apoB production is apolipoprotein A-IV [60] whose expression in humans is limited to the intestine. The physical association between apoA-IV and apoB increases nascent chylomicron residence time within enterocyte lipoprotein expansion compartments, thereby facilitating particle expansion with triglycerides [61].

HDL particles are also more involved with the absorption of cholesterol from the enterocyte than previously recognized as both FC and free phytosterols can gain systemic (plasma) entry via efflux through the ATP-binding cassette transporter A1 (ABCA1) onto a cholesterol acceptor protein like apoA-I, which is synthesized and secreted by enterocytes, or onto apolipoprotein E (apoE). Putting all of the above complexities in play, the proximal intestine thus has several options in lipoprotein-mediated absorption of cholesterol, and no doubt these in conjunction with the sterol export/efflux mechanisms work together or compensate for one another [62].

PS serve no known physiologic functions in humans and at significant concentrations can be pathological [13, 63]. Teleological forces have established homeostatic mechanisms that deny PS entrance into the plasma. ABCG5 and ABCG8 transporters are expressed in intestinal and hepatic cells, respectively [64, 65]. The ABCG transporter subfamily is composed of six half transporters with "reverse" proteins that have an ATP-binding cassette at the amino terminus and a transmembrane helix at the carboxy terminus [66]. The ABCG5-ABCG8 heterodimer is expressed exclusively in the intestine and liver and functions to efflux free sterols across membranes increasing intestinal excretion and hepatobiliary secretion of noncholesterol sterols. The genes controlling these transporters are influenced by LXRs and are located in a head-to-head orientation on chromosome 2p. ABCG5/ABCG8 actively efflux FC and PS from enterocyte to the intestinal lumen or hepatocyte to bile where they can be excreted in the stool or reabsorbed. Should a phytosterol make it to the liver as part of chylomicron or other lipoprotein surface or core, hepatocyte ABCG5/G8 will export it into the bile. It has been hypothesized that the presence of PS and phytostanols upregulates *ABCG5 and ABCG8* as a defense against phytosterol accumulation [67].

The homozygous mutation or absence of *ABCG5/G8* genes is the cause of the rare genetic condition sitosterolemia (phytosterolemia) known to be associated with premature atherosclerosis and other pathologies. Polymorphisms or sequence variants of *ABCG5* or *ABCG8* genes also exist and systemic absorption of FC and noncholesterol sterols will vary among individuals. The *ABCG5* gene is principally mutated in Asians [68] and *ABCG8* gene in Caucasians suggesting that the proteins form both hetero- and homodimers to transport the wide range of dietary sterols present in the diet [64]. Groups with elevations of noncholesterol sterol may be at risk for coronary heart disease such as those with strong family histories of premature atherosclerosis and postmenopausal women [33, 69–71]. Of interest, statin therapy is also associated with hyperabsorption of cholesterol and noncholesterol sterols such as sitosterol and campesterol levels [72] (Fig. 13.5).

Hepatocyte and Sterols

Chylomicrons transport intestinally acquired lipids (CE, FC, PS, and TG) through lymphatic channels, into plasma, to tissues, and to the liver. During passage through myocyte and adipocyte beds lipoprotein lipase (LPL) mediated hydrolysis of TG creates smaller TG-poor chylomicron remnants which are ultimately internalized at the hepatocyte by fixation with hepatic lipase, heparan sulfate proteoglycans, LDL receptors, and LDL receptor-related protein [73]. Lysosomal degradation of the chylomicron releases the sterols. CE is hydrolyzed by a cholesterol esterolase into FC [74]. Sterols are then transported by both vesicular

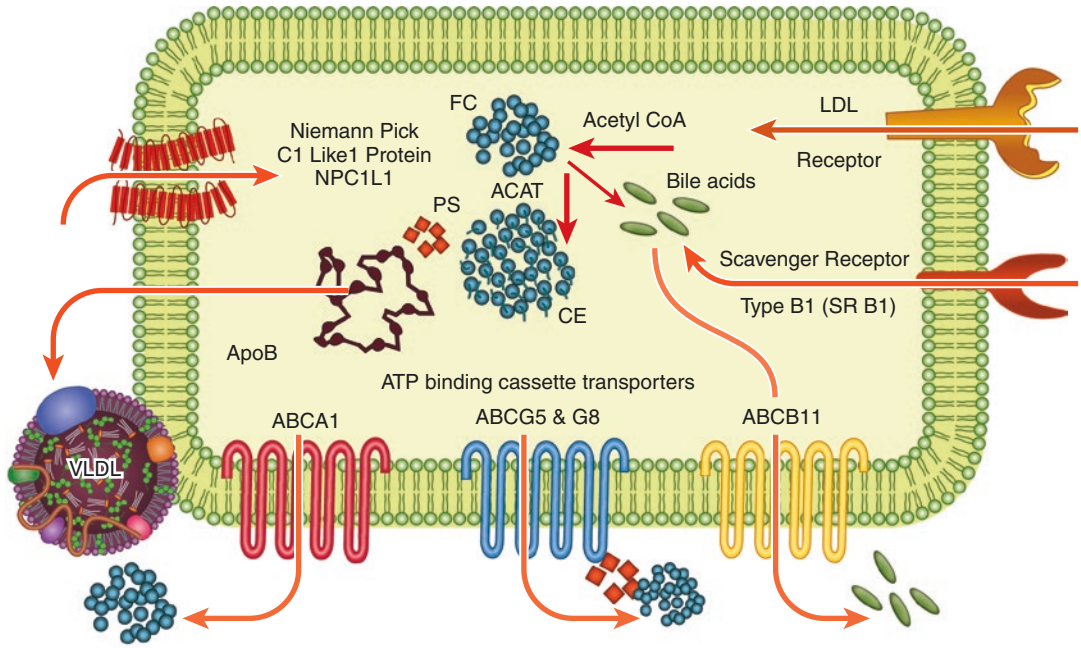

Fig. 13.5 Hepatocyte/enterocyte sterol homeostasis. Hepatocytes and enterocytes regulate cholesterol homeostasis via several membrane receptors and transporters. All of the above membrane sterol transporters ABCB11 (bile acid efflux) exist in both cells. NPC1L1 internalizes and ABCG5/G8 exports FC and PS, respectively, into the cells from gut lumen or bile and to the gut lumen and bile. LDL receptor internalizes apoB-lipoproteins, SR-B1 delipidates mature HDLs. Hepatocytes produce and export CE-containing apoB particles, such as VLDL and LDL. ABCA1 effluxes FC and PS to apoA-I. Within the cells FC can be synthesized de novo and converted to CE

(endosomal) and nonvesicular pathways for further use or excretion [75]. Depending on cholesterol balance, the increase in FC delivery may cause a downregulation of HMG CoA reductase and a decrease in hepatic FC synthesis. Several other distinct pathways exist for hepatocellular FC including conversion to bile acids, efflux to plasma HDL, and as in the intestine, esterification by ACAT2. There is a FC threshold at which ACAT2 production is upregulated [76]. MTF protein helps to package newly synthesized triglycerides (TG), PL and apoB with CE, and FC into a TG-rich low density lipoprotein (VLDL), or LDLs which after maturation are released into plasma. Lipolysis of VLDL by LPL occurs peripherally and the resultant LDL can deliver cholesterol to other tissues like the adrenals (if needed). The LDL can also acquire via the protein cholesteryl ester transfer protein (CETP) additional CE from HDL particles [77], ultimately under physiologic conditions. The LDL return its lipid content to the liver or perhaps intestine (TICE) for internalization through LDL receptors via a complex process called indirect reverse cholesterol transport [32, 39].

HDL particles are major sources of the hepatic cholesterol pool that is particularly amenable to biliary excretion [78]. Along with FC, hepatocytes can secrete any acquired PS into the bile canaliculi through ABCG5/G8 thereby limiting their incorporation into hepatic produced lipoproteins [79]. The sterols enter the biliary system and join with BA and PL and generate mixed micelles which eventually enter small intestine. The liver ABCA1 transporters can also efflux FC, PS, and PL into unlipidated apoA-I, forming a preβ-HDL [80, 81]. Most of the cholesterol in HDL particles originates in the liver [82]. The FC is then esterified by lecithin cholesterol acyl transferase (LCAT) to the more hydrophobic CE, which migrates to the HDL core forming larger, more mature HDL particles. Any PS effluxed to apoA-I is less likely to be esterified and will locate with PL on the HDL surface. The larger, CE-rich HDL particles are delipidated by SR-B1 receptors in steroidogenic endocrine glands or adipocytes. Large HDL can also transfer CE to the liver either directly (scavenger receptor B-1 delipidation) or indirectly by CETP-mediated transfer to apoB particles [77].

A less-recognized source of hepatic FC is back flux of biliary FC into hepatocytes using NPC1L1 that exists at the hepatobiliary interface [40, 44]. In effect, just as is the case with the enterocyte hepatic cholesterol homeostasis depends on intimate interplay between influx (NPC1L1) and efflux (ABCG5/G8). Therapies that interfere with the NPC1L1 protein will diminish sterol entry into both enterocytes and hepatocytes, and thus promote TICE by enhancing fecal excretion of cholesterol [83].

Hepatic excretion of FC can also involve transformation of into the synthesis of the primary BA, chenodeoxycholic, and cholic acids. The hepatic pool of cholesterol used for BA synthesis originates mostly from CE acquired by hepatic uptake of LDL particles, not from endogenous synthesis [84, 85]. BA synthesis occurs in multiple steps through two pathways: the major "neutral pathway" utilizing cholesterol 7 alpha hydroxylase (CYP7A1) or the "alternative" path utilizing mitochondrial 27-sterol hydroxylase (CYP27A1) [86].

Hepatobiliary secretion of bile acids becomes the major stimulus for bile formation. Proteins, such as steroidogenic acute regulatory protein (StAR), and Sterol Carrier Protein-2 (SCP-2) participate in cholesterol trafficking from intracellular locations to the mitochondria where BA synthesis occurs [87]. BA and PL are transported across the liver cell by a P-glycoprotein called mdr2 (multidrug resistance protein) toward the canalicular membrane where they are endocytosed into the membrane of the canalicular transporters [79, 88]. ABCB11, a sister of P-glycoprotein, acts as an ATP-dependent bile salt export pump and transports BA into the biliary tree [89]. Intestinal bile acids, after micelle delipidation, are extensively reabsorbed at the ileum via the apical sodium-dependent BA transporter (ASBAT) and then secreted into portal plasma via the basolateral BA transporter and organic solute transporter-α where they bind to albumin and return to the liver via the portal circulation [90] (Figs. 13.6 and 13.7).

In summary, sterols are present in the gut via dietary ingestion and/or biliary excretion where

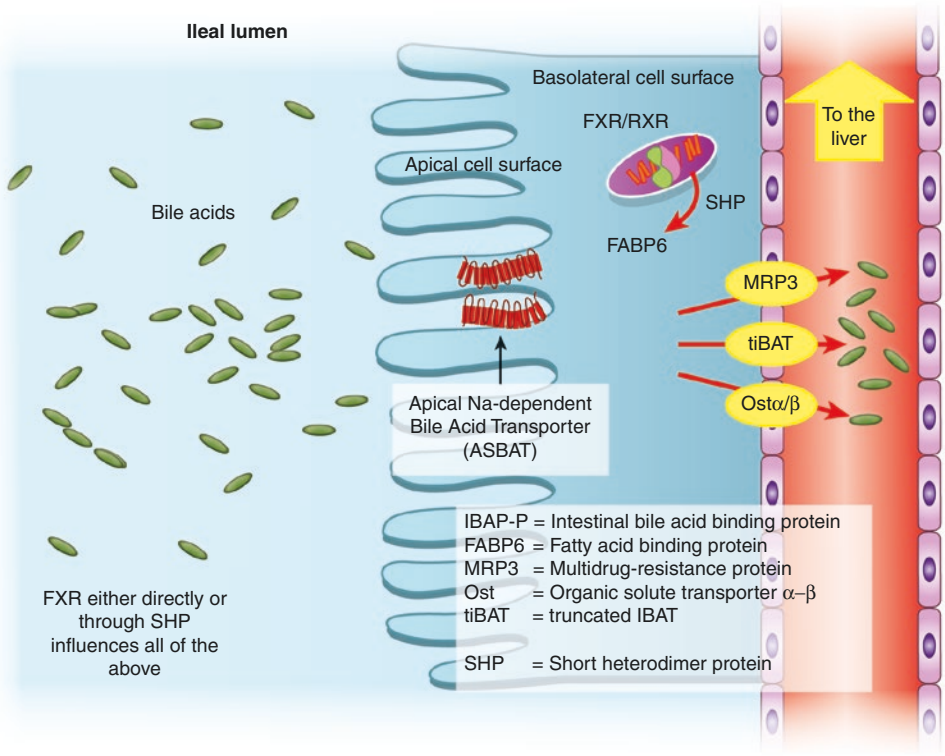

Fig. 13.6 Ileal handling of bile acids. 95% of bile acids are reabsorbed at the distal ileum and returned to the liver for reuse. BA move from the ileal lumen into enterocytes via the apical sodium-dependent bile acid transporter. Translocate thru the enterocyte via intestinal fatty acid binding proteins and then exported to the portal circulation via several other depicted transporters

they mix with hepatic secreted bile acids. The sterols are either excreted in stool or gathered for intestinal uptake in biliary micelles. Micelles are delipidated of fatty acids and sterols at enterocyte microvilli respectively by FA transport proetins and sterolpermeases including the NPC!L1 protein. Entry into the cell is influenced by lipid rafts and other proteins such as caveolin-1 and annexin and aminopeptidase N. FC can be esterified by ACAT2 and unesterified non-cholesterol sterols can be re-excreted into the gut lumen by ABCG5/ABCG8 heterodimers or incorporated into chylomicrons. FC is also effluxed to unlipidated apoA-I by ABCA1. After micelle delipidation, the majority of bile acids are reabsorbed at the ileum. FC or CE and noncholesterol trafficking in the hepatocyte is also complex and highly regulated. FC can be esterified and secreted in apoB-containing lipoproteins, effluxed to apoA-I by ABCA1, excreted into bile by ABCG5/G8 or transformed into BA, which are excreted into bile by ABCB11. Noncholesterol sterols are also effluxed to bile via ABCG5/ABCG8. Much of the above is regulated by the LXR: downregulation will increase sterol absorption and upregulation will decrease sterol excretion via LXR influence on NPC1L1, ABCA1 and ABCG5, ABCG8 (Figs. 13.8 and 13.9).

Pharmacologic Modulation of Sterol Absorption

Both cholesterol and noncholesterol sterol absorption and excretion at the intestine and liver can be manipulated by dietary modification, nonprescription food additives including plant stanols and sterols, probiotics, supple-

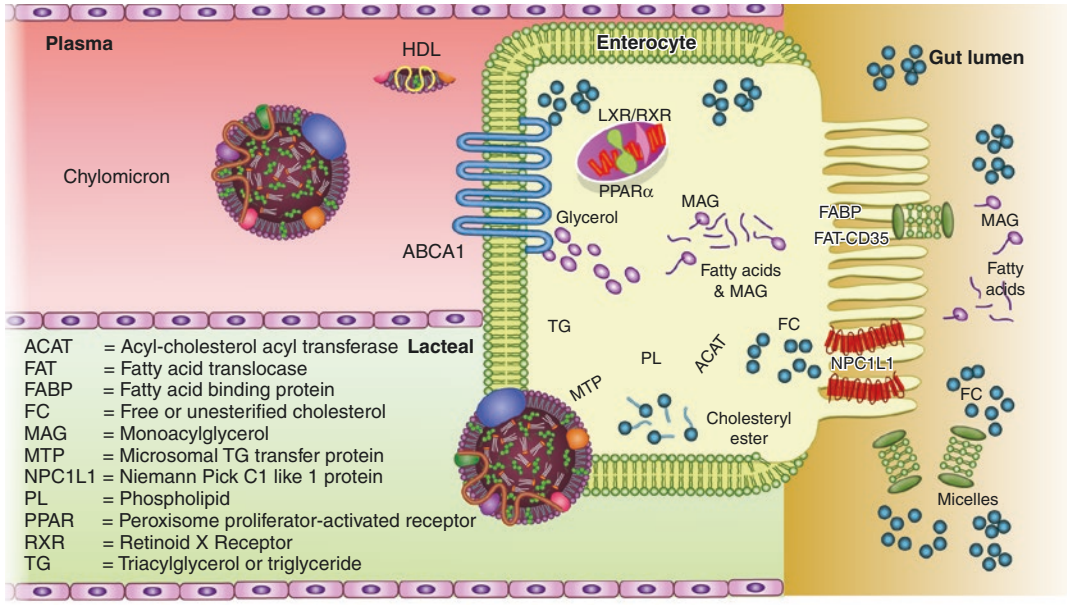

Fig. 13.7 Enterocyte handling of sterols. Gut lumen contains FC, CE, PS, stanols, FA, PL, LysoPL, MAG which are emulsified and organized with BA into mixed biliary micelles which deliver them to enterocytes in microvilli of small intestine. Sterols, much more so than PS are internalized by NPC1L1 and FA by CD34. Within enterocyte sterols are esterified and incorporated with PL and newly synthesized TG into chylomicrons, effluxed to HDL via ABCA1 or to gut lumen by *ABCG5/G8*. The chylomicrons then enter the lymphatic and ultimately the systemic circulation

ments, and prescription drugs such as ezetimibe and bile acid sequestrants. Statins can also have significant effects on cholesterol absorption.

Sterols and Stanols

As far back as the 1950s, researchers noted that oral ingestion of large amounts of plant sterols resulted in reduced cholesterol levels [91] including a decrease in LDL-C of ~10–12% (187). Today because of their inhibitory effects on intestinal cholesterol absorption, numerous PS products are used therapeutically to lower cholesterol, first receiving the recommendation of the National Cholesterol Education Program, Adult Treatment Panel III [92] and most recently by the National Lipid Association [93] and European Atherosclerosis Society (EAS) Consensus Panel [94]. Not widely appreciated is that PS supplementation can increase in plasma PS or stanol concentrations by about a factor of 2. Increases in absolute serum PS concentrations after consumption of on average 1.6 g PS per day are about 31% for sitosterol and 37% for campesterol. Although those concentrations are far lower than those in patients with phytosterolemia, safety concerns have been speculated [95]. Despite these guideline recommendations, there are no level 1 evidence from randomized, blinded clinical endpoint studies available to support PS supplements as functional food which reduces CV outcomes. Others have conjectured that PS may have benefits in other organ systems [25].

PS decreases FC incorporation in to mixed micelles thus limiting the amount FC trafficked to brush border NPC1L1. Because of NPC1L1 lower binding-affinity for PS and ABCG5/G8 higher affinity to export PS, their systemic absorption can be minimized [24]. Stanols are not absorbed and like noncholesterol sterols can interfere with the absorption of cholesterol, by displacing FC and PS from biliary micelles, and reduce plasma cholesterol concentrations. Esterified stanol products which are fat-soluble can be incorporated into a variety of substances

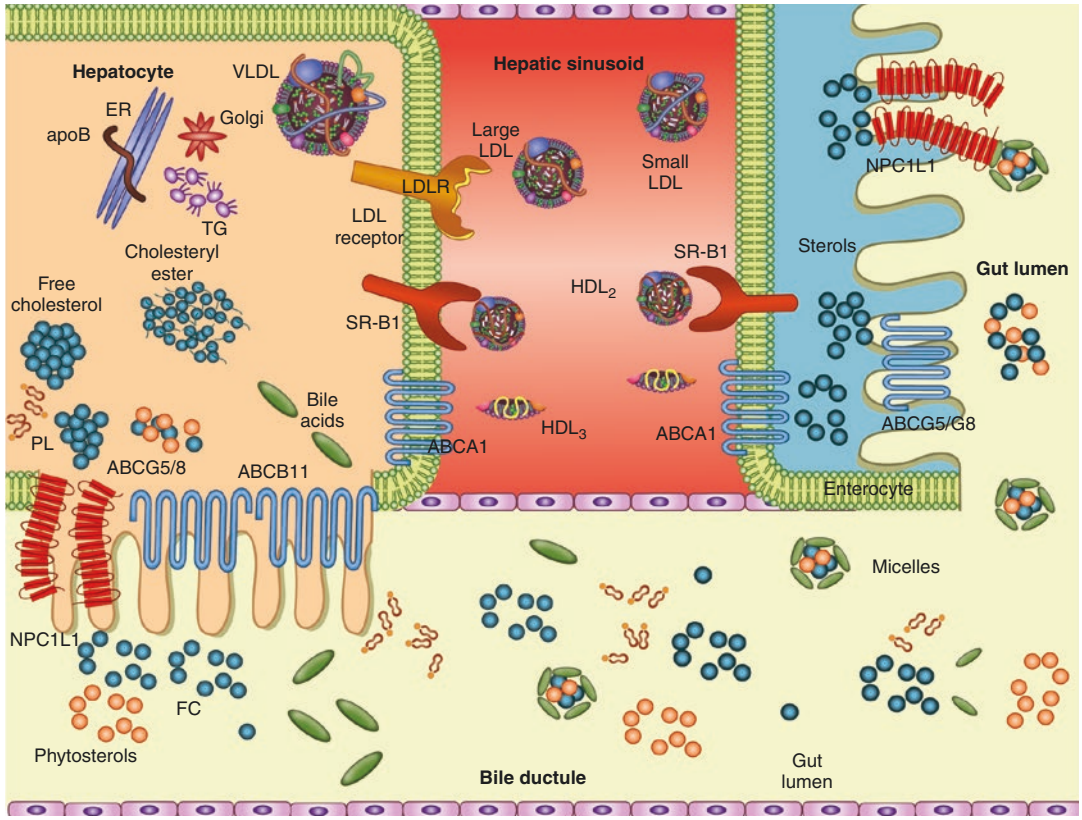

Fig. 13.8 Overview of hepatobiliary processing of sterols. Hepatocytic and enterocytic sterol homeostasis: NPC1L1 protein internalizes FC or PS from gut lumen or bile. LDLR clears LDL and its contents from plasma into hepatocytes and enterocytes. SR-B1 transfers CE from plasma HDL into hepatocytes and enterocytes. ABCA1 effluxes FC and PS to smaller plasma HDL particles. ABCG5/ABCG8 effluxes FC and PS to gut lumen or bile. ABCB11 effluxes BA from hepatocytes to bile. Not shown is the hepatobiliary PL efflux ABC-transporter ABCB4

including margarines and have been developed commercially as cholesterol-lowering agents.

Numerous double-blind studies are testament to the cholesterol-lowering benefit of PS therapy with an LDL-C reduction of approximately 10–14%, with younger persons having better responses than do elderly. The LDL-lowering benefit minimizes at doses above 2 g daily. Commercially, the use of sterols and stanols became practical when they were esterified with long chain fatty acids and added to margarines [96–99]. Single dose regimens of plant stanols are as efficacious as two or three daily doses [100]. Phytostanol esters and statins provide synergistic cholesterol lowering [101]. The polymorphisms CYP7A1-rs3808607 and APOE isoform associate with and thus might serve to predict the extent of reduction of LDL-cholesterol in response to PS consumption [102] (Fig. 13.10).

As biliary micelles are being formed, both sterols and stanols compete with cholesterol for inclusion. The cholesterol (from oral or biliary sources) that does not enter micelles is excreted in the stool. Less cholesterol is delivered to the brush border of the enterocyte, thereby reducing the amount of cholesterol that can be trafficked to the endoplasmic reticulum and incorporated into chylomicrons [96]. The overabsorption of non-cholesterol sterols and stanols may have an upregulating effect on ABCG5/ABCG8 transporters in both the intestine and liver leading to additional sterol secretion from enterocytes and hepatocytes, respectively, into the gut lumen and bile. The decreased delivery of cholesterol by

- The majority of cholesterol in the gut is of endogenous origin delivered via the biliary system
- Sterols are delivered by biliary micelles to the enterocyte microvilli where they are internalized by a varicty of complex mechanisms
- Human enterocytes typically absorb about 50-55% of intestinal cholesterol and lesser amount of PS
- Once in the enterocyte cholesterol:
  - is trafficked for esterification
  - Is incorporated into chylomicrons
  - effluxed to smaller HDL partieles
  - effluxed back to the gut via ABCG5/G8 transporters
- Xenosterols are returned (effluxed) to the gut via ABCG5/G8 transporters (enterocyte or hepatic)

Fig. 13.9 Overview of hepatobiliary processing of sterols

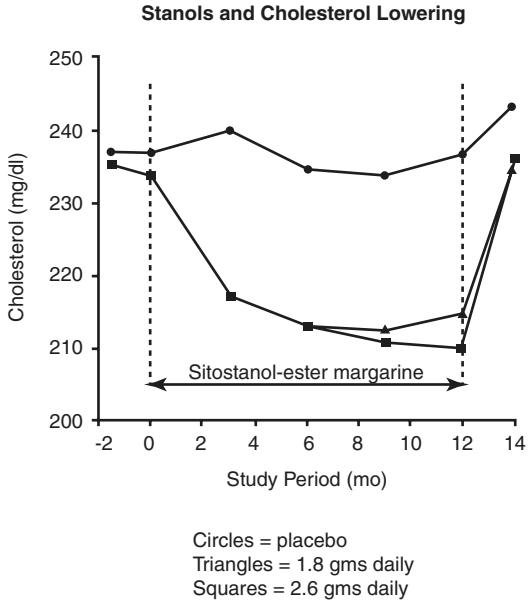

Fig. 13.10 Effect of phytostanol supplement on cholesterol levels

chylomicrons to the liver has two effects: (a) increased hepatic cholesterol synthesis and (b) upregulation of LDL-receptors (LDLR). Despite the increase in cholesterol synthesis, the increased LDLR will lead to removal of apoB-containing lipoproteins from plasma causing a net decrease in apoB and LDL-C concentrations [67]. PS but not stanols suppress bile acid synthesis and this may also lessen their LDL-C lowering efficacy over time as less hepatic cholesterol would be required for BA synthesis [103].

Because of the pathologies in those with phytosterolemia, there has been a concern that the PS that are absorbed in place of FC may be problematic. PS and stanols can interfere with all types of immune cells with unknown effects and are also incorporated into erythrocytes and platelets. Hepatic plant sterol levels can increase, and PS may enter other peripheral tissues including the lungs, the brain, into breast milk, and the vascular wall [23]. Their presence in arteries is potentially atherogenic, but the actual atherosclerosis potential is probably low because the quantity of absorbed sterols is small (5% of β-sitosterol, 15% of campesterol, and less than 1% of dietary stanols) [104]. Because sterols achieve more significant plasma levels than stanols, long-term worry over PS systemic effect has been a concern [70]. PS, but not stanols, are sensitive to oxidation, and oxidized sterols are potentially atherogenic [104]. In some studies, sterols and stanols lower blood concentrations of beta-carotene by

about 25%, concentrations of alpha-carotene by 10%, and concentrations of vitamin E by 8%, which raises concern as lack of these nutrients may adversely affect LDL-oxidation [99]. Caution with the administration of more readily absorbed PS compared to stanols may be called for in persons known to have increased plasma sterol levels, such as statin-users [105], postmenopausal women [106], and kindreds with strong family history of CHD. A study with sitostanol ester did not affect fat-soluble vitamin concentrations [107]. Through many of the mechanisms outlined in this chapter, there are significant inter-individual responses to sterol/stanol therapies [108]. In a comprehensive review of phytosterols and central nervous system function, authors have noted that phytosterols may cross the blood–brain barrier and because of the premature atherosclerosis seen in phytosterolemic patients and the potential harmful side effects of phytosterol metabolites caution may be needed with PS supplements in clinical practice [109].

To date, no clinical CV outcome endpoint studies are available to support PS use [109].

Ezetimibe

Ezetimibe was the first of a group of drugs that specifically reduce intestinal sterol absorption in humans with no effect on fat-soluble vitamins [110]. Ezetimibe is a synthetic 2-azetidinone whose full chemical name is 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone [111] which is glucuronidated by a variety of intestinal and hepatic UDP-glucuronosyltransferases [112]. Ezetimibe undergoes enterohepatic recirculation, which allows for it to be present at its site of action with negligible systemic exposure. As a result of enterohepatic recirculation, the half-life of ezetimibe is 22 hours, which thereby allows for once-daily dosing [113]. Pharmacodynamic studies, demonstrated LDL lowering efficacy was dose related, ranging from 0.25 to 10 mg with reductions in direct LDL-C by 9.9% to 18.7% within 12 weeks of treatment ($P < 0.01$). Ezetimibe had a rapid onset of activity, with ~65.0% to 80.0% of the maximum decrease from baseline LDL-C levels observed at week 1, and the maximum effect on LDL-C reduction from baseline was observed at week 2 [114].

The exact way in which ezetimibe reduces the entry of cholesterol and PS into enterocytes and hepatocytes is in part understood [44] but still being evaluated. Ezetimibe prevents the NPC1L1/sterol complex from interacting with the AP2-mediated clathrin-coated vesicles perhaps by interfering with the binding of FC to the cell membrane or by altering the structure of NPC1L1 rendering it less capable of binding to sterols [115]. Other hypotheses have been theorized such as affecting an integral membrane-bound ectoenzyme called aminopeptidase N ((alanyl)-aminopeptidase or APN) to which ezetimibe binds [116]. Ezetimibe also effectively disrupts the CAV1–ANX2 heterocomplex which traffics cytosolic FC in vivo consequently interfering with sterol absorption [52].

By reducing enterocytic sterol entry and ultimately chylomicron cholesterol content as well as back flux of cholesterol from the bile into hepatocytes, ezetimibe depletes hepatic pools of cholesterol [117] which via the action of the sterol regulatory element binding protein (SREBP) would lead to expression of the LDL receptor with inducement of the indirect reverse cholesterol transport (RCT) system in which apoB containing lipoproteins are cleared from plasma resulting in reductions of LDL-C, non-HDL-C, apoB, and LDL-P [83, 118, 119]. Ezetimibe also increases expression of ABCG5/ABCG8 which would also promote biliary excretion of sterols further contributing to RCT [120, 121]. Seemingly in a paradoxical fashion, ezetimibe monotherapy, to a variable degree, via SREBP-2 activation can increase cholesterol synthesis and via activation of PCSK9 production increase LDLR catabolism. Biomarkers of cholesterol absorption decrease but those related to synthesis, such as lathosterol and desmosterol, increase on ezetimibe monotherapy [122].

Several clinical trials using ezetimibe monotherapy in humans have revealed LDL-C-lowering effects in the range of 17–20% at a dose of 10 mg per day. The reductions in sitosterol and campesterol are even more significant at 48% for campesterol and 41% for sitosterol. Ezetimibe seems to reduce cholesterol absorption by a mean average by 54% compared to placebo. Fecal sterol excretion increased by 72%. Interestingly, cholesterol synthesis was increased by 89% compared to placebo, as indicated by the lathosterol/cholesterol ratio (an indicator of hepatic HMGCoA reductase activity). Safety and tolerability of ezetimibe have been excellent in numerous trials [122–124]. In a dose response study, 5- and 10-mg doses of ezetimibe significantly reduced LDL-C levels by 15.7% and 18.5%, respectively ($P < 0.01$ vs placebo) and significantly increased high-density lipoprotein cholesterol (HDL-C) levels by 2.9% and 3.5%, respectively ($P < 0.05$ vs placebo). A reduction in plasma TG levels was observed (P = NS) [114]. There is considerable interindividual variation in the response to ezetimibe. Pharmacogenetic results suggest that non-synonymous *NPC1L1* variation is associated with inter-individual variation in response to ezetimibe treatment [125].

Ezetimibe is most often utilized in clinical practice in combination with statins because the dual mechanisms of action, hindering cholesterol absorption and synthesis lead to synergistic LDLR upregulation and reductions in apolipoprotein B, LDL-C and C-reactive protein [126, 127].

Low-dose statin plus ezetimibe have the same cholesterol lowering effect of the high dose statins [128]. In a small pharmacokinetic study, ezetimibe 10 mg and rosuvastatin 10 mg reduced LDL-C by 61.4% within 2 weeks [129]. Ezetimibe can be particularly beneficial when administered to persons with less than predicted LDL-C lowering responses to statins or in persons known to be hyperabsorbers of cholesterol. One study of familial hypercholesterolemic patients showed poor responders to statins have decreased rates of cholesterol synthesis that may be secondary to a genetically determined increase in cholesterol absorption, associated with increased apolipoprotein *E4* genotypes [130]. In patients having a >40% additional LDL-C reduction with the addition of ezetimibe to a statin, the LDL-C response to the statin was <60% of the predicted lowering range (3–60%) – i.e., statin hypo-responders have exaggerated responses to ezetimibe [131]. In the Scandinavian Simvastatin Survival Study (4S), the LDL-C lowering ability of simvastatin was positively related to hypoabsorbers of cholesterol and negatively related in the hyper-synthesizers of cholesterol [132]. In such statin hypo-responders, statin responsiveness could be enhanced by reducing dietary cholesterol intake or inhibiting absorption with plant stanols, ezetimibe, or BAS.

Ezetimibe has been studied extensively as a monotherapy and in combination with multiple other lipid-modifying agents in clinical trials, predominantly in secondary prevention settings (see Table 13.1). The Gauging the lipid effects of RosuvAstatin plus ezetimibe Versus simvastatin plus ezetimibe TherapY (GRAVITY) study compared the efficacy, safety, and effect on biomarkers of cholesterol synthesis and absorption, inflammation (as measured by lipoprotein-associated phospholipase A2 (LP-PLA2)), and lipid/lipoprotein biomarkers in 833 adult patients with CHD or CHD risk equivalents. Patients were given simvastatin 40 mg/day or 80 mg/day plus ezetimibe 10 mg/day vs rosuvastatin 10 mg /day or 20 mg/day + ezetimibe 10 mg/day over 12 weeks. Rosuvastatin 20 mg/ezetimibe 10 mg achieved significantly ($p < 0.001$) greater reductions in TC, TG, LDL-C, Non-HDL-C, and apoB levels and TC/HDL-C, LDL-C/HDL-C, Non-HDL-C/HDL-C, and apoB/apoA-I ratios vs. either of the simvastatin/ezetimibe doses. As far as lipid goal attainment, a significantly higher percentage of patients achieved LDL-C goals of <70 mg/dl and <100 mg/dl (95.6% and 77.0%, respectively) than with either simvastatin/ezetimibe dose (87.4% and 88.6%, and 55.3% and 67.7%, respectively). As expected, biomarker studies showed that statin therapy reduced cholesterol synthesis markers and ezetimibe reduced cholesterol absorption biomarkers (including β-sitosterol). Lp-PLA2 was reduced further when ezetimibe was added to statin therapy. Safety profiles of rosuvastatin/ezeti-

Table 13.1 Clinical efficacy

	Treatment	LDL-C baseline	Total cholesterol #	LDL-C #	HDL-C #	TG #	NON-HDL-C #	apoB #	hs-CRP #	MISC.	References
Clinical Trial											
Lipid Research Clinics Coronary Primary Prevention Research Trial (LRCC)	24 g/day cholestyramine	205.3 mg/dl	13.4% decrease	20.3% decrease	5.4% increase	17% increase					[1]
	Placebo	204.5 mg/dl	0.7% decrease	3.4% decrease	2.5% increase	13.3% increase					
Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B	Lovastatin (20 mg twice a day) and colestipol (10 g three times a day)	5.08 mmol/L	33.8% decrease ($p < 0.001$)	45.5% decrease ($p < 0.001$)	16.5% increase ($p < 0.01$)	8.8% decrease					[3]
	Niacin (1 g four times a day) and colestipol (10 g three times a day)	4.92 mmol/L	22.6% decrease ($p < 0.001$)	32.1% decrease ($p < 0.001$)	40.6% increase ($p < 0.001$)	29.2% decrease ($p < 0.001$)					
Beneficial Effects of Combined Colestipol-Niacin Therapy on Coronary Atherosclerosis and Coronary Venous Bypass Grafts (CLAS Study)	30 g of colestipol hydrochloride plus 3 to 12 g of niacin daily, titrated individually (on the basis of blood cholesterol response)	171 mg/dl	26% decrease	43% decrease	37% increase	22% decrease					[4]

(continued)

Table 13.1 (continued)

	Treatment	LDL-C baseline	Total cholesterol #	LDL-C #	HDL-C #	TG #	NON-HDL-C #	apoB #	hs-CRP #	MISC.	References
	Placebo	179 mg/dl	4% decrease	5% decrease	2% increase	5% decrease					
Colesevelam, a new potent bile acid sequestrant	Placebo	5.01 mmol/L	0.3% decrease	0.4% decrease	0.7% decrease	7.4% decrease					
	1.5 g colesevelam/day	5.02 mmol/L	1.8% decrease	2.2% decrease	1.5% increase	3.0% decrease					[6]
	3.75 mg colesevelam/day	5.03 mmol/L	8.3% decrease ($p < 0.001$)	19.3% decrease ($p < 0.001$)	8.4% increase ($p < 0.05$)	11.6% increase					
Efficacy, safety, and effect on biomarkers related to cholesterol and lipoprotein metabolism of rosuvastatin 10 or 20 mg plus ezetimibe 10 mg vs. simvastatin 40 or 80 mg plus ezetimibe 10 mg in high-risk Patients: Results of the GRAVITY randomized study	Rosuvastatin 20 mg plus ezetimibe 10 mg	162/7 mg/dl	43.0% decrease	59.7% decrease	6.4% increase	28.9% decrease	54.7% decrease	46.1% decrease	25.2% decrease	A significantly greater proportion of patients achieved LDL-C goals of <100 mg/dl and <70 mg/dl with RSV20/EZE10 vs. SIM40/EZE10 and SIM80/EZE10 and with RSV10/EZE10 vs. SIM40/EZE10	[11]
	Simvastatin 40 mg plus ezetimibe 10 mg	164.8 mg/dl	39.6% decrease	55.2% decrease	3.9% increase	23.0% decrease	49.9% decrease	42.0% decrease	28.5% decrease		
	Simvastatin 40 mg plus ezetimibe 10 mg	163.1 mg/dl	41.7% decrease	57.4% decrease	4.3% increase	25.8% decrease	54.4% decrease	44.2% decrease	30.6% decrease		

Lipid-Altering Efficacy and Safety of Ezetimibe/Simvastatin Versus Atorvastatin in Patients With Hypercholesterolemia and the Metabolic Syndrome (from the VYMET Study)	Ezetimibe 10 mg/simvastatin 20 mg	139 mg/dl	33.7% decrease	49.6% decrease	6.8% increase	23.3% decrease	43.8% decrease	37.2% decrease	17.2% decrease	The percentage of patients who attained LDL-C <70 mg/dl and other prespecified levels of LDL-C and non-HDL-C were significantly greater for ezetimibe/simvastatin than for atorvastatin at all dose comparisons ($p < 0.05$)	[15]
	Atorvastatin 20 mg	137 mg/dl	28.3% decrease	39.4% decrease	5.6% increase	27.5% decrease	36.5% decrease	31.9% decrease	21.4% decrease		
	Ezetimibe 10 mg/simvastatin 40 mg	134 mg/dl	37.3% decrease	53.9% decrease	8.8% increase	29.5% decrease	48.3% decrease	41.4% decrease	27.5% decrease		
	Atorvastatin 40 mg	140 mg/dl	32.8% decrease	46.0% decrease	4.9% increase	30.0% decrease	41.4% decrease	35.8% decrease	30.0% decrease		
Impact of Dual Lipid-Lowering Strategy With Ezetimibe and Atorvastatin on Coronary Plaque Regression in Patients With Percutaneous Coronary Intervention The Multicenter Randomized Controlled PRECISE-IVUS Trial	Atorvastatin (Atorvastatin was up titrated with a treatment goal of low-density lipoprotein cholesterol (LDL-C) <70 mg/dl.)	108.3 mg/dl	18% decrease	29% decrease	11% increase	9% decrease		26% decrease	86% decrease		[16]

(continued)

Table 13.1 (continued)

	Treatment	LDL-C baseline	Total cholesterol #	LDL-C #	HDL-C #	TG #	NON-HDL-C #	apoB #	hs-CRP #	MISC.	References
	Atorvastatin plus ezetimibe 10 mg/day	109.8 mg/dl	25% decrease	40% decrease	14% increase	14% decrease		34% decrease	89% decrease		
Simvastatin with or without Ezetimibe in Familial Hypercholesterolemia (ENHANCE Trial)	Simvastatin 80 mg	317.8 mg/dl	31.9% decrease	39.1% decrease	7.8% increase	23.2% decrease		33.1% decrease	23.5% decrease		[17]
	Ezetimibe 10 mg vs simvastatin 80 mg	319.0 mg/dl	45.0% decrease	55.6% decrease	10.2% increase	29.8% decrease		49.7% decrease	49.2% decrease		
The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomized placebo-controlled trial (SHARP trial)	Simvastatin 80 mg plus 10 mg ezetimibe	2.77 mmol/L		30.3% decrease							[20]
	Placebo	2.78 mmol/L	2.9% decrease								
Intensive Lipid Lowering with Simvastatin and Ezetimibe in Aortic Stenosis (SEAS trial)	40 mg simvastatin plus 10 mg ezetimibe	139 mg/dl		53.8% decrease							[25]

	Placebo	140 mg/dl		3.8% decrease							
Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes (IMPROVE-IT Trial)	40 mg simvastatin plus 10 mg ezetimibe	93.8 mg/dl	22.7% decrease	43.3% decrease	15.7% increase	12.5% decrease	35.9% decrease	24.2% decrease	85.1% decrease		[26]
	40 mg simvastatin	93.8 mg/dl	10.0% decrease	25.5% decrease	14.0% increase	0.3% decrease	19.4% decrease	12.3% decrease	82.3% decrease		
Effectiveness and Tolerability of Ezetimibe Add-on Therapy to a Bile Acid Resin-Based Regimen for Hypercholesterolemia	Average daily doses BAS were colesevelam 3085 mg, colestipol 10,200 mg, and cholestyramine 6000 mg (most patients were on statins also)	174 mg/dl	18% decrease	19% decrease	4% decrease	14% decrease					[38]
	10 mg ezetimibe added to baseline BAS										
Lipoprotein effects of combined ezetimibe and colesevelam hydrochloride versus ezetimibe alone in hypercholesterolemic subjects: a pilot study	Ezetimibe 10 mg daily with placebo colesevelam HCl twice daily	167 mg/dl	19% decrease	24% decrease	0.9% increase	19% decrease	25% decrease	31% decrease			[40]

(continued)

Table 13.1 (continued)

	Treatment	LDL-C baseline	Total cholesterol #	LDL-C #	HDL-C #	TG #	NON-HDL-C #	apoB #	hs-CRP #	MISC.	References
	Ezetimibe 10 mg daily with 1.875 g plus colesevelam HCl twice daily	158 mg/dl	15% decrease	30% decrease	5% increase	36% increase	21% decrease	22% decrease			
Efficacy and safety of alirocumab in high cardiovascular risk patients with inadequately controlled hypercholesterolaemia on maximally tolerated doses of statins: The ODYSSEY COMBO II randomized controlled trial	Ezetimibe 10 mg/day on top of background statin therapy	2.7 mmol/L	14.6% decrease	21.8% decrease	0.5% increase	12.8% decrease	19.2% decrease	18.3% decrease			[45]
	Alirocumab 75 mg SC every 2 weeks (Q2W; plus oral placebo). Alirocumab dose was increased to 150 mg Q2W at week 12 depending on week 8 LDL-C values	2.8 mmol/L	29.3% decrease	51.2% decrease	9.6% increase	13% decrease	42.1% decrease	40.7% decrease			

Efficacy and safety of alirocumab vs ezetimibe in statin-intolerant patients, with a statin rechallenge arm: The ODYSSEY ALTERNATIVE randomized trial	Ezetimibe 10 mg/d (plus SC placebo Q2W)	193.5		14.6% decrease	6.6% increase	3.6% decrease	14.6% decrease	11.2% decrease		Lp(a) mass 7.3% decrease	[46]
	Alirocumab 75 mg SC every 2 weeks (Q2W; plus oral placebo). Alirocumab dose was increased to 150 mg Q2W at week 12 depending on week 8 LDL-C values	191.1		45% decrease	7.6% increase	9.3% decrease	40.2% decrease	36.2% decrease		Lp(a) mass 25.9% decrease	
	Subcutaneous evolocumab (420 mg monthly)	221.9 mg/dl	11.6% decrease	52.8% decrease	7.4% increase	2.9% decrease	45.7% decrease	43.5% decrease		Lp(a) mass 21.1% decrease	

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Table 13.1 (continued)

	Treatment	LDL-C baseline	Total cholesterol #	LDL-C #	HDL-C #	TG #	NON-HDL-C #	apoB #	hs-CRP #	MISC.	References
Efficacy and Tolerability of Evolocumab vs Ezetimibe in Patients with Muscle-Related Statin Intolerance: The GAUSS-3 Randomized Clinical Trial	Ezetimibe (10 mg daily)	218.8 mg/dl	36.6% decrease	16.7% decrease	2.9% increase	1.1% decrease	14.6% decrease	11.7% decrease		Lp(a) mass 0.2% increase	[47]
# = % change from baseline											
TG = triglyceride											

mibe and simvastatin/ezetimibe combinations were comparable. This study demonstrated the efficacy of ezetimibe in its additive effect in lowering various biomarkers of atherogenesis, improving lipid goal attainment and potentially in enhancing event reduction produced by statins [133].

The randomized, controlled, multicenter Vytorin vs. Atorvastatin in Patients with type 2 diabetes mellitus (T2DM) and Hypercholesterolemia (VYTAL) trial compared lipid and lipoprotein ratio alterations on 10/20 mg/day ezetimibe/simvastatin (EZE/SIMVA) to 10 and 20 mg atorvastatin/day (ATORVA), as well as comparing 10/40 mg/day EZE/SIMVA to 40 mg/day ATORVA[i]. A total of 1198 primary prevention T2DM subjects were studied for 6 weeks after randomization. LDL-C/HDL-C, TC/HDLC, NONHDL-C/HDL-C, and apoB/apoA-I ratios all showed more significant decreases from baseline in the patients receiving EZE/SIMVA when compared with ATORVA. These ratios are believed to be positively correlated with atherosclerotic cardiovascular disease risk [134]. In the VYTELD study, a 12-week, randomized, double-blind, parallel group trial, the efficacy and safety of EZE/SIMVA 10/20 mg/d vs ATORVA 10 or 20 mg/day and EZE/SIMVA 10/40 mg/d vs ATORVA 40 mg/day were examined in 1289 hypercholesterolemic moderate- or high-risk primary and secondary prevention patients >65 years of age. Patients in the EZE/SIMVA group had significantly lower LDL-C and significantly higher goal attainment of LDL-C <70 and LDL-C <100 mg/dl in all prespecified comparisons with ATORVA patients. EZE/SIMVA therapy vs ATORVA therapy resulted in significantly greater decreases in TC, NONHDL-C, apoB, and all lipid/lipoprotein ratios (LDL-C/HDL-C, TC/HDL-C, NONHDL-C/HDL-C, apolipoprotein B/apolipoprotein A-I) for all prespecified treatment comparisons. In contrast, there was no significant difference between the two therapies in high-sensitivity C-reactive protein (hs-CRP) percentage change between the prespecified comparisons. Few studies have looked at the efficacy of lipid-lowering therapies specifically in a metabolic syndrome population [135]. The VYMET study was a double-blinded, randomized, 6-week study looking at similar comparisons between EZE/SIMVA and ATORVA in 1128 primary prevention patients with metabolic syndrome and hypercholesterolemia. EZE/SIMVA was significantly superior in reducing LDL-C, NONHDL-C, apoB, attainment of LDL-C, and NONHDL-C targets, and various lipid/lipoprotein ratios, when compared with ATORVA. Together these studies indicate the utility of adding ezetimibe to a statin in reducing atherogenic lipid and lipoprotein biomarkers in both insulin-sensitive and insulin-resistant patient populations [136].

The PRECISE-IVUS study was a prospective randomized controlled study that looked at the effects of ezetimibe plus atorvastatin versus atorvastatin monotherapy on the lipid profile and IVUS-determined coronary atherosclerosis in 202 high risk Japanese patients who underwent percutaneous coronary intervention (PCI) (see Table 13.2). As expected, the combination of atorvastatin/ezetimibe resulted in lower levels of LDL-C than atorvastatin monotherapy (63.2 +/− 16.3 mg/dl vs. 73.3 +/− 20.3 mg/dl; $p < 0.001$). For the absolute change in percent atheroma volume (PAV), the mean difference between the two groups (−1.538%; 95% confidence interval [CI]: −3.079% to 0.003%) showed non-inferiority. For PAV, a significantly greater percentage of patients who received atorvastatin/ezetimibe showed coronary plaque regression (78% vs. 58%; $p = 0.004$). Side effects profiles did not differ significantly between the two therapeutic strategies. This study showed the potential benefit of a dual LDL-C lowering strategy on IVUS determined atherosclerotic disease burden and the superiority of this strategy to statin monotherapy [137].

The ENHANCE trail was a double-blind, randomized, 24-month trial comparing the effects of daily therapy with 80 mg of simvastatin either with placebo or with 10 mg of ezetimibe in 720 patients with familial hypercholesterolemia (FH). The primary outcome measure was the change in the mean carotid-artery intima–media thickness, defined as the average of the means of the far-wall intima–media thickness of the right and left common carotid arteries, carotid bulbs, and internal carotid arteries. At the end of the study, the mean change in mean CIMT did not differ between the simvastatin-only group and simvastatin-plus-ezetimibe group

Table 13.2 Clinical outcomes

	Clinical outcomes	Treatment	Primary endpoint/years of follow-up	Relative risk reduction	Absolute risk reduction	NNT	Secondary endpoint	Relative risk reduction	Absolute risk reduction	NNT	Misc.	References
Clinical Trial												
Lipid Research Clinics Coronary Primary Prevention Research Trial (LRCC)		24 g/day cholestyramine vs placebo	Definite CHD death and/or definite nonfatal MI/ 7 years	19%, $p < 0.05$	1.6%	62.5	Definite CHD death	24%			Incidence rates for new positive exercise stress test, angina and coronary bypass surgery were reduced by 25%, 20%, 21%, respectively	[1]
St. Thomas Atherosclerosis Regression Study – STARS-Atherosclerosis		Diet plus 8 g bid cholestyramine, vs. diet alone	Change in mean absolute width of the coronary segment (MAWS)				Definite nonfatal MI				MAWS of coronary segments increased by 0.003 mm in diet-only group, and by 0.103 mm in diet-plus-cholestyramine group, compared with decrease in controls.	[2]

Regression of coronary artery disease as a result of intensive lipid-lowering therapy in men with high levels of apolipoprotein B		Lovastatin (20 mg twice a day) and colestipol (10 g three times a day); niacin (1 g four times a day) and colestipol (10 g three times a day); or conventional therapy with placebo (or colestipol if the low-density lipoprotein [LDL] cholesterol level was elevated)	Progression of coronary angiographic lesions				Clinical events (death, myocardial infarction, or revascularization for worsening symptoms)	27%; (95 % confidence interval, 0.10 to 0.77)			Angiographic progression (as the only change) was less frequent among patients who received lovastatin and colestipol (21%) and those who received niacin and colestipol (25%), and regression was more frequent (lovastatin and colestipol, 32%; niacin and colestipol, 39%; *P* less than 0.005)	[3]

(continued)

Table 13.2 (continued)

	Clinical outcomes	Treatment	Primary endpoint/years of follow-up	Relative risk reduction	Absolute risk reduction	NNT	Secondary endpoint	Relative risk reduction	Absolute risk reduction	NNT	Misc.	References
Beneficial Effects of Combined Colestipol-Niacin Therapy on Coronary Atherosclerosis and Coronary Venous Bypass Grafts (CLAS Study)		30 g of colestipol hydrochloride plus 3–12 g of niacin daily, titrated individually (on the basis of blood cholesterol response), vs placebo	There was a significant reduction in the average number of lesions per subject that progressed ($P < 0.03$) and the percentage of subjects with new atheroma formation ($P < 0.03$) in native coronary arteries, over 2 years								For the absolute change in percent atheroma volume (PAV), the mean difference between the 2 groups (−1.538%; 95% confidence interval [CI]: −3.079% to 0.003%) did not exceed the pre-defined noninferiority margin of 3%, but the absolute change in PAV did show superiority for the dual lipid-lowering strategy (−1.4%; 95% CI: −3.4% to −0.1% vs. –0.3%; 95% CI: −1.9% to 0.9% with atorvastatin alone; p ¼ 0.001). For PAV, a significantly greater percentage of patients who received atorvastatin/ezetimibe showed coronary plaque regression (78% vs. 58%; p ¼ 0.004)	[4]

Impact of Dual Lipid-Lowering Strategy with Ezetimibe and Atorvastatin on Coronary Plaque Regression in Patients with Percutaneous Coronary Intervention The Multicenter Randomized Controlled PRECISE-IVUS Trial		Atorvastatin (Atorvastatin was uptitrated with a treatment goal of low-density lipoprotein cholesterol (LDL-C) <70 mg/dl), vs atorvastatin plus 10 mg ezetimibe/day	Serial volumetric intravascular ultrasound was performed at baseline and again at 9 to 12 months to quantify the coronary plaque response in 202 patients				For the absolute change in percent atheroma volume (PAV), the mean difference between the two groups (−1.538%; 95% confidence interval [CI]: −3.079% to 0.003%) did not exceed the predefined noninferiority margin of 3%, but the absolute change in PAV did show superiority for the dual lipid-lowering strategy (−1.4%; 95% CI: −3.4% to −0.1% vs. –0.3%; 95% CI: −1.9% to 0.9% with atorvastatin alone; p ¼ 0.001). For PAV, a significantly greater percentage of patients who received atorvastatin/ezetimibe showed coronary plaque regression (78% vs. 58%; p ¼ 0.004)					[16]

(continued)

Table 13.2 (continued)

	Clinical outcomes	Treatment	Primary endpoint/years of follow-up	Relative risk reduction	Absolute risk reduction	NNT	Secondary endpoint	Relative risk reduction	Absolute risk reduction	NNT	Misc.	References
Simvastatin with or without Ezetimibe in Familial Hypercholesterolemia (ENHANCE Trial)		Simvastatin 80 mg/plus ezetimibe 10 mg vs simvastatin 80 mg	Change in the mean carotid-artery intima–media thickness, which was defined as the average of the means of the far-wall intima–media thickness of the right and left				Secondary outcomes (consisting of other variables regarding the intima–media thickness of the carotid and femoral arteries) did not differ significantly between the two groups				Primary outcome was 0.0058 ± 0.0037 mm in the simvastatin-only group and 0.0111 ± 0.0038 mm in the simvastatin-plus-ezetimibe (combined-therapy) group ($P = 0.29$)	[17]
The effects of lowering LDL cholesterol with simvastatin plus ezetimibe in patients with chronic kidney disease (Study of Heart and Renal Protection): a randomised placebo-controlled trial (SHARP trial)		Simvastatin 80 mg plus 10 mg ezetimibe vs. placebo	First major atherosclerotic event (non-fatal myocardial infarction or coronary death, non-haemorrhagic stroke, or any arterial revascularisation procedure)	17% reduction ([RR] 0.83, 95% CI 0.74–0.94) log-rank $p = 0.0021$	2.1%	47.6	Major atherosclerotic events plus non-coronary cardiac deaths and haemorrhagic strokes	15% reduction (RR 0.85, 95% CI 0.77–0.94; $p = 0.0012$)	2.5%	40	Non-significantly fewer patients allocated to simvastatin plus ezetimibe had a non-fatal myocardial infarction or died from coronary heart disease (213 [4.6%] vs 230 [5.0%]; RR 0.92, 95% CI 0.76–1.11; $p = 0.37$) and there were significant reductions in non-haemorrhagic stroke (131 [2.8%] vs 174 [3.8%]; RR 0.75, 95% CI 0.60–0.94; $p = 0.01$) and arterial revascularisation procedures (284 [6.1%] vs 352 [7.6%]; RR 0.79, 95% CI 0.68–0.93; $p = 0.0036$)	[20]

Intensive Lipid Lowering with Simvastatin and Ezetimibe in Aortic Stenosis (SEAS trial)		40 mg simvastatin plus 10 mg ezetimibe	The primary outcome was a composite of major cardiovascular events, including death from cardiovascular causes, aortic-valve replacement, nonfatal myocardial infarction, hospitalization for unstable angina pectoris, heart failure, coronary-artery bypass grafting, percutaneous coronary intervention, and nonhemorrhagic stroke	4% reduction (HR = 0.96; 95% confidence interval [CI], 0.83–1.12; $P = 0.59$))	2.9%		Aortic-valve replacement	0% reduction (HR = 1.00; 95% CI, 0.84 to 1.18; $P = 0.97$)				[25]
		Placebo					Ischemic cardiovascular events	22% reduction (HR = 0.78; 95% CI, 0.63–0.97; $P = 0.02$)			Fewer patients had ischemic cardiovascular events in the simvastatin–ezetimibe group than in the placebo group mainly because of the smaller number of patients who underwent Coronary-artery bypass grafting	

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Table 13.2 (continued)

	Clinical outcomes	Treatment	Primary endpoint/years of follow-up	Relative risk reduction	Absolute risk reduction	NNT	Secondary endpoint	Relative risk reduction	Absolute risk reduction	NNT	Misc.	References
Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes (IMPROVE-IT Trial)		Simvastatin 40 mg plus 10 mg ezetimibe vs simvastatin 40 mg	Composite of cardiovascular death, nonfatal myocardial infarction, unstable angina requiring rehospitalization, coronary revascularization (≥30 days after randomization), or nonfatal stroke	6% reduction (HR = 0.936; 95% confidence interval, 0.89–0.99; $P = 0.016$)	2%	50	Composite of death from coronary heart disease, nonfatal myocardial infarction, or urgent coronary revascularization 30 days or more after randomization	5% reduction (HR = 0.95 (0.90–1.0) $p < 0.03$)			The risk of any myocardial infarction was significantly lower with simvastatin–ezetimibe than with simvastatin monotherapy (difference, 1.7 percentage points; hazard ratio, 0.87; $P = 0.002$), as was the risk of ischemic stroke (difference, 0.7 percentage points; hazard ratio, 0.79; $P = 0.008$)	[26]
Low-density lipoprotein cholesterol targeting with pitavastatin plus ezetimibe for patients with acute coronary syndrome and dyslipidaemia: the HIJ-PROPER study, a prospective, open-label, randomized trial		Pitavastatin 1–4 mg vs. pitavastatin 1–4 mg plus ezetimibe 10 mg	Composite of all-cause death, non-fatal myocardial infarction, non-fatal stroke, unstable angina, and ischaemia-driven revascularization	11% reduction (HR 0.89, 95% CI 0.76–1.04, $P = 0.152$)	4.1%		(i) Cardiovascular event (non-fatal myocardial infarction, non-fatal stroke, UA, ischaemia-driven revascularization with either PCI or CABG), (ii) all-cause death, (iii) heart failure, (iv) inflammatory markers, and (v) adverse events (including new occurrence of malignant tumour)	No significant differences were noted between standard and intensive treatment in terms of secondary outcomes			In, ACS patients with higher cholesterol absorption, represented by elevated pre-treatment sitosterol, was associated with significantly lower incidence of the primary endpoint in the statin plus ezetimibe group (HR 0.71, 95% CI 0.56–0.91)	[36]

Ezetimibe in Prevention of Cerebro- and Cardiovascular Events in Middle- to High-Risk, Elderly (75 Years Old or Over) Patients With Elevated LDL-Cholesterol - EWTOPIA 75		Ezetimibe 10 mg daily plus dietary counseling (n = 1716) or dietary counseling (n = 1695). A placebo pill was not administered in the control arm	Primary CV outcome, sudden cardiac death, myocardial infarction, percutaneous coronary intervention or coronary artery bypass grafting, and/or stroke,	34% reduction ((HR) 0.66, 95% confidence interval (CI) 0.50–0.86, p = 0.002)			Cardiac events	40% reduction (HR 0.60, 95% CI 0.37–0.98, p = 0.04)				[44]
							Cerebrovascular events	22% reductions (HR 0.78, 95% CI 0.55–1.11, p > 0.05)				
							All-cause mortality	9% increase (HR 1.09, 95% CI 0.89–1.34)	43			

($p = 0.29$), despite a 16.5% between-group difference in LDL-C which was 192.7 ± 60.3 mg per deciliter (4.98 ± 1.56 mmol per liter) in the simvastatin group and 141.3 ± 52.6 mg per deciliter (3.65 ± 1.36 mmol per liter). The difference in hs-CRP between the two groups was 25.7% lower in the combined therapy group ($P < 0.01$) [138]. The lack of effectiveness in the reduction of CIMT-tracked atherosclerotic disease despite the additional lowering of LDL-C by ezetimibe was widely viewed as questioning ezetimibe's potential for lowering CVD clinical events. Speculation about this lack of effectiveness centered around several theories: (1) a lack of CIMT improvement by ezetimibe despite the observed reduction in LDL-C may possibly have been due to ezetimibe's different mechanism of action; (2) the inability of the CIMT measurement technique to accurately reflect changes in atherosclerotic burden; and (3) the possibility that the study population had too low a risk for disease progression, perhaps due to prolonged pre-treatment with statin therapy. Counter arguments include (1) accumulating evidence that reduction of LDL-C by other non-statin therapies (e.g. BAS, ileal bypass) did reduce CVD risk; (2) the strong association between CIMT and CVD events as shown in large epidemiologic studies such as ARIC [139]; and (3) perhaps most importantly, the effects of previous statin therapy on CIMT levels and progression. Most FH patients begin to receive statin therapy early in life, and both the baseline CIMT and the rate of progression of CIMT change in ENHANCE are lower than that seen in comparable trials with less statin "pre-treatment" [140]. Therefore, "pre-treatment" with statin therapy may have limited the extent to which lowering of LDL-C levels resulted in a further decrease in the progression of CIMT. In part, because of the ENHANCE findings cardiovascular drugs now have to be judged based on the results of clinical outcome not imaging trials.

The Study of Heart and Renal Protection (SHARP) trial was a randomized double-blinded trial of 9270 primary prevention patients with chronic kidney disease (3023 on dialysis and 6247 not) followed for a median follow-up of 4.9 years. Patients were randomly assigned to simvastatin 20 mg plus ezetimibe 10 mg daily versus matching placebo. The primary outcome was first major atherosclerotic event (non-fatal MI or coronary death, non-hemorrhagic stroke, or any arterial revascularization procedure). Comparison between the simvastatin/ezetimibe group and the simvastatin-alone group showed an average LDL-C difference of 0.85 mmol/L (SE 0.02; with about two-thirds compliance) and resulted in a 17% relative risk reduction in major atherosclerotic events (526 [11.3%] simvastatin plus ezetimibe vs 619 [13.4%] placebo; rate ratio [RR] 0.83, 95% CI 0.74–0.94; log-rank $p = 0.0021$). There were significant reductions in non-hemorrhagic stroke (131 [2.8%] vs 174 [3.8%]; RR 0.75, 95% CI 0.60–0.94; $p = 0.01$) and arterial revascularization procedures (284 [6.1%] vs 352 [7.6%]; RR 0.79, 95% CI 0.68–0.93; $p = 0.0036$). After weighting for subgroup-specific reductions in LDL-C, there was no good evidence that the proportional effects on major atherosclerotic events differed from the summary rate ratio in any subgroup examined, and they were similar in patients on dialysis and those who were not. There were no clinically significant safety issues with this combination in these patients, who are at high risk for adverse events due to their illness severity and multiple medications [141]. The event reduction was consistent with the CTT trial data of reducing the risk of non-fatal MI or coronary death, stroke, or coronary revascularization by about 20% per 1 mmol/L LDL-cholesterol reduction. The SHARP trial is important because of the ability of this combination to safely and significantly reduce events in this high-risk population of patients with severe chronic illness. In comparison, two trials of statin-monotherapy regimens, one in patients on hemodialysis, the *Deutsche Diabetes DialyseStudie* [142], and AURORA which is A *Study* to Evaluate the Use of *Rosuvastatin* in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events or [143], and one other trial in patients who had undergone renal transplantation, the Assessment of LEscol in Renal Transplantation ALERT [144], failed to achieve statistically significant event reductions in their primary outcomes. The failure to achieve statistical significance in the previous renal trials has been thought to be due to factors such as the much

smaller number of patients and the much smaller proportion of modifiable vascular events in their primary outcomes.

SEAS (Simvastatin and Ezetimibe in Aortic Stenosis) was a randomized double-blinded controlled clinical trial looking at the reduction of CVD events in patients with mild to moderate, asymptomatic aortic stenosis. A total of 1873 patients were followed up for a median of 52.2 months on either 40 mg of simvastatin plus 10 mg of ezetimibe or placebo daily, resulting in an average reduction in LDL-C of at least 50%, as compared with placebo. The primary outcome was a MACE composite, including death from cardiovascular causes, aortic-valve replacement, nonfatal-MI, hospitalization for unstable angina pectoris, heart failure, coronary-artery bypass grafting, percutaneous coronary intervention, and non-hemorrhagic stroke. A small reduction in the primary outcome did not achieve statistical significance (hazard ratio in the simvastatin–ezetimibe group, 0.96; 95% confidence interval [CI], 0.83–1.12; $P = 0.59$). Similar results were found for aortic-valve replacement (hazard ratio, 1.00; 95% CI, 0.84–1.18; $P = 0.97$). However, driven by a reduction in coronary artery bypass grafting, fewer patients had ischemic cardiovascular events in the simvastatin–ezetimibe group (hazard ratio, 0.78; 95% CI, 0.63–0.97; $P = 0.02$). There was no effect on the progression of aortic stenosis as seen on echocardiography. Cancer occurred more frequently in the simvastatin–ezetimibe group (105 vs. 70, $P = 0.01$), a finding not seen in other ezetimibe clinical trials. Although this trial did not meet its primary outcome, the reduction in ischemic cardiovascular events in this asymptomatic aortic stenosis population is hypothesis generating [145].

The IMPROVE-IT trial was the first clinical trial to validate the hypothesis that adding an additional LDL-C lowering non-statin medication to patients on statin therapy would further reduce CVD events. The trial also demonstrated that lowering LDL-C to levels below established targets (e.g. LDL-C < 70 mg/dl) would reduce CVD events. A total of 18,144 patients who had been hospitalized for an acute coronary syndrome (ACS) and had LDL-C levels of 50–100 mg per deciliter (1.3–2.6 mmol per liter) if they were receiving lipid-lowering therapy or 50–125 mg per deciliter (1.3–3.2 mmol per liter) if they were not receiving lipid-lowering therapy, were enrolled and followed for a median follow-up of 6 years. Simvastatin 40 mg and ezetimibe 10 mg was compared with simvastatin 40 mg and placebo, with a primary MACE end point (a composite of first cardiovascular death, nonfatal myocardial infarction, unstable angina requiring rehospitalization, coronary revascularization (≥30 days after randomization), or nonfatal stroke.) The median time-weighted average LDL-C level during the study was 53.7 mg per deciliter (1.4 mmol per liter) in the simvastatin–ezetimibe group, as compared with 69.5 mg per deciliter (1.8 mmol per liter) in the simvastatin-placebo group (~24% LDL-C reduction, $P < 0.001$). The Kaplan–Meier event rate for the primary end point at 7 years was 32.7% in the simvastatin–ezetimibe group, as compared with 34.7% in the simvastatin-placebo group, giving an absolute risk difference of 2.0%, and a NNT of 50 (hazard ratio, 0.936; 95% confidence interval, 0.89–0.99; $P = 0.016$) [146]. The HR reduction is approximately what should be expected from the CTT trial analysis from the observed LDL-C decrease. As far as other notable prespecified endpoints, death from cardiovascular causes, MI, or stroke was reduced by 10% (1704 (22.2) 1544 (20.4) 0.90 HR (0.84–0.96), p 0.003). Significant reductions were observed in the rates of myocardial infarction and ischemic stroke (simvastatin/placebo 1118 (14.8), simvastatin/ezetimibe 977 (13.1), HR 0.87 (0.80–0.95) $p = 0.002$, and simvastatin/placebo 297 (4.1), simvastatin/ezetimibe 236 (3.4), HR 0.79 (0.67–0.94) $p = 0.008$. The HR for event reduction was very consistent with that seen by statins in the CTT trial, that is, 0.78 observed with statins in the CTT meta-analysis vs 0.80 in IMPROVE-IT [147].

The IMPROVE-IT study has multiple clinical implications. It clearly establishes a benefit of the combination therapy on CVD events in patients with acute coronary syndromes as well as chronic coronary artery disease, since it spanned at least

6 years. Furthermore, IMPROVE-IT establishes the safety of the simvastatin–ezetimibe combination. IMPROVE-IT's successful test of the benefit of two different LDL goals (70 mg/dl in the simvastatin arm and 55 mg/dl in the simvastatin plus ezetimibe arm) supports the concept of treatment based on specific lipid goals favored by some preventive cardiology groups [148]. IMPROVE-IT demonstrates that the additional LDL-C reduction achieved with ezetimibe is of the same quality in terms of CVD risk reduction, as that obtained with statins in monotherapy. This leads us to question the concept of "pleotropic effects" of statins, as well as challenging the concept of "high intensity STATIN therapy" as a target of treatment. If "high intensity CHOLESTEROL-LOWERING therapy" can achieve similar clinical results why would statins need to be pushed to maximal/high doses? Given its safety profile and greater reduction of LDL-C, combination therapy with statins and ezetimibe offers an alternative solution for patients on statins who cannot tolerate escalating doses or do not get to their goals using statins alone [149].

There are criticisms of the IMPROVE-IT trial: 42% of the patients discontinued the study medication for any reason prematurely; however, there was an equal proportion in both groups (simvastatin/placebo and simvastatin/ezetimibe). Overall treatment effect on the composite primary end point was modest considering the large sample size and relatively long follow-up period (NNT of 50 with ARR of 2%). As the trial was designed prior to contemporary guidelines, these patients were treated with a moderate-intensity statin rather than with a standard-of-care high-intensity statin. IMPROVE-IT was conducted with people within days of a myocardial infarction (MI), so the results only apply to secondary prevention. The NNT of 50 was over 7 years; the calculated NNT over *five years would be 70, compared to a NNT of approximately 44 for other statin trials* [150].

Subsequent analyses of IMPROVE-IT showed further benefits. The IMPROVE-IT analysis studied only first-time primary end points (PEP). When subsequent events were analyzed, there was an even greater difference between the simvastatin–placebo and simvastatin–ezetimibe groups (additional events HR: 0.88; 95% CI: 0.79–0.98) and total events (RR: 0.91; 95% CI: 0.85–0.97; $p = 0.007$), driven by reductions in ischemic stroke and MI. Thus, IMPROVE-IT demonstrated a reduction in not only first events but in total events over long-term follow-up with the addition of ezetimibe to statin therapy [151]. Although IMPROVE-IT did not show a reduction in mortality, additional ischemic events of stroke and MI have been associated with not only a higher mortality but also an impaired quality of life and higher costs [152]. Diabetic patients benefitted more from simvastatin–ezetimibe vs simvastatin–placebo than non-diabetics (in patients with type 2 diabetes (T2D)), the Kaplan–Meier primary end point event rate difference between groups was 5.5% absolute (hazard ratio, 0.85; 95% confidence interval, 0.78–0.94); in patients without DM, the absolute difference was 0.7% (hazard ratio, 0.98; 95% confidence interval, 0.91–1.04; $P_{int} = 0.02$) [153]. The risk reduction in patients with DM was driven by decreases in myocardial infarction (HR 24%) and ischemic stroke (HR 39%). When clinical outcomes were examined in a subset of patients who met prespecified targets of LDL-C <70 mg/dl and hs-CRP <2 mg/L vs. achieving neither target, those patients who met both targets had lower primary end point rates than those meeting neither target (cardiovascular death, major coronary event, or stroke; 38.9% versus 28.0%; adjusted hazard ratio, 0.73; 0.66–0.81; $P < 0.001$) [154]. Finally, the use of a simple 9-point risk stratification tool (the TIMI (Thrombolysis in Myocardial Infarction) Risk Score for Secondary Prevention (TRS 2P)) has been shown to identify a subset of high-risk patients who had a 6.3% (95% confidence interval: 2.9% to 9.7%) absolute risk reduction in CV death/MI/ischemic CVA at 7 years with ezetimibe–simvastatin, thus translating to a number-needed-to-treat of only16 [155].

The HIJ-PROPER study is a prospective, randomized, open-label (PROBE design) trial to assess whether intensive LDL-C lowering with standard-dose pitavastatin plus ezetimibe reduces cardiovascular events more than standard LDL-C lowering with pitavastatin monotherapy in patients with acute coronary syndrome (ACS) and dyslipidemia [156]. The primary endpoint

was a MACE endpoint consisting of all cause death, non-fatal MI, non-fatal stroke, unstable angina, and ischemia-driven revascularization. A total of 1734 Japanese patients were randomized to intensive lowering (target LDL-C < 70 mg/dl [1.8 mmol/L]; pitavastatin plus ezetimibe) or standard lowering (target LDL-C 90 mg/dl to 100 mg/dl [2.3–2.6 mmol/L]; pitavastatin monotherapy) and followed up for 3.86 years. Mean follow-up LDL-C was 65.1 mg/dl (1.68 mmol/L) for pitavastatin plus ezetimibe and 84.6 mg/dl (2.19 mmol/L) for pitavastatin monotherapy. The results showed that LDL-C lowering with statin plus ezetimibe did not reduce primary endpoint occurrence in comparison with standard statin monotherapy (283/864, 32.8% vs. 316/857, 36.9%; HR 0.89, 95% CI 0.76–1.04, $P = 0.152$). However, in, ACS patients with higher cholesterol absorption, there was a significantly lower incidence of the primary endpoint in the statin plus ezetimibe group (HR 0.71, 95% CI 0.56–0.91), suggesting a potential strategy for ezetimibe use in these "on-statin hyper-absorbers."

Because of the different mechanisms by which BAS and ezetimibe affect intestinal cholesterol absorption, the combination therapy has been investigated for additive therapeutic advantages [157]. The first report of dual therapy was of 40 patients on a stable lipid-lowering regiment that included BAS who were treated with an additional 10 mg ezetimibe and studied in a prospective chart review study over an average follow-up of 107 days. Most patients had CHD or an equivalent high risk status and were on multiple lipid-lowering medications. The BAS used were colesevelam 3085 mg [$n = 33$], colestipol 10,200 mg [$n = 5$], and cholestyramine, 6000 mg [$n = 2$]), and 31 of 40 patients were also on statins. The addition of ezetimibe 10 mg/day resulted in reductions of TC (18%), LDL-C (19%), TG (14%), and HDL-C (4%). Treatment was well tolerated [158].

Eighty six patients with FH and an LDL-C concentration >2.5 mmol/L who were receiving a maximally tolerated and stable regimen of a statin plus ezetimibe were studied for 12 weeks with the addition of 3.75 mg/day colesevelam. The addition of colesevelam was well tolerated and resulted in the following lipid and lipoprotein changes: Between-group differences (95% CI) in LDL-C, TC, HDL-C, TG, and apoB/apoA-I ratio after 12 weeks were −12.0% (−17.8 to −6.3), −7.3% (−12.0 to −2.6), +3.3% (−2.4 to +9.0), +2.8% (−10.4 to +15.9), and −12.2% (−20.2 to −4.2), respectively [159]. Other studies have shown little or no additional effects of combing BAS with ezetimibe. When 20 subjects with LDLC >130 mg/dl were studied on 10 mg ezetimibe plus standard dose colesevelam vs placebo colesevelam for 12 weeks, there was only a borderline lower LDL-C level compared to ezetimibe alone (LDL-C, 24% reduction +/− 12% vs 30% reduction +/− 11% ($P = 0.102$) [153]. The combined treatment was associated with an increase in triglyceride that was statistically significant, and no further reduction in total cholesterol, Non-HDL-C, or apoB levels [160].

The combination of ezetimibe with other non-statin agents, such as fenofibrate, niacin, thiazolidinediones, orlistat, acarbose and metformin, has been studied, though clinical trials are small and there are little or no clinical outcomes data [161]. In general, lipid and lipoprotein effects are positive, and the ezetimibe is well tolerated. There is much room for further research with these combinations.

The Cochrane analysis ("Ezetimibe for the prevention of cardiovascular disease and all-cause mortality events (Review)") is an excellent summary of 26 studies with 23,499 patients treated with ezetimibe. The findings are dominated by IMPROVE-IT study results, as this study is by far the majority of the existing ezetimibe clinical trial data. IMPROVE-IT data carried weight in the different meta-analyses ranging from 41.5% to 98.4%. Trials were selected for inclusion in the review based on the following criteria: they were randomized controlled trials (RCTs) that compared ezetimibe versus placebo or ezetimibe plus other lipid-modifying, drugs versus other lipid-modifying drugs alone in adults, with or without CVD, and which had a follow-up of at least 12 months. The main conclusions were as follows:

1. The use of ezetimibe with statins probably reduces the risk of major adverse cardiovascular events compared with statins alone (risk ratio (RR) 0.94, 95% confidence interval (CI) 0.90–0.98). This conclusion was drawn from 10 studies with 21,727 participants; the quality of the evidence was judged to be "moderate".
2. The addition of ezetimibe to statins probably reduces the risk of non-fatal MI (RR 0.88, 95% CI 0.81–0.95). This conclusion was drawn from six studies with 21,145 participants; the quality of the evidence was judged to be "moderate".
3. The addition of ezetimibe to statins probably reduces the risk of non-fatal stroke (RR 0.83, 95% CI 0.71–0.97). This conclusion was drawn from six studies with 21,205 participants; the quality of the evidence was judged to be "moderate".
4. The addition of ezetimibe to a statin might reduce the need for coronary revascularization (RR 0.94, 95% CI 0.89–0.99). This conclusion was drawn from seven studies with 21,323 participants. However, no difference in coronary revascularization rate was observed when a sensitivity analysis was limited to studies with a low risk of bias.
5. The addition of ezetimibe to a statin or fenofibrate in six studies with 19,457 participants probably had little or no effect on reducing cardiovascular death as an outcome (RR 1.00, 95% CI 0.89–1.12). This was judged to be "moderate" quality evidence.
6. The addition of ezetimibe to statin or fenofibrate had little or no effect on all-cause mortality (RR 0.98, 95% CI 0.91–1.05) based on eight studies with 21,222 participants. This was judged to be "high" quality evidence.
7. In terms of safety, ezetimibe was generally well tolerated. The addition of ezetimibe to statins did not increase the risk of hepatopathy (RR 1.14, 95% CI 0.96–1.35) based on four studies with 20,687 participants. This was judged to be "low"-quality evidence.
8. It is uncertain whether the addition of ezetimibe to statins increased or decreased myopathy risk (RR 1.31, 95% CI 0.72–2.38; 20,581 20581 participants), based on three studies. This was judged to be "very low" quality evidence given the wide CIs and low event rate. There was little or no difference in the risk of cancer, gallbladder-related disease, and discontinuation due to adverse events observed between treatment groups in these studies.

The authors overall conclusion from this meta-analysis was as follows: "Therefore, the addition of ezetimibe to statin therapy might be an alternative treatment for patients at high risk of ASCVD who are unable to tolerate the recommended statin intensities or fail to achieve their treatment goals" [162].

The Ezetimibe Lipid Lowering Trial on Prevention of Atherosclerosis in 75 or Older (EWTOPIA) trial is the first trial to look at ezetimibe alone to reduce cardiovascular events. Ezetimibe vs. dietary counseling was studied in a primary prevention setting of elderly (>75 years old) Japanese patients with an LDL-C > 140 mg/dl plus one high risk factor. The primary CV outcome was a MACE outcome consisting of sudden cardiac death, myocardial infarction, percutaneous coronary intervention, or coronary artery bypass grafting: hazard ratio (HR) 0.66, 95% confidence interval (CI) 0.50–0.86, $p = 0.002$. Secondary outcome results were as follows: cardiac events: HR 0.60, 95% CI 0.37–0.98, $p = 0.04$; cerebrovascular events: HR 0.78, 95% CI 0.55–1.11, $p > 0.05$; all-cause mortality: HR 1.09, 95% CI 0.89–1.34. This study is one of the first to show a benefit with a non-statin agent as monotherapy for primary prevention among patients with high LDL-C and adds important information about cardiovascular disease risk reduction in older patients [163].

Proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors have emerged as potent apoB lowering therapeutic agents as monotherapy or in combinations with statins or stains plus ezetimibe [164, 165].

COMBO II was a double-blind, double-dummy, active-controlled, parallel-group, 104-week study of alirocumab vs. ezetimibe in 720 patients with high cardiovascular risk and elevated LDL-C despite maximal doses of statins. Patients were randomized to subcutaneous ali-

rocumab 75 mg every 2 weeks (plus oral placebo) or oral ezetimibe 10 mg daily (plus subcutaneous placebo) on a background of statin therapy. At week 24, mean ± SE reductions in LDL-C from baseline were 50.6 ± 1.4% for alirocumab vs. 20.7 ± 1.9% for ezetimibe (difference 29.8 ± 2.3%; $P < 0.0001$); 77.0% of alirocumab and 45.6% of ezetimibe patients achieved LDL-C <1.8 mmol/L ($P < 0.0001$). Mean achieved LDL-C at week 24 was 1.3 ± 0.04 mmol/L with alirocumab and 2.1 ± 0.05 mmol/L with ezetimibe. Alirocumab and ezetimibe were generally well tolerated, with no evidence of an excess of treatment-emergent adverse events. The authors concluded that in patients at high cardiovascular risk with inadequately controlled LDL-C, alirocumab achieved significantly greater reductions in LDL-C compared with ezetimibe, with a similar safety profile [166].

The ODYSSEY ALTERNATIVE trial compared alirocumab with ezetimibe in 361 patients at moderate to high cardiovascular risk with a baseline mean LDL-C of 191.3 mg/dl and statin intolerance (defined as being unable to tolerate ≥2 statins, including one at the lowest approved starting dose) due to muscle symptoms. A placebo run-in and statin re-challenge arm were included in an attempt to confirm intolerance, and patients reporting muscle symptoms during this placebo run-in were withdrawn from the study. Continuing patients were randomized (2:2:1) to double-blind alirocumab 75 mg SC every 2 weeks (Q2W; plus oral placebo), ezetimibe 10 mg/d (plus SC placebo Q2W), or atorvastatin 20 mg/d (re-challenge; plus SC placebo Q2W) for 24 weeks. Alirocumab dose was increased to 150 mg Q2W at week 12 depending on week 8 LDL-C values. The primary end point was percent change in LDL-C from baseline to week 24 (intent-to-treat) for alirocumab vs ezetimibe. Alirocumab reduced mean (standard error) LDL-C by 45.0% (2.2%) vs 14.6% (2.2%) with ezetimibe (mean difference 30.4% [3.1%], $P < 0.0001$). Skeletal muscle-related events were less frequent with alirocumab vs atorvastatin (hazard ratio 0.61, 95% confidence interval 0.38–0.99, $P = 0.042$). The authors concluded that alirocumab produced greater LDL-C reductions than ezetimibe in statin-intolerant patients, with fewer skeletal-muscle adverse events vs atorvastatin [167].

Similar to the ODYSSEY ALTERNATIVE trial, the GAUSS-3 RCT looked at evolocumab (420 mg monthly) vs ezetimibe 10 mg/day in 511 adult hi-risk patients with uncontrolled low-density lipoprotein cholesterol (LDL-C) levels and history of intolerance to 2 or more statins. A crossover period of 24-weeks with atorvastatin or placebo was used to identify patients having symptoms only with atorvastatin but not placebo. The co-primary end points were the mean percent change in LDL-C level from baseline to the mean of weeks 22 and 24 levels and from baseline to week 24 levels. For the mean of weeks 22 and 24, LDL-C level with ezetimibe was 183.0 mg/dl; mean percent LDL-C change, −16.7% (95% CI, −20.5% to −12.9%), absolute change, −31.0 mg/dl and with evolocumab was 103.6 mg/dl; mean percent change, −54.5% (95% CI, −57.2% to −51.8%); absolute change, −106.8 mg/dl ($P < 0.001$). For weeks 22 and 24, between-group difference in LDL-C was −37.8% and absolute difference was −75.8 mg/dl. Results were very similar for the baseline to week 24 levels group. Muscle symptoms were reported in 28.8% of ezetimibe-treated patients and 20.7% of evolocumab-treated patients (log-rank $P = 0.17$). Active study drug was stopped for muscle symptoms in 5 of 73 ezetimibe-treated patients (6.8%) and 1 of 145 evolocumab-treated patients (0.7%). The authors concluded that the use of evolocumab compared with ezetimibe resulted in a significantly greater reduction in LDL-C levels after 24 weeks [168].

Statins and Cholesterol Absorption

Since cholesterol absorption, synthesis, and excretion are tightly regulated, it is not surprising that drugs which affect cholesterol homeostasis may have variable effects on cholesterol absorption and cholesterol lowering in the plasma. Short-term studies of statin use reveal an initial decrease in cholesterol and noncholesterol

sterols plasma levels [169]. After a certain time (approximately 6 weeks), noncholesterol sterol levels rise in patients on statin therapy [105, 170, 171]. LXR agonism, caused by increased cellular oxysterols, downregulates *NPC1L1* resulting in diminished sterol entry into the enterocyte. By diminishing intracellular levels of cholesterol, statins cause a downregulation of LXR-alpha. The statin induced downregulation of LXR, which in turn upregulates *NPC1L1*, increasing sterol entry into the enterocyte, and downregulates ABCA1 and ABCG5/ABCG8, reducing hepatic and intestinal excretion of sterols [45, 172]. Atorvastatin decreases cholesterol synthesis as indicated by increased lathosterol levels, and it also increases the absorption of cholesterol as indicated by increased campesterol levels (two to sixfold), and these sterols ultimately appear in lipoproteins [72]. A study in metabolic syndrome patients had similar findings [173].

The increased noncholesterol sterol absorption associated with statins may have clinical effects: in a study of carotid endarterectomy tissue in patients on statins vs those not on statins, serum plant sterols correlated with cholesterol absorption and the plaque in patients on statins was associated with increased campesterol levels. The sterols in the plaque were of dietary origin transported mostly in LDL. No definitive conclusions as to role of noncholesterol sterols in atherosclerosis can be taken from this study [174].

Hypothesis generating data from the 4S study revealed that patients with coronary atherosclerosis with hyperabsorption of sterols and low synthesis of cholesterol (as indicated by increased cholestanol: cholesterol ratio) did not respond to statin treatment. The patients with the lowest markers of absorption had the best event reduction with simvastatin and those with the highest sterol absorption had no statistically significant event reduction on simvastatin. The incidence of coronary events was unrelated to sterol levels in the placebo group [132, 175] which suggests that a patients responsiveness to statins can be identified by measuring serum a marker of cholesterol absorption such as cholestanol concentration before treatment. The lack of response to statins in the cholesterol hyperabsorbers is likely due to the decreased hepatic cholesterol synthesis in such patients. In a different analysis of the 4S trial, baseline sterol concentrations strongly indicated simvastatin suppressed the synthesis of cholesterol more effectively in subjects with high compared to low baseline synthesis but reduced respective

- PS and stanol supplements are commercially availble and recommened by guideline as an adjunct to lifestyle to lower total and LDL-cholesterol
- No outcome data exists with sterol or stanol therapy
- There is potential for increased phytosterol systemic levels in some individula (apoE4 genotype, postmenopausal women, family history of atherosclerosis and patients on statins)
- Ezetimibe, through a variety of actions reduces sterol absorption by about 50% leading to an upregulation of hepatic LDL-receptors
- Combining ezetimibe and statins induces a duo mechanism of reducing cholesterol absorption and synthesis which syncrgistically upregulates LDL receptors, dramatically reducing TC & LDL-C
- Combining ezetimibe and BAS additively reduce TC and LDL-C

Fig. 13.11 Summary of PS/stanol therapies

serum cholesterol levels less markedly ("poor response group") in those with increased marker of absorption [176] which also suggests such patients would benefit from a combination therapy of statins with stanols or ezetimibe to lower more effectively their serum cholesterol levels and reduce cholesterol absorption and prevent an increase in the levels of plant sterols [176] which also suggests such patients would benefit from a combination therapy of statins with stanols or ezetimibe to lower more effectively their serum cholesterol levels and reduce cholesterol absorption and prevent an increase in the levels of plant sterols (Fig. 13.11).

Bile Aid Sequestrants (BAS)

BAS also known as resins have been around for several decades with first-generation products launching in the 1960s and initially included cholestyramine, then colestipol which were always limited by compliance due to less than palatable powders, preparation (mixture with fluids), large and frequent dosage requirements and a high incidence of gastrointestinal side effects. BAS have often been used in children with familial hypercholesterolemia. A second-generation bile acid polymer, colesevelam which has the ability to bind to more bile acids, followed [177]. The first two BAS were once proclaimed as lipid-modulating drugs of first choice by the National Cholesterol Education program [178] because of their ability to reduce LDL-C and reduce clinical events (fatal and nonfatal MI) as seen in the Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) [179–181].

Being highly charged, chloride or hydrochlorideanion-exchange resins are not absorbable but because they can exchange the chloride for bile acids (BA), they create resin/BA complex which is excreted in feces, thereby reducing enterohepatic BA recirculation in the terminal ileum. The effect of fewer BA returning to the liver stimulates the enzyme cholesterol 7-hydroxylase and conversion of hepatic cholesterol pools to BA. In turn, LDLR upregulation and clearance of LDL particles occurs. Similar to other drugs that reduce hepatic cholesterol stores, such as ezetimibe, is a SREBP-2 activation of HMG-CoA reductase which increases FC synthesis and thus in part decreases their efficacy. BAS also can increase TG synthesis and VLDL production [182]. The earlier resins more so than colesevelam bind to other drugs and nutrients leading to interactions.

In addition to reducing apoB and LDL metrics, other lipid-modulating effects, the BA polymer colesevelam contributes to TICE enhancing fecal excretion of cholesterol [183]. Colesevelam also has an FDA indication to help achieve glycemic control likely due to the effect of bile acid sequestration which is involved in glucose regulatory signaling pathways which increase incretin release influenced by FXR or the membrane type bile acid receptor TGR5 [184–189].

The first-generation BAS were among the very first lipid-altering agents studied for the reduction of cardiovascular disease events (CVD) in a large randomized clinical trial (See Tables 13.1 and 13.2). The LRC-CPPT Study was a double-blinded, placebo-controlled retrospective study that examined the effect of cholestyramine on lowering coronary heart disease (CHD) events in 3806 asymptomatic men with type 2 hypercholesterolemia followed over 7.4 years. Participants had a total cholesterol (TC) of greater than 265 mg /dl and an LDL-cholesterol (LDL-C) of 190 mg/dl or greater. The primary outcome was CHD death or non-fatal myocardial infarction (MI). LDL-C was reduced 20.3% in the treatment group and decreased by 12.6% when compared to the diet-alone placebo group. This LDL-C reduction was accompanied by a 19% reduction in the primary outcome ($p < 0.05$). CHD death and non-fatal MI were reduced by 24% and 19%, respectively. The incidence rates for new onset positive stress test, angina pectoris, and coronary artery bypass were reduced by 25%, 20%, and 21%, respectively. Interestingly, the percentage of patients taking the full 24 g/day of cholestyramine was lowered by the drug's GI adverse effects, but LDL-C fell 35% in participants taking the full 24 g/day of cholestyramine. This full dose and LDL-C reduction would have let to a projected 49% reduction in CHD events.

The most common adverse effects noted in the treatment group were gastrointestinal effects, such as constipation, diarrhea, gas, bloating, and heartburn, but they were not severe and diminished during the course of the study. This study is the first large trial to show a safe reduction in hard CVD endpoints through therapeutic lowering of LDL-C [179–181].

The St. Thomas Atherosclerosis Regression Study (STARS) study looked at angiographic disease progression in men with mildly increased total cholesterol of 7.23 mmol/L and coronary artery disease (CAD), defined as angina or past myocardial infarction. Controls on a "usual diet" (U) were compared with a low-fat dietary intervention (D) group and with a cholestyramine (DC) group. The proportion of patients who showed overall progression of angiographic coronary narrowing was significantly reduced by both interventions (U 46%, D 15%, DC 12%), whereas the proportion who showed an increase in luminal diameter rose significantly (U 4%, D 38%, DC 33%). The Mean Absolute Width of Coronary Segments (MAWS) increased most significantly in the cholestyramine DC group and the change in MAWS was independently and significantly correlated with LDL-C concentration during the trial period. Both low fat diet (D group) and cholestyramine interventions (DC group) significantly reduced the frequency of total cardiovascular events [190].

The BAS colestipol 10 g three times/day was part of the regimen used in studying angiographic changes over 2.5 years in a double-blinded study in 146 men less than age 63 with documented CAD and elevated apolipoprotein B (apoB) atherogenic particle counts >125 mg/dl. Study arms consisted of lovastatin 20 mg /day and colestipol, niacin 4 g/day and colestipol, and placebo (or colestipol if LDLC was elevated). LDL-C fell much more in the lovastatin plus colestipol group (45%) and the niacin plus colestipol groups (32%) than the conventional therapy group (7%). Intensive therapy with colestipol helped to reduce CVD events and slow the progression of angiographic disease. Angiographic lesion progression was much less frequent in the intensely treated groups (21% for lovastatin plus colestipol; 25% for colestipol + niacin) vs. 46% progression rate in the conventional therapy group. Regression of disease was much more frequent in the intensively treated groups (32% lovastatin plus colestipol; 39% in the niacin + colestipol group) vs. a regression rate of only 11% in the conventional therapy group. Multivariate analysis indicated that a reduction in the level of apoB (or LDL-C) and in systolic blood pressure, and an increase in HDL-cholesterol (HDL-C) correlated independently with regression of coronary lesions. Death, myocardial infarction, and revascularization were followed as clinical events and were significantly less frequent in the intensive therapy groups (relative risk of an event during intensive treatment, 0.27; 95% confidence interval, 0.10–0.77). Colestipol was shown to be an effective and safe component of multi-drug therapy to reduce angiographic disease and CHD events [191].

The niacin and colestipol combination was again tested by looking at angiographic data in the Cholesterol-Lowering Atherosclerosis Study (CLAS) study. This study looked at 162 men aged 40–59 who had undergone coronary artery bypass graft surgery (CABG). The study was a randomized, placebo-controlled, 2-year angiographic trial. Total cholesterol dropped 26%, LDL-C dropped 43%, and HDL-C increased 37%. The average number of lesions per subject that progressed was reduced significantly ($P < 0.03$) and the percentage of subjects with new atheroma formation in native coronary arteries was also significantly reduced ($p < 0.3$). Atherosclerosis regression, as indicated by perceptible improvement in overall coronary status, occurred in 16.2% of colestipol-niacin treated vs 2.4% of the placebo treated ($P = 0.002$) [192]. Follow-up of 103 patients over 4 years (CLAS II) demonstrated that significantly more intensively treated patients showed non-progression on angiography (52% drug- vs 15% placebo-treated) and regression (18% drug- vs 6% placebo-treated) in native coronary artery lesions. Significantly fewer intensively treated subjects developed new lesions in native coronary arteries (14% drug- vs 40% placebo-treated) and bypass grafts (16%

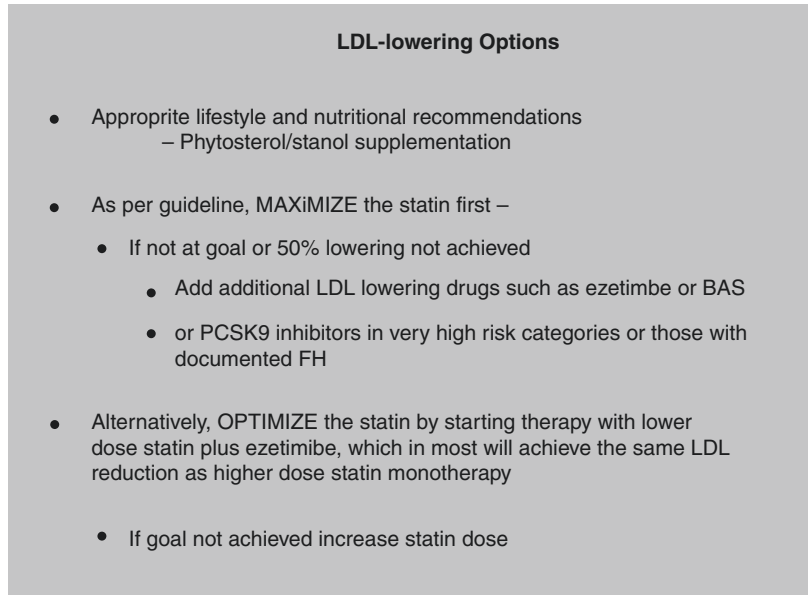

Fig. 13.12 Therapeutic options

drug- vs 38% placebo-treated). The colestipol and niacin combination was shown to be a safe and effective way to slow and even reverse angiographic CAD [193].

The BAS colesevelam offers the advantage of not being as constipating as cholestyramine and lowers LDL-C in a dosage-dependent manner. When tested in men and women with LDL-C > 4.14 mmol/L (160 mg/dl), LDL-C was lowered by 0.11 mmol/L (4.2 mg/dl) (1.8%) at 1.5 g/day and up to 1.01 mmol/L (39.1 mg/dl (19.1%) in the 3.75 g/day group [194]. Unfortunately, colesevelam was never evaluated in a clinical outcomes trial assessing clinical event reduction. There is some evidence that BAS alters the composition and qualitative characteristics of LDL particles. Colestipol hydrochloride therapy at 20–30 g/day produced LDL particles that were smaller in size, more dense, and characterized by decreased cholesterol to protein ratio. This resulted in a specific decrease in the subpopulation of larger, more buoyant LDL particles [195] (Fig. 13.12).

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14 Proprotein Convertase Subtilisin/Kexin Type 9: Functional Role in Lipid Metabolism and Its Therapeutic Inhibition

Peter P. Toth

Introduction

A proprotein convertase is a proteolytic enzyme that converts an inactive precursor molecule into an active one [1]. An example of this is the conversion of a zymogen (a proenzyme) into a mature bioactive enzyme with catalytic activity in some aspect of intermediary metabolism [2]. There is a family of nine proprotein convertase subtilisin kexins (PCSK). Eight of the nine members are catalytically active and are responsible for the proteolytic conversion of a wide variety of molecules (including receptors, hormones, enzymes, transcription factors) into their bioactive forms [3]. The ninth member of this family (PCSK9) is the most recently discovered and is atypical. PCSK9 like other PVSKs is a serine protease. Once translated in the endoplasmic reticulum (ER), its signal peptide is cleaved by a signal peptidase to form zymogen proPCSK9 [4] (Fig. 14.1). In order to leave the ER and enter the cytosol, it catalyzes the autocleavage of a prosegment from itself. Subsequent to this step PCSK9 is no longer able to engage in a catalytic activity because the cleaved prosegment remains associated and it causes steric hindrance of this enzyme's active site [5]. Enzymatically, PCSK9 has only a single molecular target: its own prosegment.

Low-density lipoprotein particles (LDL-P) are principally cleared from the systemic circulation by the LDL receptor (LDLR) [6]. LDL receptors are expressed along the surface of hepatocytes and are concentrated in clathrin-coated pits within cell membranes. Once an LDLR binds an LDL-P, it is configured within the clathrin-coated pit by LDLR adaptor protein 1 (aka clathrin associated sorting protein), though there is evidence that the protein disabled homolog adaptor protein 2 (dab-2) can also perform this role [7, 8]. An endosome forms and is covered with a clathrin polyhedral lattice [9] (Fig. 14.2). The endosome is released into the cytosol, the clathrin dissociates, and the internal milieu of the endosome is acidified. The drop in pH potentiates the dissociation of the LDLR from LDL-P. Through a mechanism that is yet to be defined, the LDL-P is specifically translocated into the lysosome for destruction by cathepsins and lipases. The LDLR is routed back to the hepatocyte cell surface to initiate another round of LDL-P uptake and catabolism (Fig. 14.3).

PCSK9 regulates the expression of the LDLR (Fig. 14.3). Once in the extracellular milieu, PCSK9 can bind to the epidermal growth factor-like repeat A domain of LDLR [10]. LDLR bound to both an LDL-P and PCSK9 are also concentrated in clathrin-coated endosomes. However, in this instance, the PCSK9 holds the

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_14

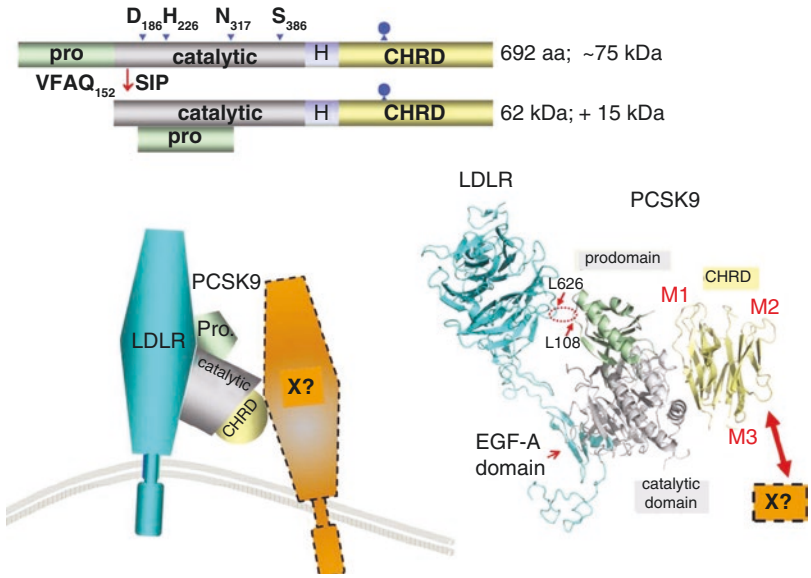


Fig. 14.1 Schematic representation of proprotein convertase subtilisin kexin 9 (PCSK9) zymogen processing and binding to the low-density lipoprotein receptor (LDLR). (**a**) The autocatalytic zymogen processing of proPCSK9 (75 kDa) at Val-Phe-Ala-Gln152↓Ser-Ile-Pro (VFAQ152↓SIP) into the (prosegment (15 kDa) ≡ PCSK9 (62 kDa)) complex is emphasized, together with the positions of the active site Asp186, His226, and Ser386 and the oxyanion hole Asn317. The C-terminal hinge domain (H) and cys-his-rich domain (CHRD) are shown. (**b**) Cartoon representation of the cell surface interaction of the catalytic domain of PCSK9 with the epidermal growth factor-A (domain of the LDLR, as well as the suspected interaction of the prosegment with the β-barrel domain of the LDLR and the CHRD with a putative membrane-bound protein X. (**c**) Crystal structure of the ectodomain of the LDLR with PCSK9 emphasizing the interaction between them and the three subdomains in the CHRD (M1, M2, and M3). The interaction of protein X is presumed to be with one of the latter subdomains, possibly M2. (From Seidah et al. [4])

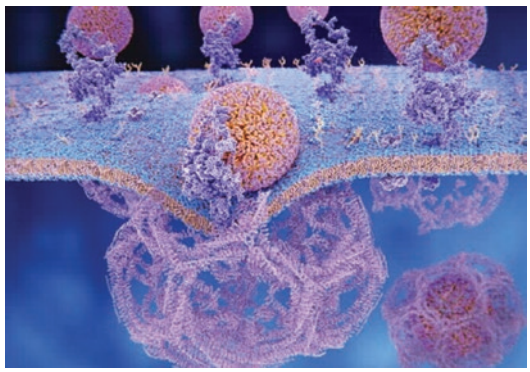


Fig. 14.2 Endosome formation and uptake into the cytosol via a polyhedral clathrin cage. (From Trialsitenews.com)

LDLR and LDL-P tightly together, and they do not dissociate as the intra-endosomal pH decreases [11]. The PCSK9 functions as a chaperone molecule of the LDLR-LDL-P complex into the lysosome. This results in LDLR catabolism and reduced cell surface expression of this receptor vital to LDL-P clearance.

The *PCSK9* gene localizes to chromosome 1 and, like the genes for *LDLR* and apoprotein B (*apo B*), is a locus for familial hypercholesterolemia [12, 13]. The expression of PCSK9 is regulated by the nuclear transcription factor sterol regulating element-binding protein-2 [14]. Gain-of-function mutations in *PCSK9* such as D374Y and R496W lead to reduced LDLR expression, increased serum levels of LDL-P, and heightened risk for ASCVD [15, 16]. Such loss-of-function (LOF) mutations in *PCSK9* as Y142X and C679X in persons of African descent [17] and Q152H in French Canadians [18] lead to increased LDLR expression, lower serum LDL-C, and reduced risk for ASCVD. Major prospective longitudinal

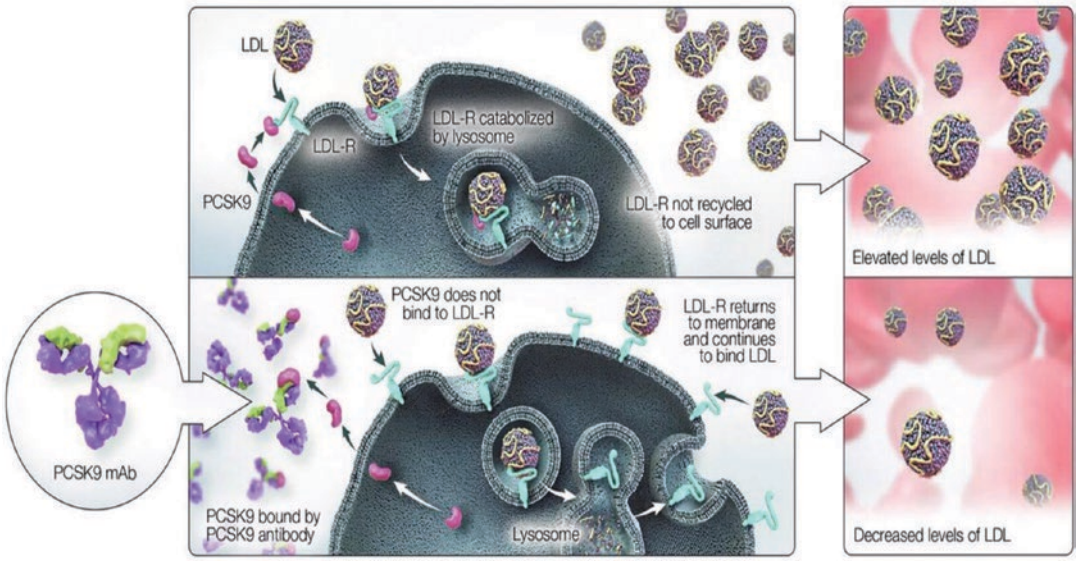


Fig. 14.3 LDL recycling, PCSK9 function, and effect of PCSK9 inhibition. Mechanism of action of PCSK9 inhibition for reduction of serum cholesterol concentration. *Top panel*: PCSK9 secreted by hepatocytes binds to LDL-R on the hepatocyte surface. Upon subsequent binding of the receptor by LDL, the PCSK9/LDL/LDL-R complex is internalized within an endosomal vesicle. The endosome fuses with a lysosome, and the PCSK9 chaperones the LDL/LDL-R complex into the lysosome for destruction. As a result, the number of LDL-Rs is decreased, resulting in less clearance of LDL from the circulation and elevated LDL concentration. *Bottom panel*: Monoclonal antibody binds to PCSK9 and prevents it from engaging the LDL-R. In the absence of PCSK9, the LDL-R is not routed to the lysosome for degradation and is returned instead to the hepatocyte surface. The recycled LDL-R is available for additional LDL binding and clearance, resulting in decreased levels of LDL. LDL, low-density lipoprotein; LDL-R, low-density lipoprotein receptor. (From Toth [96]. With permission from Elsevier)

cohorts confirm that LOF mutations in both men and women result in substantially lower LDL-C levels compared to patients with wild-type alleles for PCSK9 [19] (Fig. 14.4).

Lipoprotein receptor physiology is complex. PCSK9 also regulates cell surface expression of other lipoprotein receptors, possibly impacting serum levels of other lipoproteins and their subfractions (Fig. 14.5). PCSK9 regulates the expression of the very low-density lipoprotein receptor (VLDLR), the apolipoprotein E2 receptor, and the LDL receptor-related protein-1, all of which can participate in the clearance of various apo B-containing lipoproteins [20–23]. In addition, PCSK9 regulates the expression of a cluster of differentiation 36 (CD 36), which is a fatty acid translocase in adipocytes and hepatocytes [24]. The D374Y GOF mutation also suggests that PCSK9 upregulates Nieman Pick C1-like protein without impacting the expression of SR-BI or ABCG5/G8 [25].

Therapeutic Strategies for Inhibiting PCSK9

Monoclonal Antibodies

A monoclonal antibody is a highly specific antibody directed toward a single molecular target [26]. Given the fact that PCSK9 is a secreted protein, it can be targeted by a monoclonal antibody (mAb) in an effort to neutralize its activity. Two fully human mAbs (evolocumab and alirocumab) directed against PCSK9 have been developed for treating primary hyperlipidemia and familial hypercholesterolemia. They are fully human in an effort to reduce the risk of autoimmune responses and the generation of antibodies against them. This also reduces the risk of tachyphylaxis. These agents can be used independently of hepatic and renal function because they have no dependence on hepatic uptake and metabolism,

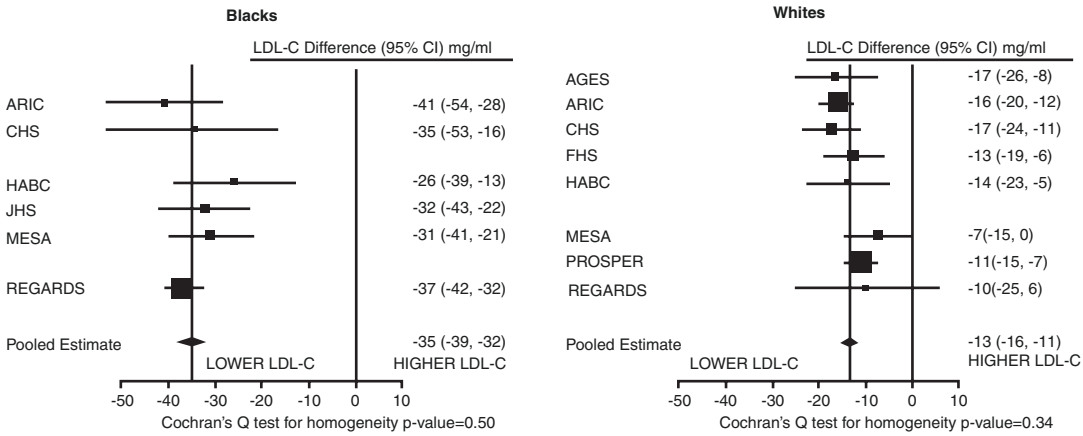


Fig. 14.4 Difference in low-density lipoprotein cholesterol (LDL-C) among participants with vs without *PCSK9* loss-of-function (LOF) variants in individual studies and pooled analyses. AGES indicates Age, Gene, Environment, Susceptibility Study–Reykjavik; ARIC, Atherosclerosis Risk in Communities Study; CHS, Cardiovascular Health Study, CI confidence interval; FHS, Framingham Heart Study; Health ABC, Health, Aging, and Body Composition Study; JHS, Jackson Heart Study; LDL-C, low-density lipoprotein cholesterol; MESA, Multi-Ethnic Study of Atherosclerosis; PROSPER, PROspective Study of Pravastatin in the Elderly at Risk for vascular disease; REGARDS, REasons for Geographic and Racial Differences in Stroke Study. LDL-C differences are comparing participants with *PCSK9* LOF variants to those without *PCSK9* LOF variants (*PCSK9* LOF variant minus no *PCSK9* LOF variant). Models for each participating study include adjustment for age, sex, region/center, and statin use. Pooled analyses are performed using inverse-variance-weighted fixed-effect models. (From Kent et al. [19])

and they do not depend on renal elimination for their clearance [27–29]. The antibody complexes formed between these agents and extracellular PCSK9 are cleared by the reticuloendothelial system (Kupffer cells, spleen, lymph nodes, bone marrow), and they do not promulgate drug interactions since they have no dependence for activity from organic anion transport proteins, the cytochrome P450 isozymes, or glucuronidation as there is no known antibody uptake pathway for the liver.

Evolocumab

LDL-C Reduction in Primary Hyperlipidemia

In patients with primary hyperlipidemia, evolocumab is a highly safe and efficacious therapy for reducing atherogenic lipoprotein burden in serum. It can be dosed at 140 mg subcutaneously (SQ) every 2 weeks or 420 mg SQ every 4 weeks. When used as monotherapy, evolocumab induces a 55%–57% reduction in LDL-C compared to placebo [30] (Table 14.1). When added to statin therapy (atorvastatin 10 mg or 80 mg; rosuvastatin 5 mg or 40 mg; simvastatin 40 mg), evolocumab provides a mean incremental reduction in LDL-C of 63% [31]. Among patients intolerant to two or more statins evolocumab provides a 38% reduction in LDL-C compared to ezetimibe [32]. For patients at various levels of ASCVD risk, the addition of evolocumab to ongoing lipid-lowering therapy (atorvastatin 10 mg or 80 mg daily or the combination of atorvastatin 80 mg daily with ezetimibe) provided a 48–62% incremental reduction of LDL-C compared to placebo [33].

LDL-C Reduction in Familial Hypercholesterolemia

Among patients with familial hypercholesterolemia (FH), as long as the patient is not a homozygote for a null mutation in *PCSK9*, mAbs directed against circulating PCSK9 would be expected to provide some degree of LDL-C reduction. In the

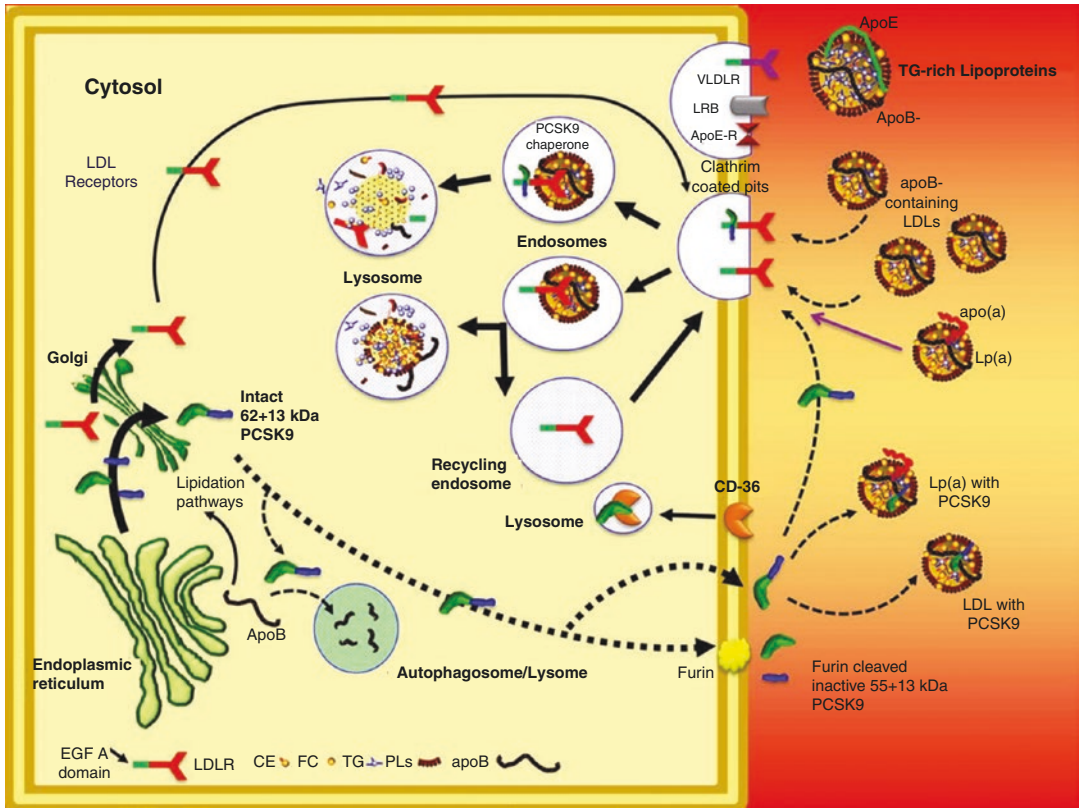


Fig. 14.5 Proprotein convertase subtilisin kexin type 9 (PCSK9) and lipoprotein trafficking. Subsequent to release from the Golgi apparatus, low-density lipoprotein receptors (LDLR) translocate to the cell membrane where they are concentrated in clathrin-coated pits. Hepatocytes secrete PCSK9 into the extracellular space which can bind to the epidermal growth-factor-like repeat A (EGF-A) domain of LDLR. Complexes composed of LDLR and LDL particles are internalized via endosomes. If the LDLR–LDL-P complex is bound with PCSK9, the complex is chaperoned into the lysosome for hydrolytic destruction. The LDLR in this case is not recycled to the cell surface. When LDLR–LDL-P complexes are not bound to PCSK9, the complex dissociates in response to a drop in pH within the endosome. The LDL-P is translocated to the lysosome for destruction, whereas LDLR is spared and recycled to the cell surface to initiate another round of LDL-P binding and uptake. The monoclonal antibodies directed against PCSK9 decrease LDLR translocation into the lysosome, increase LDLR surface expression, and significantly reduce plasma levels of LDL-P. Recent investigations demonstrate that PCSK9 can also regulate the expression of other cell surface receptors, such as a very-low-density lipoprotein receptor (VLDLR), the LDLR-related protein (LRP), an apoprotein E receptor, and cluster of differentiation 36 (CD36). In addition to its impact on cell surface receptor expression, PCSK9 potentiates the production of apo B and increases VLDL secretion by inhibiting the catabolism of apoB via an autophagosome/lysosome-dependent pathway. The serine protease furin (which is found in both membrane-bound and free forms) can cleave active, intact PCSK9 (62 kDa) into an inactive 55-kDa fragment. A small percentage of total circulating LDL and lipoprotein(a) (Lp(a)) can bind PCSK9. Although some Lp(a) is cleared by a pathway that is independent of LDLR, some is demonstrably cleared by LDLR. (From Toth [97])

reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder Study-2 (RUTHERFORD-2), evolocumab when dosed at either 140 mg every 2 weeks or 420 mg dosed every 4 weeks reduced LDL-C by 59% and 60%, respectively [34]. This result suggests that the upregulation of LDLR is so robust that patients with heterozygous FH (HeFH) respond to evolocumab with as much LDL-C reduction as patients with primary hyperlipidemia. As shown in the Trial Evaluating PCSK9 Antibody in Subjects with LDL Receptor

Table 14.1 Lipoprotein and remnant particle concentrations and percent change from baseline to week 52

Variable	Placebo		Evolocumab 420 mg QM	
	n		*n*	
LDL-P total				
Mean ± SD, (nmol/L)	246	1110.3 ± 326.2	300	609.8 ± 336.9
Percent change from baseline, mean [95% CI]	236	6.4 [2.9, 9.9]	294	–44.1* [−47.2, −40.9]
HDL-P total				
Mean ± SD, (μmol/L)	246	35.4 ± 6.1	300	37.5 ± 6.2
Percent change from baseline, mean [95% CI]	236	−0.1 [−1.6, 1.4]	294	9.4* [7.5, 11.4]
Large LDL-P				
Median (Q1, Q3) (nmol/L)	246	362.5 (231.0, 532.0)	300	91.5 (33.0, 180.5)
Percent change from baseline, median (Q1, Q3)	233	5.1 (−22.8, 43.4)	292	−73.7* (−89.8, −50.9)
Small LDL-P				
Median (Q1, Q3) (nmol/L)	246	615.0 (460.0, 775.0)	300	367.0 (274.0, 507.5)
Percent change from baseline, median (Q1, Q3)	236	3.8 (−16.9, 29.0)	294	−35.4* (−56.7, −11.4)
VLDL-P and chylomicron total				
Median (Q1, Q3) (nmol/L)	246	49.4 (32.3, 75.4)	300	35.8 (25.1, 53.7)
Percent change from baseline, median (Q1, Q3)	236	−0.3 (−26.3, 31.7)	294	−15.3** (−39.3, 15.4)
Large VLDL-P and chylomicron				
Median (Q1, Q3) (nmol/L)	246	3.1 (1.4, 6.0)	300	3.1 (1.6, 6.4)
Percent change from baseline, median (Q1, Q3)	236	1.0 (−41.8, 60.4)	294	10.5 (−26.1, 100.0)
Medium VLDL-P				
Median (Q1, Q3) (nmol/L)	246	18.2 (10.3, 31.0)	300	15.1 (8.2, 25.3)
Percent change from baseline, median (Q1, Q3)	235	7.1 (−36.3, 50.8)	292	−15.2 (−47.7, 48.3)
Small VLDL-P				
Median (Q1, Q3) (nmol/L)	246	26.2 (16.2, 37.0)	300	16.8 (10.8, 25.1)
Percent change from baseline, median (Q1, Q3)	236	−7.5 (−33.1, 30.4)	293	−29.0* (−54.1, 18.3)
IDL-P				
Median (Q1, Q3) (nmol/L)	246	74.0 (44.0, 125.0)	300	45.5 (26.0, 79.0)
Percent change from baseline, median (Q1, Q3)	236	0 (−47.4, 87.5)	294	−36.2* (−69.8, 22.0)

From Toth et al. [54]. With permission from Elsevier

P values reported are for treatment differences (evolocumab versus placebo) tested using two-sample t-test for LDL-P and HDL-P. All other parameters were analyzed using the Wilcoxon rank sum test

HDL-C high-density lipoprotein cholesterol, *IDL-P* intermediate-density lipoprotein particle concentration, *LDL* low-density lipoprotein, *LDL-P* LDL particle concentration, *Q1, Q3* first and third quartiles, *QM* once every month, *SD* standard deviation, *VLDL-P* very-low-density lipoprotein particle concentration

*$P < 0.0001$

**$P < 0.001$

Abnormalities Part B (TESLA), among patients with homozygous FH (HoFH), evolocumab reduces LDL-C 31% compared to placebo [34].

Apo B, Non-HDL-C, and Lipoprotein(a) Reduction

Apo B and non-HDL-C are important measures of atherogenic lipoprotein burden in serum and are highly predictive of risk for ASCVD events [35, 36]. Lipoprotein(a) (Lp(a)) is an LDL-P modified with the covalent addition of apoprotein(a) to apoB [37, 38]. Lp(a) is highly proatherogenic, is an important delivery platform for oxidized phospholipid into the arterial wall, is prothrombotic, and is an established risk factor for ASCVD [39–41]. The mechanism(s) by which Lp(a) is cleared from the circulation are as yet undefined [42]. In a pooled analysis that

includes 4943 participants in 15 phase 2 and phase 3 clinical trials, evolocumab dosed at 140 mg every 2 weeks or 420 mg every 4 weeks reduces median Lp(a) 22%–38% and 20%–33%, respectively [43]. Apo B is reduced by the 140 mg and 420 mg doses by 46%–52% and 40–48%, respectively. Non-HDL-C (defined as total cholesterol minus HDL-C) is reduced by the 140 mg and 420 mg doses of evolocumab by 49–56% and 48–52%, respectively. Compared to either placebo (most patients on intensive statin therapy except in patients with statin intolerance) or ezetimibe therapy, evolocumab dramatically increases the capacity to attain a non-HDL-C < 100 mg/dL (2.6 mmol/L) or an apo B < 80 mg/dL in patients with primary hyperlipidemia/mixed dyslipidemia, statin intolerance, HeFH, type 2 diabetes mellitus, and variable levels of ASCVD risk (in the DESCARTES trial) (Fig. 14.6).

Triglyceride-Enriched Lipoproteins and Lipoprotein Subfractions

Evolocumab promotes a range of changes in lipoprotein particles and subfractions. LDL-P number [44, 45] and size [46, 47], triglyceride-enriched lipoproteins and their remnants [48, 49], and HDL subfractions [50, 51] have been implicated as important risk factors for ASCVD. The apo B/apo A-I ratio is viewed as a strong predictor of CHD risk [52, 53]. Total LDL-P as well as both large and small LDL particles decrease significantly in response to evolocumab therapy [54] (Table 14.1). With the addition of evolocumab to statin therapy, the total LDL-P burden decreases from 1110 to 610 nmol/L. Total HDL particles increase significantly. In addition, the sum of chylomicron and very-low-density lipoprotein particles (VLDL-P) decrease, and both small VLDL-P and intermediate-density lipoprotein particles decrease significantly (Table 14.1). In this same study, HDL-C and apo A-I increased modestly compared to placebo by 5.7% and 2.5%, while VLDL-C and apoB/Apo A-I decreased by 13.0% and 43.2%, respectively [54]. These broad-spectrum changes in lipoproteins and their subfractions are generally viewed as beneficial.

Impact of Evolocumab on Coronary Atherosclerosis

The Global Assessment of Plaque Regression with a PCSK9 Antibody as Measured by Intravascular Ultrasound (GLAGOV) trial tested whether or not treatment of patients with evolocumab (dosed at 420 mg monthly) reduced the progression of atherosclerosis in coronary target lesions using intravascular ultrasonography [55]. When compared to placebo, evolocumab treatment reduced percent atheroma volume by −1.0% ($p < 0.001$), total atheroma volume decreased by −4.9 mm^3 ($p < 0.001$), and more patients experienced plaque regression (17% and 12.5% more experienced reductions in percent atheroma volume and total atheroma volume, respectively (both $p < 0.001$)). Among patients who achieved LDL-C < 70 mg/dL, evolocumab therapy compared to placebo had an even more favorable impact on plaque features: percent atheroma volume decreased −1.62%, and the percentage of patients with regression of percent atheroma volume was 33.2% (both $p < 0.001$). Hence, evolocumab therapy potentiates significant plaque regression, and the lower the LDL-C was reduced, the greater the improvement in atherosclerotic plaque features.

Impact of Evolocumab on Cardiovascular Events

The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial evaluated the efficacy and safety of evolocumab therapy compared to placebo in 27,564 patients with established ASCVD already being treated with moderate or high intensity statin therapy [56]. Evolocumab reduced LDL-C by a mean of 59% (from 92 to 30 mg/dL). The primary composite endpoint was comprised of CV mortality, MI, stroke, hospitalization for unstable angina, or coronary revascularization. The secondary composite endpoint was comprised of CV death, MI, and stroke. The primary and secondary composite endpoints were reduced by evolocumab compared to placebo by 15% ($p < 0.001$) and 20% ($p < 0.001$), respectively. Myocardial infarction was reduced by 27% ($p < 0.001$), stroke was reduced by 21%

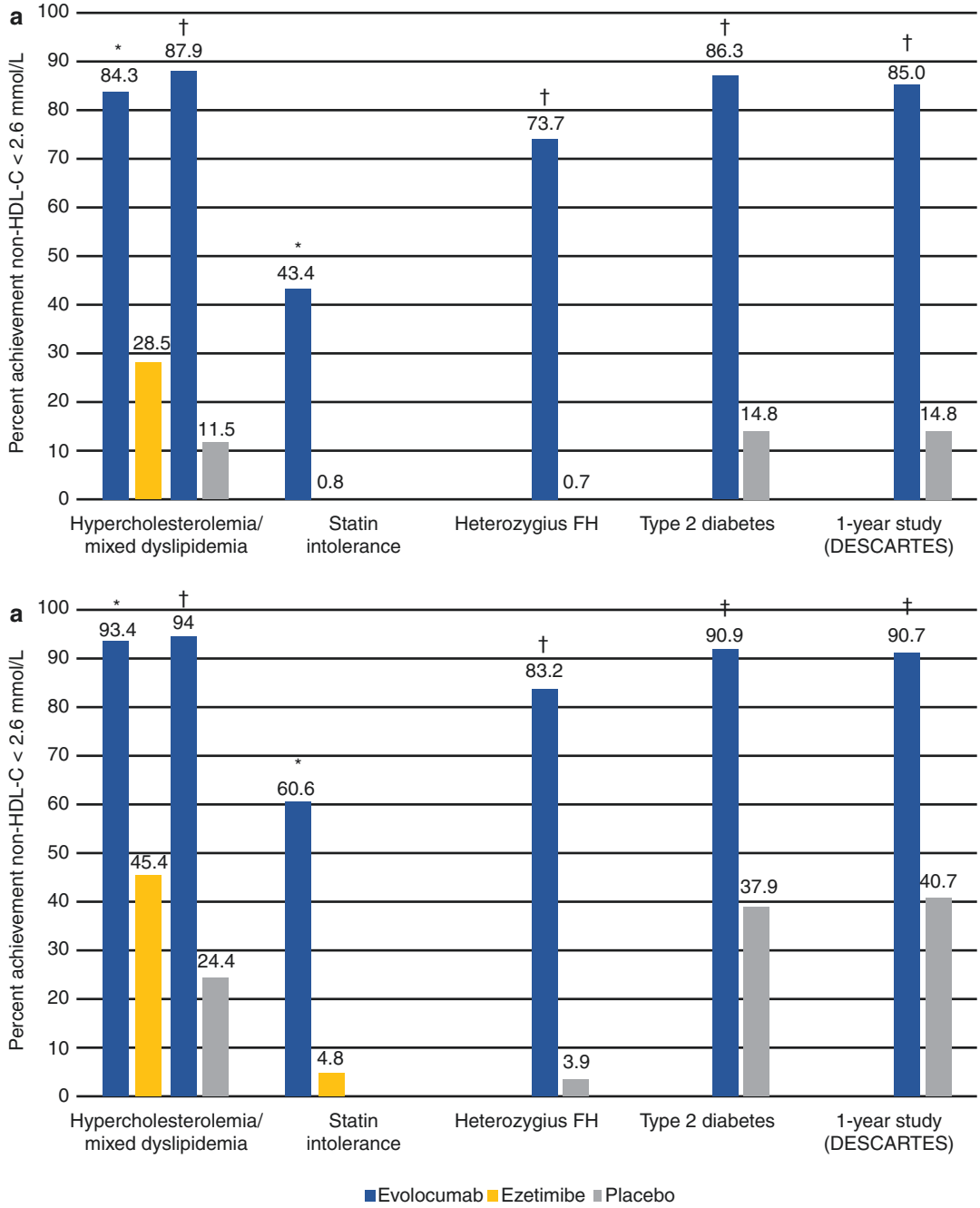


Fig. 14.6 Percent achievement in placebo or ezetimibe-controlled phase 2 and phase 3 evolocumab studies of (**a**) non-HDL-C < 100 mg/dL (2.6 mmol/L) and (**b**) ApoB <80 mg/dL. The percentages of patients who achieved non-HDL-C < 100 mg/dL (**a**) and ApoB <80 mg/dL (**b**) with evolocumab, ezetimibe, or placebo are depicted in this plot for all studies with a placebo or ezetimibe comparator. Results are shown separately for each patient population examined (hypercholesterolemia/mixed dyslipidemia, type 2 diabetes mellitus, heterozygous FH, and statin intolerance), all 12 weeks in duration, as well as for the 1-year study (DESCARTES). ApoB indicates apolipoprotein B; FH, familial hypercholesterolemia; non-HDL-C, non-high-density lipoprotein cholesterol. *Evolocumab-treated patients with ezetimibe comparator arm; †Evolocumab-treated patients with placebo comparator arm. (From Toth et al. [43])

($p < 0.01$), and coronary revascularization was reduced by 22% ($p < 0.001$). The larger the LDL-C reduction, the bigger the benefit. There was continuous benefit all the way down to <10 mg/dL of LDL-C. When comparing an attained LDL-C of <10 mg/dL to >100 mg/dL, there was a 41% relative risk reduction for mortality, MI, and stroke ($p = 0.02$). Neither cardiovascular nor all-cause mortality was reduced by evolocumab. There was no heterogeneity for benefit among prespecified subgroups. Hepatic, skeletal muscle, neurocognitive, and other adverse events in general were not different between groups with the exception of injection site reactions, which occurred more frequently in patients treated with evolocumab.

In a post hoc analysis of the FOURIER trial, the impact of evolocumab therapy on CV event rates in 22,351 patients with a prior MI was evaluated with respect to time from most recent MI, number of prior MIs, and whether or not a patient had residual multivessel CAD (≥40% stenosis in ≥2 large vessels) [57]. For patients who sustained a qualifying MI < 2 years ago or ≥2 years ago, the composite of CV mortality, MI, and stroke were reduced by 24% ($p < 0.001$) and 13% ($p = 0.04$), respectively (Fig. 14.7). For patients who sustained ≥2 previous MIs vs 1 MI, this composite was reduced by 21% ($p = 0.006$) and 16% ($p = 0.008$), respectively. Having or not having multivessel disease was associated with a 30% ($p < 0.001$) and 11% ($p = 0.055$) reduction in this composite endpoint, respectively.

In the setting of peripheral arterial disease (PAD), evolocumab appears to be particularly beneficial. When comparing evolocumab therapy to placebo in patients with and without PAD in the Fourier trial, the composite of CV death, MI, and stroke were reduced by 27% ($p = 0.004$) and 19% ($p < 0.001$), respectively [58]. For the trial as a whole, major acute limb events (defined as a composite of acute limb ischemia, major amputation (above the knee or below the knee, excluding forefoot or toe), and urgent revascularization (thrombolysis or urgent vascular intervention for ischemia) were reduced by evolocumab by 42% ($p = 0.0093$) (Fig. 14.8). Among patients with established PAD, evolocumab reduced major acute limb events by 37%, but this finding was not statistically significant. However, the event curves for the two groups separate in a compelling way (Fig. 14.8). For patients without a prior history of PAD, evolocumab reduced major acute limb events by 63% ($p = 0.0197$). In all three analyses, event curve separation is immediate and increases as a function of time. Benefit also increased as LDL-C decreased, even below 10 mg/dL.

In a subgroup analysis of 11,033 participants with diabetes in the FOURIER trial, there was an 18% ($p = 0.0021$) and 22% reduction in the composite of CV death, MI, and stroke for patients with and without diabetes [59]. Evolocumab did not increase the incidence of diabetes in patients with either no diabetes or prediabetes. In addition, hemoglobin a1c and fasting plasma glucose levels remained unchanged between groups showing no disturbance in glycemic control induced by evolocumab.

As noted above, evolocumab reduces serum levels of Lp(a). Evolocumab reduced the risk of CV death, MI, or urgent revascularization by 23% (hazard ratio, 0.77; 95% CI, 0.67–0.88) in patients with a baseline Lp(a) >median and by 7% (hazard ratio, 0.93; 95% CI, 0.80–1.08; *P* interaction = 0.07) in those ≤median [60]. Hence, it is plausible to conclude that the Lp(a) reduction promoted by evolocumab therapy contributes to overall risk reduction.

Evolocumab and Neurocognitive Impairment

There has been lingering concern that lipid lowering, especially with statins, may be associated with cognitive impairment. Although largely based on speculation, there is particular concern that if LDL-C is lowered below 50 mg/dL, this may precipitate adverse changes in the brain resulting in memory impairment or frank dementia. While it is imperative that vigilance always be maintained for adverse side effects from any pharmacologic intervention, there has historically been no prospective clinical trial evidence that aggressive LDL-C reduction or statin therapy per se cognitive impairment. Cholesterol metabolism behind the blood-brain

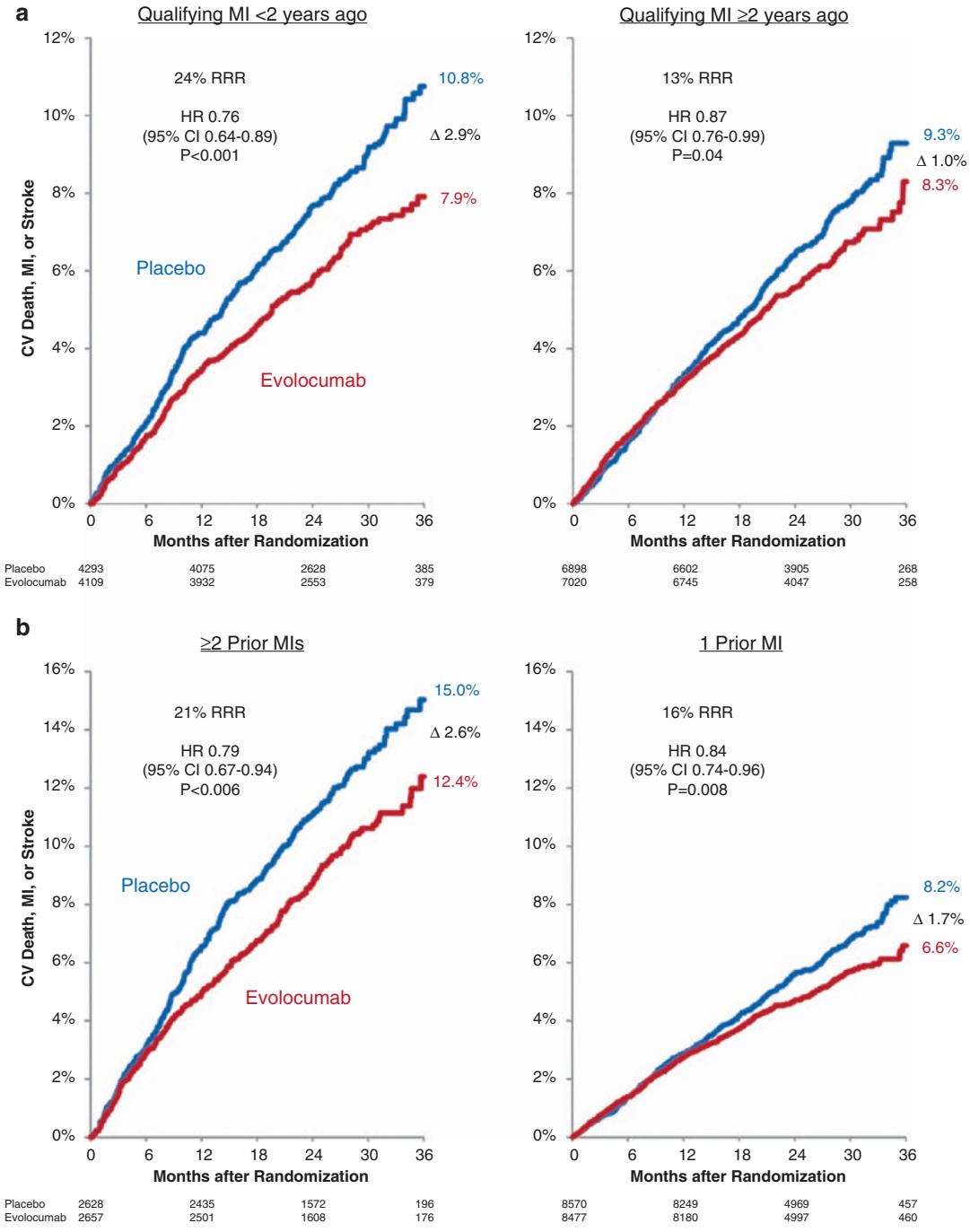


Fig. 14.7 Incidence of the key secondary end point in patients stratified by high-risk features. Cumulative incidence curves for the key secondary end point by treatment arm in patients stratified by time from qualifying myocardial infarction (MI; **a**), number of prior MIs (**b**), and presence of residual multivessel coronary artery disease (**c**). *P* values for interactions between treatment and subgroups were 0.18, 0.57, and 0.03, respectively. CI indicates confidence interval; CV, cardiovascular; HR, hazard ratio; and RRR, relative risk reduction. (From Sabatine et al. [57])

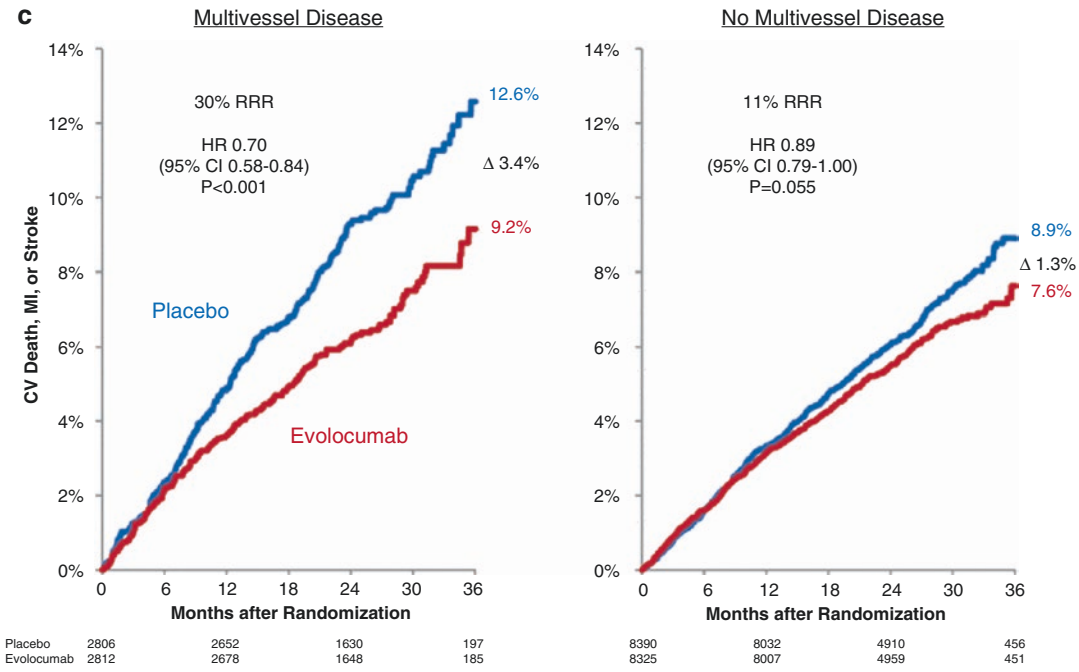


Fig. 14.7 (continued)

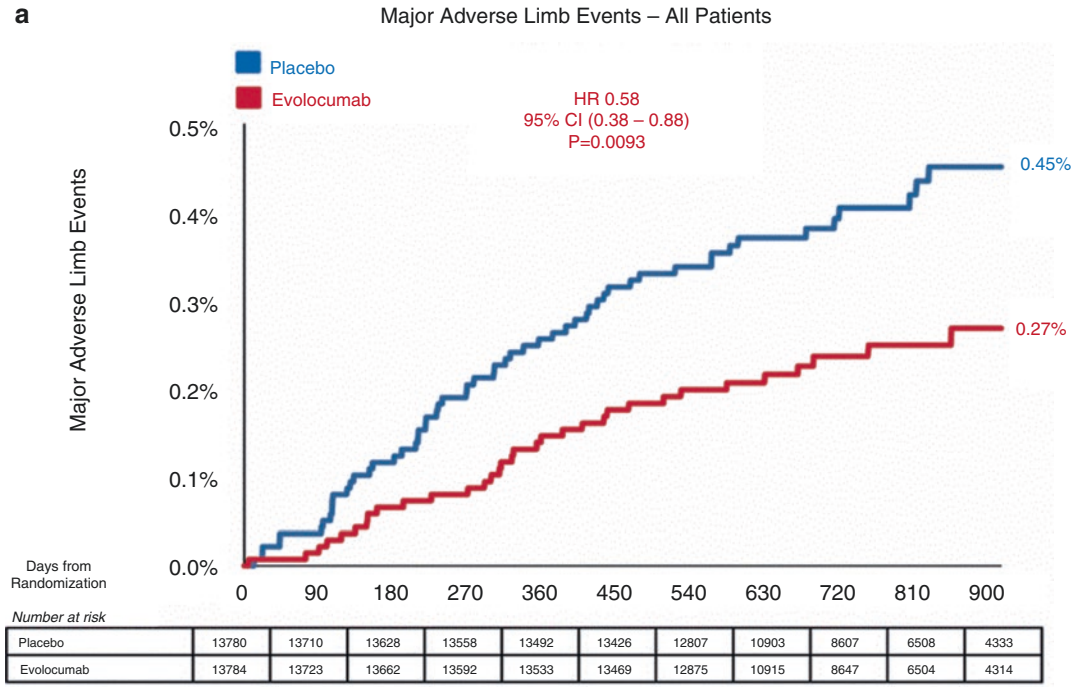
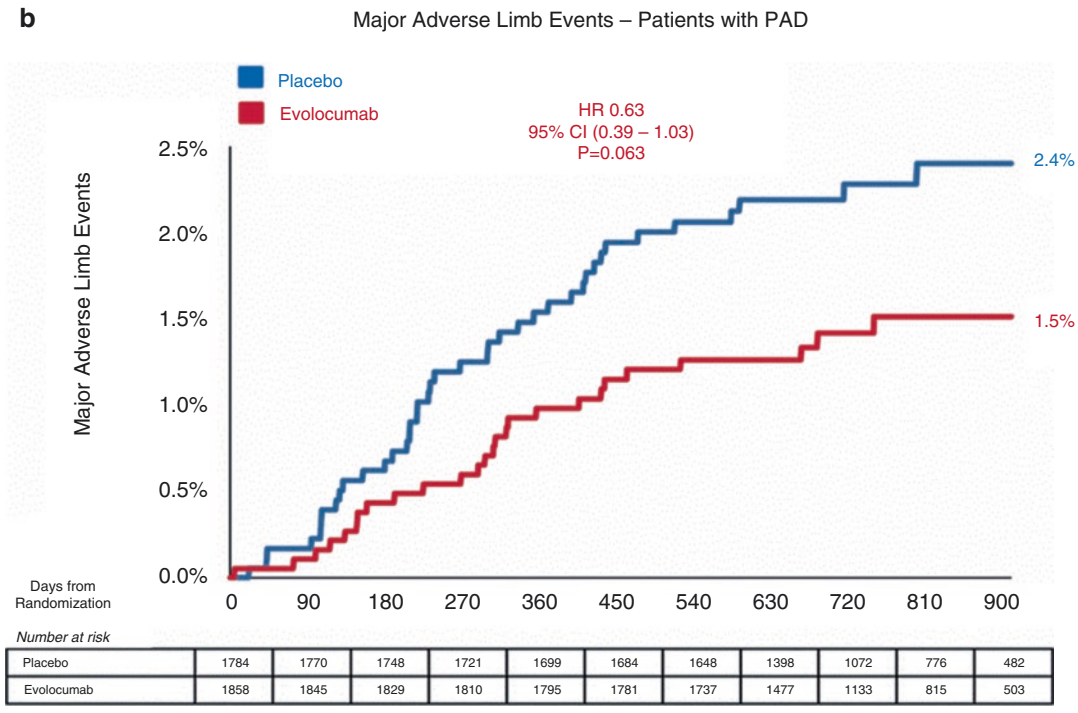


Fig. 14.8 Major adverse limb events. Major adverse limb events (composite of acute limb ischemia, major amputation, or urgent revascularization) by treatment (evolocumab in red, placebo in blue) in all randomly assigned patients (**a**), in patients with symptomatic PAD (**b**), and in patients with no known PAD (**c**). CI indicates confidence interval, HR hazard ratio, and PAD peripheral artery disease. (From Bonaca et al. [58])



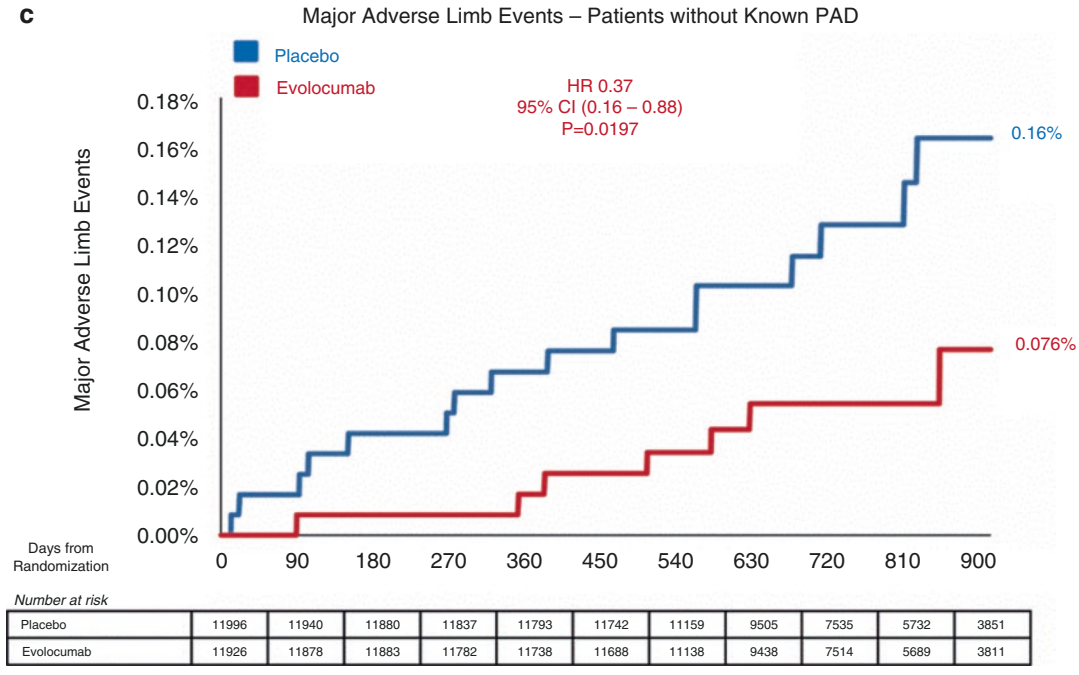


Fig. 14.8 (continued)

barrier is completely segregated from the central circulation. Within the brain, oligodendrocytes and astrocytes provide all of the cholesterol necessary for myelin formation and normal neuronal function [61, 62]. Neither cholesterol nor lipoproteins cross the blood brain barrier.

The National Lipid Association's Statin Safety Task Force concluded that statins as a class are not associated with adverse effects on cognition (strength of recommendation: A) [63]. Neither the Heart Protection Study [64] nor the Prospective Study of Pravastatin in the Elderly at Risk [65] trials were unable to demonstrate any adverse impact of statin therapy on cognitive capacity in patients with dyslipidemia. An early meta-analysis suggested that statins reduce risk of neurocognitive impairment [66]. In a prospective cohort study, after adjusting for education, smoking status, the presence of at least one APOE ε4 allele, and history of stroke or diabetes at baseline, participants treated with statins had a 48% lower risk of developing dementia compared to those who had not been treated with a statin [67]. The Cardiovascular Health Study showed a similar 44% lower risk of Alzheimer's type dementia compared to patients not taking lipid-lowering therapy [68]. Much of the concern surrounding lipid lowering and dementia relies on case reports, anecdote, and unconfirmed submissions to the adverse event reporting system of the Food and Drug Administration.

The Evaluating PCSK9 Binding Antibody Influence on Cognitive Health in High Cardiovascular Risk Subjects (EBBINGHAUS) study prospectively evaluated whether or not lipid-lowering therapy with a statin ± evolocumab very low LDL-C are associated with increased risk for cognitive impairment [69].

The Evaluating PCSK9 Binding Antibody Influence on Cognitive Health in High Cardiovascular Risk Subjects (EBBINGHAUS) study investigated whether or not lipid-lowering therapy with statins and evolocumab or low levels of LDL-C induce neurocognitive impairment. A 1204 patient subgroup of the FOURIER trial prospectively underwent assessment of their cognitive function using the Cambridge Neuropsychological Test Automated Battery (CANTAB). The CANTAB is a computer-based test that is valid independent of language and culture. Four components of cognition were evaluated: (1) spatial working memory strategy index of executive function, (2) spatial working memory between errors, (3) paired associates learning, and (4) reaction time. Patients also self-evaluated everyday cognition at the conclusion of the study. The EBBINGHAUS investigators were unable to detect any change in either individual components of the CANTAB or a global score between the beginning and end of the FOURIER trial. In addition, based on self-assessment, there was no between group differences in self-reported cognitive capacity or changes therein. These results applied even to patients who achieved ultra-low LDL-C of <10 mg/dL.

Overall Safety of Evolocumab

In addition to its efficacy, evolocumab has been shown to be safe. The most commonly occurring adverse events occurring in >5% of patients and more often than in placebo treated patients are nasopharyngitis, influenza-like reaction, upper respiratory infection, and injection site reactions [70]. In an analysis of over 6000 patients treated with evolocumab, the incidence of muscle, liver, and kidney related adverse events was similar to that observed with placebo (Table 14.2). Neurocognitive adverse events were also rare and on par with placebo, consistent with the Ebbinghaus trial (Table 14.3). Evolocumab therapy also does not increase risk for impaired glucose tolerance or diabetes mellitus [56, 70]. The side-effect profile of evolocumab is unchanged when comparing patients who achieve LDL-C levels on therapy of >40 mg/dL, 25–40 mg/dL, or <25 mg/dL [70].

Table 14.2 Laboratory investigations for muscle injury, liver function, and renal function

	Integrated parent Studies		Integrated interim extension studies Year 1 SoC-controlled period	
	Control[a]	Evolocumab	SoC	Evolocumab
	(*N* = 2080)	(*N* = 3946)	(*N* = 1489)	(*N* = 2976)
CK				
Number of patients with any post-baseline CK measurement	2055	3892	1472	2962
CK >5 × ULN, *n* (%)	14 (0.7)	27 (0.7)	17 (1.2)	17 (0.6)
CK >10 × ULN, *n* (%)	5 (0.2)	9 (0.2)	9 (0.6)	7 (0.2)
Liver function tests				
Number of patients with any post-baseline liver function test measurement	2055	3893	1477	2968
ALT or AST >3 × ULN, *n* (%)	20 (1.0)	17 (0.4)	18 (1.2)	31 (1.0)
ALT or AST >5 × ULN, *n* (%)	7 (0.3)	6 (0.2)	3 (0.2)	10 (0.3)
Total bilirubin >2 × ULN, *n* (%)	3 (0.1)	6 (0.2)	2 (0.1)	8 (0.3)
(ALT or AST >3 × ULN) and (total bilirubin >2 × ULN), *n* (%)	0	0	0	1 (<0.1)
Renal function tests				
Serum creatinine				
Baseline mean (SD), mg/dL	0.9 (0.2) (*n* = 302[b])	0.9 (0.2) (*n* = 599[b])	0.9 (0.2)	0.9 (0.2)
Number of patients evaluated at week 52	273	533	402	833
Mean (SD) change from baseline at week 52, mg/dL	−0.01 (0.1)	0.01 (0.1)	−0.01 (0.1)	−0.01 (0.1)
Blood urea nitrogen				
Baseline mean (SD), mg/dL	15.8 (4.5) (*n* = 302[b])	15.7 (4.3) (*n* = 599[b])	16.1 (4.8)	16.2 (4.4)
Number of patients evaluated at week 52	273	533	402	883
Mean (SD) change from baseline at week 52, mg/dL	0.1 (3.3)	0.2 (3.9)	−0.03 (3.8)	0.26 (3.9)

From Toth et al. [70]

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CK* creatine kinase, *SoC* standard of care, *ULN* upper limit of normal

[a]Control includes placebo and ezetimibe treatment groups

[b]For the parent trials, week 52 renal function data are available from the DESCARTES study, which enrolled 901 patients (evolocumab plus background therapy, *n* = 599; placebo plus background therapy, *n* = 302)

Alirocumab

LDL-C Reduction in Primary Hyperlipidemia

Alirocumab can be dosed at 75 mg or 150 mg SQ every 2 weeks or 300 mg SQ every 4 weeks. In patients with primary hyperlipidemia, the 75 mg and 150 mg doses induce mean LDL-C reductions of 43% and 58%, respectively. When specifically evaluated in high-risk patients on maximally tolerated statin therapy given 75 mg every 2 weeks, mean LDL-C reduction was 48.2%, and patients in the alirocumab/statin treatment arm achieved LDL-C < 70 mg/dL 75% of the time after 6 months of therapy compared to 9% for placebo treatment [71]. Among patients with type 1 and type 2 diabetes mellitus (T1DM, T2DM) treated with maximally tolerated statin therapy, alirocumab treatment (either at 75 mg or 150 mg every 2 weeks if LDL-C not controlled with the lower dose) for 6 months decreased LDL-C by 49% and 47.8% in T2DM and T1DM patients, respectively, compared to placebo [72]. In addition,

Table 14.3 Neurocognitive adverse events

	Integrated parent Studies		Integrated interim extension studies Year 1 SoC-controlled period	
	Control[a]	Evolocumab	SoC	Evolocumab
	(*N* = 2080)	(*N* = 3946)	(*N* = 1489)	(*N* = 2976)
Any neurocognitive-related AE[b], *n* (%)	6 (0.3)	5 (0.1)	5 (0.3)	27 (0.9)
Amnesia	0	2 (0.1)	2 (0.1)	8 (0.3)
Disorientation	2 (0.1)	1 (<0.1)	0	1 (<0.1)
Memory impairment	1 (<0.1)	1 (<0.1)	3 (0.2)	7 (0.2)
Delirium	0	1 (<0.1)	0	0
Cognitive disorder	1 (<0.1)	0	0	1 (<0.1)
Dementia with Lewy bodies	1 (<0.1)	0	0	0
Disturbance in attention	1 (<0.1)	0	0	0
Dementia	0	0	0	3 (0.1)
Confusional state	0	0	0	2 (0.1)
Mental impairment	0	0	0	2 (0.1)
Alzheimer's type dementia	0	0	0	2 (0.1)
Illusion	0	0	0	1 (<0.1)
Transient global amnesia	0	0	0	1 (<0.1)
Neurocognitive-related AEs leading to study drug discontinuation, n (%)	1 (<0.1)	1 (<0.1)	N/A	3 (0.1)

From Toth et al. [70]
Neurocognitive events are listed in decreasing order of frequency in the evolocumab arm of the parent trials
AE adverse event, *N/A* not applicable, *SoC* standard of care
[a]Control includes placebo and ezetimibe treatment groups
[b]Neurocognitive events were identified using deliria (including confusion), cognitive and attention disorders and disturbances, dementia and amnestic conditions, disturbances in thinking and perception, and mental impairment disorders in high-level group terms

LDL < 70 mg/dL was achieved in 76.4% and 70.2% of T2DM and T1DM patients, respectively. In both groups, fasting blood glucose and glycated hemoglobin levels remained unchanged in the two treatment groups.

LDL-C Reduction in Familial Hypercholesterolemia

Alirocumab is indicated for the treatment of HeFH. In the Efficacy and Safety of Alirocumab Versus Placebo on Top of Lipid-Modifying Therapy in Patients with Heterozygous Familial Hypercholesterolemia Not Adequately Controlled with Their Lipid-Modifying Therapy (ODYSSEY FH I and FH II), patients were treated for 78 weeks with the highest tolerated dose of a statin ± other lipid-lowering therapy and then randomized to either alirocumab (75 mg every 2 weeks and then increased to 150 mg every 2 weeks if LDL-C > 70 mg/dL) [73]. The mean LDL-C decreased from 144.7 to 71.3 mg/dL (57.9% reduction compared to placebo) in FH I and decreased from 134.6 to 67.7 mg/dL (51.4% reduction compared to placebo) in FH II [73]. These changes in LDL-C were maintained through 78 weeks of treatment. In ODYSSEY High FH (HeFH with LDL-C > 160 mg/dL despite maximally tolerated statin therapy ± other lipid-lowering therapy), alirocumab dosed at 150 mg every 2 weeks reduced mean LDL-C by 90.8 mg/dL at week 24, and this change was maintained through 78 weeks of treatment [74]. Alirocumab is not yet indicated for the treatment of HoFH.

Apo B, Non-HDL-C, and Lipoprotein(a) Reduction

In a pooled analysis of ten phase 3 ODYSSEY studies which included 4983 participants, alirocumab dose at 75 or 150 mg every week reduced non-HDL-C by approximately 42%–51% and 40% in patients who did and did not receive concomitant statin therapy, respectively [74]. Compared to statin monotherapy control arms, the addition of alirocumab to ongoing statin therapy increased the attainment of non-HDL-C < 100 mg/dL to 70–80% from 7%–10% (Fig. 14.9). Similarly, use of alirocumab at 75/150 mg every 2 weeks added to ongoing statin therapy reduced apoB by 40%–52%; among patients not treated with a statin, alirocumab decreased apoB by 36.5%. Alirocumab increased the goal attainment rate for apo B < 80 mg/dL to 78%–85% from approximately 20% on placebo (Fig. 14.9). For patients not on a statin alirocumab induced apo B <80 mg/dL in approximately 70% of patients.

Alirocumab reduces Lp(a) significantly. In a pooled analysis of ten phase 3 studies including 4915 participants, alirocumab reduced Lp(a) from baseline by 23% to 27% with alirocumab 75/150-mg Q2W and 29% with alirocumab 150-mg Q2W (both $p < 0.0001$ vs placebo) at 6 months [75]. These reductions in Lp(a) were sustained over 78–104 weeks and were independent of race, gender, the presence of familial hypercholesterolemia, baseline Lp(a) and LDL-C concentrations, or use of statins.

Triglyceride-Enriched Lipoproteins and Lipoprotein Subfractions

A comprehensive analysis of changes induced by alirocumab in lipoproteins and their subfractions as well as apo B, apoA1, ApoCII, and ApoCIII are summarized in Table 14.4 [76]. $LDL_{1–2}$ are larger, more buoyant LDL species, while $LDL_{3–4}$ are smaller, denser LDL species. Remnant lipoproteins are defined as $VLDL_3$-C + IDL-C. The ratio of apo B/apoA1 is a well-recognized marker of CV risk.

Alirocumab induces substantive reductions in triglycerides, LDL-C subfractions, VLDL-C and its subfractions, IDL-C, LDL_R-C (LDL_{real} = LDL-C – Lp(a)-C – IDL-C), and remnant lipoprotein cholesterol (RLP-C) (Table 14.4). In addition, alirocumab therapy correlates with significant reductions in apoB/apoA1 ratio, ApoCII, and ApoCIII. All of these changes would be expected to be both advantageous and beneficial with regard to ASCVD risk.

Impact of Alirocumab on Cardiovascular Events

The clinical efficacy of alirocumab was evaluated in the ODYSSEY OUTCOMES trial, which included 18,924 patients who sustained an ACS

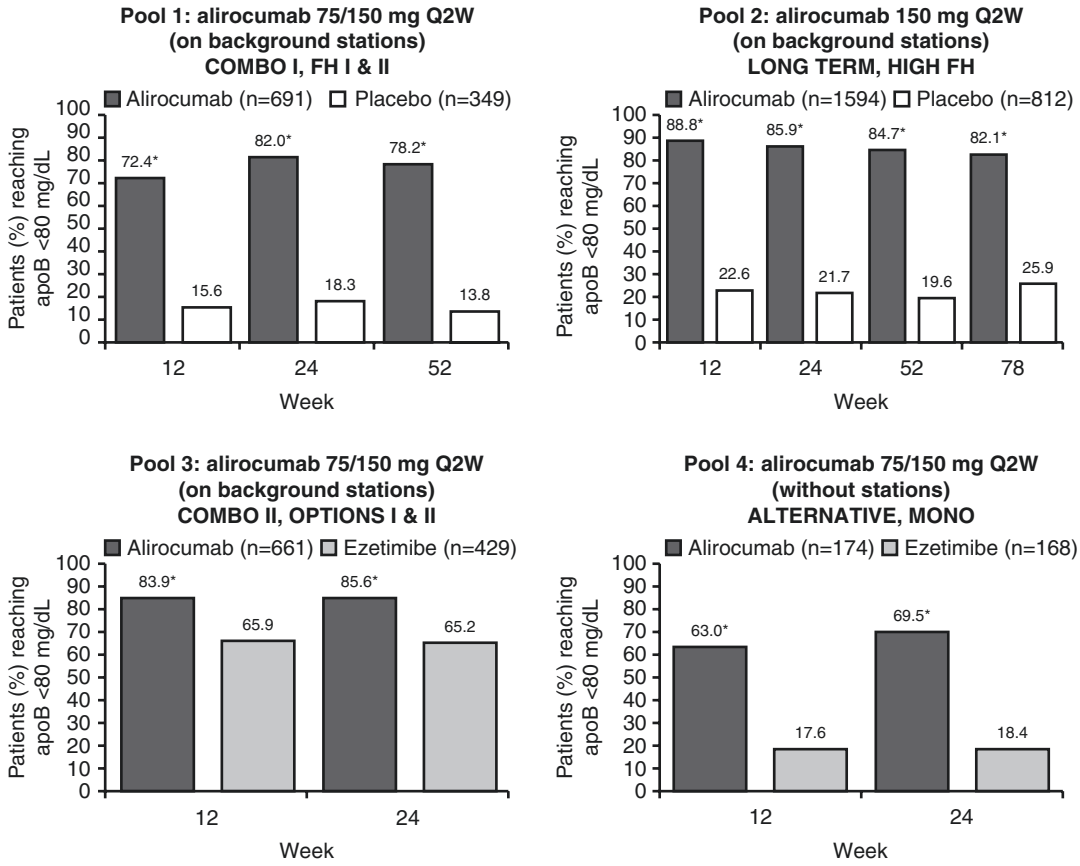


Fig. 14.9 Percent of patients achieving apoB levels<80 mg/dL during the studies (modified intention to treat population). $P < 0.0001$ vs control group at all time points and in all study pools. ApoB indicates apolipoprotein B, Q2W every 2 weeks. (From Bays et al. [98])

within the year previous to enrollment [77]. All patients were treated with either high-intensity statin therapy of the highest dose of a statin they could tolerate. Patients were randomized to either alirocumab 75/150 mg every 2 weeks with the goal of an attained LDL-C on therapy of 25–50 mg/dL. The median duration of follow-up was 2.8 years. The primary composite endpoint included death from coronary heart disease, nonfatal MI, unstable angina requiring hospitalization, and fatal or nonfatal ischemic stroke. Alirocumab reduced the primary composite endpoint compared to placebo by 15% ($p < 0.001$). The composite of all-cause mortality, nonfatal MI, and nonfatal ischemic stroke was reduced by 14% ($p < 0.001$). Other endpoint reductions included nonfatal MI 14%, fatal or nonfatal ischemic stroke 27%, unstable angina requiring hospitalization 39%, and ischemia-driven revascularization 12%. Neither coronary nor cardiovascular mortality was reduced significantly.

A variety of additional analyses of ODYSSEY OUTCOMES demonstrate broad benefit from alirocumab. The number needed to treat to prevent major adverse cardiovascular events (MACE) over a period of 3 years decreases as a function of age: 43 at age 45 years, 26 at age 75 years, and 12 at age 85 years [78]. Relative risk reduction for MACE was consistent for person above or below the age of 65 years. Alirocumab decreases risk of any stroke (28%) without increasing the risk of hemorrhagic stroke, irrespective of attained LDL-C or history of cerebrovascular disease (Fig. 14.10). This is a highly

Table 14.4 Pooled data from three studies of alirocumab for changes from baseline in cholesterol content of lipoprotein subfractions, apoB/apoA1 ratio, and levels of apo CII and CIII using vertical auto profiling (ultracentrifugation)

	Pooled data	
Lipoprotein subfractions, mg/dL	Placebo $n = 72$[a]	Alirocumab 150 mg Q2W $n = 100$[a]
LDL total		
Baseline	121.8 (29.1)	120.9 (28.8)
Posttreatment[b]	110.3 (33.1)	42.8 (22.9)
Mean (SD) change from baseline, %	−7.5 (25.1)	−64.1 (18.4)
P-value		<0.0001
LDL-R		
Baseline	95.7 (25.6)	94.4 (25.1)
Posttreatment[b]	86.1 (27.7)	27.9 (19.7)
Mean (SD) change from baseline, %	−7.2 (28.4)	−70.6 (19.4)
P-value		<0.0001
LDL_1-C		
Baseline	19.8 (7.6)	19.5 (8.5)
Posttreatment[b]	17.1 (8.8)	4.9 (4.3)
Mean (SD) change from baseline, %	−7.4 (47.6)	−64.0 (110.0)
P-value		0.0001
LDL_2-C		
Baseline	26.2 (14.3)	25.3 (14.2)
Posttreatment[b]	20.8 (14.5)	4.9 (7.5)
Mean (SD) change from baseline, %	−8.3 (98.9)	−82.8 (21.4)
P-value		<0.0001
LDL_3-C		
Baseline	39.4 (14.6)	37.9 (14.3)
Posttreatment[b]	35.9 (13.0)	11.3 (9.0)
Mean (SD) change from baseline, %	−0.2 (45.0)	−68.4 (25.7)
P-value		<0.0001
LDL_4-C		
Baseline	10.4 (7.9)	11.7 (10.0)
Posttreatment[b]	12.3 (8.6)	6.8 (3.3)
Mean (SD) change from baseline, %	78.9 (182.9)	13.7 (213.3)
P-value		0.1596
LDL_{1+2}-C		
Baseline	46.0 (19.7)	44.8 (20.2)
Posttreatment[b]	37.9 (21.4)	9.8 (11.0)
Mean (SD) change from baseline, %	−11.1 (46.4)	−62.3 (169.1)
P-value		0.0201
LDL_{3+4}-C		
Baseline	49.7 (18.5)	49.7 (21.6)
Posttreatment[b]	48.2 (17.1)	18.1 (11.1)
Mean (SD) change from baseline, %	6.0 (46.3)	−60.3 (25.5)
P-value		<0.0001
ApoB/A1		
Baseline	0.7 (0.1)	0.7 (0.2)
Posttreatment[b]	0.7 (0.2)	0.4 (0.1)
Mean (SD) change from baseline, %	−4.5 (17.2)	−47.3 (14.5)
P-value		<0.0001

(continued)

Table 14.4 (continued)

Lipoprotein subfractions, mg/dL	Pooled data	
	Placebo $n = 72$[a]	Alirocumab 150 mg Q2W $n = 100$[a]
VLDL-C		
Baseline	24.5 (20.0–32.5)	23.0 (18.0–30.5)
Posttreatment[b]	23.5 (18.0–29.5)	17.0 (14.0–20.0)
Mean (SD) change from baseline, %	−3.9 (−22.5 to 19.4)	−27.1 (−38.9 to −15.8)
P-value		<0.0001
$VLDL_{1+2}$-C		
Baseline	9.9 (7.7–13.2)	9.5 (7–12.7)
Posttreatment[b]	9.4 (7.2–12.1)	7.1 (5.5–8.8)
Mean (SD) change from baseline, %	−1.6 (−28.0 to 24.4)	−27.8 (−42.9 to −12.2)
P-value		<0.0001
$VLDL_3$-C		
Baseline	14.5 (12.0–18.0)	14.0 (11.0–17.0)
Posttreatment[b]	13.5 (11.0–18.0)	10.0 (9.0–12.0)
Mean (SD) change from baseline, %	−5.1 (−18.8 to 10.8)	−25.8 (−35.7 to −15.4)
P-value		<0.0001
IDL-C		
Baseline	16.5 (13.0–21.0)	17.0 (12.0–21.0)
Posttreatment[b]	15.0 (10.5–20.5)	7.0 (5.0–9.0)
Mean (SD) change from baseline, %	−10.6 (−28.6 to 13.9)	−57.1 (−68.6 to −42.3)
P-value		<0.0001
Triglycerides		
Baseline	135.0 (102.5–202.0)	132.0 (97.0–179.0)
Posttreatment[b]	137.0 (99.5–190.0)	101.0 (82.0–151.5)
Mean (SD) change from baseline, %	0.2 (−24.7 to 23.4)	−21.7 (−35.8 to 3.8)
P-value		0.0008
RLP-C		
Baseline	32.5 (25.5–40.0)	30.5 (23.0–36.0)
Posttreatment[b]	28.5 (23.0–37.0)	17.5 (14.0–21.0)
Mean (SD) change from baseline, %	−7.2 (−21.0 to 11.0)	−42.5 (−52.9 to −31.3)
P-value		<0.0001
ApoCII		
Baseline	4.8 (2.0)	4.9 (2.0)
Posttreatment[b]	4.8 (2.3)	3.9 (1.4)
Mean (SD) change from baseline, %	2.8 (32.5)	−17.1 (25.7)
P-value		<0.0001
ApoCIII		
Baseline	11.3 (4.3)	11.2 (4.1)
Posttreatment[b]	11.3 (4.8)	9.1 (2.7)
Mean (SD) change from baseline, %	4.0 (33.6)	−15.0 (19.3)
P-value		<0.0001
ApoCII/VLDL-C		
Baseline	0.2 (0.1)	0.2 (0.1)
Posttreatment[b]	0.2 (0.1)	0.2 (0.1)
Mean (SD) change from baseline, %	6.0 (23.5)	10.7 (25.6)
P-value		0.0866
ApoCIII/VLDL-C		
Baseline	0.4 (0.1)	0.5 (0.2)
Posttreatment[b]	0.5 (0.1)	0.5 (0.1)

Table 14.4 (continued)

Lipoprotein subfractions, mg/dL	Pooled data	
	Placebo $n = 72$[a]	Alirocumab 150 mg Q2W $n = 100$[a]
Mean (SD) change from baseline, %	7.4 (23.8)	15.5 (23.1)
P-value		0.0072

From Toth et al. [76]

Mean (SD) are reported for continuous normally distributed variables, while median (interquartile range) are reported for nonnormally distributed variables. Units are mg/dL

Q2W every 2 weeks, *Apo* apolipoprotein, *IDL-C* intermediate-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *LDLr* "LDL real" (i.e. total LDL fraction minus Lp(a) and intermediate density lipoprotein), *Lp(a)* lipoprotein (a), *RLP-C* remnant-like particle cholesterol, *SD* standard deviation, *VLDL-C* very low-density lipoprotein cholesterol

[a]Pooled data for pool of studies 565, 566, and 1003. Patients included from studies 565 and 1003 all received either placebo or alirocumab 150 mg Q2W. Patients in study 566 were randomized to one of three arms and received (1) placebo with increase in ATV dose from 10 to 80 mg at start of randomized treatment period, (2) alirocumab 150 mg Q2W plus ATV 10 mg, or (3) alirocumab 150 mg Q2W with increase in ATV dose from 10 to 80 mg at start of randomized treatment period

[b]Study 565, week 12; study 566, week 8; study 1003, week 6

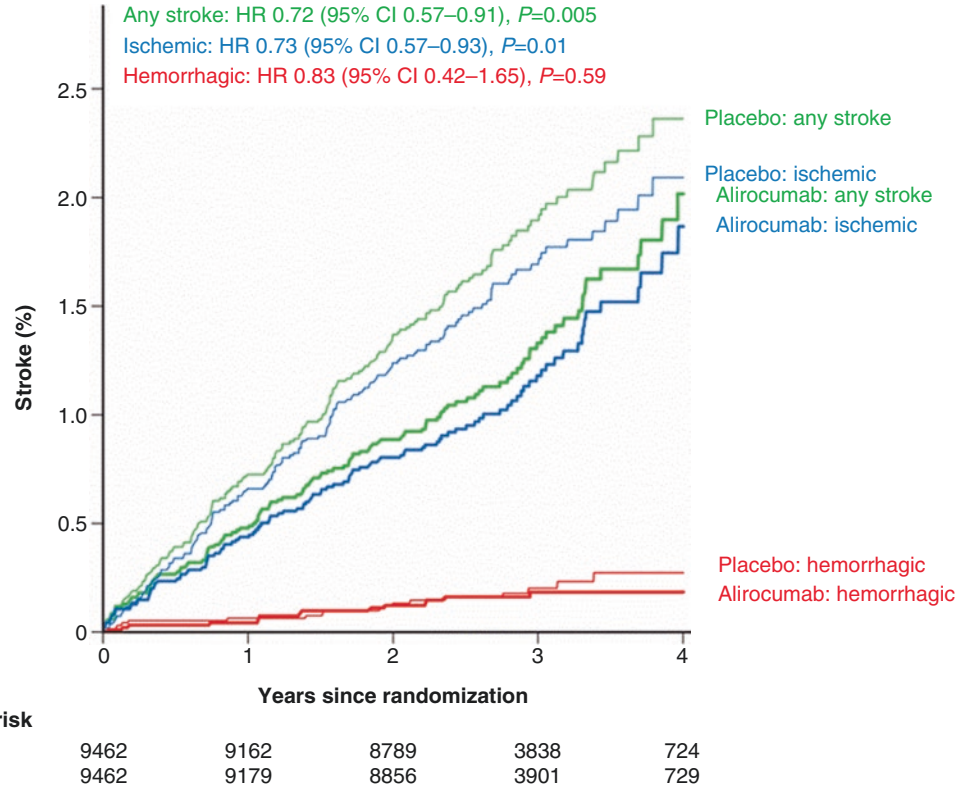


Fig. 14.10 Kaplan-Meier curves for any stroke, ischemic stroke and hemorrhagic stroke. CI indicates confidence interval and HR hazard ratio. (From Jukema et al. [99])

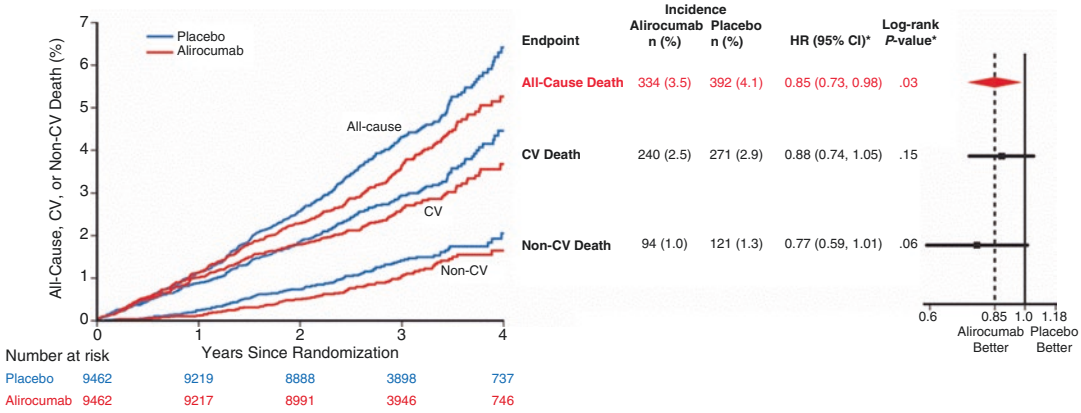


Fig. 14.11 All-cause, cardiovascular, and non-cardiovascular death (intention-to-treat population) shown as Kaplan-Meier curves (left panel) and in a forest plot (right panel). CV indicates cardiovascular and HR hazard ratio. (From Steg et al. [80])

reassuring finding given the longstanding concern that low LDL-C may correlate with increased risk for hemorrhagic stroke [79]. Although alirocumab therapy is not associated with a reduction in CV mortality, it is associated with a reduction in all-cause mortality (15%, $P = 0.03$) [80] (Fig. 14.11). This association must, however, be regarded as *nominally* significant because all-cause mortality followed CV and CHD mortality in the prespecified hierarchy of principal secondary endpoints. Among patients with a history of prior coronary artery bypass grafting prior to their qualifying ACS, alirocumab significantly reduced risk for MACE by 23% [81]. As observed in the FOURIER trial, the reduction of Lp(a) by alirocumab was shown to contribute to overall MACE reduction in the ODYSSEY OUTCOMES trial [82].

Overall Safety of Alirocumab

The side-effect profile of alirocumab is similar to that of evolocumab. The most frequently occurring adverse events are skin reactions at the injection site. Nasopharyngitis, influenza-like reaction, and diarrhea occur slightly more frequently than placebo [83]. Neurocognitive adverse events are similar to placebo. When evaluating adverse events as a function of LDL-C < 15 mg/dL, < 25 mg/dL, or > 25 mg/dL, there are no substantive differences at these different LDL-C thresholds, including for neurocognitive side-effects [84]. In ODYSSEY OUTCOMES, there were no between group differences in hepatic, skeletal muscle, or renal side effects or toxicity [77]. Risk of worsening diabetes or new onset diabetes was not different between groups.

Inclisiran

A rapidly evolving field of novel pharmacologic therapeutic agents are single-stranded and double-stranded ribonucleic acid (ssRNA and dsRNA, respectively) oligonucleotides that antagonize the translation of specific gene products. Mipomersen is an example of an ssRNA oligonucleotide) [85] (Fig. 14.12). Mipomersen enters hepatocytes and binds to a complementary nucleotide sequence according to Watson-Crick base pairing along the messenger RNA (mRNA) for apoB. This interrupts mRNA translation along the ribosome and leads to reduced apo B and, hence, VLDL production, ultimately also resulting in lower serum levels of LDL-C and Lp(a) [86]. Inclisiran is an example of a dsRNA interfering or "silencing" RNA (siRNA). DNA replication and transcription are highly regulated processes within the nucleus of a cell. However, it has become increasingly clear that gene expression is also significantly impacted by microRNAs and siRNAs that inhibit or silence gene/mRNA expression posttranscriptionally [87]. Interfering

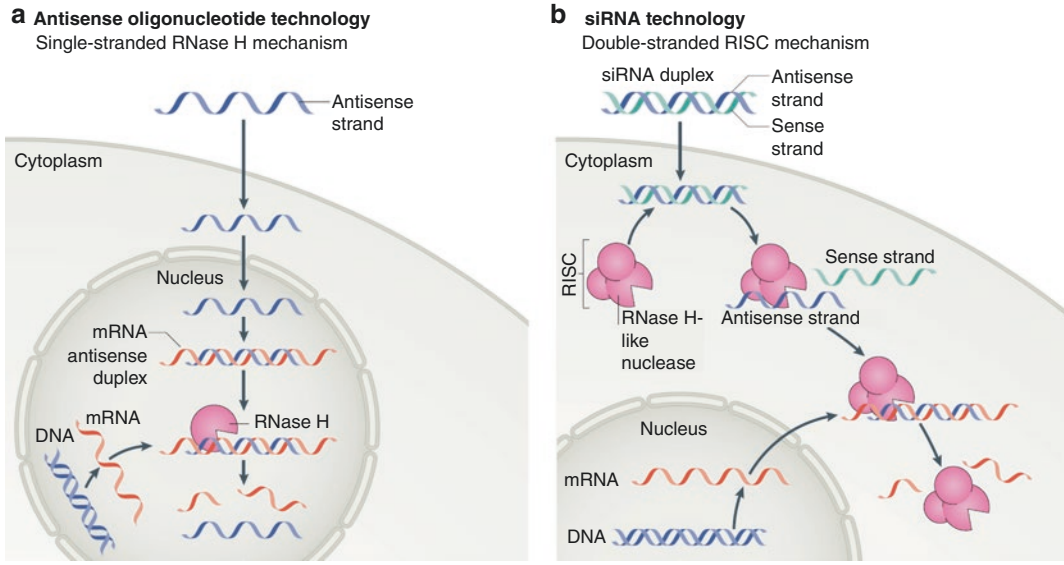


Fig. 14.12 Silencing RNA (siRNA)-based versus antisense oligonucleotide-based approaches for reducing PCSK9 expression. (**a**) Antisense oligonucleotide technology employs a single-stranded RNase H mechanism. (**b**) By contrast, small interfering RNA (siRNA) technology utilizes a double-stranded RNA-induced silencing complex (RISC) mechanism. (From Nordestgaard et al. [100])

RNAs are 20–30 nucleotides long and are composed of both an antisense strand that is complementary to a target sequence in the mRNA for a specific gene and a passenger strand [88]. The antisense strand is used to inhibit mRNA translation [89]. This, however, requires complex molecular machinery. The antisense strand is incorporated into the RNA induced silencing complex (RISC; Fig. 14.12). The RISC is a molecular complex that can be used by a cell to silence the expression of virtually any gene by three possible mechanisms: (1) interrupting mRNA translation, (2) promoting the degradation of mRNA, and (3) promoting the formation of heterochromatin or even inducing DNA elimination [87]. The antisense strand binds to an Argonaute protein which is critical for aligning the antisense strand with a target mRNA so that it can form a complementary Watson-Crick helix. Glycine-tryptophan protein of 182 kDa (GW182) promotes both translational suppression and the recruitment of CCR4–NOT deadenylase complex4 which hydrolyzes the RNA complex [89].

Inclisiran is an example of gene silencing technology that suppresses the expression of PCSK9 leading to the reduction of PCSK9 in both the intra- and extracellular compartments of the hepatocyte. Inclisiran is very specifically targeted to hepatocytes by being covalently bound to triantennary*N*-acetylgalactosamine [90]. This allows inclisiran to very specifically bind to asialoglycoprotein receptors on the hepatocyte surface with high affinity [91]. In the Trial to Evaluate the Effect of Inclisiran Treatment on Low Density Lipoprotein Cholesterol (LDL-C) (ORION-1) trial, inclisiran induced a dose-dependent reduction in serum LDL-C; inclisiran dosed at 300 mg SQ on days 1 and 90 induced the following reductions by day 180 compared to baseline and placebo: LDL-C 52.6% ($p < 0.001$), non-HDL-C 46% ($p < 0.001$), triglycerides 14.2% ($p < 0.05$), VLDL 16% ($p < 0.01$), apo B 40.9% ($p < 0.001$), Lp(a) 25.6%, and PCSK9 69% ($p < 0.001$) [92]. Injection site reactions occurred in 5% of patients receiving inclisiran. Inclisiran had a comparable rate of liver and skeletal muscle related side effects relative to placebo. On this regimen, 48% of participants achieved an LDL-C < 50 mg/dL, and 66% achieved an LDL-C < 70 mg/dL. Because of its pharmacokinetic profile and mechanism of action, inclisiran can be dosed every 6 months and provide durable, stable reductions in LDL-C [93]. Inclisiran provides identical levels of LDL-C reducing capacity to diabetics and nondiabetics

[94]. The clinical efficacy for reducing cardiovascular events by inclisiran is being evaluated in the ORION-4 trial which includes approximately 15,000 patients 55 years of age or older and with established ASCVD. It is anticipated the trial will require 5 years to complete [95].

Conclusions

1. PCSK9 is an important regulator of LDL particle uptake and catabolism.
2. PCSK9 impacts serum levels of multiple lipoprotein species and their subfractions by impacting the expression of multiple members of the LDLR family.
3. PCSK9 monoclonal antibodies (evolocumab and alirocumab) reduce LDL-C markedly and dramatically increase goal attainment rates for LDL-C, apo B, and non-HDL-C.
4. The PCSK9 mAbs have an excellent safety profile and are well tolerated.
5. The PCSK9 mAbs impact risk for CV events significantly when used in combination with statins. The risk for MI, stroke, and need for revascularization are all significantly reduced. There is no increase in risk for hemorrhagic stroke with these agents.
6. The reduction in Lp(a) by the PCSK9 mAbs contributes to ASCVD risk reduction.
7. Inclisiran suppresses the translation of PCSK9 mRNA and provides substantial capacity for reducing LDL-C as well as VLDL, apo B, and non-HDL-C. Its unique mechanism of action allows for dosing this medication twice per year.

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15 Management of Hypertriglyceridemia (Including Fibrates and n-3 Fatty Acids)

Matthew Evans and Michael Miller

Introduction

Historically, lifestyle and pharmacological interventions aimed at decreasing cardiovascular disease risk have focused on reducing low-density lipoprotein cholesterol (LDL-C), particularly through the beneficial effects of statins, or 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. However, multiple large, randomized controlled trials have demonstrated that despite achieving large reductions in cardiovascular disease risk by obtaining what are considered optimal levels of LDL-C, the incidence of major adverse cardiovascular events in these patients remains considerably elevated. Over the last several years, compelling evidence suggests a significant association between hypertriglyceridemia and cardiovascular disease, and more recent data demonstrates further reduction in adverse cardiovascular outcomes after treatment of hypertriglyceridemia with the use of omega-3 fatty acids in these patients.

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Residual Cardiovascular Disease Risk Despite LDL-C Lowering Therapy

Throughout the last 20 years, statins have become a mainstay of therapy in the primary and secondary prevention of cardiovascular disease events as demonstrated in several randomized controlled trials (Scandinavian Simvastatin Survival Study (4S) [1], Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) [2], Cholesterol and Recurrent Events (CARE) [3], Heart Protection Study (HPS) [4], West of Scotland Coronary Prevention Study (WOSCOPS) [5], Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) [6], Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) [7]). However, these trials have also demonstrated that even with LDL-C lowering, significant residual cardiovascular disease risk remains. For example, patients treated with statin therapy in the 4S trial experienced cardiovascular disease event rates approximating 20% (compared to 28% with placebo) over the 5-year study period.

Over the next several years, further analyses evaluated the effects of high-dose statin treatment for more intensive LDL-C lowering. Specifically, in the Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 Investigators (PROVE IT-TIMI 22) [8], Incremental Decrease in Events through

M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_15

Aggressive Lipid Lowering (IDEAL) [9], and Treat to New Targets (TNT) [10] trials, high-intensity (80 mg atorvastatin daily) therapy demonstrated greater cardiovascular disease risk reduction when compared to moderate intensity statin therapy, but residual risk in the high-intensity treatment arms was still noteworthy at 22.4%, 12%, and 8.7% over a median follow-up period of 4.9 years, respectively, despite mean LDL-C levels that were not elevated (62, 81, and 77 mg/dL, respectively).

Recognition of this persistently elevated cardiovascular disease risk despite high-intensity statin therapy has prompted investigation into non-statin therapies as a means for further risk reduction. In the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) trial, patients receiving ezetimibe in addition to moderate-intensity simvastatin experienced event rates of 32.7% compared with 34.7% with simvastatin alone over a 7-year period, despite mean LDL-C levels of 53.2 mg/dL [11].

In the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial, patients with elevated LDL-C (>70 mg/dL) despite moderate- or high-intensity statin therapy who received evolocumab, a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor, were found to have statistically significant risk reduction relative to placebo. Yet, they still experienced adverse cardiac events at a rate of 9.8% with median follow-up of 2.2 years, despite a median LDL-C approximating 30 mg/dL [12]. Similarly, in the Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab (ODYSSEY OUTCOMES) trial, which examined the effects of another PCSK9 inhibitor, alirocumab, in patients with a recent acute coronary syndrome (ACS) event within the previous 12 months, treated patients experienced cardiovascular events at a rate of 9.5% over median follow-up period of 2.8 years (compared to 11.1% with placebo) [13].

Accordingly, despite LDL-C optimization attained with both statin and non-statin therapies, cardiovascular disease risk remains elevated, thereby prompting investigators to search for other potential targets aimed at further risk reduction. Among the most well-established yet unexplored targets is hypertriglyceridemia.

Evidence for Hypertriglyceridemia as an Independent Risk Factor for Cardiovascular Disease

Isolating the direct contribution of hypertriglyceridemia on cardiovascular disease risk can be challenging because these patients frequently present with other comorbid conditions, which increase the risk of cardiovascular disease. They include type 2 diabetes mellitus, hypertension, hypercholesterolemia, and metabolic syndrome. In addition, the precise mechanisms linking triglyceride elevation and atherosclerosis are incompletely understood. That in part is due to the inability of large triglyceride-rich remnant particles to penetrate the vessel wall. However, because triglycerides are hydrolyzed from triglyceride-rich particles, their cholesteryl ester-enriched by-products (chylomicron and very-low-density lipoprotein remnants) can promote atherogenesis via multiple mechanisms, including direct infiltration of remnants into the vessel wall and activation of pro-inflammatory and prothrombotic signaling pathways (Fig.15.1) [14–17].

From an epidemiological standpoint, a large meta-analysis of 29 prospective studies encompassing 262,525 patients identified serum triglyceride concentration as a strong independent risk factor for cardiovascular disease events that was independent of gender and the concomitant conditions listed above [18]. Moreover, the Cholesterol Treatment Trialists' (CTT) meta-analysis encompassing 14 statin trials demonstrated that among 18,000 patients with diabetes, patients in the highest triglyceride tertile experienced cardiovascular disease events at a 26% higher rate compared to the lowest tertile; these differences persisted in the stain-treated group [19]. Finally, in the Action to Control Cardiovascular Risk in Diabetes (ACCORD)-Lipid trial, event rates in patients in the highest

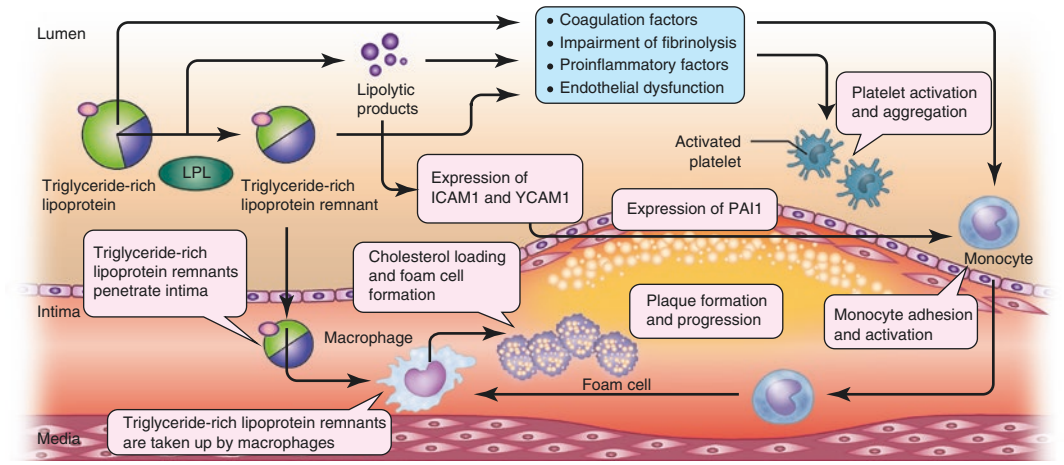


Fig. 15.1 Elevated triglyceride-rich lipoproteins and atherogenic factors driving cardiovascular disease risk. Adapted from Reiner [17]

triglyceride tertile were 21.9% higher than those taking statins alone compared with those receiving statins and fenofibrate [20].

As mentioned, post hoc analyses of several of the landmark statin trials have also demonstrated a correlation between hypertriglyceridemia and cardiovascular disease risk. In the 4S trial, patients in the highest triglyceride subgroup (> 159 mg/dL) had the highest risk for cardiovascular disease events on placebo and experienced significantly greater event reduction (52%) than either the isolated LDL-C elevation subgroup (14%) or the total study population (34%), again suggestive of hypertriglyceridemia as an important contributor to cardiovascular disease risk beyond elevations in LDL-C [21].

A similar subgroup analysis of the PROVE IT-TIMI 22 trial showed that triglyceride levels <150 mg/dL were associated with reduced cardiovascular disease risk; each 10 mg/dL decrement in serum triglyceride conferred a 1.5% reduction in the incidence of death, MI, and recurrent ACS. In addition, the combination of low LDL-C and low triglyceride levels (less than 70 and 150 mg/dL, respectively) coincided with the lowest risk of recurrent cardiovascular events [22]. In both the Myocardial Ischemia Reduction with Acute Cholesterol Lowering (MIRACL) and (dalcetrapib) dal-OUTCOMES trials, two short- and long-term post-ACS studies, elevated fasting triglyceride levels were associated with subsequent primary outcomes after adjusting for LDL-C and high-density lipoprotein cholesterol (HDL-C) [23].

In addition, a Mendelian randomization study of individuals enrolled in the Copenhagen City Heart Study (CCHS) revealed that patients with lower concentrations of non-fasting plasma triglyceride experienced lower rates of all-cause mortality [24]. Similarly, there was a causal association between elevated levels of non-fasting triglyceride and increased risk of myocardial infarction (MI) among CCHS patients with genetic variation in the apolipoprotein A5 (APOA5) gene, which codes for a protein that serves as an important determinant of plasma triglyceride levels [25]. Along with patients enrolled in the Copenhagen General Population Study (CGPS), patients with very high non-fasting triglyceride levels (e.g., >500 md/dL) were found to have a higher risk of cardiovascular disease and all-cause mortality [14].

Lifestyle Modification

For patients with borderline and high triglyceride levels (150–499 mg/dL), first-line therapy consists of adjustments to nutrition and physical activity levels (Fig.15.2). Triglyceride reduction

Diet / Lifestyle Change	TG Reduction
Weight loss (5-10% of body weight)	20%
Implement Mediterranean-style diet vs high-carb diet	10-15%
Exercise of moderate intensity (e.g. brisk walking 4-5 mph, 30 m/d)	10-20%

Fig. 15.2 Effect of lifestyle practices on triglyceride reduction. Adapted from the American Heart Association scientific statement on triglycerides and cardiovascular disease [37]

is the first and most notable effect of increased physical exercise on the lipid profile, and weight loss is the most effective non-pharmacological means of lowering serum triglycerides; a 5–10% reduction in body weight is noted to confer a 20% decrease in triglycerides [26]. Other studies have indicated for every kilogram of weight loss, one can reasonably expect a 2% reduction in serum triglyceride levels [27]. This observed reduction is mediated via upregulation of lipoprotein lipase (LPL) activity and increased utilization of triglycerides by exercising muscles, and decreases in serum triglycerides in response to aerobic exercise appear to be dose dependent [28]. In a study of middle-aged men, participants who ran 7–14 miles weekly at a mild to moderate pace experienced 20% lower fasting triglyceride levels compared to no activity, and those in the highest activity level (>20 miles weekly) experienced a 31% decline in triglycerides and the lowest observed fasting triglyceride levels [29].

Changes in dietary macronutrient composition can also contribute to further reductions in serum triglyceride levels. A Mediterranean-style diet, consisting of foods rich in monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), and dietary fiber primarily through incorporation of whole grains, vegetables, fruits, nuts, and olive oil, may result in a 10–15% lowering of triglycerides compared to a low-fat diet [30]. In the Framingham Heart Study Offspring Cohort, patients in the highest quintile of the Mediterranean-style dietary pattern were noted to have the lowest triglyceride levels (103 vs 114 mg/dL, $p < 0.001$) over a 7-year follow-up period [31].

Marine-derived omega-3 (OM3) PUFAs, primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), concentrated in marine-derived fish including anchovies, herring, salmon, and mackerel (Fig.15.3), lower triglyceride levels in part by reducing hepatic output of very-low-density lipoprotein (VLDL) [32]. PUFAs also induce peroxisome proliferator-activated receptors (PPARs), transcription factors involved in the metabolic regulation of lipid metabolism [33]. Prior studies have demonstrated a 20–30% reduction in serum triglyceride levels with incorporation of approximately 4 g of marine-derived PUFAs to the diet each day [34]; it has been estimated there is an approximate 5–10% reduction in serum triglycerides for each gram of OM3 incorporated into daily food intake [35].

Pharmacological Management

Given the robust evidence regarding reductions in cardiovascular disease risk observed with statin therapy, the American College of Cardiology/American Heart Association (ACC/AHA) cholesterol guidelines recommend statins as first-line therapy in patients with hypertriglyceridemia characterized as moderate (TG 175 to 499 mg/dL) or severe (TG ≥500 mg/dL or greater) if the atherosclerotic cardiovascular disease (ASCVD) risk score is 7.5% or higher [36]. A meta-analysis of 91 randomized clinical trials showed among patients with an average baseline triglyceride level of 177 mg/dL statins reduced mean triglyceride levels 15–20% with greater reductions (40–45%) obtained with higher baseline levels (i.e., >273 mg/dL) [37]. If triglyceride levels remain elevated despite lifestyle modifications and the maximally tolerated statin dose, some professional society recommendations advocate the use of additional triglyceride-lowering agents such as fibrates, niacin, and OM3 fatty acids [38, 39].

	EPA+DHA (mg/100 g)
Anchovy	2055
Herring, Atlantic	2014
Salmon, farmed	1966
Salmon, wild	1840
Mackerel, Atlantic	1203
Bluefish	988
Sardines, Atlantic	982
Trout	936
Goldenbass (tilefish)	905
Swordfish	899
Tuna, white (albacore)	862
Mussels	782
Striped bass	754
Shark	689
Pollock, Atlantic	542

Fig. 15.3 Selected marine sources enriched in EPA and DHA. Adapted from Bays [32]

Niacin

Niacin has been found to effectively decrease levels of small, dense LDL-C particles and increase levels of HDL-C and to inhibit the activity of hepatic microsomal diacylglycerol acyltransferase-2 (DGAT2), a key enzyme that catalyzes the final reaction in triglyceride synthesis [40].

A number of randomized studies have demonstrated that niacin monotherapy substantially reduces triglyceride levels, and a meta-analysis of 30 such trials showed that niacin was associated with an average reduction of as much as 20% [41]. Yet, despite these appreciable reductions, clinical outcomes trials of niacin-statin combination therapy have failed to demonstrate clinical benefit beyond statin monotherapy. In the AIM-HIGH trial, 3414 participants treated with simvastatin +/− ezetimibe were randomized to extended-release niacin (1.5–2 g daily) or placebo. The incidence of the primary outcome, a composite of cardiovascular death, nonfatal MI, ischemic stroke, hospitalization for an acute coronary syndrome, or symptom-driven coronary or cerebral revascularization, occurred in 16.4% in the niacin group versus 16.2% in the placebo group (p = 0.79) [42]. The trial was terminated

early after a mean follow-up of 3 years to a lack of efficacy. Similarly, in the HPS2-THRIVE study, 25,673 statin-treated patients with vascular disease were randomized to either 2 g of extended-release niacin or placebo. Once again, there was no statistically significant difference ($p = 0.29$) in major adverse cardiovascular events (MACE) defined as nonfatal MI, cardiovascular death, stroke, or arterial revascularization. In addition, the rate of adverse effects (namely diarrhea, dyspepsia, and myopathy) was significantly higher in the niacin group [43]. Consequently and not surprisingly, niacin use for treating hyperlipidemia has waned in recent years.

Fibrates

Fibrates attenuate hepatic secretion of very-low-density lipoprotein (VLDL) particles and serve as peroxisome proliferator-activated receptor alpha (PPAR-α) agonists to modulate the metabolism of triglyceride-rich lipoproteins, resulting in shifts in LDL and HDL particle size, believed to contribute to reduced cardiovascular risk [38, 44, 45].

Several randomized, placebo-controlled trials have demonstrated the triglyceride-lowering efficacy of fibrate monotherapy, in particular gemfibrozil and bezafibrate [46–48]. In addition, a meta-analysis of 53 clinical trials demonstrated that fibrate therapy lowered triglyceride levels by approximately 36% [38, 41]. However, clinical outcomes trials for fibrates have had inconsistent results with more favorable results obtained with gemfibrozil compared to bezafibrate or fenofibrate.

Specifically, in the Helsinki Heart Study, 4081 asymptomatic patients with primary dyslipidemia (non-HDL cholesterol >200 mg/dL) were randomized to receive either 600 mg of gemfibrozil twice daily or placebo. Patients receiving gemfibrozil experienced a 34% reduction in the incidence of ischemic events compared to placebo ($p < 0.02$) [46]. These findings were supported by those in the VA-HIT trial, in which 2531 men with established coronary artery disease were again randomized to 1200 mg of gemfibrozil daily compared to placebo. Patients in the gemfibrozil group experienced a primary event (defined as a composite of nonfatal MI or cardiovascular death) 22% less often compared to those in the placebo group ($p = 0.006$) [47].

In contrast, however, the BIP study randomized 3090 patients with prior MI or stable angina to either 400 mg of bezafibrate daily or placebo; after a mean follow-up of 6.2 years, there was no statistically significant reduction in the primary endpoint of fatal or nonfatal MI or sudden death ($p = 0.26$) [48]. Similarly, in the FIELD study, 9795 patients with type 2 diabetes mellitus were randomized to receive either 200 mg daily of fenofibrate or placebo. There was no statistically significant difference in the primary outcome (coronary heart disease death or nonfatal MI) between the two groups ($p = 0.16$) [49]. In both studies, there was a statistically significant increase in the use of statin therapy in the placebo groups than in the fibrate groups; this may be a confounding factor, which attenuated the treatment effects relative to previous studies [38].

Interestingly, post hoc analyses of the clinical benefit of fibrate therapy in patients with elevated triglyceride levels (>200 mg/dL) suggested a trend toward greater cardiovascular event reduction compared to those with triglyceride levels less than 200 mg/dL [41]. In the ACCORD study, 5518 patients with type 2 diabetes treated with simvastatin were randomized to receive either fenofibrate or placebo, and no statistically significant reduction in the primary outcome of nonfatal MI, nonfatal stroke, or cardiovascular death was observed during the trial period of 5 years ($p = 0.32$). However, the trial did show benefit in the subgroup with elevated triglyceride levels (>204 mg/dL) and low HDL cholesterol (<34 mg/dL) [20]. These findings suggest that fibrate therapy may be beneficial in patients with high triglyceride levels and reduced HDL cholesterol [39]. A randomized clinical outcomes trial is currently testing the selective PPAR-α agonist, pemafibrate, in diabetic patients with hypertriglyceridemia and low HDL-C [50].

Potential adverse effects associated with fibrate therapy include myopathy, cholelithiasis, and elevations in serum creatinine levels. In FIELD, creatinine levels were reversibly increased by an average of 12% [49]; although elevations have been reported in a number of clinical trials, decreases in creatinine clearance and glomerular filtration rate were not observed [51]. The incidence of myopathy is reportedly 5.5-fold greater with the use of fibrates compared with statin monotherapy and has been shown to increase further with statin-fibrate combination therapy. The incidence of muscle symptoms is reportedly greater with the use of gemfibrozil compared with fenofibrate [51]. Even when considering these adverse effects, however, in the previously mentioned meta-analysis of 53 clinical trials, the rate of discontinuation of fibrate therapy (15%) was comparable between the fibrate and placebo groups [41].

Omega-3 Fatty Acids

The cardioprotective benefits of OM3 PUFAs have been well described, as mentioned above. In addition to reducing serum triglyceride levels, EPA and DHA may attenuate atherosclerotic plaques, lower systolic and diastolic blood pressure, and improve endothelial function [52]. OM3 fatty acids serve as precursors for bioactive lipid mediators that regulate inflammation, including eicosanoids, prostaglandins, leukotrienes, protectins, and resolvins. EPA interferes with lipid oxidation by various signal transduction pathways linked to inflammation, endothelial dysfunction, and plaque instability via incorporation into cellular membranes. Due to differences in their structure, EPA and DHA associate with distinct regions of biological membranes and differential modulate membrane structure-function relationships. In particular, the lipophilic structure and space dimensions of EPA allow it to insert efficiently into lipoprotein particles and cell membranes where it scavenges free radicals [15, 53].

There are three FDA-approved omega-3 fatty acid agents available: omega-3 fatty acid ethyl esters (OM3 A EE) (marketed as Lovaza®), icosapent ethyl (IPE) (marketed as Vascepa®), and omega-3 carboxylic acids (marketed as Epanova®). In the COMBOS trial, addition of OM3 A EE to simvastatin resulted in significantly reduced non-HDL-C. In addition, treatment when compared to placebo resulted in significantly reduced triglyceride levels (27.5% vs 7.2%) [54]. The efficacy of IPE was assessed in the MARINE and ANCHOR trials, where patients experienced statistically significantly reductions in serum triglyceride levels. In MARINE, patients were randomized to receive either 4 g/daily, 2 g/daily, or placebo and experienced reductions in serum triglyceride level of 33.1% and 19.7%, respectively [55]. In ANCHOR, reductions of 21.5% and 10.2% were demonstrated with the same dosages [56].

Despite clear evidence that these products lead to statistically significant reductions in serum triglyceride levels, there has until recently been conflicting data as to whether these reductions lead to clinically significant improvements in cardiovascular outcomes.

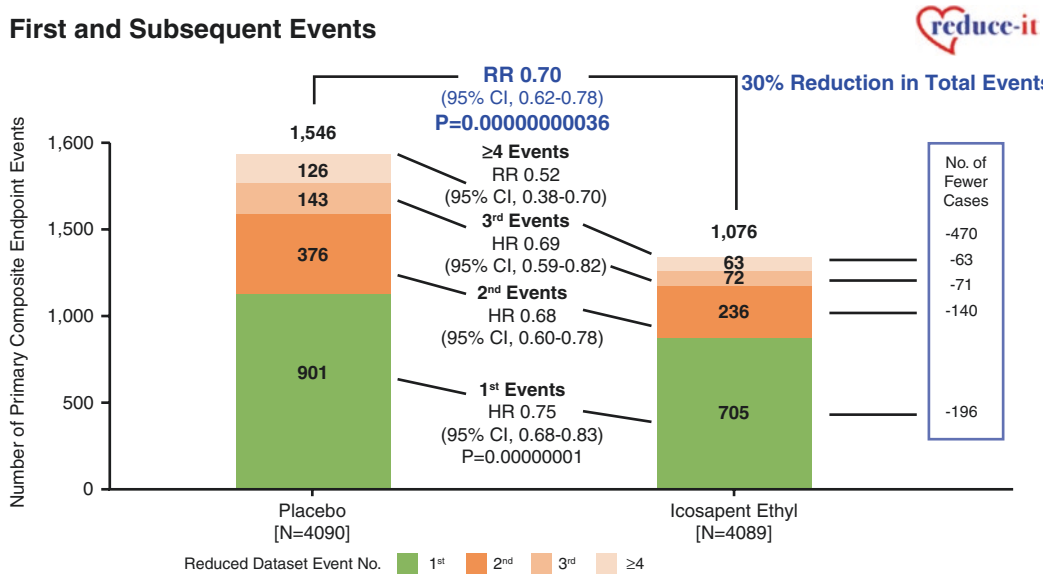
The Gruppo Italiano per lo Studio della Sopravvivenza (GISSI)-Prevenzione trial was an open-label prospective study of 11,324 patients post-MI randomly assigned to receive either OM3 fatty acid supplementation (1 g of EPA plus DHA), vitamin E (0.3 g daily), both, or none, for 3.5 years. Patients in the EPA/DHA group had a 15% reduction in the primary outcome of death, nonfatal MI, and stroke [57]. It is important to note that statins were not commonly used during the study period, and therefore, this study has minimal generalizability to current treatment paradigms. Subsequently, the Japan EPA Lipid Intervention Study (JELIS) trial randomized 18,645 Japanese patients with hyperlipidemia to receive low-dose statin (pravastatin or simvastatin) monotherapy or combination therapy with a statin and 1.8 g of EPA – patients in the EPA group experienced a 19% relative reduction in major coronary events [58].

Over the next several years, other large studies (Omega-3 Fatty Acids on the Reduction of Sudden Cardiac Death After Myocardial

Infarction (OMEGA), ALPHA OMEGA, and Outcome Reduction With Initial Glargine Intervention (ORIGIN)) failed to reproduce the results of GISSI and JELIS. However, the investigational doses in all three trials were less than the 1 g/day of OM3 fatty acids recommended by the American Heart Association (AHA) for patients with established coronary artery disease (0.84, 0.40, and 0.90 g/day, respectively) and significantly less than the doses demonstrated to be efficacious in ANCHOR and MARINE (2–4 g/d) [59–61].

The Reduction of Cardiovascular Events With EPA–Intervention Trial (REDUCE-IT) trial enrolled 8,179 patients from 11 countries with either established cardiovascular disease or diabetes and other risk factors to receive either 2 g of IPE twice daily or placebo. The primary endpoint was a composite of cardiovascular death, nonfatal MI, nonfatal stroke, coronary revascularization, or unstable angina. The risk of the primary composite endpoint was 25% lower in the icosapent ethyl (IPE) group than in the placebo group ($P < 0.0001$), corresponding to a number needed to treat 21 patients [62]. The reduction in risk of myocardial infarction, stroke, and cardiovascular death (secondary endpoint) was also significantly reduced (24%; $P < 0.0001$) as was the prespecified evaluation of endpoints that was reduced by 30% ($P < 0.0001$) [63] (Fig.15.4).

The most likely explanation for the lack of benefit seen in other contemporary OM3 fatty acid trials may be attributable to the low doses used (generally <1 g/d) or the low ratio of EPA to DHA [64]. Although the dose of EPA administered in JELIS was lower than the EPA-equivalent dose in REDUCE-IT, it resulted in a plasma EPA level (170 μg per milliliter in a Japanese population) similar to that attained in a previous 12-week lipid study, in which a total daily dose of 4 g of IPE was used in a Western population and similar to that attained in REDUCE-IT. Unlike REDUCE-IT, JELIS was an open-label design without a placebo group, used only a low-intensity stain, and was conducted in a country where fish consumption is high compared to that in the USA. In addition, at baseline, patients in JELIS had higher levels of LDL cholesterol and lower baseline triglyceride values than the patients in REDUCE-IT [62]. While the magnitude of TG reduction achieved in REDUCE-IT (~20%) is unlikely to fully account for the cardio-



Bhatt DL, Steg PG, Miller M, et al. *J Am Coll Cardiol.* 2019.

Fig. 15.4 REDUCE-IT study primary and recurrent events. Adapted from Bhatt et al. [63]

vascular benefits observed, further analyses and future studies are needed to evaluate the mechanisms underlying the benefits observed. They may include reduction of inflammation, oxidation, platelet aggregation, and restoration of endothelial function [65, 66].

It is also unclear to what extent the highly concentrated EPA compound, IPE, might be clinically superior to DHA vis-à-vis cardiovascular disease events. The soon to be completed secondary prevention STatin Residual Risk Reduction With EpaNova in HiGh CV Risk PatienTs With Hypertriglyceridemia (STRENGTH) study testing the combination of EPA/DHA carboxylic acids in patients with hypertriglyceridemia and low HDL-C will undoubtedly provide further insight regarding the use of OM3 for cardiovascular disease protection [67].

Gastrointestinal side effects (nausea and diarrhea/loose stools) appear to be the most common adverse events, occurring in up to 27% of patients at doses of 4 g daily [68]. In a meta-analysis of 29 clinical trials of OM3 fatty acid therapies, the risk of treatment discontinuation was again similar between treatment and placebo groups [69].

Summary

Despite maximally tolerated therapy targeting LDL-C reduction, a considerable amount of residual cardiovascular disease risk remains in most patients. Several genetic, observation, and post hoc analyses have provided evidence for hypertriglyceridemia as a potential target for further minimizing this risk. First-line therapy for patients with mild to moderate hypertriglyceridemia includes lifestyle modification in the form of carbohydrate reduction, weight loss, and implementation of a Mediterranean-style diet rich in unsaturated fatty acids. For patients with elevated triglyceride levels, despite these modifications, pharmacotherapy including statins, fibrates, and omega-3 fatty acids may be indicated. Ongoing randomized controlled trials continue to assess the effects of these classes on clinical cardiovascular outcomes.

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16

Pathophysiology and Management of Dyslipidemias Associated with Insulin-Resistant States

Kevin C. Maki, Lane Benes, and Mary R. Dicklin

Introduction

Insulin resistance (IR), defined as an impaired ability of a given circulating concentration of insulin to promote the clearance of glucose from the blood, is common in the United States and other developed countries. It is present in a large majority of people with obesity, polycystic ovary syndrome, impaired glucose tolerance, and type 2 diabetes mellitus [1]. In addition, IR is present in approximately half of patients with hypertension [2].

IR has both genetic and environmental determinants. Increased adiposity, sedentary lifestyle, and cigarette smoking all appear to contribute causally to its development [3]. However, some individuals have a strong genetic component and may thus manifest IR in the absence of acquired factors. For example, first-degree relatives of patients with type 2 diabetes mellitus are insulin resistant compared with controls matched for body mass index (BMI) [1]. Nevertheless, the degree of IR is further increased when genetic disposition is combined with lifestyle factors that promote IR.

Obesity and Type 2 Diabetes Mellitus

The incidence and prevalence of overweight and obesity have increased dramatically in the United States during the last generation [4–7]. Figure 16.1 shows the prevalence of obesity among US adult men and women, based on representative samples of the population, from 1960 to 2014. The prevalence of obesity nearly tripled during this time; the increases have been mainly attributable to the period since 1980. In 2015–2016 the prevalence of obesity (BMI ≥30 kg/m^2) among US adults was 39.8% [8]. Obesity is the strongest risk factor for the development of type 2 diabetes mellitus, with estimates from Mendelian randomization analyses suggesting an increased risk of type 2 diabetes per unit increase in BMI of ~26% [9]. Therefore, it is not surprising that the incidence of type 2 diabetes mellitus has been rising in concert with that of obesity. Data released by the Centers for Disease Control and Prevention indicate that approximately 84 million adult Americans (~34% of the adult population) have pre-diabetes and 30 million (~9% of the population) have diabetes [10].

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_16

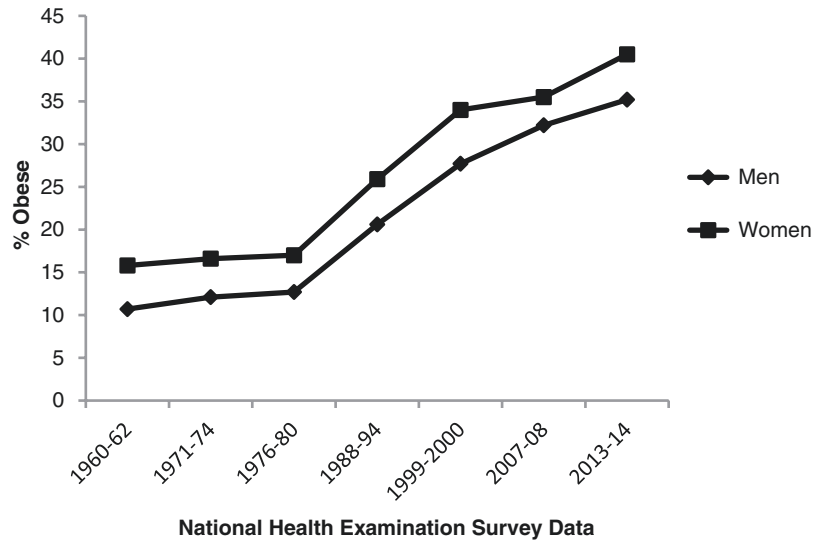

Fig. 16.1 Prevalence of obesity among US adult (≥20 y of age) men and women, based on representative samples of the population, from 1960 to 2014 [4–6]

Metabolic Syndrome

IR is thought to play a central role in the development of a cluster of interrelated metabolic abnormalities that predispose to the development of atherosclerotic cardiovascular disease (ASCVD) and occur together more often than would be predicted by chance. Over the years, this clustering of risk factors has been referred to by such terms as the insulin resistance syndrome, syndrome X, metabolic syndrome, and the cardiometabolic risk syndrome. The commonly acknowledged features include increased adiposity, atherogenic dyslipidemia (discussed in detail below), glucose intolerance, and hypertension, although more recent evidence indicates that other abnormalities are also associated with this syndrome, including hyperuricemia, low-grade inflammation, and a prothrombotic state [11]. The focus of this chapter will be the pathogenesis and management of the characteristics of dyslipidemia associated with insulin-resistant states.

Lipid Abnormalities Associated with IR

Insulin-resistant states are often accompanied by three major disturbances in the lipid profile, which have been collectively defined "atherogenic dyslipidemia" [12]. The features of atherogenic dyslipidemia include the following:

1. Elevated circulating triglycerides (TG)
2. Reduced high-density lipoprotein cholesterol (HDL-C) concentration
3. A predominance of small, dense low-density lipoprotein (LDL) particles

It should be noted that the term "lipid triad" has also been used in the literature for this group of disturbances, as well as to describe a separate, but related, set of lipid abnormalities (a TG concentration ≥ 200 mg/dL in combination with an LDL-C/HDL-C ratio > 5.0) [13]. It is important to distinguish between these lipid disturbances, because elevated LDL-C is not a feature of the dyslipidemia associated with IR per se, although it may be present concurrently.

Functions of Insulin

There are multiple molecular mechanisms that can produce cellular IR and therefore contribute to the metabolic disturbances that contribute to elevated ASCVD risk in obesity, type 2 diabetes mellitus, polycystic ovarian syndrome, and other insulin-resistant states. The interested reader is referred to recent reviews for more detailed

descriptions of the relevant metabolic pathways (Fig. 16.2) [14, 15]. Prior to summarizing the influence of IR on lipid metabolism, it is instructive to briefly review some of the functions of insulin in its role as the "master metabolic hormone." Insulin has a number of actions beyond the promotion of cellular uptake of glucose. It suppresses hepatic gluconeogenesis and shifts the metabolic state of tissues, particularly skeletal muscle, toward the oxidation and storage (as glycogen) of energy from carbohydrate. At the same time, insulin suppresses the activity of hormone-sensitive lipase. This, in turn, reduces the release of free fatty acids (FFAs) from adipose tissues into the circulation and thereby lowers the availability of FFAs as a substrate for oxidation. Insulin also stimulates lipoprotein and hepatic lipases, enhancing the hydrolysis of TGs in circulating lipoproteins and allowing their FFAs to move into cells. In the liver, insulin stimulates the catabolism of apolipoprotein (Apo) B.

Not all of the actions of insulin may be impaired to the same degree in IR. In patients with IR who do not have diabetes, normal glucose levels are maintained through increased insulin secretion (compensatory hyperinsulinemia). Thus, some of the metabolic abnormalities associated with IR may not result from impaired insulin action, but rather from overstimulation of insulin-mediated processes that are not impaired or are less impaired than insulin-stimulated cellular glucose uptake.

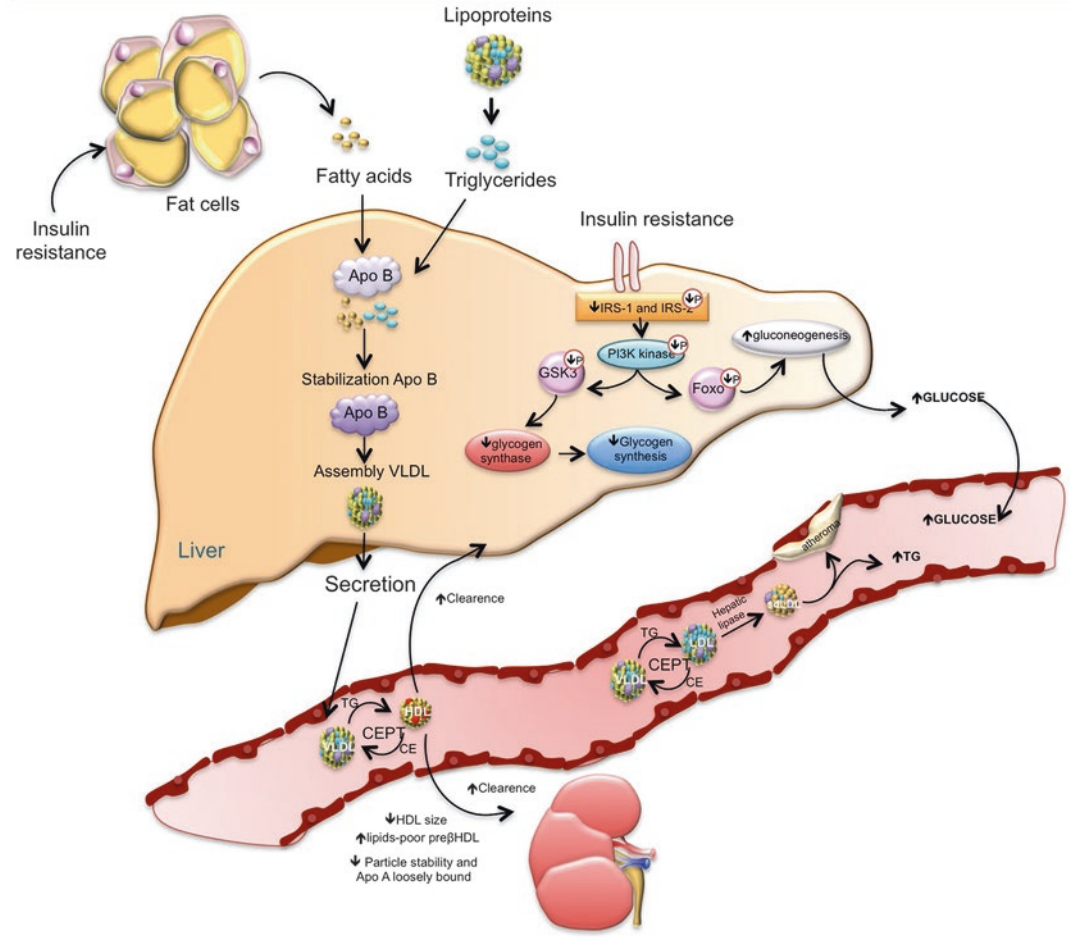

Fig. 16.2 Model of insulin resistance. Taken from Ormazabal et al. [14]

Excessive Production and TG Enrichment of Very-Low-Density Lipoprotein (VLDL): The Primary Lipid Abnormality in the Insulin-Resistant State

The primary metabolic abnormality associated with insulin-resistant states is overproduction of VLDL. In insulin-resistant states, VLDL particles are rich in TG and Apo C-III, resulting in mild-to-moderate hypertriglyceridemia. If the overproduction of VLDL is accompanied by other defects, such as Apo C-II deficiency (which lowers lipoprotein lipase activation), or a defect in the hepatic clearance of TG-rich Apo B-containing lipoproteins, the result can be more severe hypertriglyceridemia and/or mixed dyslipidemia, involving elevations in TG and LDL-C.

Two features of the insulin-resistant state are centrally involved in the pathogenesis of VLDL overproduction: elevated circulating levels of FFAs and hyperinsulinemia. It appears that both must be present to generate overproduction of VLDL. For example, in subjects with normal insulin sensitivity, a glucose infusion will not only increase the plasma insulin concentration but also lower levels of FFAs and reduce hepatic VLDL secretion [1]. In contrast, patients with poorly controlled type 1 diabetes have low insulin levels and high concentrations of FFAs, but do not have elevated VLDL secretion [1]. However, among insulin-resistant subjects with abdominal obesity, fasting and postprandial levels of circulating insulin are elevated, but this hyperinsulinemia does not sufficiently suppress FFA release into the circulation; thus, both insulin and FFA levels are elevated, resulting in VLDL overproduction.

Increased hepatic exposure to FFAs inhibits Apo B degradation and leads to increased VLDL synthesis [16]. Insulin promotes lipogenesis, which contributes to the TG pool available for incorporation into VLDL particles. In addition, insulin's ability to enhance Apo B degradation may be impaired in the insulin-resistant patient [16].

Reasons for Elevated FFA Levels in Insulin-Resistant States

The release of FFAs into the circulation is directly proportionate to the size of a fat cell. Thus, increasing adiposity is accompanied by adipocyte hypertrophy and greater release of FFAs into the circulation. A chronically elevated FFA level is believed to be a cause of IR. This is illustrated by the observation that IR can be induced in normal subjects by the infusion of a lipid emulsion for several hours, which raises the FFA concentration, mimicking the obese state [17]. Conversely, the niacin analog acipimox suppresses FFA release into the circulation and enhances insulin sensitivity [18]. The mechanisms that are responsible for the effect of a chronically elevated FFA level on insulin sensitivity are beyond the scope of this review, but the interested reader is referred to a recently published review on this topic [19].

FFA turnover in adipose tissues varies according to location. The abdominal visceral fat depots are the most metabolically active and contribute disproportionately to the circulating FFA level. For example, it has been estimated that an abdominally obese male with 20% of his body fat in the visceral stores will have a 50% contribution of these stores to the circulating FFA concentration [20]. Upper body subcutaneous fat is less metabolically active than abdominal visceral fat, and lower body subcutaneous fat is least metabolically active. For this reason, an abdominally obese woman is likely to have greater lipid disturbances than a woman of similar BMI with a "gynoid" pattern of obesity, who carries most of her excess adiposity on the hips and thighs. Some ethnic groups (e.g., South Asians) tend to have a greater proportion of their body fat carried in the abdominal visceral depots and thus may display IR and other metabolic abnormalities at relatively low BMI.

It should be noted that an elevated circulating FFA concentration may be present in the absence of obesity [1]. In some individuals, the primary metabolic defect responsible for IR may be impaired "fat trapping." When TGs in lipoproteins are hydrolyzed, they enter cells (primarily

adipose and muscle) through the action of acylation stimulating protein [21]. In some individuals, this mechanism is impaired, resulting in an abnormally large escape of FFAs back into the circulation. Such people may have circulating FFA levels that are much higher than would be predicted by their degree of adiposity, and they may be thought of as being "metabolically obese" [21]. In addition, some medications, particularly antiretroviral drugs, may cause peripheral lipodystrophy with a resulting inability of subcutaneous adipose tissues to take up FFAs released by lipoprotein lipase-catalyzed hydrolysis of TGs, resulting in excess return of FFAs liberated to the circulation.

Formation of Small, Dense LDL Particles

Overproduction of VLDL increases the plasma TG concentration. VLDL particles compete with chylomicron particles for the available lipoprotein lipase (Fig. 16.3) [22]. The result is a prolongation of the residence time of TG-rich lipoprotein particles in the circulation and increases in the concentrations of partially delipidated remnant particles (i.e., chylomicron remnants, small VLDL particles, and intermediate-density lipoproteins [IDL]). Evidence from a variety of sources supports the view that these TG-rich remnant lipoprotein particles are atherogenic [23, 24].

An increase in the circulating TG concentration provides additional substrate for cholesteryl ester transfer protein (CETP), which catalyzes the exchange of TG from TG-rich lipoproteins to LDL and HDL particles in exchange for cholesteryl esters (Fig. 16.2) [14, 25]. CETP activity is reportedly enhanced by an elevation in FFA concentration [16, 26]. The result is that the LDL and HDL particles become relatively TG rich and cholesterol poor. The TG in these particles can be hydrolyzed by hepatic lipase to form smaller, denser particles.

In hypertriglyceridemia, there is also excessive production of a species of VLDL that is Apo C-III rich, but ApoE deficient (Fig. 16.4) [27]. ApoE is needed to bind to high-affinity hepatic receptors for clearance, and Apo C-III delays the clearance of these VLDL particles by inhibiting the actions of both lipoprotein lipase and hepatic lipase, giving time for their TG to be hydrolyzed before removal from the circulation, enhancing the formation of small, dense LDL particles.

Small, dense LDL particles are believed to have enhanced atherogenicity for several reasons [28, 29]. They have less affinity for the Apo B receptor, resulting in extended circulation in the

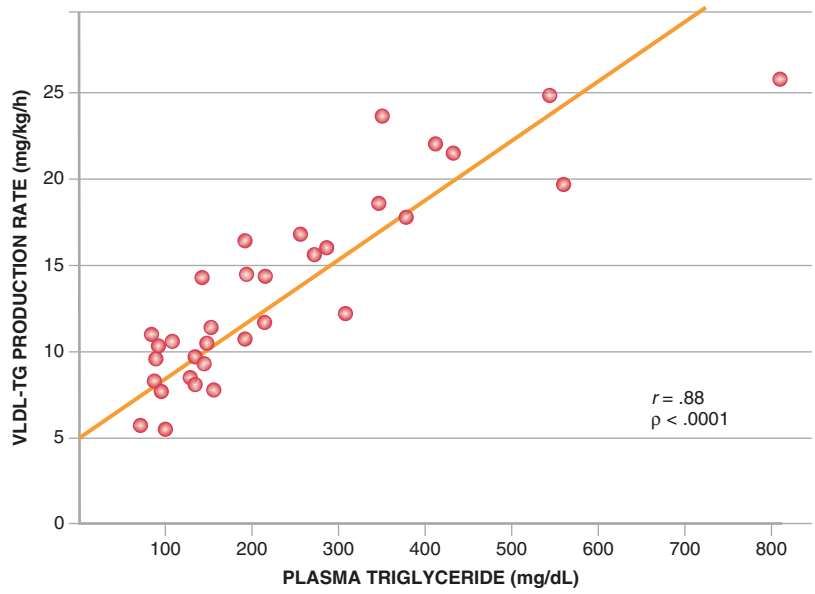

Fig. 16.3 Correlation between very-low-density (VLDL) triglyceride (TG) production rate (mg/kg/h) and plasma TG concentration (mg/dL). Adapted from Olefsky et al. With permission from Elsevier [22]

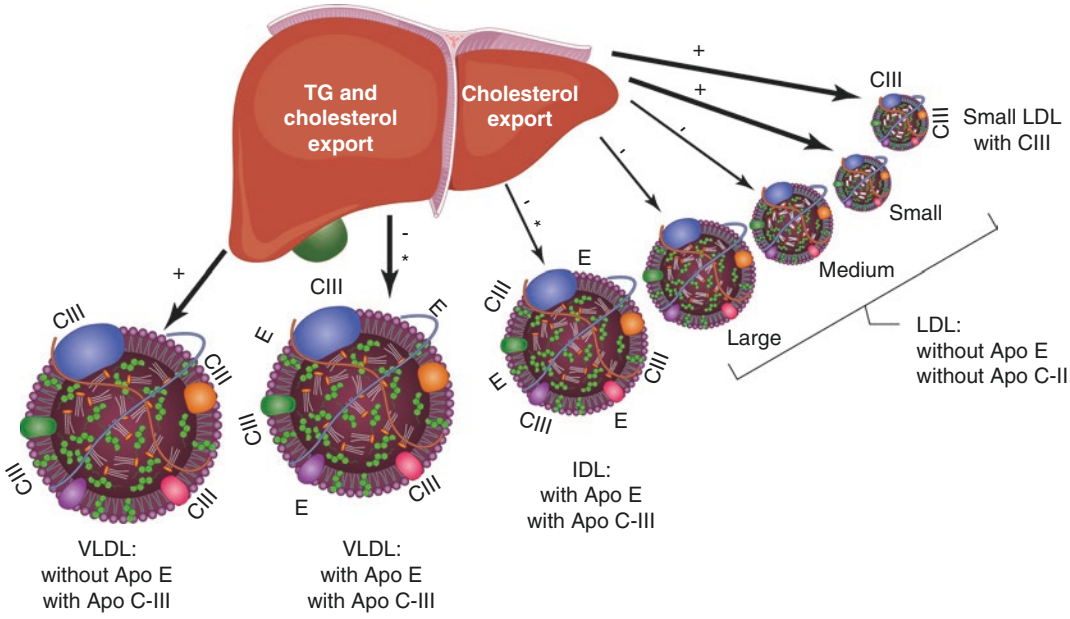

Fig. 16.4 Shift in lipoprotein subspecies in hypertriglyceridemia. Adapted from Sacks [27]

plasma before hepatic clearance. Small, dense LDL particles also have greater interactivity with intra-arterial proteoglycans, which can lead to longer residence time within the arterial wall. Moreover, once in the arterial wall, they are more susceptible to oxidative modification, in part because smaller particles carry fewer fat soluble anti-oxidative compounds such as tocopherols and polyphenols [30, 31]. This can lead to unregulated uptake by macrophages, contributing to foam cell formation.

The term LDL subclass pattern A is used to denote a predominance of larger, more buoyant LDL particles, whereas LDL subclass pattern B is a predominance of small, dense LDL particles. The prevalence of LDL subclass pattern B increases progressively with the fasting plasma TG concentration. For example, Austin and colleagues found that at a TG concentration of 100 mg/dL, the prevalence of pattern B was approximately 18%, whereas at a TG concentration of 250 mg/dL, it was approximately 88% [12]. The conversion from pattern A to B appears to be a threshold phenomenon that depends mainly on the circulating TG concentration [28, 29, 32]. Those with a high propensity to form pattern B (e.g., due to high CETP activity) will convert to pattern B at a lower TG level.

This principle was demonstrated with TG reductions induced by fenofibrate or omega-3 acid ethyl esters [32, 33]. In both studies, substantial TG lowering (30–50%) had no effect on LDL particle size or subclass distribution, as long as the on-treatment TG concentration remained above 250 mg/dL. However, at TG concentrations below 250 mg/dL, significant increases in LDL particle size were observed. Furthermore, the proportion of subjects who converted from pattern B to A increased as the on-treatment TG concentration declined below 250 mg/dL.

Mechanisms Linking IR to Low HDL-C

As discussed earlier, an elevation in the TG concentration increases the CETP-mediated exchange of cholesteryl esters for TG between TG-containing lipoproteins and HDL particles. This results in smaller HDL particles that are relatively enriched with TG and depleted of cholesterol. The TG-enriched particle primarily

undergoes catabolism by hepatic lipase, the activity of which is increased by hypertriglyceridemia and hyperinsulinemia. After TG hydrolysis by hepatic lipase, the particle is further reduced in size and more easily dissociates from Apo A-I [34]. Small HDL particles and free Apo A-I are filtered at the nephron and renally excreted. Apo A-I also appears to dissociate from these TG-enriched HDL particles prior to catabolism, allowing clearance by the kidney (Fig.16.2) [14, 16, 25, 35]. Thus, insulin-resistant individuals typically have lower levels of both HDL-C and Apo A-I.

In addition to decreased concentration, the compositional changes of HDL associated with atherogenic dyslipidemia appear to decrease its anti-atherogenic properties. HDL particles lacking Apo A-I have decreased affinity for ATP-binding cassette transporter 1 (ABCA1), a cell surface protein that transfers cholesterol from cells to HDL. It has been observed that HDL-mediated cholesterol efflux is directly proportional to Apo A-I level [36]. Changes to proteins that interact with HDL may also contribute to decreased HDL concentration and function. For example, glycation of ABCA1 in hyperglycemia has been suggested to reduce transfer of cholesterol from cells to HDL [37].

Activities of Lipoprotein and Hepatic Lipases and Their Relationships with Atherogenic Dyslipidemia

Although overproduction of VLDL appears to be the primary lipid disturbance associated with IR and atherogenic dyslipidemia, the TG clearance rate is also generally reduced. In part, this may relate to an impairment of the ability of insulin to activate lipoprotein lipase. In addition, greater FFA levels in the interstitium may reduce lipoprotein lipase activity through end-product inhibition of lipoprotein lipase activity [21]. Finally, elevated levels of Apo C-III, resulting in an increased ratio of Apo C-III to Apo C-II, reduce the activation of lipoprotein lipase and hepatic lipase, contributing to slower clearance of TG through hydrolysis [27].

Angiopoietin-like 3 (ANGPTL3), which is an inhibitor of lipoprotein lipase and endothelial lipase, has also recently been found to play a major role in promoting uptake of circulating TGs into adipose tissue postprandially [38, 39]. Genetic loss-of-function variants that lower activities of ANGPTL3 or lipoprotein lipase, or decrease Apo C-III production, result in TG reduction and are associated with decreased ASCVD risk [40, 41].

Lifestyle Management for Atherogenic Dyslipidemia

A twofold approach is recommended for the management of dyslipidemia associated with IR [42, 43]. The first is to reduce the underlying lifestyle factors that contribute to the development of the insulin-resistant state, and the second is to treat the individual lipid and non-lipid risk factors associated with IR. Lifestyle changes are widely recognized by national health organizations as the first step in the management of IR and its related risk factors, including atherogenic dyslipidemia [42, 43]. The two cornerstones of lifestyle changes for insulin-resistant patients are increased physical activity and loss of excess body fat. Both exercise and weight loss improve insulin sensitivity and all of the risk factors associated with IR.

Current recommendations for physical activity include at least 150 min of moderate-intensity physical activity each week or at least 75 min per week of vigorous-intensity activity [43]. It appears that most of the benefits of exercise can be achieved by lower-intensity activities such as walking, which, for most people, is the form of exercise that is most easily incorporated into their daily routine [44]. Loss of 5–10% of body weight can produce significant improvements in metabolic risk factors for coronary heart disease (CHD), including IR and atherogenic dyslipidemia [43]. Weight loss of this magnitude will typically lower the fasting TG concentration by 10–20% in those with elevated TG levels [45, 46].

Cigarette smoking has also been shown to reduce insulin sensitivity and contribute to atherogenic dyslipidemia, adding to the myriad of reasons that smoking cessation should be encouraged. In addition, excessive alcohol intake will increase the TG concentration. Therefore, moderation (or cessation) of alcohol consumption should be encouraged, if applicable [42, 43].

Dietary recommendations for ASCVD risk reduction include consumption of a diet low in saturated fatty acids (5–6% of energy) while minimizing intakes of dietary cholesterol and *trans* fatty acids, all of which raise the level of LDL-C. The American Heart Association/American College of Cardiology primary prevention guidelines encourage a diet emphasizing intakes of whole grains, nuts, fruits, vegetables, and legumes and minimizing intakes of processed meats, refined carbohydrates, and sweetened beverages [43, 47]. Individuals at risk for IR are advised to avoid excessive carbohydrate intake and consume diets that include relatively more unsaturated fats, because a diet high in carbohydrate will increase the TG level [42]. Low saturated fat diets that are relatively high in unsaturated fatty acids have been shown to reduce the TG concentration by 10–15% compared to high carbohydrate diets with similar saturated fat and cholesterol levels [45, 46].

In addition to the lifestyle changes outlined above, clinicians should be aware that some drug therapies can worsen the components of atherogenic dyslipidemia and should thus be avoided or minimized, if possible. Drugs that may cause elevated TGs include oral estrogens (transdermal estrogens generally have minimal effects on the lipoprotein lipid profile), glucocorticoids, bile acid sequestrants, protease inhibitors, retinoic acid, anabolic steroids, sirolimus, raloxifene, tamoxifen, beta-blockers, and thiazide diuretics [48].

Lipid Targets in Atherogenic Dyslipidemia

The term atherogenic cholesterol has been used to refer to all cholesterol carried in Apo B-containing lipoproteins. LDL-C is the dominant form of atherogenic cholesterol and, as such, is the primary target of lipid management. However non-HDL-C (LDL-C + TG-rich lipoprotein cholesterol) has been shown to be more predictive of adverse ASCVD events than LDL-C alone and, thus, is another target of lipid management [49, 50].

HDL-C is not a target for lipid-altering therapies, although both lifestyle and pharmacologic therapies that lower IR and TG levels will tend to raise the HDL-C concentration, which might contribute to the benefits of these interventions [51].

Because VLDL, LDL, IDL, lipoprotein (a), and chylomicron particles each contain one molecule of Apo B (Apo B-100 for those of hepatic origin and Apo B-48 for chylomicron particles), the Apo B concentration is an indicator of the total number of circulating atherogenic particles. Apo B and non-HDL-C concentrations are highly correlated, and because measurement of Apo B is an extra laboratory expense, it is not routinely measured in the United States [49]. Nevertheless, some researchers believe that changes in Apo B are superior to those of LDL-C and non-HDL-C for the clinical management of dyslipidemia [52, 53]. If Apo B is measured and found to be elevated, it is considered an ASCVD risk-enhancing factor. Mendelian randomization studies evaluating the associations of variants in genes that produce lower TG and LDL-C levels have demonstrated similar degrees of reductions in CHD or ASCVD risk for a 10-mg/dL reduction in LDL-C and a 50-mg/dL reduction in TG [50]. Because the level of TG-rich lipoprotein cholesterol per mg/dL is approximated by the concentration of TG/5, this suggests a similar association for each 10-mg/dL increase in each component of non-HDL-C (LDL-C and TG-rich lipoprotein cholesterol). Both LDL-C and TG lose statistical significance as predictors after adjustment for the Apo B concentration, suggesting that the total circulating concentration of particles with atherogenic potential (LDL and TG-rich lipoprotein particles) is the main driver of lipoprotein-associated ASCVD risk [50]. IR is mainly associated with elevation in the circulating concentrations of TG-rich lipoprotein particles.

Approaches to Lowering Non-HDL-C

There are two approaches to lowering non-HDL-C levels in patients with elevated TG: targeting additional lowering of LDL-C and focusing on additional lowering of TG-rich lipoprotein cholesterol. Lifestyle therapies are recommended for all patients and are a cornerstone of ASCVD prevention efforts [43, 49]. Statin therapy is considered the first-line pharmacologic approach for ASCVD risk reduction. To further lower LDL-C in a statin-treated patient, the statin dose can be increased, and/or adjunctive therapy can be used, including a cholesterol absorption inhibitor and/or a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor [43, 49, 54, 55]. The other is to add an agent, such as fibrate, niacin, or omega-3 fatty acids, which lowers VLDL-C and TG-rich remnant lipoproteins. Current guidelines emphasize use of lifestyle intervention and maximally tolerated statin therapy, followed by use of a cholesterol absorption inhibitor and/or PCSK9 inhibitor therapy for those with sufficiently high risk who do not have an adequate response to statin therapy [49]. However, there is evidence to support the efficacy of other pharmacologic therapies, including icosapent ethyl (an eicosapentaenoic acid [EPA] omega-3 fatty acid concentrate) and fibrate therapy in high-risk patients with elevated TG concentrations, particularly if accompanied by below-average levels of HDL-C [56–58].

Drug Therapies Aimed at Reducing LDL-C

Moderate- and high-intensity statins are recommended as first-line therapy for patients with elevated LDL-C who are in a statin benefit group [49]. The use of statins is supported by a greater quantity of clinical trial data than any other class of lipid-altering agent. The results from large event trials have consistently shown that statin therapy reduces ASCVD morbidity and mortality. These effects are present at all levels of baseline lipids studied, as well as in groups that would be expected to be enriched with insulin-resistant individuals, such as subjects with diabetes mellitus, hypertriglyceridemia, and hypertension [59, 60].

Statins also markedly reduce concentrations of both TG and TG-rich lipoproteins in hypertriglyceridemic subjects [61–63]. The hypotriglyceridemic effects of statins have been widely underappreciated, largely because, until recently, few studies of statin therapy had been undertaken in hypertriglyceridemic subjects. TG lowering with statins is modest in normotriglyceridemic subjects, but more pronounced in those with hypertriglyceridemia.

Kinetic studies have shown that statins increase the fractional catabolic rate of Apo B-containing lipoproteins (VLDL, IDL, and LDL) while having little effect on VLDL production [62, 64]. The result is that the circulating levels of TG, TG-rich lipoprotein cholesterol (VLDL-C and IDL-C), and LDL-C all decline. Reductions in TG of 30–50% and non-HDL-C of 50–60% have been reported when hypertriglyceridemic patients are treated with the maximum approved doses of higher efficacy statins (atorvastatin and rosuvastatin). The magnitude of TG lowering differs among statins [65]; atorvastatin and rosuvastatin have been shown to result in greater TG reductions, compared to simvastatin, at doses that produce equivalent LDL-C reduction [66].

Statin therapy is safe and well tolerated in many patients; however, some experience statin intolerance and cannot achieve adequate LDL-C lowering with statins alone. Another important consideration is that statins have been shown to increase the incidence of type 2 diabetes, especially with higher-intensity statins and in patients with major type 2 diabetes risk factors, such as pre-diabetes or metabolic syndrome and its components [67]. For these reasons, it is not unreasonable to consider using a non-statin therapy.

Additional agents that may be considered for further lowering of LDL-C include non-drug therapies (plant sterols/stanols and viscous fibers), a cholesterol absorption inhibitor, and/or a PCSK9 inhibitor. Bile acid sequestrants also reduce LDL-C when added to statin therapy, but tend to modestly increase the plasma TG concentration, and, thus, are not ideal for patients

with atherogenic dyslipidemia. Plant sterols/stanols and viscous fibers can be expected to provide an additional 5–10% reduction in LDL-C and non-HDL-C when added to statin therapy [55, 68]. Coadministration of a cholesterol absorption inhibitor (10 mg ezetimibe) will generally lower the LDL-C and non-HDL-C concentrations by a further 10–20% [69]. PCSK9 inhibitors reduce LDL-C by an additional 50–60% beyond statins and do not appear to increase the risk of new-onset diabetes or worsen glycemia [70–73].

At the time of this writing, a new cholesterol-lowering drug is under evaluation by the US Food and Drug Administration. Bempedoic acid is a pro-drug that when activated in the liver inhibits ATP-citrate lyase, an enzyme upstream of 3-hydroxy-3-methylglutaryl coenzyme A reductase in the cholesterol biosynthesis pathway [74, 75].

Therapies That Target TG

Fibrates, niacin, and omega-3 fatty acids all lower VLDL-C and other TG-rich lipoproteins [63]. Fibrates work by stimulating peroxisome proliferator-activated receptor (PPAR) alpha. This results in enhanced lipoprotein lipase expression, reduced hepatic production of Apo C-III, and enhanced hepatic fat oxidation. In addition, fibrates increase the production rates of Apo A-I and Apo A-II. Studies of lipoprotein kinetics have shown that fibrate therapy increases the clearance rates for VLDL, IDL, and LDL particles, but, surprisingly, appears to have little effect on VLDL secretion [62]. At the usual dosages, fibrates typically lower the TG concentration by 30–50% and increase HDL-C by 10–25%. The LDL-C response to fibrate therapy is dependent on baseline TG and LDL-C concentrations. In patients with very high TG concentrations (≥500 mg/dL), the LDL-C level may rise. In patients with less severe hypertriglyceridemia, particularly those with concomitantly elevated LDL-C, the LDL-C concentration may decline by as much as 20%.

The dramatic effects of niacin on the blood lipid profile were noted more than a half-century ago. The effects of niacin on lipid metabolism are due to its ability to suppress FFA release from adipose tissues as well as its ability to inhibit hepatic diacylglycerol acyltransferase (DGAT) [76]. The latter is an enzyme involved with TG synthesis and VLDL production. The result of these changes is enhanced hepatic Apo B degradation and reduced VLDL production. Niacin also markedly increases the number of circulating HDL particles by selectively inhibiting the uptake of Apo A-I by hepatocytes, thus reducing the fractional catabolic rate of HDL. At approximately 2 g/day, extended-release niacin will reduce the plasma TG concentration by 20–50%, increase HDL-C by 15–35%, and lower LDL-C by 5–25%. Although niacin improves all of the features of atherogenic dyslipidemia, two issues raise concerns about its clinical usefulness in such patients. The first is flushing, which is experienced to some degree by most patients and may limit compliance. Its intensity is diminished, but not eliminated, by use of a prescription, extended-release preparation. The second issue associated with niacin use is the development of IR. One might predict that the reduced FFA release from adipose tissues would lead to improved insulin sensitivity. However, the reverse appears to be true, although the mechanisms responsible for this effect are not fully understood [35]. The use of niacin can cause people with mild glucose intolerance to convert to frank diabetes by worsening the degree of IR, thereby increasing demand on the pancreatic beta-cells [35, 77]. For this reason, niacin should be used with caution, if at all, in patients with IR or glucose intolerance, particularly in light of the stronger evidence for efficacy of omega-3 fatty acids (mainly icosapent ethyl at present) and fibrates (based on subgroup analyses from multiple trials) for ASCVD risk reduction [56–58].

The long-chain omega-3 polyunsaturated fatty acids EPA and docosahexaenoic acid (DHA), found in high concentrations in the oils of cold water fish, have been known for years to have a hypotriglyceridemic effect when consumed in

high doses (1–4 g/day of EPA + DHA). Prescription preparations of concentrated omega-3 fatty acids are available and, compared to fish oil supplements, require fewer capsules to be taken to achieve a therapeutic dose of omega-3 fatty acids. These prescription formulations provide either EPA alone or EPA + DHA in ethyl ester or FFA (carboxylic acid) forms.

Omega-3 fatty acids reduce VLDL production by inhibiting DGAT, and possibly through a mild stimulatory effect on PPAR alpha, thus stimulating hepatic fat oxidation [64, 78]. The result is reduced hepatic synthesis and secretion of VLDL, with no apparent effect on hepatic uptake of Apo B-containing particles [64, 78]. Omega-3 fatty acids therefore reduce circulating levels of TG and VLDL-C (25–50%). They also generally produce a small rise in HDL-C (3–10%). As with fibrates, omega-3 fatty acids sometimes lower LDL-C modestly (5–10%), particularly among subjects with higher baseline LDL-C. However, in patients with more severe hypertriglyceridemia, the LDL-C concentration may rise with omega-3 products that contain DHA [79]. Nevertheless, the reduction in VLDL-C is typically larger than the increase in LDL-C, so the net result is a reduction in cholesterol carried by atherogenic, Apo B-containing lipoproteins (non-HDL-C).

Clinical outcome trials with fibrate therapy have been generally supportive of a protective cardiovascular effect in hypertriglyceridemic patients [80, 81], particularly in subgroups of subjects with high TG and low HDL-C [82], but the evidence base is not as robust as that observed in trials with statins or other adjunctive therapies (ezetimibe, PCSK9 inhibitors, or icosapent ethyl). Until recently the patients enrolled in outcome trials of TG-lowering therapies were not specifically selected for hypertriglyceridemia, and, in the case of omega-3, the dosages administered were often low (<1 g/day).

The results from the Reduction of Cardiovascular Events with Icosapent Ethyl—Intervention Trial (REDUCE-IT) were released in 2018. REDUCE-IT examined the effect of 4 g/day EPA ethyl esters on risk of ischemic events in patients with established ASCVD, or with diabetes and ≥ 1 risk factor, with a TG concentration of 135–499 mg/dL and an LDL-C level of 41–100 mg/dL while receiving statin therapy [58]. The composite of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, or unstable angina was 25% lower with EPA ethyl esters compared with placebo [58]. Thus, at the time of this writing, the strongest evidence favoring use of an agent that mainly lowers TG is for icosapent ethyl [58, 83]. Two large-scale trials are underway with other agents, including the Outcomes Study to Assess Statin Residual Risk Reduction with Epanova in High CV Risk Patients with Hypertriglyceridemia (STRENGTH) with EPA + DHA carboxylic acids and the Pemafibrate to Reduce Cardiovascular Outcomes by Reducing Triglycerides in Patients with Diabetes (PROMINENT) trial with pemafibrate [84, 85]. It should also be noted that the risk reduction in REDUCE-IT and another trial of EPA ethyl esters completed in Japan (Japan EPA Lipid Intervention Study [JELIS]) suggest larger benefits than are likely to be explained solely on the basis of changes in the lipoprotein lipid profile [86]. Thus, other effects of EPA such as those related to platelet function, hemodynamics, inflammation, oxidation, fibrosis, and/or membrane fluidity and cardiac electrical stabilization may have also contributed to the observed benefits in these trials [83, 87].

Management of Dyslipidemia in Diabetes

ASCVD is the leading cause of morbidity and mortality for patients with type 2 diabetes, and the ASCVD risk elevation in such patients is partly attributable to atherogenic dyslipidemia. Even if LDL-C levels are not elevated in patients with type 2 diabetes, IR changes lipid metabolism and lipoprotein composition in ways that lead to more pathogenic forms of LDL-C and an elevation in TG-rich lipoproteins. Atherogenic dyslipidemia in diabetes may be affected by the degree of glycemic control and relative degree of insulin deficiency.

Lifestyle modifications including weight loss, dietary modification, and aerobic exercise are crucial to the management of dyslipidemia in diabetes. In addition to use of statin therapy and other pharmacologic agents to manage LDL-C and non-HDL-C levels, ASCVD risk in diabetes can also be reduced with some antihyperglycemic medications. Sodium-glucose co-transporter 2 inhibitors and glucagon-like peptide-1 agonists have been shown to improve cardiovascular outcomes in patients with type 2 diabetes [88]. Pioglitazone, a potent insulin-sensitizing drug in the thiazolidinedione class of PPAR-gamma agonists, has also been shown to reduce the risk of cardiovascular events in patients with IR, the effects of which may be partly mediated through improving aspects of atherogenic dyslipidemia [89].

Summary

IR is common in the United States and other developed countries. It is present in a majority of individuals with obesity, impaired glucose tolerance or type 2 diabetes mellitus, and polycystic ovary syndrome. IR is also present in approximately half of those with hypertension.

The combination of elevated circulating FFAs and compensatory hyperinsulinemia that are characteristic of insulin-resistant states leads to VLDL overproduction, which is the primary abnormality of lipid metabolism in "atherogenic dyslipidemia" (elevated TG, reduced HDL-C, and a predominance of small, dense LDL particles). VLDL overproduction, particularly Apo C-III-rich/Apo E-deficient particles, results in an elevated circulating TG concentration, which drives the CETP-catalyzed exchange of TG from TG-rich lipoprotein particles for cholesteryl esters from LDL and HDL particles. This results in relatively TG-rich and cholesterol-poor LDL and HDL particles.

Apo A-I on TG-enriched HDL particles dissociates and is cleared by the kidney, contributing to low levels of circulating HDL-C and HDL particles. TG-enriched LDL particles undergo delipidation by lipase enzymes (primarily hepatic lipase), forming smaller, more dense particles, which are believed to have increased atherogenicity. LDL subclass pattern B is a predominance of small, dense (more atherogenic) LDL particles. A shift from pattern A (a predominance of larger, more buoyant LDL particles) to pattern B occurs when the TG concentration exceeds a threshold. The threshold varies from person to person, but is between 100 and 250 mg/dL for most individuals.

The approach to the management of atherogenic dyslipidemia is twofold: addressing the underlying causes of IR (obesity and physical inactivity) and treating the specific lipid and nonlipid risk factors associated with the insulin-resistant state. Loss of excess body fat and increased physical activity will reduce IR and improve all the associated ASCVD risk factors, including atherogenic dyslipidemia. A diet with a moderate intake of carbohydrates that emphasizes whole grains, nuts, seeds, fruits, vegetables, legumes, and non-tropical oils will also contribute to improvement of atherogenic dyslipidemia. For patients who are unable to adequately control atherogenic dyslipidemia with lifestyle changes, drug therapy may be indicated.

The primary targets of lipid management in atherogenic dyslipidemia are LDL-C and non-HDL-C (LDL-C + TG-rich lipoprotein cholesterol). Non-HDL-C lowering may be approached by intensifying efforts to lower LDL-C. This can be accomplished pharmacologically by use of statin therapy with or without adjunctive treatment such as a cholesterol absorption inhibitor or a PCSK9 inhibitor. An alternative, or complementary, approach to lower the non-HDL-C level is to use an agent (fibrate, omega-3 fatty acids, or, rarely, niacin) that primarily lowers the concentrations of TG and TG-rich lipoprotein cholesterol.

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17 Therapeutic Management of Obesity

George A. Bray

Introduction

Increased body weight poses health risks and increases health-care costs [1]. The body mass index [BW (kg)/Ht $(m)^2$] is one of the most widely used methods of assessing the degree of overweight and is used as a surrogate of obesity, which is technically an increase in body fat. The prevalence of obesity has risen steadily over the past 40 years as the epidemic of obesity has spread worldwide [2, 3, 4, 5].

Obesity results from an imbalance between energy intake and expenditure, but it is the connection between these two components that may provide the clues about how we should understand, prevent, and treat this problem [6]. While food is, of course, the ultimate "source" of energy for a positive energy balance, food is much more than energy. Moreover, many other factors, other than food, impinge on whether an individual develops obesity.

The pathology of obesity can best be understood as an enlargement of fat cells and, in some individuals, an increased number of fat cells [7, 8]. These enlarged fat cells release more fatty acids and a variety of cytokines, including leptin, and tumor necrosis factor-α that can provide a basis for understanding how obesity produces insulin resistance and changes in the inflammatory, thrombotic, and coagulation systems. In contrast to products whose secretion increases as fat cells increase in size, adiponectin, the most abundant product of the fat cell which is related to insulin sensitivity, declines as body fat increases.

There is a large industry offering various forms of treatment for obesity. Each year sees new additions to the diet books, which promise amazing results. The reality, however, is somewhat different, since the prevalence of obesity has been on the increase from the time "Dr. Atkins Diet Revolution" was published in 1972 [9]. Each year people who have regained the weight they lost with the last weight loss effort will try the next one under the delusion of the "false hope syndrome," i.e., that having failed last time, they are sure to succeed this time [10]. Weight loss programs all experience a plateau in body weight during treatment, which is often followed by a regain of body weight. [11, 12, 13]. Lifestyle strategies, including diet, exercise, and behavioral modification are often the first line of treatment. The five pharmacologic agents currently approved by the Food Drug Administration for long-term use produce additional modest weight loss. Surgical intervention, which includes among others the gastric bypass, sleeve gastrectomy, and gastric banding, offers the most effective long-term results, but at an increased risk of mortality and with substantial morbidity.

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_17

Prevalence and Cost of Obesity

Body Mass Index

Over the past 50 years, there has been a steady rightward shift in the distribution curve for body weight, although some people suggest the weight curve may be bimodal. A normal BMI is between 18.5 and 24.9 kg/m^2. A BMI between 25 and 29.9 is operationally defined as overweight, and individuals with BMI >30 are obese, after taking into consideration other factors such as people who are muscle builders [1].

Central Adiposity

The location of excess fat in ectopic locations, such as hepatic fat or visceral fat cells, is a particularly hazardous form of excess fat. Measurement of centrally located fat can be done with MRI or CT scans, but waist circumference is a practical measure for the clinician. If the BMI is between 20 and 35 kg/m^2, the waist circumference provides a clinically valuable index of increasing risk for diabetes, heart disease, and cancer. In the USA, a waist circumference of >40 in. in men and > 35 in. in women is a high-risk category, but most of the rest of the world uses considerably lower cut points (90–94 cm [35.5–37 in.] for men and 80 cm [31.5 in.] for women). When BMI and waist circumference were used to predict the risk of hypertension, dyslipidemia, and the metabolic syndrome, the waist circumference was shown to be a better predictor than the BMI [1, 14].

Prevalence of Obesity

Using BMI as the criterion, the worldwide epidemic of obesity began in the 1980s and continues today. It affects children as well as adults [5]. For example, among young people aged 2 to 19, about 31.8% are considered to be either overweight or obese, and 16.9% are considered to be obese. More than two in five black and Hispanic youth are considered to be overweight or obese.

The rising prevalence of type 2 diabetes in adolescents, as well as adults, is directly related to obesity. Obesity has a higher prevalence in the Latino and African-American populations as well as in the Native Americans. Both height and weight have increased in adults aged 20–74 years between 1960 and 2000 but may have leveled off in adults between 2000 and 2010 [1, 5].

Cost of Obesity

Obesity is expensive, costing between 3% and 8% of health-care budgets [15]. Hospital costs and use of medication increase with increasing BMI. In a large health-maintenance organization, mean annual costs were 25% higher in participants with a BMI between 30 and 35 and 44% higher in those with a BMI > 35, compared to individuals with a BMI between 20 and 25. Costs for lifetime treatment of hypertension, hypercholesterolemia, type 2 diabetes, heart disease, and stroke in men and women with a BMI of 37.5 were $10,000 higher than for men and women with a BMI of 22.5, according to data from the National Center for Health Statistics and the Framingham Heart Study (see Ref. [7]).

Etiology of Obesity

Energy Imbalance

We become obese because we ingest more energy in the food we eat than we need for our daily activities. Voluntary overeating (by subjecting individuals to repeated ingestion of energy exceeding daily energy needs) can increase body weight [16]. When these individuals stop overeating, they invariably lose all or almost all of the excess weight. The use of overeating protocols to study the consequences of food ingestion has shown the importance of genetic factors in the pattern of weight gain [16].

Epidemiologic Model

An epidemiological model provides a useful framework to conceptualize obesity as a disease process. In an epidemiologic model, environmental agents act on the host to produce a disease. Disease is a function of the virulence of the agent and the susceptibility of the host. For obesity, the principal environmental agents are food and physical inactivity. Other agents include drugs, toxins, viruses, the microbiome, and alter-ego interactions. In Western affluent societies, foods, particularly tasty, inexpensive, and convenient foods high in fat, are abundant and provide the energy for fat storage. Portion sizes have increased, providing more energy to people with each portion, and people tend to eat more when larger portions are provided. Obesogens are an interesting potential group of chemical agents used in various products related to food and where more research is needed. Viruses are known to produce obesity and their potential role in obesity in humans needs to be studied further. Physical activity within the general population has gradually been reduced, thereby decreasing energy expenditure [17]. Some have described the current "environment" as a "virulent" or "toxic" environment that has heightened the risk for obesity. For the genetically susceptible host, this excess of food energy, environmental toxins, and viruses, along with the reduced level of physical activity, may lead to an accumulation of fat in fat cells. Genetics loads the gun; environment pulls the trigger (*see* Ref. [18]).

Environmental Agents Causing Weight Gain

Intrauterine Factors

Several intrauterine events influence postnatal weight and lifetime weight gain and fatness and offer the health professional an opportunity to intervene at an early stage [19]. These include maternal smoking, which should be eliminated; maternal weight gain, which should be modulated; maternal diabetes, and intrauterine undernutrition, which can be treated. All of these factors heighten the individual's risk for increased body weight and diabetes later in life.

Drug-Induced Weight Gain

In our current medicated society, it would not be surprising to find that drugs can cause weight gain, and this is another place where the health professional can intervene. Knowing which drugs can lead to weight gain opens the door to selecting alternatives that do not have this effect. Several receptors, especially the histamine H_1, adrenergic α_{1A}, and serotonin (5-HT_{2C} and 5-HT_6) receptors, explain much of the weight gain associated with atypical antipsychotic drugs (*see* Ref. [7]).

Diet as a Cause of Obesity

Many aspects of diet contribute to obesity, and once again there are opportunities for the health professional to intervene. The sugar or high-fructose corn syrup (HFCS), which sweetens soft drinks, fruit drinks, and sports drinks, have been implicated in the epidemic of obesity. The sugar in soft drinks and fruit drinks provides "invisible" energy, which is not readily detected physiologically by the body. Thus, individuals do not "compensate" after drinking these beverages, by reducing the intake of other sources of food energy, and beverage calories become "add-on" calories [20].

Infant and Child Environment

Infancy and early childhood are also periods where prevention can be put into practice by the health-care professional. Infants who are breast-fed for more than 3 months may have a reduced risk of future obesity. These advantages of breast-feeding may be counterbalanced by the types of foods a mother eats. The percentage of linoleic acid n-6 fatty acids in breast milk has increased over the past 50 years, whereas the amount of n-3 linolenic acid has remained unchanged [21]. This increased load of arachidonic acid may alter the rate of adipose tissue fat storage. In addition, children who sleep less have a higher risk for weight gain during school years. Children are in part a dietary product of their parental role models, and the parental dietary and exercise patterns that lead to parental obesity predict childhood obesity.

Fat Intake as a Cause of Obesity

Epidemiologic data suggest that a high-fat diet is associated with obesity [22]. For example, the relative weights in several populations are directly related to the percentage of fat in their diet. A high-fat diet provides high energy density (i.e., more calories for the same weight of food), which makes overconsumption more likely. Differences in the storage capacity for various macronutrients may also be involved. The capacity to store glucose as glycogen in the liver and muscle is limited, so glucose must be continually replenished. In contrast, fat stores contain more than 100 times as many calories as provided in the daily intake of fat. This difference in storage capacity for glucose and fat makes eating carbohydrates a more important physiologic need that may lead to overeating when dietary carbohydrate availability is limited, and carbohydrate oxidation cannot be reduced sufficiently. In addition to the quantity of fat, there are qualitative aspects of fat, which may affect weight gain. Saturated fats have more detrimental effects during weight gain than polyunsaturated fats [23].

Physical Inactivity as a Cause of Obesity

Low levels of physical activity correlate with weight gain. In a 10-year study of individuals aged 20–74 years in the National Health and Examination Survey (NHANES I), those with low levels of recreational activity gained more weight than did those with higher levels. The decline in moderate activity and increase in light and sedentary activity are correlated with the rising prevalence of obesity [17]. Low levels of baseline energy expenditure predicted weight gain in Pima Indians. Time spent watching television correlates with percent of overweight children (*see* Ref. (7))

Smoking and Its Cessation Affect Body Weight

Smokers have a lower body weight, and cessation of smoking is generally associated with significant weight gain. Here is where efforts at smoking cessation run into the societal demands for "thinness" challenges the health-care professional who needs to provide support for their patients who are trying to quit smoking [7].

Host Agents Causing Obesity

Genetic Causes

There are several rare clinical forms of obesity. The Prader–Willi syndrome is one of the most common. It results from chromosomal abnormality on chromosome 15 and is characterized by a "floppy" baby who has difficulty feeding. These children are mentally slow, short in stature, and obese [24]. The Bardet–Biedl syndrome is due, in at least one pedigree, to a defect in the chaperonin-like gene [24].

The leptin gene, the leptin receptor, the melanocortin-4 receptor gene, the proopiomelanocortin (POMC) gene, and agouti gene have significant effects on body fat and fat stores. MC4-receptor defects may account for up to 6% of obesity in early-onset, severely obese children [25]. Treatment of leptin-deficient children with leptin decreased body weight and hunger, indicating the importance of leptin for modulation of these processes in normal subjects. Heterozygotes for leptin deficiency have low but detectable serum leptin and have increased adiposity, indicating that low levels of leptin are associated with increased hunger and gain in body fat. Leptin can also increase energy expenditure and during reduced calorie intake, leptin attenuates the fall in thyroid hormones and the fall in 24-h energy expenditure [25].

The epidemic of obesity is occurring on a genetic background that does not change as fast as the epidemic has been exploding. Genome-wide association studies have found well over 100 genes that have small effects on body weight, but they account for only a small part of the variation in BMI. The fat and obesity gene (FTO)` has the largest effect and produces an additional 3 kg of body weight in those homozygous for the susceptibility variant [25].

Physiologic Factors

The discovery of leptin in 1994 opened a new window on the control of food intake and body weight [6]. The response of leptin-deficient chil-

dren to leptin indicates the critical role that this peptide plays in the control of energy balance. Leptin enters the brain, probably by transport across the blood–brain barrier. It then acts on receptors in the arcuate nucleus to regulate, in a conjugate fashion, the production and release of at least four peptides: leptin inhibits the production of neuropeptide Y (NPY) and agouti-related peptide (AGRP), both of which increase food intake; it enhances the production of proopiomelanocortin (POMC), the source of α-melanocyte-stimulating hormone (α-MSH), which reduces food intake.

At least two other brain peptide systems have also been linked to the control of feeding. Melanin-concentrating hormone (MCH) is found in the lateral hypothalamus and decreases food intake when injected into the ventricular system of the brain. Orexin (also called hypocretin) was identified in a search of G protein-linked peptides that affect food intake. It increases food intake and plays a role in sleep.

Endocannabinoids are derived from arachidonic acid. The endogenous cannabinoids (anandamide and arachidonoyl 2-glycerol) increase food intake by acting on CB-1 receptors in the brain. Antagonists to the CB-1 receptor reduce food intake.

Gut peptides, including glucagon-like peptide-1, polypeptide YY oxyntomodulin, and cholecystokinin, reduce food intake, whereas ghrelin, a small peptide produced in the stomach, stimulates food intake [6, 7].

Pathology and Pathophysiology of Obesity

Fat Is an Endocrine and Inflammatory Organ

Two mechanisms can explain the pathophysiology of obesity: the first is increased fat mass, which allows obesity to be easily recognized and stigmatized. Fat mass may also play a role in the accompanying osteoarthritis and sleep apnea [8]. The second mechanism is the increased secretion of adipokines by the enlarged fat cells that act on distant organs. The discovery of leptin catapulted the fat cell into the arena of endocrine cells. In addition to leptin, there are increased amounts of adipsin (complement factor D), angiotensinogen, etc., and metabolites such as free fatty acids and lactate. In contrast to the other fat cell products, adiponectin release is decreased in obesity. The products of the fat cell in turn modify the metabolic and inflammatory processes in other organs of the host. For the susceptible host, these metabolic and inflammatory changes increase fatty acids and estrogens, leading to a variety of other processes, including hyperinsulinemia, atherosclerosis, hypertension, and physical stress on bones and joints [1].

Complications of Obesity

Death

Obesity is associated with shortened life span and contributes between 100,000 and 400,000 excess deaths per year in the USA. Both the NCHS data and the Framingham data show that a BMI of 30 or more decreases life span by 3–5 years. compared to normal weight [8]. Obesity is also associated with increased health-care costs and diminished quality of life particularly during the last years of life in persons with obesity, which results from the comorbidities associated with obesity (i.e., sleep apnea, type-2 diabetes, osteoarthritis, heart disease, etc.) [1].

Diseases

The curvilinear "J"-shaped relationship of BMI to risk of complications has been known for 100 yrs. As obesity increases, so, too, do the risks of type 2 diabetes, CVD, hypertension, arthritis, cognitive impairment, and some cancers. In the USA, diagnosed diabetes increased from 7.8 million cases in 1993 to 29 million in 2017; >8 million additional cases remain undiagnosed, and an estimated 71 million adults have prediabetes. Population-based

studies have suggested that ~75% of cases of hypertension can be attributed to obesity, and approximately one-third of cancer deaths are linked to poor nutrition, excess weight, and sedentary lifestyle. Worldwide, 44% of the diabetes burden, 23% of ischemic heart disease, and 7–41% of certain cancers are attributable to excess weight. Obesity also decreases both health-related quality of life and life expectancy [26].

Treatment

Realities of Treatment

The first step in treating a patient with obesity is to evaluate them and their needs and wishes [7, 13, 27]. The Guidelines for Obesity provide an algorithm to help in making this assessment [27]. It is a useful framework on which to hang basic clinical information that is collected during the examination.

Realism on the part of the health-care provider and the patient is an important element to establish at the beginning of any treatment program for obesity. For most treatments, including behavior therapy, diet, and exercise, the weight loss, measured as percentage loss from the baseline weight, usually plateaus after a loss of <10%. For many patients, this is a frustrating experience since they want to reach a "dream weight," which is a weight loss of nearly 30%! A loss of <17% is considered by many patients to be a disappointment. It is thus important for the patient and health-care provider to recognize that an initial weight loss of 10% is a success and is one that will produce health benefits [28].

After completing the evaluation, treatment options can be identified. The cornerstone of treatment of most patients with obesity is a comprehensive program involving diet, exercise, and behavioral therapy aimed at helping the patient lose and maintain weight loss. The elements of such a program are outlined in the Guidelines (2013) for managing overweight and obesity in adults [27].

Dietary Treatment for the Patient with Obesity

Diets Low in Fat, Carbohydrate, or Energy Density

A variety of diets, including low-fat foods, low-carbohydrate foods, or a balanced reduction of all macronutrients, have been used to treat obesity. New diets and strategies appear at the beginning of each year as patients, ignoring their own past failures, embark on a new trial of obtaining their weight goals. Several evaluations have been done of weight loss diets [1, 29, 30]. The most recent of these is published by US News and World Report in 2019 [31]. The list of diets is based on the expert evaluation by a group of health-care professionals with knowledge of obesity. In 2019, the top-ranked diet was the Mediterranean diet [32], which was tied with the DASH diet [33] which had been number 1 for the previous 8 years. The third was a flexible version of the vegetarian diet called the Flexitarian diet, in which meat is allowed occasionally [34]. The diets and the scores provided by the reviewers are shown in order: No. 1 Mediterranean Diet - Overall score: 4.2 out of 5; No. 2 DASH Diet - Overall score: 4.1 out of 5; No. 3 The Flexitarian Diet - Overall score: 4 out of 5; No. 4 (tie) MIND Diet - Overall score: 3.9 out of 5; No. 4 (tie) WW (Weight Watchers) Diet - Overall score: 3.9 out of 5; No. 6 (tie) Mayo Clinic Diet - Overall score: 3.8 out of 5; No. 6 (tie) Volumetrics Diet - Overall score: 3.8 out of 5; No. 8 TLC Diet - Overall score: 3.7 out of 5; No. 9 (tie) Nordic Diet - Overall score: 3.6 out of 5; No. 9 (tie) Ornish Diet - Overall score: 3.6 out of 5; No. 11 (tie) The Fertility Diet - Overall score: 3.5 out of 5; No. 11 (tie) Jenny Craig Diet - Overall score: 3.5 out of 5; No. 11 (tie) Vegetarian Diet - Overall score: 3.5 out of 5; No. 14 Asian Diet - Overall score: 3.4 out of 5; No. 15 (tie) Anti-Inflammatory Diet - Overall score: 3.3 out of 5; No. 15 (tie) Flat Belly Diet - Overall score: 3.3 out of 5; No. 15 (tie) Nutritarian Diet - Overall score: 3.3 out of 5; No. 15 (tie) Spark Solution Diet - Overall score: 3.3 out of 5 - No. 19 Engine 2 Diet - Overall score: 3.2 out of 5; No. 20 (tie) Eco-Atkins Diet -

Overall score: 3.1 out of 5; No. 20 (tie) South Beach Diet - Overall score: 3.1 out of 5; No. 20 (tie) Vegan Diet - Overall score: 3.1 out of 5; No. 23 (tie) Biggest Loser Diet - Overall score: 3 out of 5; No. 23 (tie) Glycemic-Index Diet - Overall score: 3 out of 5; No. 23 (tie) Nutrisystem Diet - Overall score: 3 out of 5; No. 23 (tie) Zone Diet - Overall score: 3 out of 5; No. 27 (tie) Abs Diet - Overall score: 2.9 out of 5; No. 27 (tie) Macrobiotic Diet - Overall score: 2.9 out of 5; No. 27 (tie) Slimfast Diet - Overall score: 2.9 out of 5; No. 30 HMR Program - Overall score: 2.8 out of 5; No. 31 Optavia Diet - Overall score: 2.7 out of 5; No. 32 Alkaline Diet - Overall score: 2.5 out of 5; No. 33 (tie) The Fast Diet - Overall score: 2.4 out of 5; No. 33 (tie) Paleo Diet - Overall score: 2.4 out of 5; No. 33 (tie) Raw Food Diet- Overall score: 2.4 out of 5; No. 33 (tie) Supercharged Hormone Diet - Overall score: 2.4 out of 5; No. 37 Atkins Diet - Overall score: 2.2 out of 5; No. 38 (tie) Keto Diet - Overall score: 2.1 out of 5; No. 38 (tie) Whole30 Diet - Overall score: 2.1 out of 5; No. 40 Body Reset Diet - Overall score: 2 out of 5; No. 41 Dukan Diet Overall score: 1.9 out of 5.

Assessing the value of diets is best done with a meta-analysis or head-to-head comparisons. For low-fat diets, a meta-analysis comparing a low-fat diet vs. conventional diets identified five studies lasting up to 18 months. In comparing the weight loss at 6, 12, and 18 months, there were no statistically significant differences from control, leading the authors to conclude that low-fat diets produce weight loss, but not more so than other diets. In a meta-analysis comparing "named" diets, including many of those listed above, Johnston et al. (34 Johnston) concluded that there were no consequential differences in weight loss at the end of 1 year.

The Volumetrics diet received a relatively high rating of No. 6 in the US News and World Report list. It focuses on energy density as a guide to selecting foods. If a diet is high in fat or low in water content, then it will have a high energy density (i.e., more calories per gram). In one trial, Ello-Martin et al. [35] reported a weight loss of 7.9 kg after 1 year, by feeding a diet with a low energy density. The diet was low in fat and rich in fruits and vegetables with a high-water content. This underscores the role of energy density in a diet as a factor in weight loss. It is important to appreciate that little weight loss will occur unless the diet induces an energy deficit, but there may be a number of different ways to do that.

Several controlled trials showed more weight loss with a low-carbohydrate diet than the control diet in the first 6 months but no difference at 12 months. In two head-to-head comparisons of four popular diets, the average weight loss at 6 and 12 months was the same [36, 37]. The best predictor of weight loss for each of the diets was the degree of adherence to the diet [36, 37]. The most recent comparison was a two-arm trial of low-carbohydrate and low-fat diets [38]. At the end of 1 year, there was no difference, and there was no prediction of response based on insulin gene expression. In a meta-analysis comparing low-fat and low-carbohydrate diets, where the two diets had the same amount of protein, Hall and Guo [39] concluded that there was no clinically significant difference. A more recent trial [40] arguing that a low carbohydrate diet might help in maintaining weight loss has been criticized [41], because when the analysis was done as originally proposed by the authors, there was no difference between diets – only when a shift in endpoint was done did the authors claim to show a difference [40]. It was also surprising that this study found no difference in resting energy expenditure or physical activity with weight loss between the two diet groups. Only the doubly labeled water method of measuring energy expenditure showed a difference [40] that may have been an artifact of the assumptions made in its use [41].

Portion-Controlled Diets

Portion control is one dietary strategy with promising long-term results. A trial in diabetic patients using portion-controlled diets as part of the lifestyle intervention (Look AHEAD Program) found that weight loss was increased across each quartile of portion control product use [42].

Behavior Modification and Lifestyle Interventions

Behavioral modification in lifestyle programs has been an important part of programs for weight loss for more than a quarter of a century [42, 43]. Weight losses have been in the 5–10% range [27]. Behavior modification has a number of components. First, it is a strategy designed to help people understand their eating behavior, from the triggers that start it to the location, speed, and type of eating, through the consequences of eating and the rewards that can change it. In addition, it consists of strategies to help people develop assertive behavior, learn cognitive techniques for handling their internal discussions, and ways of dealing with stress. The newest innovation in the use of lifestyle intervention is to implement it over the Internet, which has shown some promising results [1, 44].

Exercise

Exercise is important for maintaining weight loss, but when used alone, it does not generally produce much weight loss [45]. Comparisons of people who successfully maintain weight loss and those who do not show a critical role for exercise. More than 200 min/wk. provides greater likelihood of maintaining weight loss than lower levels of exercise. Using a pedometer or wrist activated devices allows counting of steps. Working toward 10,000 steps per day is a good goa and one that the health-care provider should encouraged.

Medications

Five medications are currently approved for long-term treatment of obesity along with four other for short-term use [1, 46, 47].

Noradrenergic Drugs

Diethylpropion, phentermine, benzphetamine, and phendimetrazine are approved by the US FDA for short-term use, usually considered to be up to 12 wk. These drugs probably work by blocking reuptake of norepinephrine into neurons. Phentermine is among the most widely prescribed appetite suppressants. Clinical trials with these drugs are usually short term. [1].

Orlistat

Orlistat is a potent and selective inhibitor of pancreatic lipase that reduces intestinal digestion of fat. One clinical trial resulted in a 5.5% in the placebo group compared to 9% of body weight in the orlistat group at 1 year [48]. Another study achieved a weight loss of 11% compared to 6% in the placebo-treated group arm and reported a 37% reduction in the development of T2DM in patients who had impaired glucose tolerance at baseline [49]. In a meta-analysis of 31 studies using orlistat (Table 17.1), the maximal weight loss (by modeling) was −6.65 kg, and half the maximal effect occurred by 35.4 weeks [47].

Orlistat is the only medication the US FDA has approved for weight management in adolescents with obesity. Adherence to orlistat use falls off rapidly after initial prescription [50]. Orlistat can cause small but significant decreases in fat-soluble vitamin levels, and clinicians are advised to recommend that patients to take vitamin supplements. Rare cases of severe liver injury have been reported in patients taking orlistat, but a causal relationship has not been established. However, patients who take orlistat should contact their health-care provider if itching, jaundice, pale color stools, or anorexia develops.

Lorcaserin

Lorcaserin is a selective agonist for the serotonin-2c receptors, which is its mechanism for reducing food intake [50]. It has low affinity for the serotonin-2b receptors found on heart valves.

Three clinical studies provide the basis for lorcaserin's approval [51, 52, 53]. In a meta-analysis of five studies using lorcaserin (Table 17.1), the maximal weight loss (by modeling) was −5.39 kg, and half the maximal effect occurred by 19.3 weeks [47]. They also showed improvements in cardiovascular risk factors [51, 52, 53].

Preclinical toxicology studies in rats found an increased number of brain and mammary tumors, but these have not been observed in human beings. This may reflect the fact that the drug

Table 17.1 Some features of interventional surgery for treatment of obesity

	Gastric bypass	Adjustable gastric banding	Sleeve gastrectomy
30-day mortality	0.08%	0.11%	0.50%
Complications	21.0%	13%	13%
Reoperations	2.56%	12.23%	9.05%
BMI change from baseline at 1 year	−14.5 kg/m2	−10.5 kg/m2	−16.2 kg/m2
% excess body weight loss	72%	33.4%	69.7%
Diabetes remission	95%	73.9%	83% (Obs studies)
Hypertension remission	81%	53.5%	83% (Obs studies)
Dyslipidemia remission	80%	39%	–
Sleep apnea remission	95%	94%	–

does not reach the high concentrations in the central nervous system of human beings as it does in rats [54].

Liraglutide

Liraglutide is a glucagon-like peptide-1 (GLP-1) agonist with a 97% homology to GLP-1. These molecular changes extend the circulating half-life from 1–2 minutes to 13 hours. Clinicians should prescribe this drug in combination with a reduced-calorie diet and increased physical activity for chronic weight management in adult patients who have an initial BMI of >30 kg/m^2 or in adult patients with a BMI >27 kg/m^2 who have T2DM, hypertension, or dyslipidemia.

The administration of daily subcutaneous injections of liraglutide at doses of 1.2, 1.8, 2.4, or 3.0 mg produced mean weight losses of 3.8, 5.4, 6.1, and 7.8 kg, respectively, after 1 year of treatment, compared to a loss of 2.0 kg in the placebo-treated group and 3.9 kg in the orlistat-treated comparator group [55]. Another larger 56-week trial reported that liraglutide reduced body weight by 8.4 kg compared to 2.8 kg in the placebo-treated group (on average) [56]. In another trial [57], those receiving liraglutide for weight maintenance (after initially losing weight with a low-calorie diet) lost an additional 6.8 kg compared to no additional weight loss in the placebo group. Only about half of the placebo group was able to maintain the weight they lost as a result of dieting. In a meta-analysis of three studies using liraglutide (Table 17.1), the maximal weight loss (by modeling) was −7.68 kg, and half the maximal effect occurred by 12.7 weeks [47].

A history of medullary thyroid carcinoma or multiple endocrine neoplasia syndrome type 2 is a contraindication to liraglutide. Clinicians should not prescribe liraglutide for patients with a history of pancreatitis and should discontinue liraglutide if acute pancreatitis develops. If weight loss doesn't exceed 4% after 16 weeks, liraglutide should be stopped [54]. There have been two cardiovascular outcome trials with liraglutide (1.8 mg/d) [58] and the long-acting version, semaglutide (0.5 or 1.0 mg weekly) [59]. In patients with T2DM, liraglutide significantly lowered the rate of the first occurrence of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke [59]. Semaglutide lowered the rate of cardiovascular death, nonfatal myocardial infarction, or nonfatal stroke [59].

Combination of Topiramate and Phentermine: Extended Release

The combination of phentermine/topiramate ER (PHEN/TPM ER) uses lower doses of phentermine than are usually prescribe when phentermine is used alone. Phentermine acts to reduce appetite through increasing norepinephrine in the hypothalamus. Topiramate may reduce appetite through its effect on GABA receptors.

Efficacy and safety of this drug combination came from two clinical studies [54, 60, 61]. Patients in two of these studies had higher risk profiles due to excess weight. PHEN/TPM ER produced weight losses of 9.3% and 10.7% with the middle and high doses (respectively) compared to 2.2% in the placebo group [62]. In a meta-analysis of six studies using phentermine/topiramate (Table 17.1), the maximal weight loss

(by modeling) was 15.6 kg, and half the maximal effect occurred by 29.8 weeks (some of which was related to the titration schedule) [47].

Improvements in blood pressure, glycemic measures, HDL cholesterol, and triglycerides occurred with both the recommended and the top doses of the medication in these trials [60, 61]. Improvements in risk factors related to the magnitude of weight loss. In patients with obstructive sleep apnea (OSA), this combination reduced the severity of symptoms [62].

Taking topiramate in the first trimester of pregnancy may increase risk of cleft lip/cleft palate in infants. Therefore, clinicians must inform women of childbearing potential of this risk and conduct a pregnancy test before prescribing PHEN/TPM ER. Glaucoma is a rare side effect of topiramate, and the drug is contraindicated in glaucoma, as it is in patients with hyperthyroidism and within 14 days of treatment with monoamine oxidase inhibitors and in patients with hypersensitivity to any of the ingredients in the medication. Topiramate is a carbonic anhydrase inhibitor that often produces tingling in the fingers and may make carbonated beverages taste different. Other potential issues include a risk for kidney stones (associated with topiramate) and increased heart rate in patients susceptible to phentermine.

Combination of Naltrexone-Bupropion: Sustained Release

Bupropion alone is approved for treatment of depression and for smoking cessation. It reduces food intake by acting on adrenergic and dopaminergic receptors in the hypothalamus. It has a modest effect on weight loss. Bupropion stimulates the pro-opiomelanocortin neurons in the hypothalamus to produce pro-opiomelanocortin, which is further processed to α-melanocyte-stimulating hormone (which reduces food intake) and β-endorphin (which stimulates feeding). Naltrexone blocks this effect of β-endorphin on appetite, thus allowing the inhibitory effects of α-melanocyte-stimulating hormone to reduce food intake, by acting on the melanocortin-4 receptor system [63].

FDA approval of the combination drug naltrexone/bupropion was based on three trials (63. 64. 65). In one study [63], weight loss at 56 weeks was 5.0% for the lower dose of naltrexone/bupropion (16 mg per day/360 mg per day) and 6.1% for a higher dose (32 mg per day/360 mg per day), compared to placebo. Treatment also improved waist circumference, fasting glucose, fasting insulin, homeostasis assessment model of insulin resistance (HOMA-IR), and HDL cholesterol, but there was a transient increase in BP.

A second study included an intensive behavioral modification program [64] and produced weight loss at 56 weeks of 5.1 ± 0.6% for naltrexone/bupropion (32 mg per day/360 mg per day) versus 9.3 ± 0.4% for placebo. The study also reported significant improvements in weight, waist circumference, insulin, HOMA-IR, HDL cholesterol, triglycerides, and quality of life.

Weight loss at week 56 in a third study was 6.4% with naltrexone/bupropion (32 mg per day/360 mg per day) compared to 1.2% with placebo [65]. As in the other studies, there were improvements in cardiometabolic risk markers, weight-related quality of life, and control of eating.

In patients with type 2 diabetes, naltrexone/bupropion resulted in significantly greater weight reduction (5.0% versus 1.8% in the placebo group) and significantly greater reductions in HbA1c (−0.6 vs. -0.1%; $P < 0.001$) [66]. There was also improvement in triglycerides and HDL cholesterol compared with placebo.

Efficacy of weight loss with the naltrexone/bupropion combination at 1 year is higher than lorcaserin but not as high as PHEN/TPM ER and is associated with improvements in risk factors [63–66]. In a meta-analysis of six studies using naltrexone/bupropion, the maximal weight loss (by modeling) was −13.2 kg, and half the maximal effect occurred by 35.2 weeks (probably related to the titration schedule) [47].

Because bupropion increases pulse and both bupropion and naltrexone increase BP, an ongoing study is examining cardiovascular outcomes [67].

Comparison of Medications Approved for Chronic Weight Management

There are no head-to-head comparisons of the medications approved for long-term use. However, there is an analysis of 28 RCTs of weight-loss medications that included trials with orlistat, lorcaserin, liraglutide, naltrexone/bupropion, and PHEN/TPM ER [68]. The inclusion criteria and background lifestyle interventions differed across studies. Attrition rates were 30–45% across these trials. All five agents were associated with significantly greater weight loss at 1 year than placebo. Combined, these studies reported a weight loss of >5% in 23% of patients treated with placebo, 44% of patients treated with orlistat, 49% of patients treated with lorcaserin, 55% of patients treated with naltrexone/bupropion SR, 63% of patients treated with liraglutide, and 75% of patients treated with PHEN/TPM ER. The highest odds ratio for treatment-related discontinuation of the trial was with liraglutide and naltrexone/bupropion [68].

Using data from these trials, Dong et al. [47] used computer modeling to identify the maximal weight loss, the time to achieve maximal weight loss and the effect of dropouts.

Best Practices for Medications Approved for Weight Management

Criteria for using these medications approved for long-term use were agreed between the 2013 American Heart Association/American College of Cardiology/the Obesity Society Guideline for the Management of Overweight and Obesity in Adults [27] and the 2015 Endocrine Clinical Practice Guideline on Obesity Pharmacotherapy [46] . Clinicians may consider prescribing weight-reducing drug therapies for patients who: (1) struggle to achieve weight goals, (2) meet label indications (BMI >30 kg/m^2 or > 27 kg/m^2 with comorbidity), and (3) need to lose weight for health reasons (such as osteoarthritis, prediabetes, fatty liver, or other conditions). Furthermore, the American Association of Clinical Endocrinologists/American College of Endocrinology Comprehensive Clinical Practice Guidelines for Medical Care of Patients With Obesity from 2016 [69] indicate that clinicians may consider pharmacotherapy as a first-line treatment for weight reduction if patients present with one or more severe comorbidities and would benefit from weight loss of 10% or more. Those guidelines don't require that patients fail lifestyle therapy before clinicians prescribe medications.

Bariatric Surgical Procedures for the Treatment of Obesity

Surgical strategies to treat obesity began more than 50 years ago and have now risen to over 200,000 procedures in the USA [1, 70]

There are three principal procedures now widely used. Sleeve gastrectomy (SG) is the most common procedure, and Roux-en-Y gastric bypass (RYGB) is second with laparoscopic adjustable gastric banding being less commonly done. The technically complicated biliopancreatic diversion is occasionally performed, but there is insufficient controlled data to include it. Data on the response to these three procedures are summarized in Table 17.1 [71].

Adapted from Chang et al. [71] The data from randomized controlled trials were used when available. Where they were not reported, data from observational studies were used.

Criteria for Bariatric Surgery

The National Institutes of Health Consensus Panel in 1991 established the initial criteria for surgical interventions for obesity [72]. The panel concluded that individuals with BMI ≥35 kg/m^2 with a related comorbidity or BMI ≥40 kg/m^2 were appropriate candidates for bariatric surgery. An additional criterion was failure of medical treatment to accomplish sustained weight loss. These criteria have been variably interpreted over many years but have remained essentially unchanged until the present. This is the result of a recent joint statement by international diabetes organizations that has indicated that bariatric or metabolic surgery procedures are a consideration

for patients with poorly controlled T2DM and a BMI of 30–35 kg/m^2 [73]. The Endocrine Society has also released pediatric guidelines for bariatric surgery [74]. The reason for this is the high rate of reversion of diabetes to normal glucose tolerance as shown in Table 17.1.

Outcomes of Bariatric Surgery

Bariatric surgery carries more risk than nonsurgical strategies. In addition to a low rate of mortality (see Table 17.1), there are also serious complications that occur in 4.1% of all patients undergoing this surgery. The experiences of both the surgeon and the surgical center are predictors of surgical outcomes [75]. Longer-term complications can include intestinal obstruction, marginal ulcer, ventral hernia, and gallstones. Additional metabolic complications include nephrolithiasis, osteoporosis, and hypoglycemia. Mineral and vitamin deficiencies and weight regain occur in variable numbers of patients. Micronutrient deficiencies following gastric bypass include: iron, 33% to 55%; calcium/vitamin D, 24% to 60%; vitamin B12, 24% to 70%; copper, 10% to 15%; and thiamine, <5% [76]. Established guidelines recommend that the health-care provider routinely give nutrient supplementation to include multivitamins, vitamin B_{12}, iron, minerals, calcium, and vitamin D [77].

In contrast to the disadvantages of surgery, there are clear benefits that outweigh the risks and potential complications. Weight loss is significant and a major benefit. But like other forms of treatment for obesity, there is a considerable variability in response. In a large follow-up study of patients undergoing a Roux-en-Y gastric bypass, the trajectories of initial weight loss in the first year tended to be retained for up to 7 years and varied from 12 to 45% of initial weight [78]. Weight loss following laparoscopic banding is similarly variable, but weight losses are only about half that seen with gastric bypass.

The single best predictors of sustained postoperative weight loss (identified by the LABS Consortium) are postoperative eating and lifestyle behaviors. Specifically, subjects who self-monitor by weighing themselves frequently and who avoid eating when full and avoid snacking between meals appear to experience the greater weight loss [79]. The poorest weight loss following gastric bypass is comparable to the best reported weight loss for nonsurgical interventions [80]. Changes in weight from baseline to 5 years in the surgical groups were superior to the changes seen with medical therapy. Body weight decreased 23% with gastric bypass, 19% with SG, and 5% with drug therapies [81].

The remarkable remission of T2DM following bariatric operation is shown in Table 17.1 and was originally noted by Pories et al. [82]. The durability of the remission was sustained for up to 7 years in many participants. A meta-analysis found no difference in the remission of type 2 diabetes following sleeve gastrectomy and gastric bypass [83]. Overall, there is considerable evidence favoring surgical procedures for control of, or inducing remission in, type 2 diabetes, versus intense medical treatment [73]. As a result, the term "metabolic" surgery has become popular. The concept that clinicians should consider surgical intervention for patients with poorly controlled type 2 diabetes and patients with less severe obesity with type 2 diabetes, rather than having BMI be the primary indication for surgery, has gained broad support. Remission of dyslipidemia, sleep apnea, and hypertension can also occur [71].

The Swedish Obese Subjects Study is one of the longest and most thoroughly evaluated studies of gastrointestinal operations for patients with obesity [80]. The control group comprised obese patients who were treated with the best available clinical alternatives in the Swedish health-care system. Weight loss for many patients with gastric bypass exceeded 50 kg. Mortality was significantly reduced by 29% in the operated patients [84], who also showed a reduction in myocardial infarction, stroke, and reduced incidence of diabetes mellitus. Cancer was significantly reduced in women [85].

In addition to the major gastrointestinal procedures described above, there are several other surgical strategies which have been used. Vagal blockade is one of them. A vagal blockade can be produced by activating electrodes placed around the vagal trunks at the diaphragm in order to

produce intermittent vagal blockade. Weight loss occurs by reducing appetite and inducing early satiety. Weight loss is modest but is superior to sham-treated controls yet less successful than conventional surgical procedures described above [86]. Vagal blockade is safe but has limited efficacy. Another technique is to use gastrointestinal endoscopic interventions with one of several devices, placed either by gastrointestinal endoscopy or suturing procedures. The US FDA approved two gastric balloons in 2015 and another in 2016. Clinicians can fill the Orbera Intragastric Balloon System with 400 to 700 mL of saline. The ReShape Integrated Dual Balloon System contains two connected, saline-filled balloons. In 2016 the FDA approved the Obalon Balloon System, which expands with air after insertion. Technical improvements to these devices have resulted in a favorable safety profile [87]. The present protocol requires removal of the intragastric balloon 3–6 months after placement, which is a limitation to the long-term efficacy of this intervention. The balloon can be replaced for those who regain weight [88]. In August 2017, the US FDA sent a letter to healthcare providers, noting seven deaths associated with liquid-filled intragastric balloon systems used to treat obesity. Four of the reports involved the Orbera Intragastric Balloon System and one with the ReShape Integrated Dual Balloon System. Two earlier deaths were also noted. A specially designed percutaneous gastrostomy tube and apparatus, called the AspireAssist device, has also been evaluated. It allows patients to directly remove ingested food from the stomach. In a clinical trial lasting 1 year using this device, patients lost 12.1% compared to 3.6% in the control group. This aspiration technique requires available facilities to discard the aspirated food and is not for everyone. Finally, endoscopic placement of a duodenojejunal luminal sleeve is under evaluation [89]. In a study that examined endoscopic ablation of duodenal mucosa to enhance glycemic control of T2DM, reduction of HbA1c persisted 6 months after ablation. Liposuction (also known as lipoplasty or suction-assisted lipectomy) is the most common esthetic procedure performed in the USA, with over 400,000 cases performed annually [90]. Although not generally considered a bariatric procedure, clinicians remove and contour subcutaneous fat by aspiration after injecting physiologic saline. As techniques have improved, it is now possible to remove significant amounts of subcutaneous adipose tissue without affecting the amount of visceral fat. In a study to examine the effects of this procedure, Klein et al. [90] examined seven women with diabetes who were also overweight and eight women with normal glucose tolerance that were overweight before and after liposuction. One week after assessing insulin sensitivity, the subjects underwent large volume tumescent liposuction, which consists of removing more than 4 liters of aspirate injected into the fat beneath the skin. There was a significant loss of subcutaneous fat but no change in the visceral fat. Subjects were reassessed 10–12 weeks after the surgery. The control women lost 6.3 kg of body weight and 9.1 kg of body fat, which reduced body fat by 6.3%. The women with diabetes had a similar response with a weight loss of 7.9 kg, a reduction in body fat of 10.5 kg, and a reduction in percent fat of 6.7%. Waist circumference was also significantly reduced. In spite of these significant reductions in body fat, there were no changes in blood pressure, lipids or cytokines (tumor necrosis factor-α, interleukin-6), or C-reactive protein. There was also no improvement in insulin sensitivity, suggesting that removal of subcutaneous adipose tissue without reducing ectopic fat depots has little influence on the risk factors related to being overweight.

Conclusion

The epidemic of obesity over the past 50 years has increased by threefold the number of individuals with obesity. Since no one chooses to be fat, this has led to the search for cures for the patient with obesity. This chapter has outlined the development of obesity and its associated problems. It is a chronic relapsing disease process for which bariatric surgery is the most effective treatment. However, many people don't want surgery,

and for them, there are a variety of diets, exercise programs, and behavioral programs, which can be supplemented by the use of pharmacological treatment in many cases. The difficulty of reversing obesity is well recognized and poses a major challenge for those working on this obstinate problem.

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High-Density Lipoproteins

18

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Introduction

In 1929, Michel Macheboeuf, from the Pasteur Institute in Paris, precipitated the first lipoprotein from animal serum [1]. It was a lipid-rich 'fraction A' isolated by ultracentrifugation several decades later and termed high-density lipoprotein (HDL) by the laboratory of John Gofman at the University of California in Berkley [2]. In the very first studies of isolated lipoproteins, reduced cholesterol content was observed in HDL from a small group of patients with atherosclerotic cardiovascular disease (CVD) [3]. Based on this and other findings, the HDL hypothesis was proposed by Miller and Miller to postulate that 'reduction of plasma HDL concentration may accelerate the development of atherosclerosis, and hence ischaemic heart disease, by impairing the clearance of cholesterol from the arterial wall' [4]. When confirmed later in large-scale prospective epidemiological studies [5], the inverse relationship between circulating HDL-cholesterol (HDL-C) and CVD fuelled innumerable studies of HDL over the last decades. This work resulted in detailed characterization of both normal HDL metabolism and its abnormalities associated with CVD. As a possibly too straightforward corollary of the HDL hypothesis, HDL-C raising was broadly accepted as a promising approach to reduce CVD risk, opening a way to the development of HDL-targeting therapies. Quite unexpectedly, such therapies largely failed to provide clear benefits when added to standard treatment regimens involving statins, revealing the incompleteness of our understanding of HDL. This chapter reviews major aspects of current knowledge of this extensively studied but still enigmatic lipoprotein.

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Normal Plasma HDL

HDL is a small, dense, protein-rich lipoprotein, possessing a mean size of 8–10 nm and density of 1.063–1.21 g/ml [6]. HDL particles are composed predominantly of polar lipids solubilized by apolipoproteins but equally contain numerous other proteins, including enzymes, lipid transfer proteins, acute-phase response proteins, complement components and proteinase inhibitors, and may contain small amounts of non-polar lipids.

M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_18

Structure

HDL particles are plurimolecular, quasi-spherical or discoid, pseudomicellar complexes. HDL structure is predominantly defined by apolipoprotein A-I (apoA-I), the major HDL protein.

Reconstituted Discoid HDL

The amino acid sequence of apoA-I contains periodically repeating units organized in amphipathic α-helices which are responsible for the potent detergent-like ability of the protein, allowing it to avidly bind lipids and to solubilize them into stable lipoprotein particles. As a result of this rare property, apoA-I spontaneously forms *discoid HDL* in the presence of minute amounts of phospholipids, with the α-helices arranged around the circumference of HDL and their long axes perpendicular to the acyl chains of the phospholipids. According to such *belt model* [7], each discoid HDL particle contains two molecules of apoA-I together with 150–200 molecules of phospholipid, and is 9–10 nm in diameter and 4.7 nm in thickness [8]. Other amphipathic apolipoproteins, including apoA-II, apoE and apoM, are also capable of forming discoid HDL. Similarly, discoid HDLs which contain small amounts of cholesterol and some other lipids are present at low concentrations of <5% of total apoA-I in human plasma.

The belt model was later developed to the *double belt model*, in which two ring-shaped apoA-I molecules encapsulate a lipid membrane leaflet in an antiparallel orientation in which helices 5 of the both molecules directly oppose each other according to a left-to-left (LL) 5/5 interface [9]. In a refined *belt and buckle model*, the N- and C-terminal residues of apoA-I double back on the molecule [10], while a *looped belt model* proposes an existence of a looping region within helix 5 that causes a localized opening between the parallel belts, which may include a site of the action of lecithin–cholesterol acyltransferase (LCAT). An alternative *solar flares* refinement of the double belt model represents the N-termini of apoA-I molecules as globular nodules [11].

Reconstituted Spherical rHDL

The majority of HDL particles in human plasma are however spherical and contain a neutral lipid core composed of cholesteryl ester and triglyceride surrounded by a polar lipid monolayer composed of phospholipids and free cholesterol. The fundamental interactions of apoA-I helices with phospholipid acyl chains do not change markedly in reconstituted spherical HDL relative to the discs, resulting in the similarity in apoA-I structure between the two shapes. According to the *trefoil model* of spherical HDL [12], three or four apoA-I molecules, bent 120° on the kinks in helices 5 and 10, are organized in antiparallel belts as per the double belt model, forming a three-dimensional cage-like structure. This cage serves to encapsulate neutral lipid core and supports surface polar lipids in the intervening open spaces between the molecules. An alternative organization of apoA-I molecules in spherical HDL involves a *helical dimer with hairpin,* with two molecules in a double belt and the third as a separate hairpin [13], being similar to the trefoil model from a molecular perspective.

Native HDL Particles

Native spherical HDL particles contain from three to five molecules of apoA-I, with their surface being dominated by protein , which may account up to 87% of the surface of small, dense HDL. General features of apoA-I structure appear to be similar between native and reconstituted HDL (rHDL), extending across lipid-associated forms of all sizes, shapes and origin [8]. Indeed, similar cross-linking patterns of apoA-I molecules were found in human plasma HDL particles containing predominantly apoA-I, in reconstituted spheres and in reconstituted discs irrespective of the particle size or density [14]. HDL particle size appears thereby to be modulated via a twisting motion of the resident apoA-I molecules. However, subtle conformational adaptations including appearance of loop regions in helix 5 of apoA-I [15] may occur in localized regions of the protein in response to changes in either particle diameter or surface packing density.

Heterogeneity

Conformational flexibility of apoA-I forms a basis for the heterogeneity of HDL particles in physical properties and chemical composition. Analytic ultracentrifugation separates HDL in two subclasses, light, lipid-rich *HDL2* (d 1.063–1.125 g/ml) and dense, protein-rich *HDL3* (d 1.125–1.21 g/mL) [16]. HDL2 and HDL3 can be further fractionated by non-denaturing polyacrylamide gradient gel electrophoresis [16], which distinguishes between large *HDL2b* (size 9.7–12.0 nm) and *HDL2a* (8.8.–9.7 nm) and small *HDL3a* (8.2–8.8 nm), *HDL3b (*7.8–8.2 nm) and *HDL3c* (7.2–7.8 nm). Equivalent HDL subclasses can be isolated by isopycnic density gradient ultracentrifugation [16].

Agarose gel electrophoresis of HDL allows analytical separation of HDL according to surface charge and shape, into *α-migrating particles*, which represent the majority of circulating HDL, and *pre-β-migrating particles*, consisting of nascent discoid and poorly lipidated HDL [16]. The agarose and the gradient gel electrophoresis can be combined into a two-dimensional method which allows separation of up to 12 distinct apoA-I-containing HDL subclasses, including $preβ_1$, $preβ_2$, $α_1$, $α_2$, $α_3$, $α_4$, $preα_1$, $preα_2$ and $preα_3$ particles [16].

Finally, NMR can quantify three distinct HDL subclasses differing in size, notably *large* (8.8–13.0 nm), *medium* (8.2–8.8 nm) and *small* (7.3–8.2 nm) *HDL* [16].

Composition

HDL contains a variety of protein and lipid components at a wide range of concentrations, together with microRNAs (miRs), which can be transported by HDL to various tissues.

Proteome

Proteins form the key structural and functional moiety of HDL. Elevated protein content of approximately 50 wt% entails high complexity to the protein composition of HDL which is enriched in different proteins as compared to other lipoprotein classes. Proteomic analyses identify a large number of HDL-associated proteins; thus, The HDL Proteome Watch at http://homepages.uc.edu/~davidswm/HDLproteome.html provides a list of 95 proteins that are reliably located on HDL as of 29 March 2019.

Apolipoproteins *Apo A-I* is the major structural and functional HDL protein which accounts for approximately 70 wt% of total protein in HDL [6, 8]. Major functions of apoA-I involve interaction with cellular receptors, activation of LCAT and endowing HDL with multiple antiatherogenic activities. *ApoA-II* is the second-major HDL apolipoprotein which represents approximately 15–20 wt% of total HDL protein. ApoA-II is more hydrophobic than apoA-I and circulates as a homodimer composed of two identical polypeptide chains connected by a disulphide bridge.

ApoCs form a family of small exchangeable apolipoproteins. *ApoC-I* is involved in the activation of LCAT and inhibition of hepatic lipase (HL) and cholesteryl ester transfer protein (CETP). *ApoC-II* functions as an activator of several triacylglycerol lipases, including lipoprotein lipase (LPL). *ApoC-III* inhibits LPL and HL and decreases the uptake of chylomicrons by hepatic cells. *ApoE* is an essential structural and functional glycoprotein component of HDL, which serves as a ligand for apoB/apoE receptors and ensures lipoprotein binding to cell-surface glycosaminoglycans. *ApoM* specifically binds small hydrophobic molecules, primarily sphingosine-1-phosphate (S1P). Other physiologically important HDL apolipoproteins include apoA-IV, apoD, apoF, apoJ and apoL-I.

Enzymes *LCAT* is a highly glycosylated enzyme which catalyses the esterification of cholesterol to cholesteryl esters in plasma lipoproteins, primarily in HDL (carrying approximately 75% of plasma LCAT activity) but also in apoB-containing particles. Human *paraoxonases* (PON) are calcium-dependent lactonases PON1, PON2 and PON3. In the circulation, *PON1* is almost exclusively associated with HDL. Hydrolysis of homocysteine thiolactone has been

proposed to represent a major physiologic function of PON1 [17]. *Platelet-activating factor acetyl hydrolase (PAF-AH),* equally termed lipoprotein-associated phospholipase A_2 (*Lp*PLA_2), degrades PAF and cleaves phospholipid substrates with a short residue at the sn-2 position, such as proinflammatory oxidized short-chain phospholipids.

Lipid transfer proteins *CETP* is a glycoprotein that shuttles between HDL and apoB-containing lipoproteins to facilitate a heteroexchange of cholesteryl esters and triglycerides. The structure of CETP includes a hydrophobic tunnel filled with two cholesteryl ester molecules and plugged by an amphiphilic phosphatidylcholine molecule at each end [18]. *Phospholipid transfer protein (PLTP)* is primarily associated with HDL and exchanges phospholipids between HDL particles, converting them into larger and smaller subspecies.

Other proteins Positive acute-phase response proteins, including *serum amyloid A (SAA)* isoforms, whose plasma concentrations are much lower as compared to apolipoproteins but can be markedly elevated by acute inflammation, form a large family of HDL-associated proteins [19]. SAA1, the major member of the family, is predominantly carried by HDL. LPS-binding protein (LBP) is an acute-phase glycoprotein capable of binding the lipid A moiety of LPS and facilitating LPS diffusion [20]. Several *proteins involved in complement regulation*, including complement component 3 (C3), C4b binding protein, C9 and vitronectin, are equally present in isolated HDL. A family of HDL-associated *serpin* proteins exemplified by α-1-antitrypsin contains serine proteinase inhibitor domains [19].

Heterogeneity Proteins are non-uniformly distributed across HDL subpopulations. Thus, HDL can be separated into *particles containing apoA-I with (LpA-I:A-II) or without (LpA-I) apoA-II* by electro-immunodiffusion in agarose gels [16].

Furthermore, proteomic analysis of HDL particle subpopulations isolated from normolipidemic subjects by density gradient ultracentrifugation, immunoprecipitation or FPLC identifies distinct patterns of distribution of individual proteins across the particles [21]. Distinct clusters of proteins bundled into HDL particles can be distinguished by similar functions including lipid metabolism, antioxidative/anti-inflammatory activity and haemostasis [22]. Specific protein–protein interactions appear to drive formation of such complexes which include lipid-poor particles dominated by PLTP, apoJ and proteins implicated in host defence and inflammation [23]. The diversity of molecules which bind to HDL suggests that the lipoprotein can serve as a versatile adsorptive surface for proteins and peptides to form complexes with distinct functionalities.

Lipidome

Phospholipids prevail in the HDL lipidome, accounting for 40–50 mol% of total lipid, with lesser proportions of cholesteryl esters (30–40 mol%), triglycerides (3–5 mol%) and free cholesterol (5–10 mol%) [24]. *Phosphatidylcholine,* the principal plasma phospholipid that accounts for 32–35 mol% of total lipids in HDL, is a structural lipid, consistent with its even distribution across HDL subpopulations *Lysophosphatidylcholine* is another important phospholipid subclass in HDL (1.4–8.1 mol % of total lipids) derived from regulated degradation of phosphatidylcholine by phospholipases, including LCAT. *Phosphatidylethanolamine* is moderately abundant in HDL (0.7–0.9 mol % of total lipids). *Plasmalogens* are minor phospholipids which contain a vinyl ether–linked fatty acid essential for their antioxidative properties [25]. *Phosphatidylinositol*, *phosphatidylserine*, *phosphatidylglycerol*, *phosphatidic acid* and *cardiolipin* are negatively charged minor (0.8 mol % of total lipids) phospholipids present in HDL which may impact its net surface charge and modulate lipoprotein interactions with lipases, extracellular matrix and other protein components [26].

Sphingomyelin, a structural lipid which enhances surface rigidity, is the major HDL sphingolipid (5.6–6.6 mol% of total lipids), which

largely originates from triglyceride-rich lipoproteins (TGRL) [25]. *Ceramide* is a minor (<0.1 mol% of total lipids) sphingolipid intermediate implicated in cell signalling, apoptosis, inflammatory responses, mitochondrial function and insulin sensitivity. Both sphingomyelin and ceramide are enriched in large, light relative to small, dense HDL [26]. Among lysosphingolipids, *S1P* is particularly interesting, reflecting its key role in vascular biology [27]. S1P is associated preferentially with small, dense HDL particles [25], consistent with their elevated content of apoM [21].

Unesterified (free) *sterols* are located in the surface monolayer of HDL particles and regulate its fluidity. HDL sterols are dominated by cholesterol, reflecting the pivotal role of lipoproteins in the cholesterol transport through the body. *Cholesteryl esters* are largely (up to 80%) produced in HDL and form its lipid core [8]. Most of cholesteryl ester in HDL is accounted for by cholesteryl linoleate. HDL-associated *triacylglycerides* are dominated by species containing oleic, palmitic and linoleic acid moieties [8]. Minor HDL lipids include diacylglycerides, monoacylglycerides and free fatty acids.

Glycome

Protein moiety of HDL is covered by a carbohydrate coating accounting for up to 3.3 wt% of total protein [28, 29]. HDL carbohydrate residues form N-glycan antennas protruding outside of the particle and carrying N-acetylneuraminic (sialic) acid at their termini underlain by N-acetylglucosamine, fucose, mannose and galactose. Several HDL proteins, including LCAT, CETP, apoCs, fetuin A and α-1-antitripsin, are glycosylated, while the glycosylation of apoA-I remains controversial [28, 30, 31]. The presence of sialic acids at the termini of N-glycans may play a role in HDL metabolism.

Metabolism

Production

HDL production combines several multistep processes that rely on membrane-bound and plasma proteins and predominantly involves apoA-I, the major HDL component. ApoA-I is mainly produced and secreted by the liver and the intestine (Fig. 18.1). The liver is traditionally considered as the principal site of apoA-I production in humans; strikingly however, the amounts of apoA-I produced by the liver and the intestine are comparable [32].

ApoA-I secreted by hepatocytes is either lipid-free or lipid-poor and needs to be lipidated in order to form HDL. Such HDL assembly typically occurs at the cell surface and begins with the transfer of membrane phospholipids and cholesterol to apoA-I. This process involves interaction of apoA-I with cell-surface ATP-binding cassette transporter A1 (ABCA1; Fig. 18.1). *ABCA1* is a ubiquitous transmembrane protein which functions as a membrane phospholipid translocase whose enzymatic activity leads to the transfer of phospholipid molecules across a cell plasma membrane [33]. The presence of active ABCA1 promotes binding of apoA-I to the cell surface, predominantly to lipid domains in the membrane that are created by the activity of ABCA1 and only in a minor part to ABCA1 to stabilize the transporter. As a result of such interactions, heterogeneous populations of discoid *nascent HDL* particles are formed. ABCA1 thereby promotes the transfer of phospholipid and cholesterol from plasma membrane to lipid-free apolipoproteins or lipid-poor HDL that readily interact with ABCA1 in a process which is critical for plasma HDL metabolism [33]. Indeed, ABCA1-mediated lipidation of apoA-I increases the stability of the apolipoprotein in the circulation, preventing its rapid elimination through the kidney. Apolipoproteins other than apoA-I, including apoA-II, apoA-V, apoC-I, apoC-II, apoE and apoM, are also capable of forming nascent HDL upon interaction with ABCA1, indicating that they are also important in the HDL formation.

In the next step, free cholesterol is transferred from the polar surface monolayer to the non-polar hydrophobic core as a result of esterification by LCAT, thereby creating *spherical HDL* possessing a hydrophobic core (Fig. 18.1). This reaction delays catabolism of discoid HDL, allowing progression to HDL maturation. As a

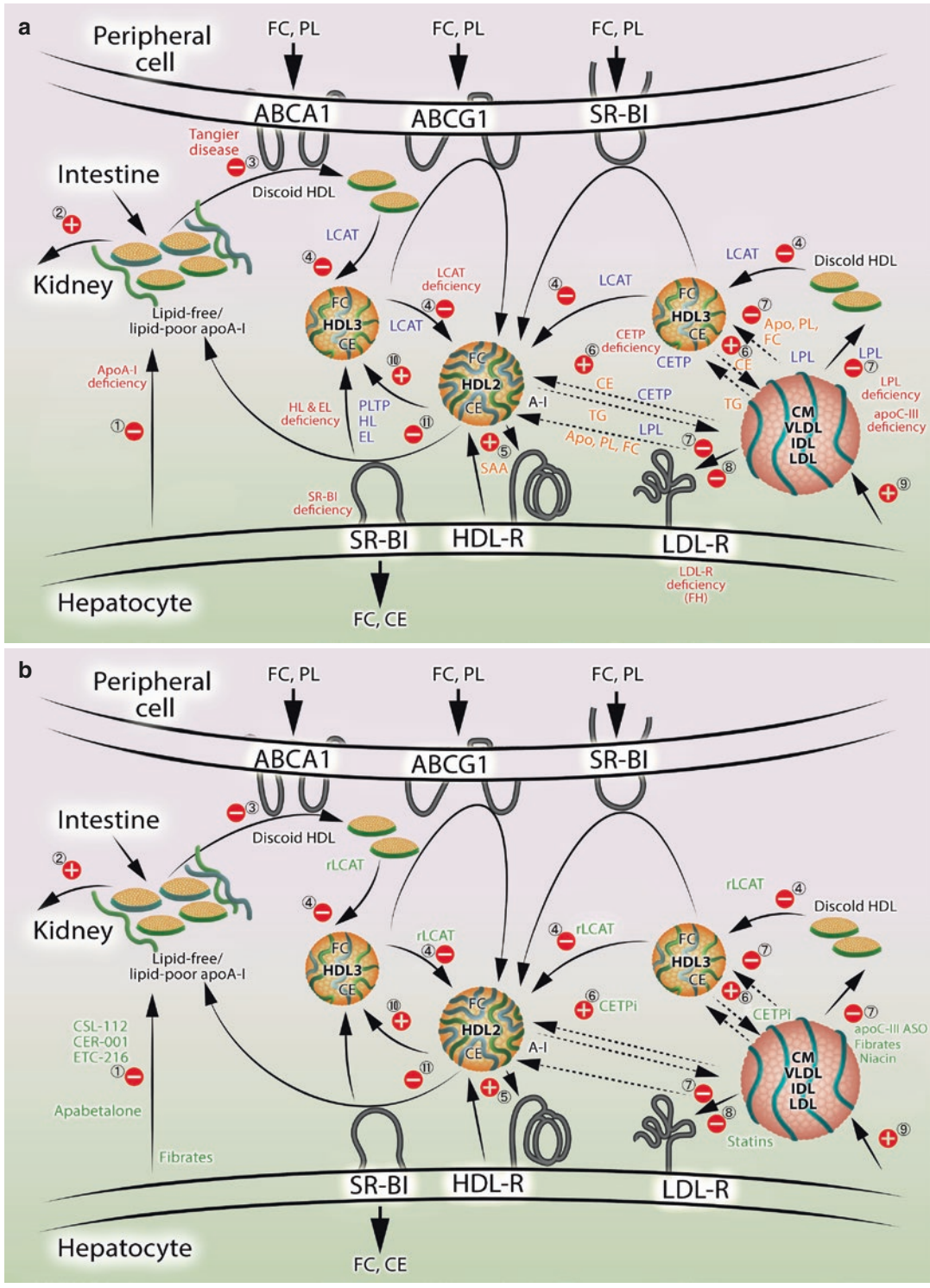

Fig. 18.1 Major pathways of normal and altered HDL metabolism (**a**), together with sites of action of HDL-targeting agents (**b**). (1) ApoA-I is secreted by hepatocytes and intestinal cells, in a lipid-free and/or lipid-poor (pre-beta) form. This pathway can be impaired in apoA-I deficiency, leading to low HDL-C and can be rescued by

(continued)

rHDL infusions to expand plasma pool of lipid-poor particles, or by agents enhancing apoA-I production. (2) Lipid-free and/or lipid-poor HDL are catabolised via kidneys. This pathway can be accelerated in dyslipidemic and inflammatory states, leading to low HDL-C. (3) ApoA-I lipidation occurs upon interaction with ABCA1, resulting in the formation of lipid-poor, discoid HDL particles. This pathway can be impaired in ABCA1 deficiency (Tangier disease), leading to low HDL-C. (4) Esterification of discoid HDL cholesterol catalysed by LCAT leads to the formation of HDL lipid core primarily composed of cholesteryl ester; HDL particles grow in size and become small, dense, lipid-poor spherical HDL3 and large, light, lipid-rich spherical HDL2. This pathway can be impaired in LCAT and apoA-I deficiency, leading to low HDL-C and can be rescued by infusions of recombinant LCAT. Both HDL3 and, particularly, HDL2 can accept cellular cholesterol and phospholipid via ABCG1- and SR-BI-mediated pathways, increasing HDL lipid load. (5) SAA produced by the liver displaces apoA-I in HDL under inflammatory conditions, with loss of apoA-I by the renal catabolism. This pathway can be accelerated in dyslipidemic and inflammatory states, leading to low HDL-C and can be normalized by anti-inflammatory agents. (6) Spherical HDL2 and HDL3 are remodelled by CETP which ensures cholesteryl ester transfer from HDL to TGRL and reciprocal triglyceride transfer; as a result, HDL particles become depleted in cholesteryl ester and enriched in triglyceride. This pathway can be accelerated in dyslipidemic and inflammatory states, leading to low HDL-C and can be normalized by CETP inhibitors. This pathway can also be delayed in CETP deficiency, leading to extremely high HDL-C. (7) Hydrolysis of TGRL by LPL induces transfer of surface apolipoproteins, phospholipid and free cholesterol to the plasma HDL pool. This pathway can be inhibited in LPL deficiency, leading to low HDL-C and can be rescued by apoC-III inhibition and LPL activation. This pathway can also be accelerated in apoC-III deficiency, leading to high HDL-C. (8) LDL and TGRL remnants are removed from the circulation via the LDL receptor (LDL-R). This pathway can be delayed in dyslipidemic states, including familial hypercholesterolemia (FH), and can be normalized by statins. (9) VLDL are produced and secreted by the liver. This pathway can be accelerated in dyslipidemic states, leading to hypertriglyceridemia and can be normalized by niacin. (10) Lipids in mature spherical HDL are hydrolysed by HL and EL to produce smaller HDL particles. This pathway can be accelerated in dyslipidemic states, leading to low HDL-C. This pathway can also be delayed in HL and EL deficiency, leading to high HDL-C. (11) Selective uptake of HDL cholesteryl ester via SR-BI recycles small, lipid-poor HDL subspecies and lipid-free/lipid-poor apoA-I. This pathway can be delayed in SR-BI deficiency, leading to extremely high HDL-C. Particle transformations and mass transfers are shown as solid and dotted lines, respectively. HDL components are listed in orange, enzymes and lipid transfer proteins are in blue, HDL-targeting therapies are in green and genetic deficiencies are in red. Abbreviations: A-I apoA-I, CE cholesteryl ester, CETPi CETP inhibitors, FC free cholesterol, FH familial hypercholesterolemia, HDL-R holoparticle HDL receptor, LDL-R LDL receptor, PL phospholipid, rLCAT recombinant LCAT, TG triglyceride

result, spherical HDL particles displaying slow catabolic rate comprise the major fraction of plasma HDL. Interestingly, other HDL subpopulations are similarly metabolized within discrete, stable-size pools with little interconversion between them [34].

ApoA-I secreted by the intestine is predominantly present in chylomicrons; some part of intestinally synthesized apoA-I is carried by discoid HDL [35]. Together with apoB-48 and apoCs, apoA-I represents a major component of chylomicrons, accounting for up to 20 wt% of their protein [35]. This frequently neglected observation emphasizes physiologic importance of intestinally derived apoA-I in the HDL metabolism.

LPL is expressed in heart, muscle and adipose tissue and hydrolyses triglycerides carried by chylomicrons and very low-density lipoproteins (VLDL), producing free fatty acids for tissue cells. No other metabolic pathway can substitute for the LPL-catalysed lipid hydrolysis, rendering proper LPL functionality essential for energy production. In this pathway, LPL adds phospholipid, free cholesterol and apolipoproteins from the surface of TGRL to the circulating HDL pool (Fig. 18.1). Indeed, apoA-I and several other apolipoproteins readily move between lipoprotein particles and can be exchanged between TGRL and HDL. These processes are active in the both post- and interprandial phases, providing a quantitatively major contribution to HDL-C. Consistently, LPL activity is strongly correlated with HDL-C concentrations in healthy individuals [36].

Remodelling and Catabolism

Remodelling and catabolism of HDL are ensured by interactions with cellular receptors, transporters, plasma proteins and enzymes. Mature spherical HDL can further grow via efflux of cellular cholesterol and phospholipid through *ATP-binding cassette transporter G1 (ABCG1;* Fig. 18.1) [37]. In addition, cells possessing

scavenger receptor class B type I (SR-BI) can transfer membrane cholesterol to spherical HDL in a process of passive diffusion according to concentration gradients (Fig. 18.1) [38].

In the circulation, HDL is extensively remodelled by lipid transfer proteins. Plasma *CETP* primarily transfers cholesteryl ester from mature spherical HDL to apoB-containing particles, particularly VLDL, in exchange for triglyceride [39]. As a result of CETP activity, HDL becomes depleted of cholesteryl ester and enriched in triglyceride, which accelerates HDL catabolism (Fig. 18.1). *PLTP* converts spherical HDL into both larger and smaller particles as well as transfers to HDL phospholipids which are removed from apoB-containing lipoproteins during lipolysis by LPL [39].

Both surface and core lipids of HDL are extensively modified by lipases. *HL* breaks down primarily tri- and diglycerides but also phospholipids of HDL, resulting in the reduction of HDL particle size (Fig. 18.1). *Endothelial lipase (EL)* primarily hydrolyses HDL phospholipids but also triglycerides. Upon interaction with the surface of hepatocytes, cholesteryl esters are transmitted from cholesterol-rich HDL to the liver by their selective uptake via *SR-BI* (Fig. 18.1). The activity of SR-BI in the selective cholesterol uptake leads to the formation of small, dense HDL particles depleted of cholesterol. Albeit extensively studied, this pathway represents a minor route of cholesteryl ester elimination from the circulation in humans, whereas the transfer of cholesteryl ester to apoB-containing lipoproteins by CETP with subsequent hepatic uptake of LDL quantitatively prevails [6]. Finally, large apoE-containing HDL can be internalized via interaction with apoB/apoE receptors [40].

Following removal through the liver, HDL-derived cholesterol is secreted into the bile [41]. In a non-biliary pathway, direct excretion of cholesterol occurs in the proximal small intestine. The major sites of catabolism of the protein HDL components are the liver and the kidney [6]. HDL particles can be removed from the circulation by holo-particle HDL receptors, such as cubilin present in kidney proximal tubules and the ectopic β-chain of ATP synthase at the surface of hepatocytes (Fig. 18.1).

Biological Activities of Normal Functional HDL

Plasma HDL displays multiple cardioprotective activities which may act cooperatively to ensure normal vascular function. However, it is challenging to determine their relative contributions to the vascular health. HDL is predominantly thought of as a lipid-carrying particle which is crucial in cholesterol clearance and metabolism [42]. Indeed, as peripheral mammalian cells cannot catabolize cholesterol to keep it at non-toxic levels, HDL can be essential for maintaining healthy levels of cholesterol in peripheral tissues through a pathway of reverse cholesterol transport (RCT), whereby HDL effluxes superfluous cholesterol from cells to transport it to the liver for excretion. However, diverse HDL components endow HDL with multiple cardioprotective activities beyond its role in RCT through inhibiting inflammation, thrombosis, cell death, acute-phase response and oxidative damage [6].

As a consequence, HDL particles display protective effects towards the endothelium. HDL-mediated removal of cellular cholesterol contributes to vasodilation. HDL particles equally protect the endothelium by suppressing superoxide production and expression of proinflammatory cytokines in both macrophages and endothelial cells [43]. Anti-thrombotic actions of HDL particles are largely attributed to their effect on platelets [44]. Through enhancing the production of nitric oxide and prostacyclin, HDL can attenuate platelet aggregation [43, 44].

Next, HDLs display anti-apoptotic effects, inhibiting apoptosis of endothelial and vascular smooth muscle cells on the one hand, and, on the other hand, enhancing their proliferation and migration [43]. SR-BI-initiated signalling pathway plays an important role in these protective effects.

HDLs beneficially influence insulin sensitivity. This effect may be underlain by enhanced glucose uptake in adipose tissues and accelerated insulin secretion from the pancreas [45].

HDL may protect against infections, such as via LBP and a specific apoL-I-containing lytic

factor for Trypanosoma brucei [6]. This role of HDL is consistent with the hypothesis that HDL has evolved as a component of innate immunity [46].

As biological activities of HDL are defined by its composition, revealing relationships between composition, structure and function of HDL is critical for our understanding of HDL biology. ApoA-I provides a key contribution to multiple biological activities of HDL, which involve lipid binding [47]. Other apolipoproteins, including apoA-II, apoA-IV, apoC-I, apoE and apoM, can also ensure cellular cholesterol efflux. Several HDL apolipoproteins, particularly apoE, apoA-IV, apoA-V, apoJ, apoC-I, apoC-II, and apoC-III, display antioxidative activity, whereas apoJ and apoE protect endothelial cells from dysfunction and apoptosis. Cholesterol efflux capacity and cholesterol transport to the liver for excretion are largely related to proteins harboured by HDL [6].

HDL particles are heterogeneous in structure and composition; as a consequence, their biological activities equally differ across HDL subpopulations. Importantly, small, dense, protein-rich HDL display potent anti-atherogenic activities, consistent with their distinct content of multiple protein and lipid components [26].

Abnormal HDL Metabolism

Epidemiology of HDL

Altered HDL metabolism is manifested in abnormal levels of HDL-C, which can be either reduced or elevated as compared to healthy subjects. Low circulating levels of HDL-C are widely accepted as a strong and independent predictor of CVD as shown in large-scale cross-sectional and prospective studies, including the Framingham Heart Study and the PROCAM Study [48, 49]. Low HDL-C is a risk factor for multiple vascular diseases, including myocardial infarction, peripheral artery disease, ischemic stroke and deep vein thrombosis [6]. The inverse relationship between HDL-C and CV risk persists across multiple populations, ethnicities and disease states. Reflecting such robust data, HDL-C levels are included in all major algorithms to estimate CV risk, such as the Framingham risk prediction tool, the PROCAM score and the SCORE approach.

Low HDL-C levels are frequently observed in general populations and persist despite statin treatment [6]. By contrast, extremely high HDL-C is a rare condition; this observation hampered large-scale epidemiological studies of high HDL-C levels. Recently, several studies reported deleterious role of extremely high HDL-C for overall and CV mortality, resulting in the U-shape of the relationship between CV risk and HDL-C [50–52].

Epidemiologic studies of HDL are however limited by extensive confounding. Indeed, HDL-C levels are strongly linked to obesity, insulin resistance, exercise, and alcohol consumption, all of which feature elevated plasma triglyceride levels. It is important in this regards that genetic epidemiology does not necessarily support causal relationships between HDL-C and CV risk. Indeed, no association between HDL-C levels and CVD was observed in a large study using Mendelian randomization [53], suggesting that low HDL-C is not causatively related to atherosclerosis [54].

Measurement of apoA-I constitutes an acceptable alternative to the use of HDL-C in assessing CV risk. Evidence supporting the preferential use of apoA-I over HDL-C is however not yet conclusive [55].

Circulating concentrations of HDL subpopulations can also be employed to evaluate CV risk. Thus, both HDL2- and HDL3-cholesterol constitute strong predictors of coronary heart disease [16]. Plasma levels of large α1-HDL are consistently associated with protection from atherosclerosis [16]. Similarly, levels of large HDL measured by NMR typically display negative correlations with CV risk [16]. In addition, elevated levels of apoC-III-containing HDL may represent an important determinant of CVD [56]. However, clinical value of HDL subpopulations relative to that of plasma HDL-C remains to be firmly established.

Pathways of Abnormal HDL Metabolism

Studies of both rare genetic disorders and genetic associations are useful for identifying factors involved in HDL metabolism. HDL-C concentrations are under strong genetic control; effects of the majority of common gene variants on HDL-C are small, whereas effects of some rare variants can be pronounced.

Low HDL-C States

Homozygous or compound heterozygous *apoA-I deficiency* is a rare condition which results in complete absence of apoA-I from plasma accompanied by a marked decrease in HDL-C and increased risk of premature CVD [57]. Subjects with heterozygous forms of apoA-I deficiency feature plasma HDL-C and apoA-I levels that are about 50% of normal. HDL biogenesis is disrupted in apoA-I deficiency as a result of abnormal HDL production and deficient LCAT activation by apoA-I (Fig. 18.1). Remarkably, some rare variants of apoA-I, including apoA-I Milano and apoA-I Paris, are paradoxically associated with low HDL-C levels and reduced risk of CVD paralleled by greater longevity [58].

Most known *ABCA1 mutations* result in Tangier disease associated with the deficiency in cellular cholesterol efflux to lipid-free and lipid-poor apolipoproteins (Fig. 18.1) [59]. Low HDL-C is a common characteristic of ABCA1 deficiency, frequently resulting in elevated CV risk [59].

Naturally occurring mutations in LCAT are another common cause of low plasma HDL-C (Fig. 18.1) [60]. *Familial LCAT deficiency* results from the complete loss of LCAT activity, while *fish-eye disease* is associated with a change in the substrate specificity of LCAT that becomes inactive towards HDL, while retaining its cholesteryl ester-generating activity towards apoB-containing lipoproteins. The latter property can be important in accelerating atherosclerosis in fish-eye disease relative to familial LCAT deficiency [61]. In these diseases, plasma HDL-C and apoA-I levels are reduced, while plasma-free cholesterol is elevated.

Genetic defects of LPL may lead to hypertriglyceridemia and low HDL-C [62]. Thus, familial *LPL deficiency* is a rare disorder characterized by severe hypertriglyceridemia and marked reductions in HDL-C and LDL-C levels. Reduced LPL activity contributes to HDL-C lowering by reducing the availability of surface constituents of TGRL (Fig. 18.1) [63].

Genetically determined *elevated CETP activity* leads to decreased concentrations of HDL-C and hypertriglyceridemia (Fig. 18.1) [64]. HDL metabolism is substantially altered in dyslipidemic states of *hypertriglyceridemia* and *insulin resistance*, reflecting rapid removal from the circulation of small HDL particles which result from the intravascular lipolysis of triglyceride-enriched HDL. In *hypercholesterolemia*, abnormalities in HDL metabolism are primarily observed as moderate reductions in plasma apoA-I and HDL-C levels.

Finally, decrease in circulating HDL-C levels, increase in triglyceride levels and HDL enrichment in SAA at the expense of apoA-I are typical components of *inflammatory states* and the acute phase reaction [65].

High HDL-C States

Mutations that reduce CETP activity lead to elevated plasma HDL-C (Fig. 18.1) [66]. Homozygous *CETP deficiency* can be associated with complete loss of CETP activity, leading to the accumulation of large, cholesteryl ester-rich HDL and elevation of HDL-C levels up to fivefold, which do not reduce CV risk. By contrast, common CETP genotypes, including TaqIB, I405V and 629C > A, associated with lower CETP activity and higher HDL-C levels are inversely related to coronary risk [66]. However, concomitant reductions in apoB-containing lipoproteins (which may also occur in other states of abnormal HDL metabolism) complicate proper evaluation of the role of HDL-C raising in CETP deficiency. Loss-of-function *mutations in SR-BI* similarly result in markedly increased HDL-C levels (Fig. 18.1) which may [67], or may not [68], be associated with elevated risk of CVD.

Loss-of-function *mutations in apoC-III* lead to increased HDL-C and reduced triglyceride and LDL-C concentrations [69]. These effects are associated with reduced risk of CVD [69, 70].

Complete *HL deficiency* is a rare autosomal recessive condition resulting in elevated plasma concentrations of HDL-C and apoA-I with the accumulation of large HDL [62]. *EL deficiency* may equally induce hyperalphalipoproteinaemia [62]. However, HDL-C raising resulting from such mutations is not necessarily atheroprotective [53, 71].

Altered Structure, Composition and Function of HDL

All major atheroprotective functions of HDL, including cholesterol efflux capacity as well as other activities, can become deficient under conditions favouring accelerated development of CVD. Such proatherogenic conditions primarily involve atherogenic dyslipidaemia, insulin resistance, inflammation and infection, which often, but not always, feature low HDL-C levels [6]. Under these conditions, structure and particle profile of HDL are modified in such a way that functionally deficient small particles possessing abnormal composition and conformationally altered apoA-I preferentially accumulate at the expense of their large counterparts [72].

The capacity of the plasma HDL pool to remove cholesterol from peripheral cells can be reduced as a result of diminished circulating levels of HDL particles (i.e., diminished HDL quantity). The link between reduction in plasma HDL levels, impairment of normal clearance of cholesterol from the arterial wall and acceleration of atherosclerosis was proposed by Miller and Miller in 1975 [4]. An assay that quantifies clinically relevant functional properties of HDL (i.e., diminished HDL quality) rather than its plasma concentrations can however be more appropriate for the evaluation of CV risk. A considerable body of evidence points to cellular cholesterol efflux from macrophages as a biomarker of beneficial effects of HDL on CV health [73, 74]. The negative relationship between cholesterol efflux capacity of HDL and both the presence of CVD and the risk of future CV events is frequently independent of HDL-C concentrations [73, 74], additionally suggesting that low levels of HDL-C can represent a crude biomarker of impaired HDL function rather than be causally related to CVD.

Monogenetic forms of low HDL-C dyslipidaemia, such as apoA-I or LCAT deficiency, can be equally characterized by the presence of HDL with defective intrinsic cholesterol efflux capacity. HDL particles are also deficient in antioxidative activity in dyslipidemic states involving low HDL-C levels, often in association with insulin resistance [72].

Altered composition of HDL, primarily depletion of apoA-I paralleled by its oxidation and glycation as well as alterations in the HDL lipidome, typically underlies functional deficiency of HDL [6]. Furthermore, functionally relevant alterations in the HDL proteome occur under pro-atherogenic conditions [75], while inflammation-induced modifications of HDL composition may further contribute to HDL dysfunction [76]. In particular, SAA can replace apoA-I in HDL, attenuating anti-inflammatory properties of the lipoprotein, whereas deficiency in PON1, PAF-AH and/or LCAT can contribute to the impairment of HDL capacity to reduce oxidation [76]. In addition, HDL glycome can be altered under pathological conditions, bearing a potential to aggravate functional HDL deficiency. Such alterations may include desialylation which decreases size and negative charge of HDL and alters interactions with lipases and cellular proteins, resulting in diminished cholesterol efflux capacity and reduced LCAT activity [29].

Importantly, clinical relevance of impaired antiatherogenic activities of HDL in cardiometabolic diseases largely remains indeterminate. Indeed, the very concept of HDL dysfunction has been developed using ex vivo assays; it is unclear whether HDL particles are also dysfunctional in a setting of a living organism.

Therapeutic Correction of Abnormal HDL Metabolism

Reflecting growing knowledge of HDL, development of HDL-targeting therapeutics has been concentrated around several major directions including inhibition of CETP, infusions of rHDL, activation of LPL and use of nicotinic acid, in addition to a broad variety of other strategies (Table 18.1) (Fig. 18.1).

CETP Inhibition

The first clinical trial attempting to intervene into this pathway of HDL metabolism was conducted with *torcetrapib* which was administrated to patients prone to coronary events whose HDL-C levels were increased by +72% and LDL-C levels decreased by −25% [77]. Unexpectedly, both overall and CV mortality were significantly elevated in the treatment arm relative to placebo.

Table 18.1 Overview of HDL-targeting therapies

Type of therapeutics	Drug (developer)	Properties	Status
CETP inhibitors	Torcetrapib (Pfizer)	CETP inhibitor	Failed in phase III trial owing to excess all-cause mortality, abandoned
	Dalcetrapib (Hoffman La Roche)	Weak CETP inhibitor	Failed in phase III trial owing to lack of efficacy, development stopped
	Evacetrapib (Eli Lilly)	Potent CETP inhibitor	Failed in phase III trial owing to lack of efficacy, development stopped
	Anacetrapib (Merck)	Potent CETP inhibitor	Successful phase III trial but development stopped owing to relatively weak clinical efficacy and drug accumulation in adipose tissue
HDL infusions	MDCO-216 (The Medicines Company; former ETC-216)	Recombinant apoA-I Milano complexed to a phospholipid	Under development, showed efficacy and safety in phase I trials
	CSL111 (CSL Behring)	Purified human plasma apoA-I reconstituted with a phospholipid	Successful clinical trial but development discontinued (see CSL112)
	CSL112 (CSL Behring)	Purified human plasma apoA-I reconstituted with a phospholipid	Superseded CSL111, safe and efficacious. Ongoing clinical trial AEGIS-II in ACS patients
	CER-001 (Cerenis)	Recombinant apoA-I complexed to sphingomyelin and a phospholipid	Did not reduce total atheroma volume in CVD patients in phase III trial but reduced carotid mean vessel wall area in HoFH patients in phase III trial
Delipidated HDL		Lipid-poor HDL produced by selective delipidation of native human HDL subsequently used for autologous reinfusion	No current development reported
ApoA-I mimetics	APP018 (D-4F, Bruin Pharma, Novartis)	Oral APOA1 mimetic peptide. Nascent HDL formation, cholesterol efflux, antioxidative and anti-inflammatory activities	Inconsistencies between in vitro and in vivo functional properties, no current development reported
Recombinant LCAT	ACP-501 (AlphaCore Pharma recently acquired by MedImmune)	Recombinant human LCAT	Successfully tested in phase I trial, currently in phase II trials

(continued)

Table 18.1 (continued)

Type of therapeutics	Drug (developer)	Properties	Status
LPL activators	Pemafibrate (Kowa Pharmaceuticals)	PPARα agonist, pleiotropic effects involving upregulated production of apoA-I and apoA-II	Successful phase III clinical trials
	Volanesorsen (apoC-III ASO, Ionis, Akcea)	Inhibitor of apoC-III production	Development halted due to safety concerns
Niacin	Nicotinic acid, vitamin B3	HDL-C raising and triglyceride-lowering effects, prolonged exposure may lead to diabetes	Failed to produce clinical benefits in combination with statins. No current development reported
BET inhibitors	Apabetalone (RVX-208, Resverlogix Corp.)	Upregulation of hepatic secretion of apoA-I, anti-inflammatory actions	Undergoing clinical trials
LXR agonists		Upregulation of ABCA1 and ABCG1 expression	Several specific LXRα-targeting formulations under development
FXR agonists		Acceleration of cholesterol excretion via HDL	Efficiency disputed, reduced HDL-C despite increased faecal cholesterol excretion
miR-33 antagonists		Upregulation of ABCA1 and ABCG1 expression	Efficiency in humans unclear

This deleterious effect was proposed to reflect off-target hypertensive effects of the drug [78].

Dalcetrapib, the second CETP inhibitor which entered clinical trials, raised HDL-C by +35% without affecting LDL-C levels. The development of the drug was discontinued due to the lack of positive effects on overall and coronary events–associated mortality [79]. The increase in HDL-C was suggested to be too modest for potential clinical benefits.

Evacetrapib, another CETP inhibitor, was investigated in high-risk patients with vascular disease and did not result in a lower rate of CV events, whilst showing large increases in HDL-C (+133%) and reductions in LDL-C (−31%) concentrations [80].

Anacetrapib, the most recent addition to the class of CETP inhibitors, was thoroughly tested for safety and was found to be clinically effective, decreasing incidence of major coronary events in the absence of increased risk of death, cancer, or other serious adverse events [81]. The beneficial effect of the drug was however modest (−9%) despite large increase in HDL-C (+104%); in addition, anacetrapib was found to accumulate in adipose tissue [82] and its development was stopped. The clinical benefit of anacetrapib could be accounted for by the reduction in non-HDL-C, and more specifically, small VLDL levels, suggesting little clinical role of the HDL-C raising [82]. Therefore, despite considerable initial promise, CETP inhibition provides insufficient CV benefit for clinical use.

rHDL Infusion

The first drug of this class entering clinical trials was *ETC-216* comprised of apoA-I Milano protein supplied with a phospholipid [83]. The infusions of ETC-216 were able to decrease the mean atheroma volume in patients with acute coronary syndrome (ACS) [83]. However, the drug was not pushed into further clinical development primarily due to high production costs compared to its moderate clinical benefit. Furthermore, ETC-216 was responsible for a dose-dependent increase in neutrophils [84].

Seeking improvement in safety and efficacy, a formulation called *MDCO-216* comprised of apoA-I Milano and phosphatidylcholine was introduced [85] which did not induce adverse immunostimulation [84], but neither showed effectiveness in a clinical setting [86].

The initial success with the trials of ETC-216 was followed by the development of *CSL-111* consisting of normal human apoA-I combined with soybean phosphatidylcholine. Short-term infusions of CSL-111 did not reduce atheroma volume and were slightly hepatotoxic at high concentrations, but they did improve the plaque characterization index and coronary score [87]. The drug was reformulated as *CSL-112* that did not contain hepatotoxic cholate and was essentially homogeneous in the particle size. In a phase II trial, CSL-112 was well tolerated and acutely enhanced cholesterol efflux in patients with CVD [88], probably acting via remodelling the circulating HDL pool through formation of small particles possessing enhanced anti-atherogenic activities [89].

Another infusion agent named *CER-001*, a negatively charged rHDL containing human recombinant apoA-I, sphingomyelin and phosphatidylglycerol, was somewhat efficient in patients with extreme conditions including homozygous familial hypercholesterolemia [90] and extensive plaque-burdened ACS [91], but was eventually proven to be ineffective in patients with more typical presentation of ACS [92, 93].

A modification of HDL infusion therapy involved reinfusion of autologous *delipidated HDL* after their selective delipidation ex vivo [94]. This approach converted large, lipid-rich HDL to small, lipid-poor particles with enhanced atheroprotective activities [26] and tended to reduce total atheroma volume after seven weekly injections in ACS patients undergoing cardiac catheterization as compared to control plasma apheresis treatment [94].

ApoA-I-mimetic peptides structurally resemble apoA-I in the way that they all contain amphipathic α-helical structures albeit often do not share any sequence homology to apoA-I yet possessing similar biological function [95]. These agents can beneficially impact HDL metabolism upon infusion. Unlike recombinant or purified apoA-I proteins, apoA-I mimetics are short-chain and easy to produce. Moreover, they can be administered orally, though their oral bioavailability is limited [96]. Initial reports were promising on the effectiveness of these agents in animal models, but a recent report of aggregated results shows inconsistencies between in vitro and in vivo functional properties of seven apoA-I mimetic peptides [97].

Another therapeutic strategy features infusions of *recombinant LCAT*, a single HDL protein. A formulation called *ACP-501* favourably modified HDL metabolism after a single intravenous infusion [98]; an approach involving multiple intravenous infusions is currently being assessed in a phase 2 trial.

To sum up, none of the HDL infusions has proven to be both effective and safe up to date; furthermore, intravenous infusions appeared to be too impractical for everyday therapy.

LPL Activation

Upregulation of LPL activity is normally considered as a means to reduce plasma triglycerides but this approach is highly effective in elevating HDL-C levels and can be added to the list of HDL-targeting therapies. ApoC-III represents a promising therapeutic target for the treatment of hypertriglyceridemia, as it inhibits TGRL hydrolysis by LPL [99]. Recent phase I trial of the administration of *volanesorsen*, an apoC-III antisense oligonucleotide in healthy subjects revealed reductions in plasma apoC-III and triglyceride levels accompanied by elevated HDL-C [99]. This approach is currently entering clinical trials [100].

Fibrates is a class of compounds that work by activating peroxisome proliferator-activated receptor α (PPARα). PPARα is active in hepatocytes where it stimulates production of apoA-I and apoA-II and inhibits production of apoC-III, thereby enhancing LPL activity [101]. As a result, fibrates increase concentrations of HDL-C and small pre-β1-HDL. Fibrates are normally considered for treatment of hypertriglyceridemia and provide moderate clinical benefits [102] but their side-effects include formation of cholesterol gallstones, rhabdomyolysis and myopathy. A novel member of this drug class, pemafibrate, did not show major adverse effects and recently passed phase 3 of clinical trials [103].

Another approach to combat hypertriglyceridemia employs an adenoviral vector *AMT-011* expressing human LPL, with an intent to replace a faulty LPL gene with a properly functioning copy [104]. If successful, this strategy should equally be able to increase HDL-C.

Nicotinic Acid

In the pre-statin era, *nicotinic acid*, also known as niacin and vitamin B3, was considered as a promising candidate to prevent CVD, reflecting its ability to raise HDL-C, decrease triglycerides, alleviate carotid intima-media thickening and reduce incidence of stenosis [101]. However, recent large-scale studies performed in patients on statins failed to demonstrate clinical benefits of niacin treatment [105, 106]. In addition, Cochrane meta-analysis of long-term clinical trials did not find evidence for niacin's efficiency in patients already treated with statins [107]. Prolonged exposure to niacin was also found to be associated with increased risk for onset of diabetes [107]. Despite these limitations, nicotinic acid may still be helpful for individuals not tolerating statins. Indeed, a meta-analysis reveals that niacin is capable of reducing CV events in the absence of statin treatment [108].

Other Agents

Other agents employed within the paradigm of HDL-targeting therapy can be classified into bromodomain and extra-terminal domain (BET) inhibitors, nuclear receptors agonists and miR inhibitors.

BETs are protein-interaction modules involved in chromatin organization and modulation of gene transcription. *Apabetalone (RVX-208)*, a small *BET inhibitor* designed to directly upregulate hepatic secretion of apoA-I, moderately raised HDL-C and showed promising results in the early stages of clinical trials [109], but later failed to present significant clinical benefits compared to placebo [110]. In recent trials, vulnerability of atherosclerotic plaques was however favourably modified by apabetalone [111]. Pooled together, results from available clinical trials of apabetalone reveal a reduction in CV events relative to placebo [112].

Nuclear receptor agonists are represented by those acting on liver X receptor (LXR) and farnesoid X receptor (FXR). *LXR agonists* are activated by oxidized sterols, raise HDL-C and exert a multitude of antiatherogenic actions including beneficial effects on cholesterol metabolism, inflammation, proliferation and insulin sensitivity [113]. Major adverse effects of LXR agonists however involve hepatic steatosis and nervous system deficiency [114]. There are two LXR types, notably LXRα expressed in the liver, kidney, and several other organs, and LXRβ expressed ubiquitously [115]. Importantly, specific activation of LXRα receptors retains beneficial effects on HDL whilst lacking lipogenic effects and may represent a promising strategy of dyslipidaemia treatment.

FXR agonists are bile acid-activated nuclear receptors implicated in the regulation of cholesterol metabolism [116]. FXR activation reduces lipid synthesis and storage and ameliorates insulin resistance. As a result, FXR agonists increase faecal cholesterol excretion and reduce intestinal cholesterol reuptake, positively influencing net cholesterol metabolism. The major side effects of FXR activation are represented by the inhibition of bile acid synthesis and reduction of HDL-C [117].

*MiR*s are small RNAs that do not code proteins, and, hence, were earlier considered as 'junk RNA' to be later discovered to play important biological role in the development, repair and homeostasis. HDL transports miRs, one of which, miR-33, inhibitsABCA1 expression [118, 119]. Although animal models support the role of miR-33 as a potential therapeutic target for HDL biogenesis, the efficacy of this approach in humans remains to be established.

Conclusions

The research of HDL passed through a full cycle of discovery, data accumulation, drug development and clinical application. Yet after almost a

century of studies, the available knowledge is still controversial. Most importantly, it remains unclear why HDL-targeting therapies do not provide expected clinical benefits despite robust epidemiological findings linking low HDL-C to CVD.

The simplest explanation is that HDL-C-raising resulted in deleterious off-target effects as observed with torcetrapib which increased blood pressure in atherosclerotic patients [77]. Other therapies did not however reveal major adverse effects, discounting this possibility. It is also possible that potential clinical benefits were abolished by the statin treatment. Indeed, all recent studies of HDL-targeting agents were performed in patients treated by statins which markedly modify lipoprotein metabolism. For example, statin therapy interferes with ABCA1-mediated macrophage cholesterol efflux and may thereby diminish anti-atherogenic function of HDL [120]. Furthermore, combined exposure to gene variants that encode targets of CETP inhibitors and statins was associated with discordant reductions in LDL-C and apoB levels and a corresponding risk of CV events that was proportional to the attenuated reduction in apoB but was significantly less than expected per unit change in LDL-C, weakening potential benefits of CETP inhibition [121].

Another possibility involves confinement of benefits to specific subgroups. Thus, patients homozygous for a variant in the ADCY9 gene responded positively to dalcetrapib and experienced a significant reduction in CV event under the drug [122]. In the torcetrapib trials, patients with highest HDL-C on-treatment levels were less likely to suffer major CV events or death, or to display atherosclerosis progression [123]. In the AIM-HIGH niacin trial, a significant 33% decrease in primary events in the niacin group beyond that conferred by statins alone was observed in patients with elevated triglyceride and reduced HDL-C levels [105]. Given that thorough subgroup analyses were performed in the large-scale studies, this possibility still looks remote.

Accumulation of dysfunctional HDL particles was invoked as another explanation of the failure of HDL-targeting therapeutics. It is however highly unlikely that mechanistically distinct HDL-C-raising agents all result in the formation of dysfunctional HDL. Consistent with this notion, available data do not detect dysfunctional HDL on treatment [124].

Given the high heterogeneity of HDL particles, it is possible that clinical studies did not target right HDL populations. Indeed, while therapeutic HDL-C-raising predominantly increases levels of large HDL, elevated anti-atherogenic activities are ascribed to small, dense, protein-rich particles [26]. Data directly supporting this hypothesis is however lacking.

The recent discovery of the U-shape relationship between HDL-C and CVD [50, 51] suggest an intriguing possibility that HDL-C-raising achieved on treatment was excessive. Epidemiology of genetically determined extremely high HDL-C is in part consistent with this suggestion which requires rigorous testing.

The last possibility is that HDL-C is not causatively related to CVD and represents a biomarker for elevated triglycerides [54], implying that HDL-C-raising per se is meaningless. In support of this hypothesis, Mendelian randomization studies often do not detect causal relationships between HDL-C and CVD [54]. Complex metabolic network underlying HDL metabolism is however not taken into account by these considerations.

Following the largely disappointing results of the clinical studies, the focus of HDL therapeutics is now shifting from the mere increase in HDL-C concentrations to the normalization of cardioprotective HDL functionality, primarily of cellular cholesterol efflux capacity of HDL. This shift of paradigm implies that a continuous, slow, steady-state flux through the dynamic RCT pathway is more important for the relationship between HDL-C and CVD than static levels of HDL-C, rendering HDL-C of secondary clinical significance [73, 74]. However, key metabolic links between HDL-C and triglycerides [54] are eloquently absent from this hypothesis, clearly demonstrating that HDL remains an exceedingly intriguing therapeutic target to reduce CV risk despite decades of intense research. Ongoing

controversies between classic epidemiology, genetic epidemiology and clinical trials need to be urgently resolved in order to allow further development of HDL-targeting therapies.

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Lipoprotein (a): Principles from Bench to Bedside

19

Marlys L. Koschinsky and Michael B. Boffa

Lipoprotein(a) in Context

Although lipoprotein(a) (Lp(a)) was first reported in 1963 as an antigenic variant of low-density lipoprotein (LDL) [1], there remain many outstanding questions regarding the role of this enigmatic lipoprotein in health and disease. It has become clear, however, that Lp(a) is the single most prevalent genetically determined risk factor for coronary heart disease (CHD) [2, 3]: elevated plasma Lp(a) levels (above 50 mg/dL; 100 nmol/L) occur in approximately 20% of the global population [4]. Challenges in Lp(a) measurement led to results from several large prospective trials in the early 1990s that cast doubt on the predictive value of Lp(a) for CHD risk [5–7]. However, elegant genetic studies in 2009 using both Mendelian randomization and genome-wide association studies (GWAS) demonstrated almost conclusively that Lp(a) is a causal risk factor for CHD [8, 9]. This served to propel the field forward by recognizing the importance of elevated Lp(a) as a contributor to the disease rather than merely a passive marker of the disease process.

A major breakthrough in understanding the role of Lp(a) in atherosclerosis was the demonstration that Lp(a) is a preferential carrier of oxidized phospholipid (oxPL) species compared to low-density lipoprotein (LDL) [10]. oxPL species were previously recognized as potentially proatherogenic, reflecting their highly proinflammatory properties [11, 12]. Delivery, deposition, and preferential retention of Lp(a) at the sites of growing lesions provide a mechanism to deliver oxPL to this milieu, thereby increasing local inflammation [13]. Indeed, many of the proposed proinflammatory responses arising from oxPL-modified Lp(a) overlap with proatherogenic properties of Lp(a) [14]. Of note, the identification of Lp(a) as a strong risk factor for the progression of calcific aortic valve disease also hinges on the proinflammatory properties of this lipoprotein [15, 16]. The role of Lp(a) in hemostasis, owing to the high degree of similarity of its distinguishing apolipoprotein(a) (apo(a)) component to that of the fibrinolytic proenzyme plasminogen [17], has been less clear *in vivo*, and may reflect a more nuanced effect in this complex process [18].

Lp(a) presents challenges for both researchers and clinicians. For bench researchers, studies of the function of Lp(a) have been hampered owing to the lack of a physiologically relevant animal

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_19

model [19]. This reflects the interesting evolutionary history of Lp(a) in which it is only found in Old World Monkeys, apes, and humans [20]. From the viewpoint of practicing clinicians, challenges related to the lack of widespread availability of a standardized assay for Lp(a) measurement, coupled with the lack of direct evidence that lowering Lp(a) is of clinical benefit, make Lp(a) a difficult parameter to include as part of a standard CHD risk assessment. However, the development of new pharmaceutical approaches designed to specifically lower Lp(a) through significant reduction in its hepatic apo(a) production are on the horizon [21]. The next decade of Lp(a) research will see definitive answers on the benefit of Lp(a)-specific reduction on CHD events, coupled with ever-increasing advances in our understanding of fundamental Lp(a) biology and pathophysiology.

Structure–Function Relationships in Lp(a)

Lp(a) is a unique lipoprotein class that consists of two components: an LDL-like particle containing apolipoproteinB-100 (apoB-100) and a single molecule of apolipoprotein(a) (apo(a)) which is covalently linked to apoB in the Lp(a) particle by a single disulfide bond [22] (Fig. 19.1). The lipid

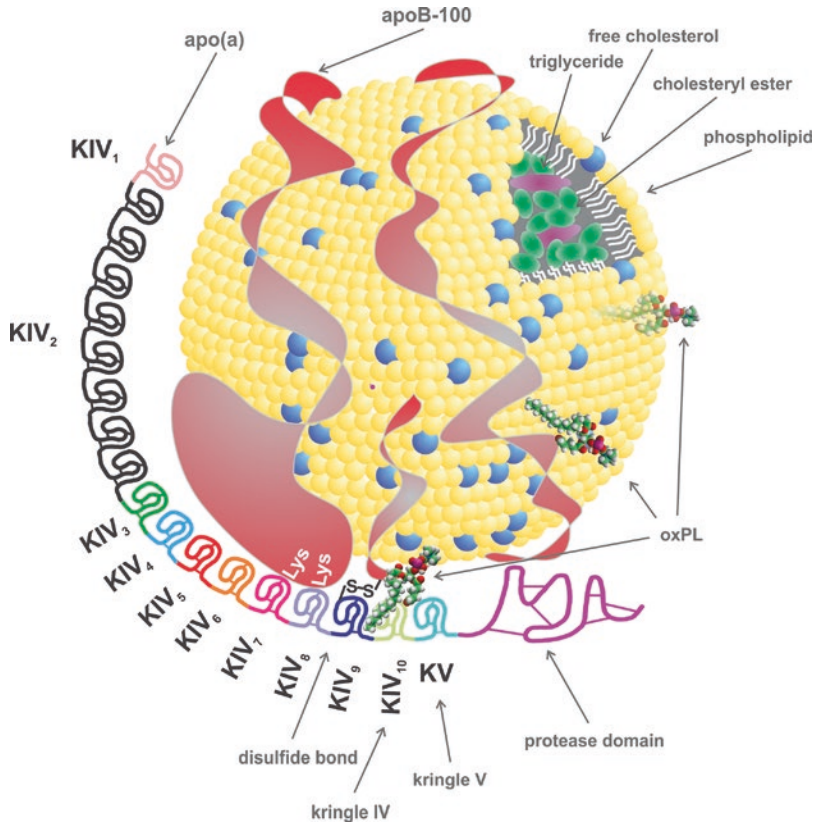

Fig. 19.1 Structure of Lp(a). Lp(a) consists of an LDL-like particle which consists of a core of neutral lipids (cholesteryl esters and triglycerides) surrounded by an amphipathic shell of phospholipids and free cholesterol as well as a single molecule of apoB-100. The apoB-100 component is covalently linked to the unique glycoprotein apo(a) by a single disulfide bond. Apo(a) consists of repeated structural domains called kringles, with multiple copies of kringle IV (KIV) and a single copy of a kringle V (KV). Assembly of Lp(a) requires an initial noncovalent interaction between lysine binding sites on KIV_7 and KIV_8 with specific lysines in the amino-terminus of apoB-100. Also depicted is the presence of oxPL on Lp(a), which can be covalently linked to apo(a) or apoB-100, or present within the lipid moiety

composition of the LDL component of Lp(a) is generally similar to that of LDL [23], although the lipidome of Lp(a) has not been extensively studied.

Apo(a) is the hallmark of Lp(a) structure and contains numerous copies of a kringle domain (KIV) that is very similar to the sequence of plasminogen kringle 4, followed by sequences with a high level of similarity to the corresponding kringle 5 and protease domains of plasminogen [17] (Fig. 19.2). There are 10 types of KIV domains in apo(a) [24]: sequence differences between these domains result in unique features including a strong lysine-binding site in KIV type 10 [25], as well as weak lysine-binding sites in each of KIV types 5–8 [26–28] (Fig. 19.2). Some of the weak lysine binding sites have been found to be critical in the noncovalent binding between apo(a) and apoB-100 [29–33], with KIV type 9 containing the free cysteine that is required for disulfide bond formation with apoB in the covalent Lp(a) particle [22] (Figs. 19.1 and 19.2). There has been a great deal of focus on the apo(a) KIV type 2 domain, which is present in a variable number of identical copies encoded by *LPA* – the gene that encodes apo(a) – that gives rise to Lp(a) isoform size heterogeneity in the population [34] (Fig. 19.2). The number of identically repeated copies of KIV_2 varies from 3 to greater than 30, resulting in large variations in the size of the apo(a) component of Lp(a). This size variability is also found in apo(a) from Old World Monkeys and apes which are the only other species that contain *bona fide* Lp(a) particles [35–37]. The functional significance of small versus large Lp(a) isoforms with respect to relative contributions to Lp(a)-associated risk is unclear and remains an active area of research interest [38–40].

The strong lysine binding site in apo(a) KIV_{10} (Fig. 19.2) has been proposed to facilitate the interaction of Lp(a) with cellular receptors and biological substrates such as fibrin [41, 42]. The presence of this binding site in apo(a) has long been considered a mechanism whereby Lp(a) can interfere with the functions of plasminogen in fibrin clot breakdown [43, 44], especially given the lack of a functional protease domain in apo(a) [45] (Fig. 19.3). This mechanism has been difficult to demonstrate *in vivo*, where the

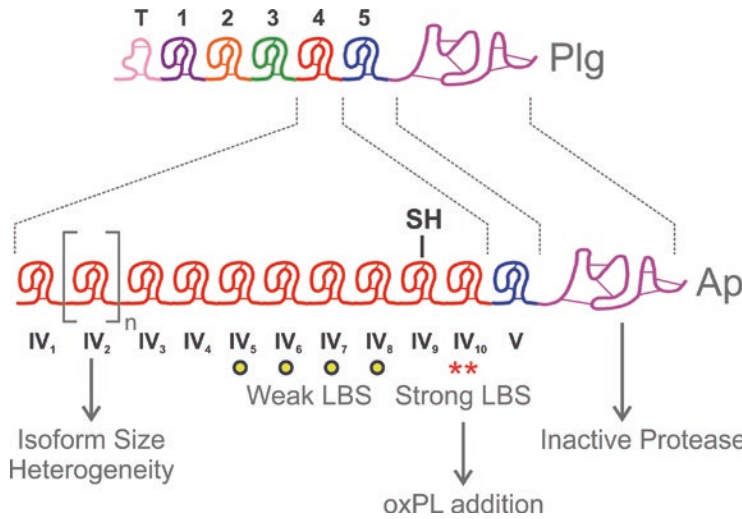

Fig. 19.2 Structural organization of apo(a). Apo(a) is the product of the gene *LPA*, which arose from duplication of the gene encoding plasminogen (Plg). Plasminogen consists of an amino-terminal tail domain (T), five kringles (1–5) and a trypsin-like protease domain. Apo(a) lacks the tail domain and kringles 1–3 and consists of multiple copies of a kringle 4-like sequence (IV_1 to IV_{10}), a kringle 5-like sequence (KV), and an inactive protease domain. KIV_2 is present in different number of copies based on differences in the number of KIV_2-encoding exons in *LPA*, and accounts for Lp(a) size heterogeneity seen in the population. KIV_5 to KIV_8 contain weak lysine binding sites, while KIV_{10} contains a strong lysine binding site and harbors the oxPL covalently attached to apo(a)

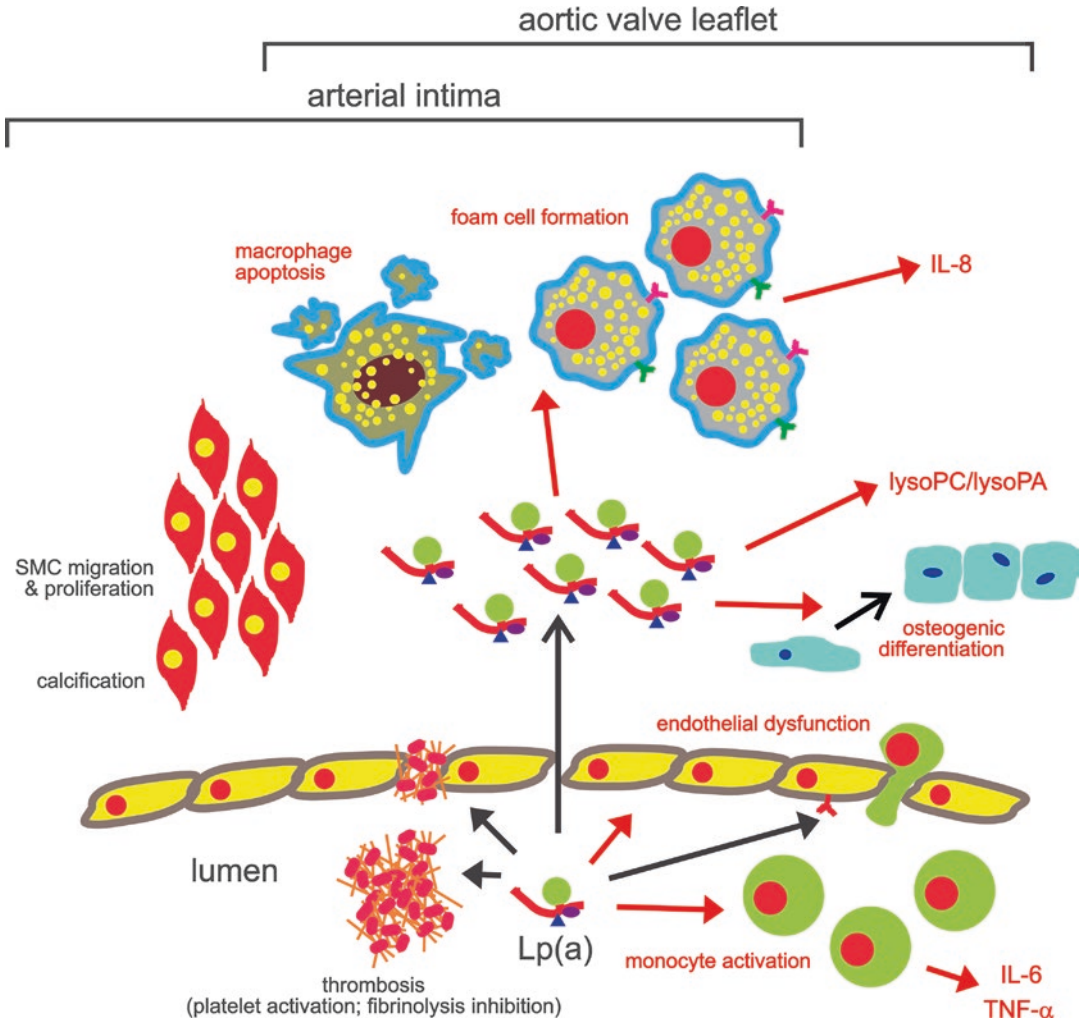

Fig. 19.3 Pathogenic mechanisms of Lp(a). Lp(a) promotes endothelial damage by causing mural thrombosis and endothelial dysfunction; the latter promotes Lp(a) passage through the protective layer of endothelium. Lp(a) can also activate monocytes in the circulation. The roles of Lp(a) in the arterial intima in atherosclerosis and in the valve interstitium in the aortic leaflet are overlapping and involve promotion of lipid deposition, inflammation and calcification. The activities in red have been shown to be mediated by oxPLs on apo(a)/Lp(a). IL interleukin, lysoPC lysophosphatidylcholine, lysoPA lysophosphatidic acid, SMC smooth muscle cell, TNF tumor necrosis factor. (From Boffa and Koschinsky [161])

role of Lp(a) in the inhibition of blood clot breakdown or the promotion of a prothrombotic state remains unclear [18, 46–48]. However, it has been recently shown that the presence of a strong lysine binding site in apo(a) KIV_{10} is an absolute requirement for the covalent addition of oxidized phospholipid in this kringle [49] (Fig. 19.2). This, in turn, has been suggested to enable a proinflammatory role for Lp(a) that hints to the role of this lipoprotein in disease [14] (Fig. 19.3). For example, a recombinant form of apo(a) (17K)) containing covalently linked oxPL in KIV_{10} has been shown to upregulate IL-8 expression in cultured human monocytes *in vitro* [50], to facilitate the upregulation of levels of IL-6 and TNF-α in peripheral blood monocyte cultures primed with β-glucan and 17K apo(a) [51], and to induce osteogenic differentiation of valvular interstitial cells [52] (Fig. 19.3). In all cases, the stimulatory effect of

the 17K apo(a) was blunted by the disabling of the strong lysine binding site in KIV_{10}.

Compared to plasminogen, apo(a) is extensively glycosylated (approximately 28% carbohydrate by weight [23]) with both *N*- and *O*-linked glycans associated with each kringle domain and interkringle sequence, respectively [17, 23, 53]. The extent of glycosylation of apo(a) is heterogeneous even within a given individual. The significance of this extensive carbohydrate modification to apo(a)/Lp(a) function remains unclear at this time.

Variation in Plasma Lp(a) Levels in Human Populations

Plasma Lp(a) levels vary dramatically within the population. There is a more than 1000-fold difference between the highest and lowest levels, which range from <1 to greater than 250 mg/dL, and Lp(a) levels are highly positively skewed [54]. Lp(a) levels are primarily genetically determined, with estimates of heritability topping 90% [55, 56], and hence are relatively stable within an individual during their lifetime [57]. The main driver of this is sequence variation is within *LPA* itself, and in turn the major determinant within *LPA* is variation in the number of KIV_2-encoding exons between different alleles [58–60]. There is a general inverse correlation between *LPA* gene size (and hence apo(a) protein size) and Lp(a) levels [61]. Additional genetic variants, both inside and outside *LPA*, have been reported to impact Lp(a) levels [57]. The strong inheritance of Lp(a) levels has allowed ground-breaking genetic studies that have definitively established the role of Lp(a) as a cardiovascular risk factor [8, 9, 62] while also confounding attempts to lower Lp(a) levels through healthy behavioral interventions such as diet and exercise or conventional lipid-lowering therapy [57].

Interestingly, the distribution of Lp(a) levels and the relationship between *LPA* allele size and Lp(a) levels differs between certain ethnic groups, reflecting differences in *LPA* genetic architecture between these groups [56, 59, 60]. Most striking, people of sub-Saharan African descent have higher median Lp(a) levels, a much less skewed distribution of levels, and higher Lp(a) levels associated with medium-sized *LPA* alleles compared to the global population [63]. On the other hand, people of Chinese or Japanese descent have lower Lp(a) levels and an increased preponderance of large *LPA* alleles while South Asians have Lp(a) levels intermediate between Caucasians and Africans [57]. The extent to which these differences might underlie population differences in cardiovascular risk or Lp(a)-attributable cardiovascular risk remains unclear.

Lp(a) as a Risk Factor for Vascular Disease

Coronary Heart Disease

An exhaustive number of studies since the 1970s have shown that elevated plasma Lp(a) (greater than 30–50 mg/dL; 75–100 nmol/L) is a risk factor for CHD. Within the past decade, the large meta-analysis performed by Erqou and colleagues (reporting on 126,634 subjects corresponding to 36 studies from 1970–2009) reported that Lp(a) is an independent risk factor for CHD and ischemic stroke, with a continuous relationship to risk beginning at Lp(a) levels of ~30 mg/dL [64]. A subsequent meta-analysis showed that having a small Lp(a) isoform doubled the risk for CHD [38]. Kamstrup and colleagues reported the results of a Mendelian randomization study, using the Copenhagen City Heart Study and Copenhagen General Population Study, showing the association of genetically elevated Lp(a) levels (as predicted by quartiles of KIV_2-encoding repeats in the genome) with increased risk for myocardial infarction [8]. Also in 2009, Clarke and colleagues reported the identification of the *LPA* locus as the strongest predictor of CHD in a genome-wide association study [9]; furthermore, using the PROCARDIS cohort, they showed that two *LPA* single nucleotide polymorphisms (SNPs) (rs10455872 and rs3798220) predicted elevated Lp(a) levels and increased CHD risk in an additive fashion [9]. Although these three landmark studies are often cited, there are many

additional publications that support an independent, and possibly causal role for Lp(a) in CHD risk [65].

Most studies of Lp(a) as a cardiovascular risk factor have been conducted in primarily Caucasian subjects. As Lp(a) concentrations and the genetic architecture differs substantially between ethnic groups, it has been of interest to assess if Lp(a)-attributable risk similarly varies. A recent study in a multiethnic population found that Lp(a)-attributable risk for myocardial infarction is highest in South Asians, Southeast Asians, and Latin Americans, but is absent in Arabs and Africans [4]. However, the power to detect an association between Lp(a) and myocardial infarction was low for Africans in this study; indeed, previous studies showed a similar association between Lp(a) levels and CVD events in whites and blacks [66]. A very recent report from the Multi-Ethnic Study of Atherosclerosis (MESA) found that the contribution of Lp(a) to risk for carotid atherosclerosis was higher than for whites than blacks, and borderline for Hispanics [67, 68].

Secondary Prevention

Although the risk for Lp(a) in primary prevention settings is well established, the role of Lp(a) in secondary prevention remains controversial [69]. A related issue is the question of whether Lp(a) can confer residual risk upon aggressive lowering of LDL. There are a number of studies to support a role for Lp(a) as a risk factor in the context of low (less than 70 mg/dL) LDL [70, 71]. Indeed, there is evidence for residual risk for clinically elevated Lp(a) even in the context of extreme lowering such as can be obtained in the era of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Recent outcome data from PCSK9 inhibitor trials suggest that Lp(a) levels in the highest quartile (>165 nmol/L) predict risk for a composite endpoint of coronary heart disease death, myocardial infarction, or urgent revascularization [72]. Moreover, in the entire study population of 25,096 subjects, those with the lowest achieved levels of both Lp(a) and LDL-cholesterol (LDL-C) had the lowest risk [72]. As this large study enrolled patients only with preexisting CHD, it is very strong evidence that Lp(a) is an important risk factor in secondary as well as primary prevention.

In a recent study by Willeit and colleagues, data from seven randomized, placebo-controlled, statin outcomes trials were harmonized to calculate hazard ratios (HRs) for cardiovascular events, defined as fatal or nonfatal coronary heart disease, stroke, or revascularization procedures [73]. HRs for cardiovascular events were estimated within each trial across four Lp(a) groups (15 to <30 mg/dL, 30 to <50 mg/dL, and ≥50 mg/dL, vs <15 mg/dL), prior to pooling estimates using multivariate random-effects meta-analysis. Initiation of statin therapy reduced LDL cholesterol (mean change −39% [95% confidence interval (CI)-43 to -35]) without a significant change in Lp(a) [73]. Associations of baseline and on-statin treatment Lp(a) with CVD risk were approximately linear, with increased risk at Lp(a) values of 30 mg/dL or greater for baseline Lp(a) and 50 mg/dL or greater for on-statin lipoprotein(a) [73]. This study clearly shows that Lp(a) is an independent risk factor for CVD, with residual risk attributable to Lp(a) observed in the context of LDL-lowering with statins. Importantly, Lp(a) was a significant risk factor at all levels of LDL-C, clearly indicating that addressing risk attributable to high Lp(a) cannot be mitigated solely by lowering LDL-C [73].

Peripheral Arterial Disease

Several studies have shown that elevated Lp(a) levels are a risk factor for peripheral arterial disease (PAD). In the Linz Peripheral Arterial Disease (LIPAD) study, 213 Austrian patents with PAD were matched to controls [74]. Lp(a) concentrations above the 75th percentile (OR 3.73), as well as low molecular weight apo(a) isoform size (OR 2.21) were significant predictors of PAD. These observations were confirmed in other studies including the Cardiovascular Disease in Intermittent Claudication (CAVASIC) [75] as well as MESA [68]. The prospective risk

of elevated Lp(a) for the development of PAD has been reported in several studies including EPIC-Norfolk (European Prospective Investigation of Cancer-Norfolk) [76], with a twofold risk observed in the highest Lp(a) quartile compared to other quartiles (adjusted HR 2.06); this was unchanged by adjustment for LDL-cholesterol and other potential confounders and exceeded that observed for coronary artery disease (CAD) (adjusted HR of 1.33). Additionally, it has been reported using a Spanish cohort of 1503 outpatients with symptomatic PAD followed for 3 years that in patients with established PAD, elevated Lp(a) concentrations are a risk factor for subsequent arterial events (MI, stroke, limb amputation) and mortality [77]. Most recently, large GWAS analysis in the Million Veteran Program has shown a strong role for elevated Lp(a) in PAD [78]. In this study, 32 million DNA sequence variants with PAD (31,017 cases and 211,753 controls) were tested; these included veterans of European, African, and Hispanic ancestry. The results were replicated using 5117 PAD cases and 389,291 control samples from the UK biobank. In these, 19 PAD loci (18 of which are novel) were reported: 11 of the loci (including *LPA* and *LDLR*) were correlated with disease in coronary, cerebral, and peripheral vasculature [78]. Four of the loci were specific for PAD including the Factor V Leiden variant (*F5* p.R506Q), suggesting a potential role for thrombosis in the disease pathology in the peripheral vasculature. Of note, the *LPA* gene was the strongest predictor of PAD relative to all other genes identified [78]. The mechanism of action of Lp(a) as a strong, specific predictor of PAD risk requires further study, including the potential link to thrombotic events.

Ischemic Stroke

Compared to the relationship between Lp(a) and CHD risk, the role of elevated Lp(a) levels as a risk factor for stroke remains less clear [64, 66, 76, 79, 80]. Although a strong association exists between Lp(a) and ischemic stroke in children [81–85], the association is less consistent in adults. Additionally, data related to the underlying cause of ischemic stroke (embolic, lacunar, or atherothrombotic) is not always reported, making interpretation of a role for Lp(a) as a risk factor for stroke challenging. A recent large meta-analysis by Nave and colleagues has shed some light on the association of Lp(a) with ischemic stroke [80]. A total of 20 studies were included in the meta-analysis, with a total of 90,904 subjects and 5029 stroke events. The pooled estimated odds ratio (OR) for 11 case-control studies comparing high versus low Lp(a) levels was 1.41 (95% CI, 1.26–1.57), while the pooled estimate for relative risk (RR) was 1.29 (95% CI, 1.05–1.58) for nine prospective studies; interestingly, ischemic stroke subtype contributed to observed heterogeneity in the analyses and individuals with a mean age less than 55 had an increased RR in the prospective studies [80]. These observations are notable given that cryptogenic stroke accounts for ~40% of ischemic stroke events in this age group and may be linked to as-yet unidentified risk factors such as elevated Lp(a).

A recent publication from Langsted and colleagues [86] provides new information and exciting directions for future research on the role of Lp(a) in ischemic stroke. Using the Copenhagen General Population study (CGPS; 49,699 individuals) and the Copenhagen City Heart Study data (CCHS; 10,813 individuals), these investigators showed increased causal risk for stroke in in the CGPS for individuals with Lp(a) levels in the 96th to 100th percentile was 1.6 (CI: 1.24–2.05); the highest absolute 10-year risk for elevated Lp(a) was observed in individuals over 70 years old who also had additional risk factors of hypertension and active smoking. Of note, the elevated LDL-C was associated with a greater risk for stroke in younger individuals compared to the >70 year old group with Lp(a) in the top 5%; the latter showed a comparatively minor contribution of LDL-C to ischemic stroke risk [86]. Unlike younger populations whose stroke events are most commonly linked to coronary atherosclerosis, a larger proportion of ischemic strokes in older age patients are lacunar infarcts, caused by arteriolosclerosis (small vessel disease) in the brain [87]. The mechanism underly-

ing the observations of Langsted clearly requires further study. Additionally, more extensive study of the relationship between elevated Lp(a) in non-Caucasian populations should be pursued. For example, increased Lp(a) levels may be a greater risk factor for stroke in blacks [66, 88].

Calcific Aortic Valve Disease

Studies over the past decade have established elevated Lp(a) as an important inherited risk factor for calcific aortic valve disease (CAVD) and its clinical manifestation as aortic stenosis (AS). CAVD is a distinct pathophysiological process from atherosclerosis, although it does share some features and risk factors [89] (Fig. 19.3). The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium was the first to show that a SNP in the *LPA* gene associated with elevated plasma Lp(a) levels (rs10455872) was the only one of 2.5 million SNPs considered that was associated with CAVD and the need for aortic valve replacement in multiple cohorts [62]. This seminal study spawned a considerable body of evidence indicating that elevated Lp(a) is a causal risk factor for AS [90]. The contribution of Lp(a) to the process is likely mediated in large part by the oxPL modification of apo(a) which can cause proinflammatory, proapoptotic and procalcific effects in the valve [14] (Fig. 19.3). This has been recently demonstrated in an important study by Zheng and colleagues [52] in which they showed the ability of Lp(a) as well as a 17 kringle-containing recombinant apo(a) (17K) to cause osteogenic differentiation of cultured vascular interstitial cells through alterations in gene expression; increased expression of proinflammatory IL-6 was also observed (Fig. 19.3). These effects were mitigated by either the addition of an antibody that blocks the covalent oxPL in Lp(a) (E06) or use of a 17K variant that lacks covalently linked oxPL [52]. The causal role of oxPL in AS has been demonstrated in a nested case-control study of the Copenhagen General Population Study which showed that levels of oxPL/apoB (largely representing oxPL on Lp(a)), oxPL/apo(a), and Lp(a) levels predicted AS risk at a level comparable to that observed for *LPA* genotype [91]. In patients with preexisting disease, it has been shown in a follow-up analysis of the Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin (ASTRONOMER) trial that elevated Lp(a) levels as well as measures of oxPL/apoB and oxPL/apo(a) in the highest tertile were strong predictors of progression rate of the disease as well as the need for valve replacement over a median 3.5-year follow-up [92].

Lipoprotein(a) in Familial Hypercholesterolemia

Whether or not Lp(a) is specifically elevated in familial hypercholesterolemia (FH) patients has been a point of controversy, with elevated Lp(a) observed in some kindreds but not others [93–96]. The largest of these studies, conducted in 1960 patients with FH and 957 relatives without FH in the Spanish Familial Hypercholesterolemia Cohort Study (SAFEHEART), found that median Lp(a) was significantly higher in FH 23.6 mg/dL (intraquartile range (IQR) 9.6–59.2) versus 21.0 mg/dL (IQR 7–47.2) and that Lp(a) levels ≥50 mg/dL were significantly more common in FH (29.3% versus 22.2%) [93]. Regardless, there is strong evidence to suggest that elevated Lp(a) levels contribute to atherothrombotic CVD (ASCVD) events in patients with genetic FH. In a recent study [97], family members from 755 index FH cases enrolled in SAFEHEART were tested for elevated Lp(a) (greater than 50 mg/dL) and genetic FH. Over 5 years, it was found that FH and elevated Lp(a) each contributed to ASCVD and risk for death (HR 2.47; $p = 0.036$, and 3.17; $p = 0.024$ respectively), with the greatest risk seen in relatives with both FH and elevated Lp(a) (HR 4.40; $p < 0.001$). These findings mirror previous cross-sectional studies in the SAFEHEART population [93], where not only were Lp(a) levels significantly elevated in patients with receptor-negative mutations in LDLR, but Lp(a) > 50 mg/dL was also an independent risk factor for CVD in these patients.

The Role of Lipoprotein(a) in Thrombosis and Hemostasis

The extensive homology between apo(a) and plasminogen reported in 1987 [17] has suggested a role for Lp(a) in promoting thrombosis through inhibition of fibrinolysis (Fig. 19.3). Despite strong evidence of an antifibrinolytic effect of apo(a) from *in vitro* and animal model studies [98–100], there is a paucity of direct evidence for the significance of this effect in cardiovascular disease in humans [18]. In the event of a myocardial infarction or ischemic stroke, it is difficult to disentangle the effects of Lp(a) on the acute thrombosis from on the underlying atherosclerosis. Notably, however, elevated Lp(a) does not appear to impact the efficacy of thrombolytic therapy [101, 102]. One study using Doppler ultrasound imaging of the carotid arteries found an effect of elevated Lp(a) on the extent of stenosis and occlusion (presumably reflecting ongoing plaque rupture and thrombosis) but not on total plaque area [47]. This result could also be compatible with elevated Lp(a) promoting more vulnerable plaque features. It should be noted that inherited thrombophilia itself (for example Factor V Leiden) is not a strong risk factor for ASCVD [103], further calling into question the relevance of the antifibrinolytic effects of Lp(a)/apo(a).

Indeed, many studies have examined if elevated Lp(a) is a risk factor for venous thromboembolism. While some smaller early studies provided conflicting results [18], the more recent, larger meta-analyses found a small but significant increased risk for higher Lp(a) levels [104, 105] while Mendelian randomization studies did not find elevated Lp(a) to be a significant risk factor [46, 106, 107]. Yet, in studies of pediatric stroke, also a "pure" thrombotic disorder in that underlying atherosclerosis is not a factor, elevated Lp(a) is a consistent risk factor (see above) and in this population also interacts with inherited thrombophilia [82, 85, 108]. Clearly, the question of the impact of Lp(a) on coagulation and fibrinolysis in the context of ASCVD and pediatric stroke requires further mechanistic studies – potentially in animal models – if the goal is to develop a therapeutic to impede the harmful effects of Lp(a).

Regulation of Lp(a) Production and Catabolism: Current State of Knowledge

Studies from the Rader laboratory established that plasma Lp(a) levels are determined primarily at the level of Lp(a) production rather than catabolism [109, 110]. Biogenesis of Lp(a) encompasses the following processes: transcription of the *LPA* gene, protein translation and movement through the secretory pathway, and the assembly of Lp(a) particles (Fig. 19.4). Each process is potentially regulated, thereby contributing to circulating levels of plasma Lp(a).

As outlined above, the major genetic determinant of plasma Lp(a) levels is *LPA* allele size. The inverse relationship between isoform sizes and levels of plasma Lp(a) has been demonstrated to arise, at least in part, from differences in the secretion efficiency of different apo(a) isoforms (Fig. 19.4). Biochemical experiments in cultured cell models have indicated that larger apo(a) isoforms are less readily secreted from hepatocytes and undergo more extensive intracellular degradation, likely explaining the observations in human populations [111, 112]; these findings have recently been corroborated by *in vivo* metabolic studies in humans [113]. However, additional possible mechanisms of regulation of Lp(a) biosynthesis by *LPA* allele size, such as the efficiency of Lp(a) assembly, have not been studied.

Our current understanding of Lp(a) assembly is that it is a two-step process: The initial, non-covalent step requires lysine binding sites within apo(a) KIV types 7 and 8 [32, 33], which bind to specific lysines in the amino-terminal domain of apoB [33] (Fig. 19.1). Following this, covalent bond formation occurs between apo(a) and apoB100, likely catalyzed by a specific oxidase-like activity [114].

While the biochemical details of the non-covalent and covalent Lp(a) assembly steps are well-understood, what remains in dispute is the location of these events (Fig. 19.4). Until recently, it was generally accepted that Lp(a) was covalently assembled extracellularly from newly-secreted apo(a) and either newly-synthesized or

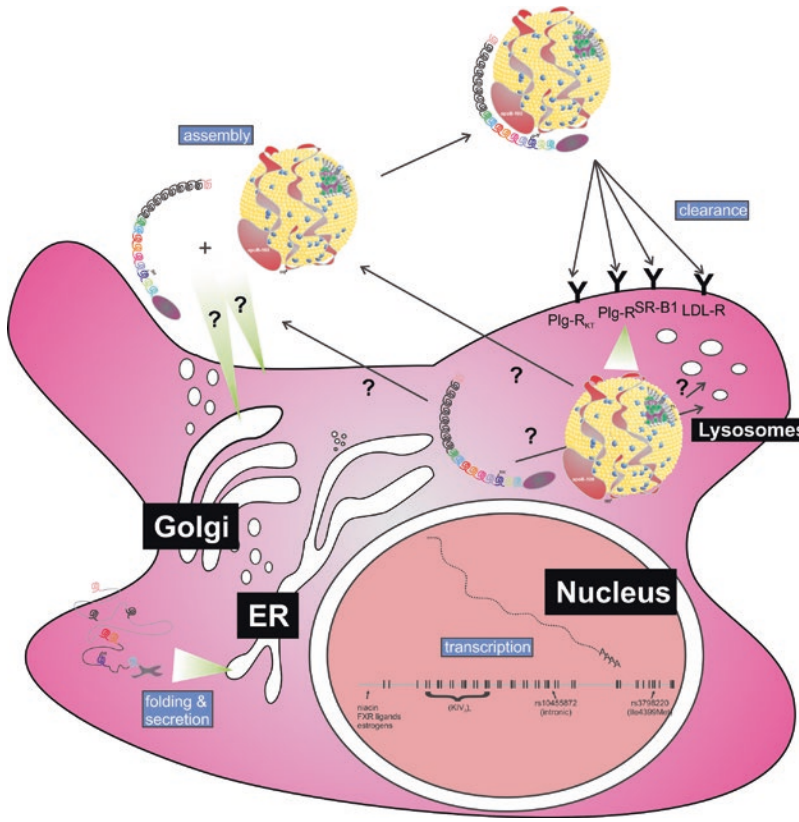

Fig. 19.4 Biosynthesis and catabolism of Lp(a). Apo(a) is encoded by *LPA*, and transcription of these gene can be controlled by niacin as well as FXR ligands and estrogens. Apo(a) biosynthesis is controlled also at the level of folding in the endoplasmic reticulum (ER) and subsequent secretion, with larger apo(a) isoforms being subject to increased presecretory degradation and hence less efficient secretion. Assembly of Lp(a) may occur either on the surface of the hepatocyte or within the secretory pathway, most likely from an apoB-100-containing lipoprotein particle specific for Lp(a), but also possibly from circulating LDL or from internalized and recycled apoB-100 or apo(a). Receptors for Lp(a) on hepatocyte appear to be Plg-R_{KT} and other plasminogen (Plg) receptors, SR-B1, and LDLR, of which all but SR-B1 lead to internalization of Lp(a) and its degradation (or recycling) in lysosomes

circulating LDL [22, 115, 116]. More recent *in vivo* kinetic studies in humans have come to varying conclusions, likely owing to differences in study design and analytic methodology; evidence for extracellular assembly of Lp(a), in part from circulating LDL [117–119], as well as almost entirely intracellular assembly [120] has been reported. In the latter case, however, it was not possible to rule out rapid Lp(a) formation from a specific pool of apoB100-containing protein on the cell surface or in the space of Disse. The identity of the apoB100-containing lipoprotein that covalently assembles with Lp(a) is currently unknown and could represent a special pool of apoB100-containing lipoprotein destined for Lp(a) [120]. Evidence has also been presented from *in vivo* kinetic studies for assembly of Lp(a) from either recycled apo(a) or apoB100 derived from internalized Lp(a) [118, 121, 122] (Fig. 19.4).

Until recently, Lp(a) was not considered to impact metabolism of any other lipoprotein classes. This changed with the report in 2016 of an extensive genome-wide study that identified Lp(a) as an important modulator of very low-density lipoprotein (VLDL) metabolism [123]. Specifically, the Lp(a)-raising rs10455872-G allele was strongly associated with smaller diam-

eter VLDL particles and with lower levels of extra-large, large, and medium-sized VLDL particles. Interestingly this association did not carry over to any measures of high-density lipoprotein (HDL), which often vary inversely with those of triglyceride-rich lipoproteins [124]. The Lp(a)-raising allele was also associated with larger diameter LDL particles [123]. These findings make sense in that the larger, more triglyceride-rich VLDL1 particles tend to give rise to larger amounts of small, dense LDL [125]. Crucially, Mendelian randomization analysis demonstrated that the effects of increased Lp(a) synthesis on VLDL metabolism were causal [123]. However, a molecular mechanism to explain this phenomenon remains lacking.

The primary pathway for removal of Lp(a) from the circulation remains unclear. Indeed, no definitive Lp(a) receptor has been identified [126]. Evidence has been provided for contributions from plasminogen receptors [127, 128], scavenger receptor type B1 [129], and members of the LDLR family including VLDLR (present only in very low levels on hepatocytes) [130], LRP1 [131], and LDLR itself [127, 132, 133] (Fig. 19.4). The last is particularly controversial with evidence both for [127, 132–134] and against [135–137] its involvement in Lp(a) binding and internalization published. The LDLR may only be relevant under conditions where plasma LDL levels are very low, LDL-receptor is upregulated, and Lp(a) levels are high; this has been demonstrated *in vitro* [132, 134] and using *in vivo* human kinetic studies [138, 139].

Pharmaceutical Approaches to Lowering Lp(a)

Until recently, there were no approaches to specifically lower Lp(a). Indeed, compounds such as niacin [140], the monoclonal antibody PCSK9 inhibitors alirocumab [141] and evolocumab [142], the microsomal triglyceride transfer protein (MTP) inhibitor lomitapide [143], cholesteryl ester transfer protein (CETP) inhibitors including anacetrapib [144], and the apoB antisense oligonucleotide mipomersen [145] all lower Lp(a) as well as LDL and in some cases also affect HDL and/or triglycerides as well. Additionally, the effects of these compounds on Lp(a) levels are quite modest, in the range of 20–30% lowering. Niacin failed to reduce ASCVD events in two large outcomes trials [146, 147] and hence is no longer recommended for Lp(a) lowering [148]. CETP inhibitors have been withdrawn from the approval process. The side effects profiles of lomitapide and mipomersen make them inappropriate for Lp(a) lowering in isolation. Although the PCSK9 inhibitors do not have a large effect on Lp(a), there is some evidence from cardiovascular outcomes trials that those with higher Lp(a) might derive additional cardioprotection from these agents [72, 149].

Lipoprotein apheresis, which is generally restricted to patients with homozygous FH or extreme hypercholesterolemia that does not respond to other treatments, is very effective in lowering Lp(a) as well as LDL (each by 60–75% acutely, with mean interval concentrations amounting to a 40% reduction [150]). However, this approach is expensive and invasive. In Germany, lipoprotein apheresis is approved for reimbursement in cases of isolated elevated Lp(a) where Lp(a) is >60 mg/dL [150]. In the USA, it is currently only used in specialized centers for extreme cases where significantly elevated Lp(a) levels are directly linked to recurrent events such as pediatric stroke [151]. Because lipoprotein apheresis lowers all classes of atherogenic lipoproteins, and because of ethical and practical issues in performing randomized controlled trials of this approach, it has been difficult to assess whether the dramatic reductions in event rates after initiation of apheresis can be attributed to Lp(a)-lowering [152–155].

The most recent and exciting development has been of antisense oligonucleotide drugs targeted at reducing apo(a) expression [21]. These compounds bind to *LPA* mRNA in the liver and cause degradation of the mRNA by RNase H1, thereby preventing its translation into protein and reducing Lp(a) biosynthesis. Two factors make these drugs exceptionally promising: first, they very dramatically reduce Lp(a) (more than 90% [156]); second, they are highly specific to Lp(a)

[156, 157] including reducing apoB-associated oxPL levels [157]. Thus, they promise to at last directly address in cardiovascular outcome trials the hypothesis that lowering Lp(a) prevents ASCVD events, and they are potent enough to reduce Lp(a) below the risk threshold in virtually every patient. The IONIS-APO(a)-L_{Rx} compound is the more potent of the two reported variants because of the presence of an *N*-acetyl galactosamine residue that targets the compound to the liver via the asialoglycoprotein receptor [21]. IONIS-APO(a)-L_{Rx}, now renamed pelacarsen, has been licensed by Novartis for the initiation of a phase 3 randomized controlled cardiovascular outcomes trial.

Current Thinking on the Use of Lipoprotein(a) in Clinical Practice

The National Lipid Association (NLA) has recently released a statement on Lp(a) [148]. It is a comprehensive document that summarizes the evidence base for Lp(a) as a risk factor for vascular disease and serves as a guideline for clinicians who seek to understand how to incorporate lipoprotein(a) into clinical practice. With respect to measurement, it is recommended that Lp(a) be measured, where possible, as particle concentrations (nmol/L) versus mass concentrations (mg/dL). The latter is a mass measurement that is biased by the size variability of the lipoprotein(a) particle, which is primarily dictated by isoform size heterogeneity of apo(a). It is not valid to use a factor to convert mg/dL measurements to nmol/L measurements, again due to the isoform size heterogeneity of Lp(a). Assays used should be isoform size independent and traceable to the World Health Organization/International Federation of Clinical Chemistry and Laboratory Medicine (WHO/IFCCLM) secondary reference material [158, 159]. Generally, Lp(a) levels above either 50 mg/dL or 100 nmol/L have been identified as reasonable values to identify individuals with clinically elevated Lp(a). It is important to note, however, that these values have been determined based largely on Caucasian populations; specific risk cut-offs for Lp(a) different ethnic groups have not been determined. Additionally, risk cut-offs for Lp(a) in the context of different disease states (e.g. FH, diabetes mellitus, renal disease) have not been established. It is recommended that the 50 mg/dL or 100 nmol/L cut-offs be tentatively applied to all populations [148].

Two International Classification of Diseases (ICD)-10 codes have been added for Lp(a) testing (E78.41 = elevated Lp(a) and Z83.430 = family history of elevated Lp(a)). The relative stability of Lp(a) levels over a lifetime supports the perspective that repeat measurement is generally unnecessary, provided that the initial blood sample was not obtained during an acute illness [57] or that Lp(a)-lowering drugs have been taken. Based on the 2019 NLA statement [148], measurement of Lp(a) is suggested for use for the following:

1. *To refine risk assessment for ASCVD events in adults with the following:*
   (a) First-degree relatives with premature ASCVD (<55 years of age in men or <65 years of age in women)
   (b) A personal history of premature ASCVD
   (c) Primary severe hypercholesterolemia (LDL-C ≥ 190 mg/dL) or suspected familial hypercholesterolemia
2. *To aid clinical decision-making in the following circumstances:*
   (a) To maximize management of modifiable ASCVD risk factors
   (b) To identify a possible cause for a less-than-anticipated LDL-C lowering to evidence-based LDL-C lowering therapy
   (c) For cascade screening of family members with severe hypercholesterolemia.
   (d) To identify those at risk for progressive CAVD

However, in light of the ever-increasing evidence of the significance of Lp(a) as a causal and independent risk factor for a variety of vascular diseases, interest in the universal measurement of Lp(a) is gaining momentum [87, 160]. At this time, there is no way to specifically lower Lp(a).

However, since Lp(a) levels are relatively constant throughout the lifespan, a strong argument can be made for the measurement of Lp(a) at least once in an individual's lifetime as a component of optimal CVD risk management. In cases where Lp(a) is known to increase such as in postmenopausal women [57], a second measurement in individuals close to the risk cut-off for Lp(a) should be considered.

Looking to the Future of the Lp(a) Field

Owing to the ongoing development of therapeutics designed to specifically lower Lp(a) and the imminent assessment of an apo(a) antisense oligonucleotide in a large phase 3 clinical trial, it will be possible, likely within the next 4–5 years, to address the Lp(a) hypothesis directly (i.e., that lowering Lp(a) will reduce cardiovascular events). In the interim, much work remains to be done to further our understanding of this fascinating lipoprotein [159]. Efforts to standardize and harmonize the measurement of Lp(a) will continue to be key, as well as determining ethnic group-specific and comorbidity-specific risk cut-offs for Lp(a). Additionally, there are still many gaps in our understanding of fundamental aspects of Lp(a) biology and mechanisms of its pathophysiology. Clearly, more work is required to fully understand the key regulatory steps in Lp(a) production and the route of its catabolism *in vivo*. These processes also need to be interrogated in pathophysiological conditions, and in response to lipid-lowering therapies. We continue to expand our understanding of the mechanism of Lp(a) pathogenicity at the molecular and cellular levels. There remain, however, questions about the nature of the contribution of Lp(a) to plaque vulnerability and consequent atherothrombotic events. As well, the relative contributions of Lp(a) to risk for vessel disease in different vascular beds (e.g. peripheral versus carotid and cerebral arteries) and the corresponding underlying mechanisms merit further investigation.

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Management of Homozygous Familial Hypercholesterolemia

20

Raul D. Santos

- Homozygous familial hypercholesterolemia (HoFH) is an autosomal codominant disease.
- HoFH affects between 1/300,000 and 1/1,000,000 in areas where founder effects are not present.
- HoFH is characterized by LDL-C usually >300–500 mg/dL, early onset xanthomata, diffuse atherosclerosis, and aortic and supra-aortic valve disease.
- Severity of the HoFH phenotype and complications depend on molecular defects mostly occurring on the LDL receptor.
- Cardiovascular complications of HoFH must be diagnosed early and managed accordingly.
- Lipid-lowering treatment must be started at time of diagnosis.
- High dose statin therapy and ezetimibe therapy alone are inadequate to control LDL-C in HoFH.
- PCSK9 inhibitors (Evolocumab) reduce LDL-C by 20% on average and response depends partially on LDL receptor expression.
- Lomitapide may reduce LDL-C up to 50% in HoFH and liver transplantation may be a therapeutic option.
- Lipoprotein apheresis when available is the treatment of choice in HoFH.
- Available treatments prolong life free of events in HoFH; however, early death is still frequent.

Introduction

Homozygous familial hypercholesterolemia (HoFH) is a devastating codominant autosomal disease affecting between 1/300,000 and 1/1,000,000 individuals in areas where founder effects are not encountered [1–4]. HoFH is much more frequent when a founder effect is present, for example, 1/30,000 and 1/275,000 as encountered respectively in Afrikaners and in French Canadians [5]. HoFH is characterized by extremely elevated plasma low-density lipoprotein-cholesterol (LDL-C) concentrations (usually >300–500 mg/dL or 7.7 and 12.8 mmol/L, respectively) and onset of cutaneous and tendinous xanthomas before 10 years of age. Furthermore, HoFH individuals usually develop diffuse atherosclerotic cardiovascular disease (ASCVD) mainly in the ostia of coronary arteries in association with aortic valve or supra-aortic valve disease occurring usually in the first and second decades of life [1, 5, 6]. In most situations HoFH is caused by loss of function variants in the LDL receptor gene (*LDLR*), seldom by defects in apolipoprotein B (*APOB*) or proprotein convertase subtilisin kexin type 9 (*PCSK9*) genes,

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_20

and even less frequently the phenotype can be caused by variants in the Low-Density Lipoprotein receptor adaptor protein 1 (*LDLRAP1*) gene (autosomal recessive hypercholesterolemia–ARH) [7, 8]. Phenotype severity depends mostly on the molecular defects, but it is gravest in those with two null alleles in the *LDLR* [7]. HoFH is usually refractory to standard pharmacological lipid-lowering therapies like high dose statins and ezetimibe and treatment with other medications like PCSK9 monoclonal antibody (evolocumab) and microsomal transfer protein (MTP) inhibitors (lomitapide) are necessary. Lipoprotein apheresis (LA) is the gold standard of HoFH treatment but, unfortunately, not available universally [8, 9]. Despite its severity and lack of adequate control of dyslipidemia, lipid-lowering therapy has increased event-free survival in HoFH individuals; however, ASCVD and especially aortic valve or supra-aortic valve disease still causes early morbidity and mortality in this disease.

Genetics of HoFH and Phenotypical Consequences

The severity of the HoFH phenotype (i.e., plasma LDL-C elevation and its consequences) depend on the genetic defects causing this disease as seen in Fig. 20.1. HoFH subjects develop the disease by a combination of inheritance of defects in canonical genes (i.e., *LDLR*, *APOB*, and *PCSK9*). FH individuals can be true homozygotes (having the same variant in both *LDLR* or *APOB* alleles), compound heterozygotes (having different pathogenic variants in the same gene, i.e., *LDLR* or *APOB* alleles one coming from each parent), or double heterozygotes having different genes causing the phenotype (e.g., *LDLR* and *APOB* or *LDLR* and *PCSK9* variants one coming from each parent). Approximately 95% of FH cases where a genetic defect is encountered are due to pathogenic variants in the *LDLR*. The latter can be classified as either defective (LDLR residual activity 2–25%) or null, also called negative, LDLR residual activity (LDLR activity <2%) [7]. Figure 20.1 shows that the most severe cases are due to combinations of null variants in either *LDLR* alleles, where less severe forms are caused by association of defective *LDLR* variants or when *APOB* and *PCSK9* genes are involved either alone or in double heterozygosity with the *LDLR*.

The HoFH phenotype can also be caused by defects in the *LDLRAP1* and affected individuals usually have a milder phenotype [10]. A similar phenotype to HoFH can be caused by pathogenic autosomal recessive variants in ATP Binding Cassette Subfamily G Member 5 (*ABCG5)* or ATP Binding Cassette Subfamily

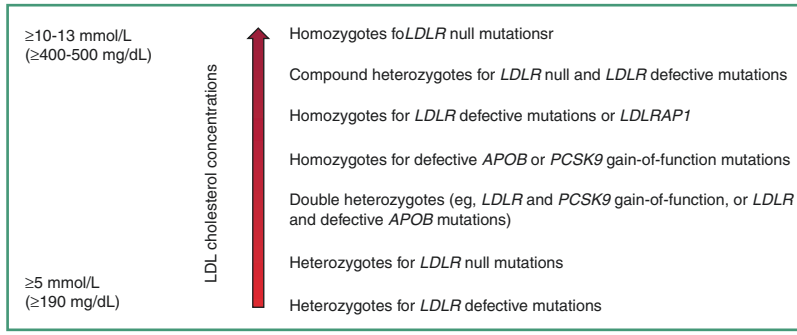

Fig. 20.1 Severity of FH phenotype and molecular defect. Severity of the FH phenotype depends mostly on genetic defects. LDL-C concentrations might overlap in individuals with different genetic defects. They might also vary according to the presence or absence of small-effect gene variants. Homozygotes have the same mutation in two alleles of the same gene. Double heterozygotes have different mutations, one on each allele of the same gene. Compound heterozygotes have mutations in two different genes. LDLR null mutations defined as LDL receptor activity <2% in fibroblasts. LDLR defective mutations defined as LDL receptor activity 2–25% in fibroblasts. (From Santos et al. [7]. With permission from Elsevier)

G Member 8 (*ABCG8*) genes as in β-sitosterolemia. Another phenocopy of HoFH, especially in children, can occur due to defects in the lysosomal acid lipase (LAL) gene (*LIPA*) [10, 11].

Severity of the genetic defect modulates not only the phenotype and ensuing cardiovascular risk, but also the response to pharmacological therapy [12, 13]. In 97 Spanish HoFH patients, all with molecular diagnoses, those with null *LDLR* alleles had higher LDL-C and developed ASCVD earlier than those with defective ones: 23 ± 19 years old versus 39 ± 11 years old, respectively [13]. Those with null alleles will also respond less to therapies that increase the expression of the LDLR like statins and PCSK9 inhibitors [7, 12, 14, 15]. These individuals will have a greater need to use medications that reduce Very Low-Density Lipoprotein (VLDL) or apoB production like lomitapide [16] or mipomersen [17]. On the other hand, individuals bearing defective alleles will have a greater response to statins [15] and PCSK9 inhibitors as seen in the TAUSSIG study with the PCSK9 inhibitor evolocumab [14, 18, 19] (Fig. 20.2).

Factors other than the genetic defects per se appear to influence the expression of LDLR and consequent response to lipid-lowering therapy in HoFH individuals [14]. Thedrez et al. showed that there were marked differences in LDLR expression at the lymphocyte surface of HoFH individuals bearing the same genetic defect [14]. Also, for the same molecular defect there were differences in expression of the LDLR after incubation with a PCSK9 inhibitor suggesting a modulatory role of nongenomic or post-translational factors [12]. However, how much other genes and epigenetics may influence the severity of the HoFH phenotype as it occurs in heterozygous FH is still a matter of investigation [20].

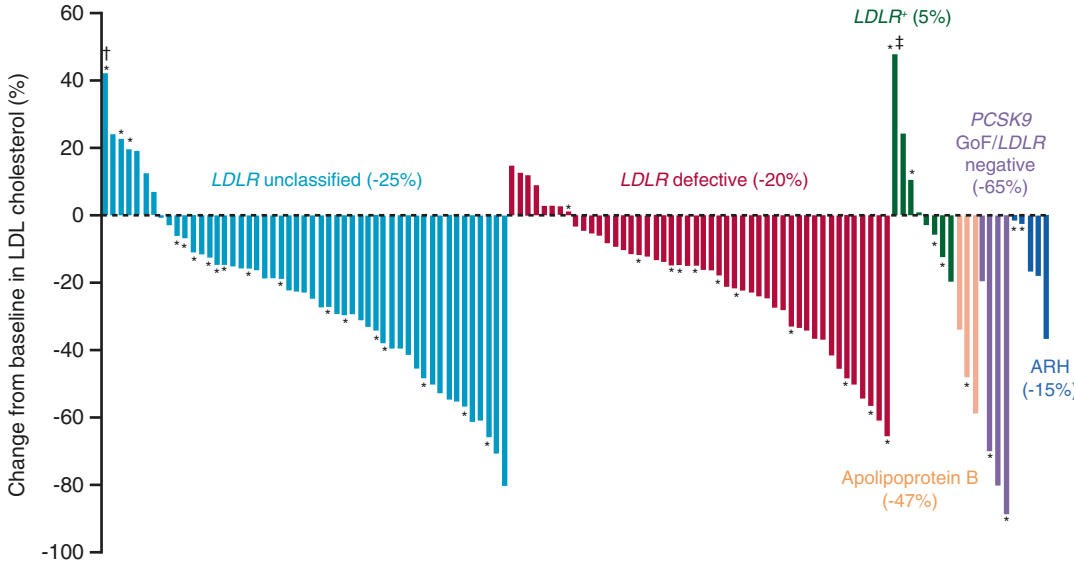

Fig. 20.2 Variability in response to PCSK9 inhibition and genetic defects in the TAUSSIG study. There is wide variability in the response to PCSK9 inhibitors in HoFH that in part depends on the type of genetic defect as shown in the TAUSSIG study [18]. Even in individuals with the same genetic defect the response is variable and in part explained by expression of the LDLR on hepatocyte cell surfaces [14]. Those bearing null/null variants on the *LDLR* will not respond. LDL cholesterol change from baseline to week 12, by underlying genetic abnormality. Mean change in LDL cholesterol is shown in parentheses after each genetic abnormality category. GoF gain of function. *Apheresis patient. †Patient missed apheresis before week 12 blood draw due to snowstorm. ‡Week 12 immediately after vacation; dietary indiscretion suspected. ARH autosomal recessive hypercholesterolemia. (Raal et al. [18]. With permission from Elsevier)

Diagnosis of HoFH and the Severe FH Phenotype

Table 20.1 shows two proposed clinical and genetic criteria for HoFH diagnosis. Clinically, HoFH is suspected by the presence of extremely high LDL-C concentrations usually >500 mg/dL (12.8 mmol/L) or >300 mg/dL (7.7 mmol/L), respectively, in individuals receiving or not lipid-lowering therapy, onset of cutaneous or tendinous xanthomas before 10 years of age, and when there is a history of high cholesterol in the parents and or consanguinity [5]. However, more recent evidence shows that HoFH despite being a severe disease is much more heterogeneous than previously thought. Raal et al. [21] described a group of 167 HoFH individuals mostly from South Africa and the Netherlands and confirmed previous Dutch data [2] showing great heterogeneity in LDL-C levels: ranging from 170–785 mg/dL (4.4–20.1 mmol/L) to 101–785 mg/dL (2.6–20.1 mmol/L) in those receiving or not lipid-lowering therapy. In this study, the subjects age ranged from 1 to 75 years old, and 21 (12.5%) patients were older than 50 years. In the cohort, 26.7% and 30.6% had respectively untreated and treated LDL-C < 500 mg/dL (12.8 mmol/L) and <300 mg/dL (7.7 mmol/L). Despite the important genetic influence on LDL-C levels, a great part of variability was not explained by the different genotypes. Considering this, the criteria described by Gidding et al. [6] and updated by Defesche et al. [8] proposed lower LDL-C thresholds (LDL-C > 400 mg/dL or >10.3 mmol/L) than previously done for clinical suspicion of HoFH [1, 5]. Actually, recent evidence shows an overlap of LDL-C levels between many homozygous and heterozygous FH individuals and those with LDL-C > 400 mg/dL (10.3 mmol/L), or >300 mg/dL (7.7 mmol/L) with an additional ASCVD risk factor or >190 mg/dL (4.9 mmol/L) with two other risk factors should be included in the so called severe FH phenotype [7]. Of course, the higher the LDL-C the greater the risk of ASCVD events. Molecular diagnosis should be offered to all suspected HoFH patients and their first-degree relatives [10].

Table 20.1 Proposed diagnostic criteria for homozygous familial hypercholesterolemia (HoFH)

Cuchel et al. [1]	Gidding et al. [6]
Genetic confirmation of two mutant alleles at the LDLR, APOB, *PCSK9*, or *LDLRAP1* genes Or Untreated LDL-C > 500 mg/dL (>13 mmol/L)[a] or treated LDL-C > 300 mg/dL (≥8 mmol/L)[a] together with either: Cutaneous or tendon xanthoma before age 10 years Or Untreated elevated LDL-C levels consistent with heterozygous FH in both parents	LDL-C > 400 mg/dL (>10 mmol/L) and one or both parents with clinically diagnosed FH or one or both parents with positive genetic testing for two identical (homozygous FH) or nonidentical (compound or double heterozygous FH) LDL-C-raising gene defects in *LDLR*, *APOB* or *PCSK9* or one or both parents with autosomal recessive hypercholesterolemia (ARH) HoFH is highly probable if the individual has LDL-C > 560 mg/dL (>14 mmol/L or LDL-C cholesterol >400 mg/dL (10 mmol/L) and aortic valve disease or xanthomas at <20 years of age. Occasionally, individuals with HoFH have LDL-C < 400 mg/dL (<10 mmol/L).

[a]These LDL-C levels are only indicative, and lower levels, especially in children or in treated patients, do not exclude HoFH

In addition to high cholesterol levels, HoFH patients usually present with cutaneous and tendinous xanthomas as well as corneal arcus and xanthelasmas of early onset (Fig. 20.3). Many will present with a systolic or diastolic heart murmur at the aortic region due to aortic valve disease (either stenosis or insufficiency) or supra-aortic valve stenosis.

It is important to emphasize that the above-mentioned phenotype is not exclusive to FH and that β-sitosterolemia can indeed have a similar presentation. The latter must be suspected when parents do not show a phenotype compatible with heterozygous FH (since defects in *ABCG5* and *ABCG8* are autosomal recessive) and when no genetic defects are encountered in the three canonical genes (*LDLR*, *APOB*, and *PCSK9*) as well as in *LDLRAP-1* [10]. Usually sitosterolemia responds well to a diet poor in plant sterols and ezetimibe [22].

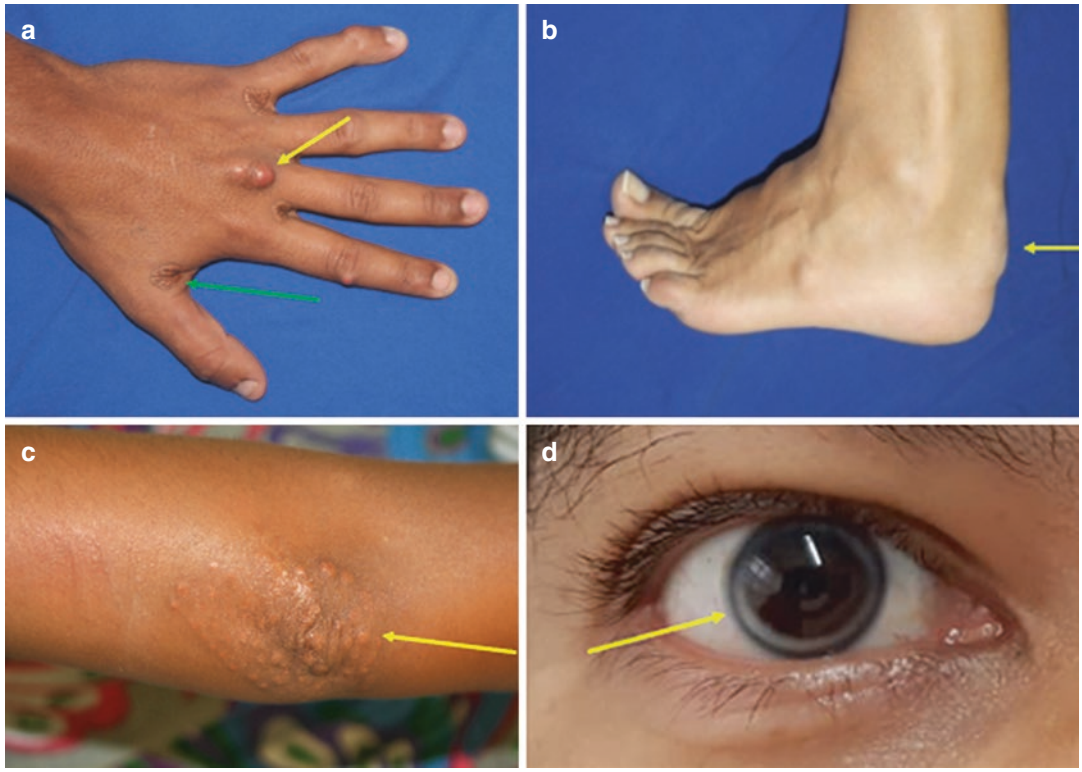

Fig. 20.3 Cutaneous, tendinous, and eye manifestations of severe familial hypercholesterolemia (either HoFHor heterozygous FH). (**a**) Interdigital xanthomas (green arrow); Tendinous xanthomas (yellow arrow); (**b**) Achilles heel thickening due to tendinous xanthoma; (**c**) cutaneous elbow xanthomas; (**d**) corneal arcus

Natural History of HoFH

Due to the extremely elevated LDL-C from conception, HoFH individuals usually develop xanthomas, corneal arcus, early ASCVD, and aortic or supra-aortic valve disease as shown in Fig. 20.4. Raal et al. [23] presented data from historical cohorts from South Africa (n = 149, 54% female) and showed that before the statin era (<1990) the mean ± standard deviation (SD) age of the first ASCVD event was 12.8 ± 5.9 years. After 1990 it changed to 28.3 ± 10.8 years (Fig. 20.5). Data from the UK showed that if left untreated HoFH individuals would have a life expectancy of 18–32 years [24]. Recently with statin treatment and advances in molecular diagnosis there is evidence that disease is more heterogeneous than thought with some HoFH individuals living more than 50 years [21]. Indeed, early and intensive LDL-C lowering can make the difference, even if HoFH patients seldom attain proposed LDL-C goals compatible with a lower risk of ASCVD. Retrospective data from 133 UK and South African patients followed for 25 years (1990–2014) suggest that LDL-C lowering prolongs their lives [25]. Figure 20.6 shows that in the study of Thompson et al. 34% died and 60% had an ASCVD event during follow-up. However, those who persisted with total cholesterol >320 mg/dL (8.2 mmol/L) had a 3.6 hazard ratio for all-cause mortality than those with lower LDL-C values. One important characteristic of the patients was unfortunately the young age at time of death (mean 31.4 ± 14.5 years).

ASCVD has usually a malignant course in HoFH. Usually atherosclerotic plaques develop in the aorta and adjacent to the coronary ostia [26], as shown in Fig. 20.7, a fact that may lead to

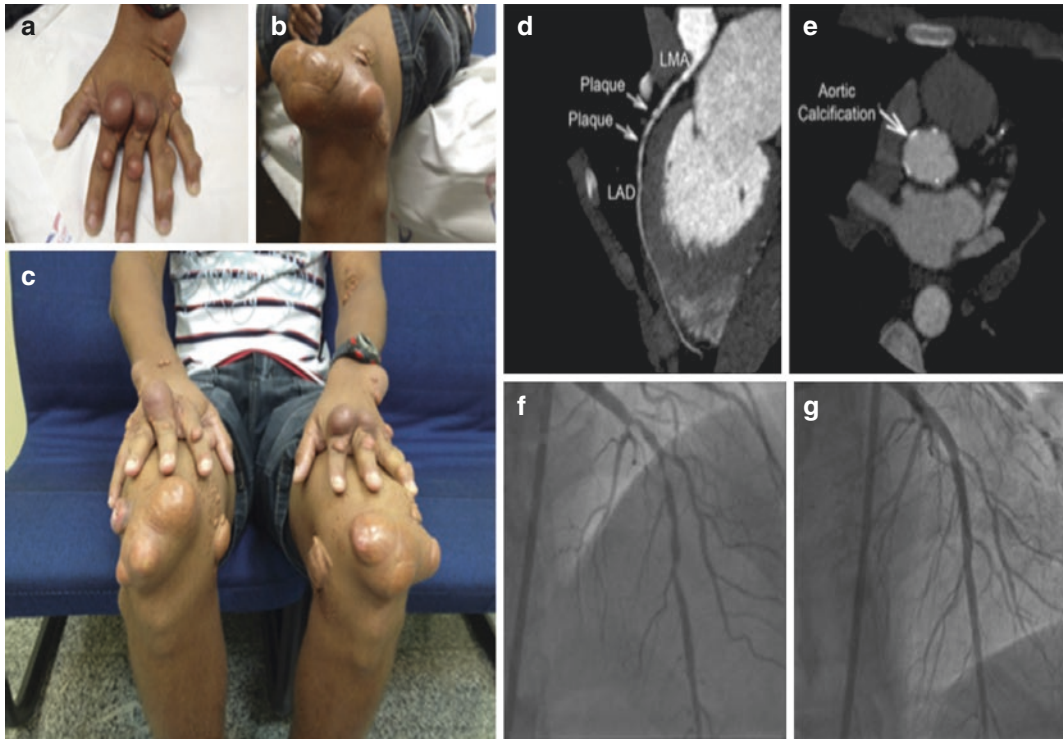

Fig. 20.4 Giant tendinous xanthomas and severe subclinical coronary atherosclerosis in an asymptomatic 20-year-old individual with HoFH that had never been treated until diagnosis. Patient was asymptomatic, had an untreated LDL-C of 710 mg/dL (18.2 mmol/L), genetic study showed homozygosis for a pathogenic variant on the *LDLR* Genotyping (A540T in exon 11). Giant tendon xanthomas on hands and knees (**a**–**c**), computed tomography angiography showed a plaque in the proximal left anterior descending (LAD) coronary artery causing severe stenosis (**d**, upper arrow), a moderate plaque in the ventricular posterior coronary artery, and aortic calcification (**e**, arrow). Conventional angiography showed a plaque involving the proximal LAD coronary artery and 80% stenosis (**f**). Angioplasty of the LAD coronary artery with two drug-eluting stents was performed without incident (**g**). Lack of adequate diagnosis and treatment led to this extreme phenotype. (From Rocha et al. [33]. With permission from Elsevier)

severe ventricular ischemia, heart failure, acute coronary syndromes, and sudden death [1]. In addition, other vascular beds such as the cerebrovasculature peripheral or renal arteries may be affected.

HoFH patients may also develop aortic valve disease (initially as valve regurgitation and then stenosis) and/or supra-aortic valve stenosis. Valve fibrosis, calcification, and inflammation are frequent. These unfortunately may progress due to hemodynamic stress even after cholesterol lowering [1]. In addition, severe calcification in the whole aorta is also frequently encountered [27]. Less frequently calcification of the mitral valve may lead to regurgitation.

Clinical and Laboratory Evaluation of HoFH Individuals

Due to its severity and early risk of ASCVD and aortic/supra-aortic valve complications, HoFH individuals must be seen by specialists in lipidology and vascular medicine. Secondary causes of severe dyslipidemia like hypothyroidism, nephrotic syndrome, severe jaundice, and biliary obstruction, use of steroids and antiacne (isotretinoin) medications must be ruled out. Family history of dyslipidemia, early ASCVD, and consanguinity must be evaluated. First-degree relatives of an index case must be evaluated for lipids and cascade screening performed.

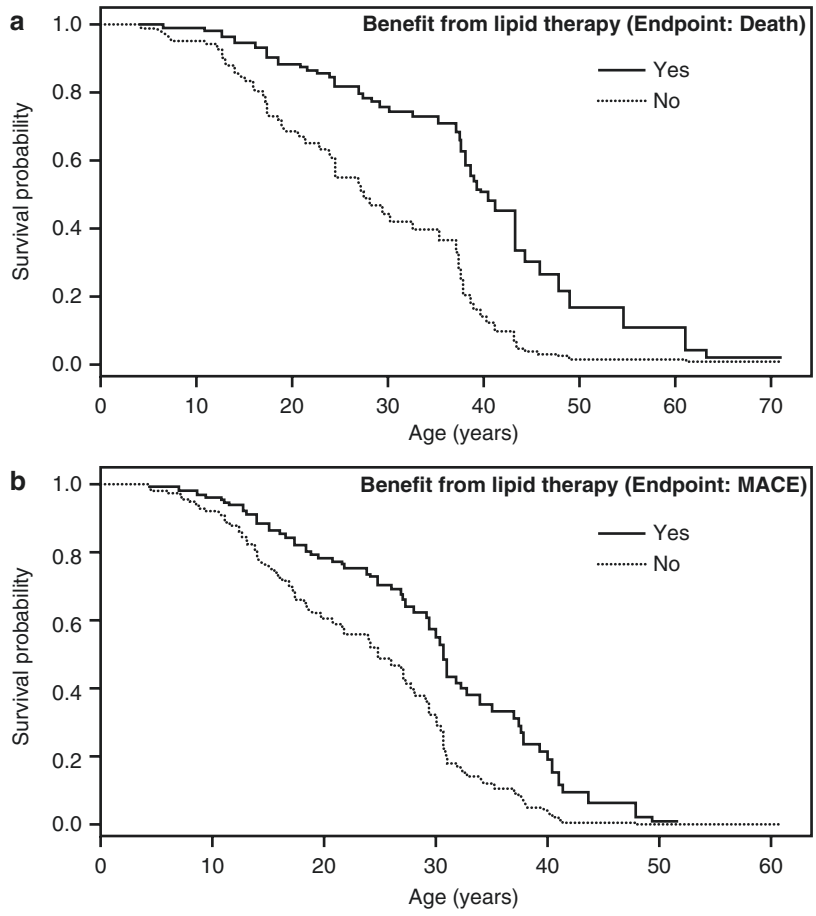

Fig. 20.5 Impact of standard lipid-lowering treatment on natural history of HoFH in South African patients. Cox proportional hazards model with time-varying benefit from statin therapy comparing treated and untreated person-years for (**a**) survival and (**b**) first major adverse cardiovascular event (MACE) in patients with HoFH, with year of birth fixed as mean year of birth. On average a 25% reduction in LDL-C was associated with a 14-year free of event survival. However, age of first ASCVD event or mortality was 32 years. (Raal et al. [23])

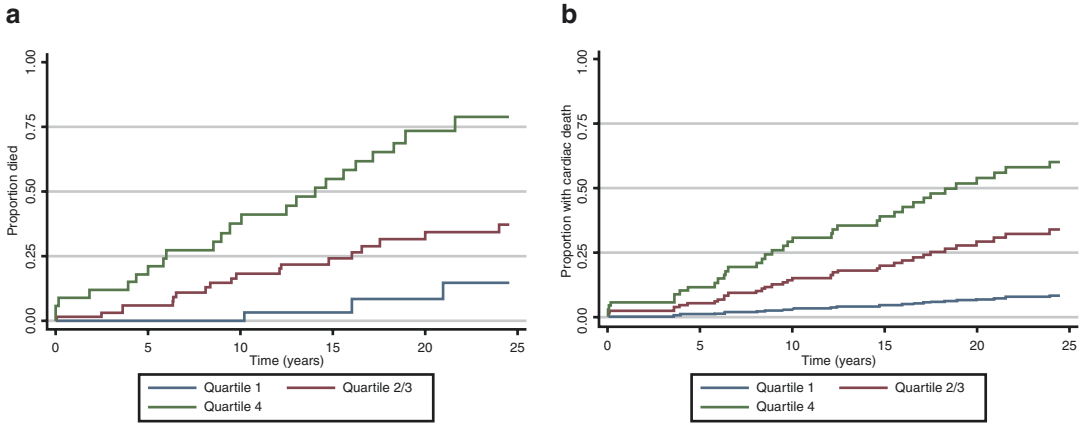

Fig. 20.6 Impact of lipid-lowering therapy on all cause (**a**) and cardiovascular (**b**) mortality during a mean 25-year follow-up in HoFH patients from the UK and South Africa. (**a**) Kaplan–Meier plot of time to death from any cause after the start of treatment, according to quartile of on-treatment serum cholesterol; (**b**) Kaplan–Meier plot of time to death from a cardiovascular cause after the start of treatment, according to quartile of on- treatment serum cholesterol. Quartile 1, <8.1 mmol/L (315 mg/dL); Quartiles 2 to 3, 8.1–15.1 mmol/L (315–590 mg/dL); and Quartile 4, >15.1 mmol/L (>590 mg/dL). (Thompson et al. [25])

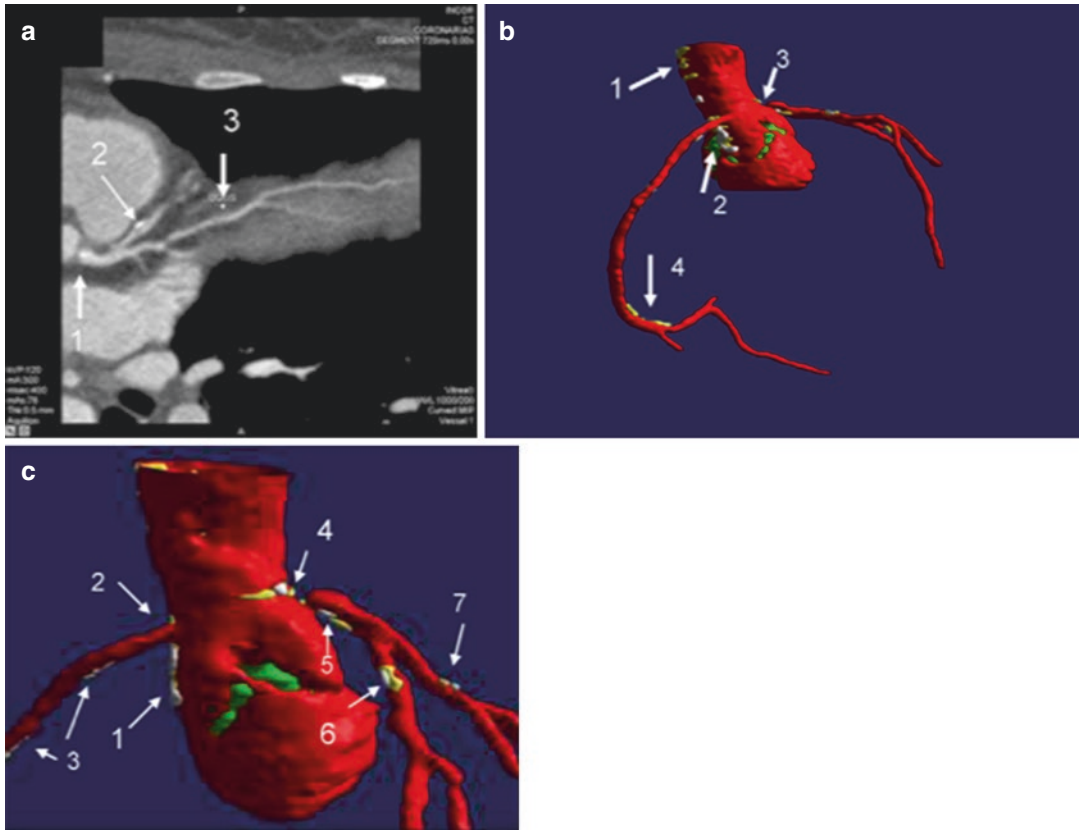

Fig. 20.7 Subclinical coronary atherosclerosis detected by cardiac-computed tomography in angiography in HoFH patients. An oblique coronal view in 2D multiplanar reformatted image. Mixed atherosclerotic plaques compromising the origin of the left main coronary artery (arrow #1), left anterior descending artery (arrow #2), and left ramus artery (arrow #3) are shown. (**b**) Anterior view of a three-dimensional model built from 2D images, using segmentation based on Hounsfield units. Non-calcified plaque in yellow (arrow #1) and calcified plaques in white compromising ascending aorta, right coronary artery ostium (arrow #2) and proximal left main artery (arrow #3) can be seen in this view. Arrow 4 depicts mixed plaques compromising the distal third of right coronary. (**c**) Detailed and zoomed image depicting proximal ascending aorta and coronary origins. Mixed plaques (yellow for non-calcified and white for calcified) are seen adjacent to the origin of right coronary artery (arrows 1 and 2). Arrow 3 depicts mixed plaques compromising proximal and mid segments of the right coronary artery. Arrow 4 indicates mixed plaques (yellow and white) in left coronary artery ostium, and arrow 5 mixed plaques (yellow and white) in left main artery. Mixed plaques are also seen in left anterior descending artery (arrow 6) and left ramus (arrow 7). (Santos et al. [26]. With permission from Elsevier)

Molecular diagnosis must be offered to confirm diagnosis and rule out possible phenocopies like β-sitosterolemia or LAL deficiency. Next-generation sequencing to identify pathogenic variants on *LDLR*, *APOB*, *PCSK9*, and *LDLRAP-1* should be performed. Multiplex ligation-dependent probe amplification (MLPA) should be used to detect copy number variants of the *LDLR*. If results are negative, further evaluation of phenocopies should be pursued for sitosterolemia (autosomal recessive pathogenic variants in *ABCG5* or *ABCG8*) and LAL deficiency (autosomal recessive pathogenic variants on *LIPA*) [10]. Indeed, the higher the LDL-C concentration greater the chance of encountering an autosomal dominant cause for hypercholesterolemia [28, 29]. For those with LDL-C >310 mg/dL (7.9 mmol/L) in a Canadian population, test positivity was approximately 90% in comparison with only 2% for those with LDL-C >190 mg/dL

(4.9 mmol/L). Results of molecular testing must, however, be evaluated by specialists due to an elevated number of variants of unknown significance (VUS) that can be encountered [29].

Considering the early and high risk of ASCVD and aortic valve disease, patients should be interviewed for symptoms such as angina pectoris and dyspnea. On physical examination, active evaluation for xanthomas, corneal arcus, valvular, and vascular murmurs should be performed. Lipoprotein(a) [Lp(a)] has been independently associated with a higher ASCVD risk in heterozygous FH [30, 31]; however, its role in atherosclerosis and valvular disease in HoFH deserves to be better evaluated. At any rate, an Lp(a) determination is recommended since for a clinical diagnosis Lp(a) cholesterol may be accounted as total cholesterol and LDL-C, since 30–45% of Lp(a) mass is cholesterol, and may confound the diagnosis as previously shown [31].

Considering the elevated frequency of subclinical coronary atherosclerosis in these individuals [26, 32, 33], cardiac computed tomography angiography is recommended as routine evaluation even in children at least every 5 years [1]. Myocardial stress tests to detect ischemia can also be performed on a patient-by-patient basis. Doppler echocardiographic evaluation of the heart and aorta are recommended annually, and magnetic resonance imaging can also evaluate atherosclerosis, the severity of calcification burden, and also reduced vascular lumen in the aorta [27]. The latter may have implications on possible myocardial revascularization as well in aortic/supra-aortic valve stenosis surgery [34]. Carotid Doppler should also be performed to detect atherosclerosis. Finally, Doppler ultrasound of the peripheral arteries may be necessary to detect atherosclerotic plaques in those territories.

Treatment of HoFH

General Measures

Due to the great severity of hypercholesterolemia, HoFH patients will have very little response to a low saturated fat diet, however, a healthy diet (rich in fruits, vegetables, whole grain, low saturated fat meat, and dairy products) is recommended [1]. Smoking cessation is of extreme importance for FH individuals [35] and professional care must be offered if necessary.

Physical activity is recommended, however, evaluation of myocardial ischemia and aortic valve and supra-aortic valve disease need to be performed before more intensive exercise is recommended [1]. The presence of valvular disease also opens the possibility of infectious endocarditis and must be considered with preventive measures especially in those with a valve prosthesis. Despite being rare in young populations, other risk factors like hypertension or type 2 diabetes should be adequately controlled if present.

There is no evidence that aspirin reduces ASCVD events in either heterozygous or homozygous FH patients without previous clinical manifestations of ischemic heart disease. The HEART UK guidelines recommend 75 mg aspirin daily in primary prevention of HoFH individuals after the age of 16 years due to elevated frequency of subclinical atherosclerosis and the extremely high risk of myocardial infarction and death due to ischemic heart disease [36].

LDL-C Goals in HoFH

The main objective of treatment in HoFH patients is to reduce LDL-C as much as possible. A minimum recommend LDL-C reduction is 50%. However, for most individuals, plasma LDL-C will still be quite elevated. If possible, LDL-C < 100 mg/dL (<2.5 mmol/L) in adults and <135 mg/dL (<3.5 mmol/L) in children are recommended [1, 6, 7]. More recently ESC/EAS guidelines recommend LDL-C < 70 mg/dL (<1.8 mmol/L) for FH patients overall and even <55 mg/dL (<1.4 mmol/L) in those with previous ASCVD events [37]. Unfortunately, due to the severity of LDL-C elevation in HoFH, these goals are rarely attained. However, even moderate LDL-C reduction in HoFH can change the natural history of ASCVD [23, 25]. An approximately 25% reduction in LDL-C with statins, bile acid binding resins, and ezetimibe is able to gain

14 years of life free from events in South African patients [23]. Moreover, data from a 25-year follow-up from the UK and South Africa suggest that at least a total plasma cholesterol <320 mg/dL (8 mmol/L) should be achieved. In that study cardiovascular death rates were around 10%, 30%, and 60% in 25 years, respectively, for those with total cholesterol <320 mg/dL (8.2 mmol/L); 320–600 mg/dL (8.2–15.4 mmol/L) and >600 mg/dL (15.4 mmol/L) (Fig. 20.6). Those achieving the lowest LDL-C values were treated with statins, ezetimibe, evolocumab, and LA when available. More recently, data from the open label extension of the TAUSSIG study which tested the PCSK9 inhibitor evolocumab on top of maximally tolerated statin/and or ezetimibe therapy (and in many cases LA) showed a 2.6% event rate per year in 106 HoFH patients followed for a median of 4.1 years [19]. That rate is much lower than that previously reported for this very high-risk population [25].

Lipid-Lowering Pharmacological Therapy

Figure 20.8 depicts the mechanisms of action of drugs approved to treat HoFH. Due to the complex molecular etiology and consequent extremely high plasma LDL-C concentrations in HoFH, pharmacological treatment is composed of many drugs working by different mechanisms. The most frequent defect in HoFH is a severe reduction of LDL plasma clearance by the hepatocytes due to very low or absent expression of the LDLR [1, 12]. More rarely defects in binding of apoB to the LDLR or reduction in LDLR/LDL internalization from the hepatocyte surface as may occur in the case of ARH, which may cause the FH phenotype. Therefore, considering different molecular defects and mechanisms leading to the extreme severe hypercholesterolemia, there is need of different classes of drugs that work by either increasing expression of the LDLR (statins,

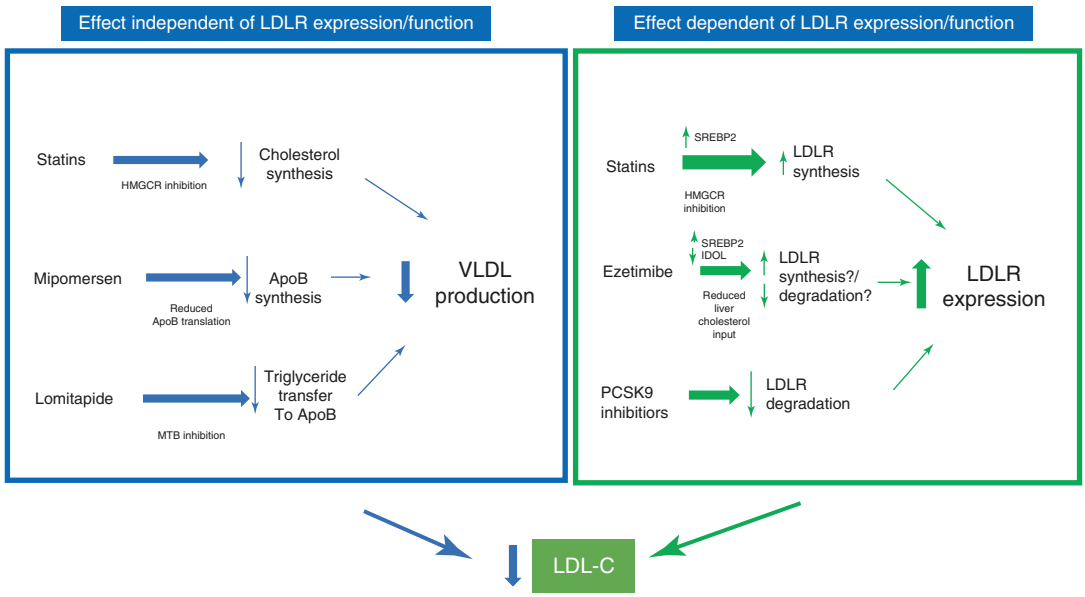

Fig. 20.8 Mechanism of action of approved pharmacological lipid-lowering treatments for HoFH. Mechanisms involved with low-density lipoprotein cholesterol (LDL-C) lowering by medications approved for homozygous familial hypercholesterolemia and their possible associations with LDLR (LDL receptor) expression/function. apoB indicates apolipoprotein B, HMGCR 3-hydroxy-3-methylglutaryl-coenzyme reductase, IDOL inducible degrader of LDL receptor, MTP microsomal triglyceride transfer protein, PCSK9 proprotein convertase subtilisin kexin type 9, SREBP2 steroid regulatory element binding protein-2, and VLDL very-low-density lipoprotein. (Santos [12]. Mipomersen license was withdrawn in late 2019)

ezetimibe, bile acid sequestrants, and PCSK9 inhibitors) or by reducing the synthesis of LDL precursors (lomitapide, mipomersen), that is, VLDL [12]. Despite use of the different medications, HoFH patients in most situations still persist with very high LDL-C levels and extracorporeal removal of LDL by LA is frequently needed.

As a general rule pharmacological therapy must be started at the time of diagnosis even in young children with the highest tolerated doses of available medications [1, 6]. Indeed, due to disease severity and absence of LA in most parts of the world, in children treatment is usually done with either "off label" use of statin doses higher than recommended or by prescription of medications approved for adults only [38, 39].

Contraception is extremely important for women with HoFH at reproductive ages and must be promptly instituted since with the exception of bile acid binding resins and LA [4], all other therapies are contraindicated in pregnancy. Of interest is a recent retrospective case series of 39 HoFH women who became pregnant and inadvertently used statins, ezetimibe, and even evolocumab at initial phases of pregnancy showed neither fetal nor pregnancy complications [40]. LA is the treatment of choice in HoFH women who want to become pregnant [41].

Statins

Table 20.2 provides information about pharmacological therapies preferentially used to reduce LDL-C in HoFH patients. Statins reduce plasma LDL-C and circulating apoB containing lipoproteins mainly by increasing the expression of LDLR on the liver surface. In addition, by inhibiting HGMGCo-A reductase, statins reduce intrahepatic cholesterol synthesis and VLDL production [1]. The latter mechanism explains effects of statins in reducing plasma LDL-C in HoFH patients with null/null alleles in the *LDLR* and also in those where the phenotype is caused by *APOB, PCSK9*, and *LDLRAP1* defects. Considering their highest potency in comparison with the other drugs of the class [42] either atorvastatin or rosuvastatin should be used preferentially to reduce LDL-C in HoFH. On average the highest daily doses of these medications (80 mg atorvastatin or 40 mg rosuvastatin) reduce LDL-C by 20%; however, the response is highly variable [15, 43]. Stein et al. evaluated the response to high dose rosuvastatin treatment in 53 children and adults with molecular defined HoFH [15]. On average LDL-C was reduced by 20.3 ± 13.6%, but individual responses varied from 0% to 54%. In those with defective and null *LDLR* defects, the mean reductions were 21.3 ± 13.1% and 12.9 ± 12.5%, respectively. In the two patients with ARH mean LDL-C reduction was 33.5 ± 9.7%. Despite these relatively modest LDL-C reductions, observational data show that statins may reduce mortality in HoFH patients [23] and must be used in their highest tolerated doses even in children [6, 44].

Ezetimibe

Ezetimibe acts mainly by reducing intestinal cholesterol absorption by the Niemann–Pick C1-Like 1 (NPC1L1) transport protein. Consequently, less cholesterol is transported to the liver by chylomicrons and remnants, and reduction of cholesterol concentration in the hepatocyte may lead to reduced VLDL synthesis and or greater expression of the LDLR [12]. On average 10 mg ezetimibe/day adds a 21% plasma LDL-C reduction on top of high dose statin therapy in HoFH patients [45]. Ezetimibe therapy will be particularly useful in those with sitosterolemia, a phenocopy of HoFH due to defects in *ABCG5* and *ABCG8*, where plant sterols and/or cholesterol excretion is reduced [22].

Bile acid sequestrants (BAS)

There are no clinical trials of BAS (cholestyramine, colestipol, and colesevelam) in HoFH patients [36]. However, these medications may help reduce LDL-C by a mechanism similar to ezetimibe (i.e., by reducing bile acid absorption, hepatocytes upregulate expression of LDLR in order to increase production of bile acids via 7-α-hydroxylase). The BAS may be useful especially during pregnancy since they are not absorbed, but their impact as an isolated drug is expected to be modest. One study evaluated the effects of adding colesevelam 3.75 g/day on top

Table 20.2 Medications preferentially utilized for LDL-C reduction in HoFH patients

Medication	Mode of action	Usual dose/route	Average impact on LDL-C	Most frequent adverse events
Statins[a]				
Atorvastatin	Inhibit hepatic cholesterol synthesis/ Increase LDLR expression	10–80 mg/day oral (20 mg/day max >6 years children for HoFH)	20.3 ± 13.6% [15, 43], response depends on type of genetic defect	Muscle pain, rarely elevations in AST/ALT
Rosuvastatin		5–40 mg /day oral (20 mg max 7–17 children years of age)		
NPC1L1 inhibitor				
Ezetimibe	Reduce intestinal cholesterol absorption/ Increase LDLR expression?	10 mg/day oral adult (children and adolescents 7–17 years old)	Average 21% on top of high dose statin therapy [45]	Fatigue, abdominal pain, diarrhea
Bile acid bind resins				
Colestiramine	Reduce intestinal cholesterol absorption/ Increase LDLR expression?	4–8 g oral bid before meals (pediatric 2–4 g bid before meals)	Not tested specifically in HoFH patients	Gastrointestinal adverse events (nausea, vomiting, bloating, diarrhea, obstipation). May bind other oral, medications and reduce their effectiveness
Colestipol		5 g powder or 4 g oral tablets bid before meals (not available pediatric)		
Colesevelam		6 × 625 mg oral tablets or 3.75 g packet/day, or 3 × 625 mg tablets or 1.875 g packet bid; 3.75 g packet every day or 1.875 g packet bid (10–17 years for Heterozygous FH) not tested in HoFH		
PCSK9 inhibitor				
Evolocumab[b]	Increase LDLR expression	420 mg subcutaneous injection once a month (not approved for pediatric patients). In TAUSSIG 420 mg subcutaneous injection every 2 weeks average LDL-C went from −19.6% at week 12 to −29.7% at week 24 (nonapproved dosage) [18, 19]	Average 20–24% on top of statin/ ezetimibe or LA therapy; wait 8–12 weeks for LDL-C at least 15% [18, 19]. Response depends on type of genetic defect	Nasopharyngitis, influenza, upper respiratory tract infection, headache, myalgia, and diarrhea and injection-site reactions [18, 19].

(continued)

Table 20.2 (continued)

Medication	Mode of action	Usual dose/route	Average impact on LDL-C	Most frequent adverse events
MTP inhibitor				
Lomitapide	Inhibit VLDL synthesis and plasma apoB and LDL production	5–60 mg/oral day (not approved for pediatric patients)	38–50% on top of statin/ezetimibe therapy or LA therapy [16]	Nausea, diarrhea, abdominal cramps and elevation in liver enzymes, e.g., 34% and 14% had reversible AST and ALT levels respectively >3× and 5× the upper limit of normal and steatosis [16]

[a]Other statins may be used however with less effectiveness
[b]Alirocumab not yet approved for HoFH but clinical trial in adults already completed (clinicaltrials.gov NCT03156621)
[c]Medications used preferentially to reduce LDL-C in HoFH according to their potency and mechanism of action

of statin/ezetimibe therapy in heterozygous FH patients who persisted with elevated LDL-C levels [46]. After 12 weeks there was an additional 12% LDL-C reduction in comparison with placebo.

PCSK9 Inhibitors

PCSK9 is expressed mainly in the liver, intestine, and kidney. Synthesized PCSK9 is secreted into plasma and binds to the LDL–LDLR complex on hepatocytes and reduces the recycling of the LDLR to cell surface leading it to lysosomal degradation [47]. Indeed, gain of function variants of PCSK9 are a rare cause of heterozygous [8, 48] FH and even less frequently of HoFH [49] due to reduced expression of the LDLR in the hepatocyte membrane [48]. Fully human monoclonal antibodies (MAb) against plasma PCSK9 (evolocumab and alirocumab) reduce LDL-C by binding circulating PCSK9. The MAb–PCSK9 complex is removed by the reticuloendothelial system. Reduced availability and consequent binding of PCSK9 increases LDLR recycling to the hepatocyte surface and, therefore, LDLR expression and LDL removal from plasma. Either alirocumab or evolocumab lead to intensive (on average 50–55%) plasma LDL-C reduction in heterozygous FH (HeFH) [19, 50, 51]. This robust effect occurs probably because in HeFH only one of the LDLR alleles is affected and therefore half of the LDLR are normal in this form of the disease [52]. Indeed, on average, responses to PCSK9 inhibitors in HeFH patients do not differ from individuals with other causes of hypercholesterolemia [53]. However, considering that the HoFH phenotype is usually secondary to severe defects on the LDLR codified by either *LDLR* alleles and that in many circumstances other genes may be involved, less robust and heterogeneous responses are encountered [19, 52, 54, 55] to evolocumab treatment (at the moment this chapter was being written only evolocumab was approved for reduction of plasma LDL-C in HoFH patients, in 2020 first results of alirocumab were set to be presented).

In a small open label single arm study, Stein et al. tested the effect of two different doses of evolocumab in HoFH patients [55]. Eight patients with LDLR negative or defective defects on stable drug therapy were treated with subcutaneous 420 mg evolocumab every 4 weeks for ≥12 weeks, followed by 420 mg every 2 weeks for an additional 12 weeks. Mean change from baseline in LDL-C at week 12 was −16.5% (range, 5.2% to −43.6%) and −13.9% (range, 39.9% to −43.3%); with 4- and 2-week dosing, respectively. No reduction was seen in the two LDLR negative patients. Over the treatment periods, mean ± SD LDL-C reductions in the six LDLR defective patients were 19.3 ± 16% and 26.3 ± 20% with 4- and 2-week dosing, respectively (ranging from 4% to 48% with 2-week dosing).

In TESLA B, a double-blind placebo controlled study, Raal et al. [54] evaluated the effect of evolocumab 420 mg once a month versus placebo for 12 weeks in HoFH patients undergoing maximally tolerated standard lipid-lowering ther-

apy. In a 2:1 randomization design, 33 patients received evolocumab and 13 placebo, evolocumab reduced LDL-C at 12 weeks by 30.9% (95% CI –43.9% to −18.0%; $p < 0.0001$). Actually, the reduction of LDL-C with evolocumab was 23.1% (95% CI –30.7 to −15.4) while there was a mean 7.9% non-significant increment with placebo (95% CI –2.7 to 18.5). The absolute reduction in LDL-C was 96 mg/dL (95%CI −148 to 44 mg/dL) [2.5 mmol/L (95% CI –3.8 to −1.1)]. Despite this, the residual mean on treatment LDL-C was still very high, that is, 280 mg/dL (7.2 mmol/L) [52]. The drug was well tolerated and there were no differences in adverse events versus placebo.

Further data on the impact of evolocumab on LDL-C levels in HoFH came from two publications of the open label extension TAUSSIG study [18, 19]. In that study 106 HoFH patients aged 12 years or older using maximally tolerated standard lipid-lowering therapy received evolocumab 420 mg subcutaneously monthly, or if on LA every 2 weeks. Dosing could be increased to every 2 weeks after 12 weeks at investigator discretion. Mean change in LDL-C from baseline (329 ± 137 mg/dL or 8.4 ± 3.5 mmol/L) to week 12 was 21 ± 25% and was sustained until week 216 (−24 ± 41%) [19]. This variability was partially explained by the genetic defect causing the HoFH phenotype (null vs. defective variants) as previously discussed [14, 18]. Of 48 patients with HoFH who were up-titrated to 420 mg every 2 weeks, mean change in LDL-C improved from −19.6% at week 12 to −29.7% at week 24 [18]. From weeks 12 through 216, LDL-C reductions ≥ 15% were observed in 56.7–72.2% of studied patients [19]. Evolocumab was well tolerated and the most common adverse events were nasopharyngitis, influenza, upper respiratory tract infection, headache, myalgia, and diarrhea. Injection-site reactions were mostly minor and did not lead to evolocumab discontinuation. In the study of Santos et al. [19], no neutralizing antibodies were detected during a median follow-up of 4.1 years. The annual rate of major cardiovascular events was 2.8%, less than previously reported for this population [18, 25] suggesting that the additional LDL-C lowering may have reduced ASCVD events. Evolocumab is being tested in HoFH children/adolescents 10–17 years of age (clinical trials.gov NCT02624869). Alirocumab inhibitor is not yet approved for HoFH but was being tested at the time this chapter was written in both adults and children/adolescents with HoFH (clinical trials.gov NCT03156621 and NCT03510715, respectively).

Despite the favorable impact of evolocumab on top of statins and ezetimibe on LDL-C, most patients with HoFH still persist with very high LDL-C levels and other treatments are needed to adequately reduce the levels of pro-atherogenic lipoproteins.

MTP-Inhibition (Lomitapide)

Lomitapide reduces the production of chylomicrons and VLDL by inhibiting the microsomal triglyceride transfer protein (MTP) in the intestine and liver, respectively [56]. Reduction of VLDL assembly at the liver is associated with diminished apoB and LDL production [57] on an LDLR independent mechanism. Therefore, this drug is indicated especially for the severest HoFH forms that are refractory to medications that increase expression of the LDLR like statins and PCSK9 inhibitors [12].

The efficacy and safety of lomitapide was tested initially in an open label single arm 78-week duration study [16] enrolling 29 HoFH patients on standard lipid-lowering therapy or LA. Lomitapide dose ranged from 5 to 60 mg/day (median 40 mg). After 26 weeks there was a mean 50% reduction in LDL-C from (347 ± 118 mg/dL or 8.9 ± 3.0 mmol/L to 174 ± 99 mg/dL or 4.5 ± 2.5 mmol/L). Similar LDL-C lowering effects were encountered in patients that were being submitted to LA treatment [58]. Of the initial 29 patients 23 (79%) completed the study up to 78 weeks where the mean LDL-C was 38% versus baseline [16].

Blom et al. evaluated the efficacy and safety of lomitapide (20–60 mg) in 19 individuals of the original study that were followed for a median of 5.1 years [59]. In 17 individuals who participated for at least 4 years there was a mean 45% (95% CI, −61.6% to −29.4%) reduction in LDL-C. Of

importance at least 58% of studied subjects reached an LDL-C < 70 mg/dL (1.8 mmol/L) at least once during the study.

The LOWER registry (Lomitapide Observational Worldwide Evaluation Registry clinical trials.gov NCT02135705) is evaluating patients treated with lomitapide from different countries. Blom et al. [56] presented partial results from 163 patients using a mean dose of 10 mg daily. The mean LDL-C reduction was 47.2% at 36 months and approximately two thirds of participants had LDL-C reductions greater than 50%. More results from this study are expected in late 2020.

The main adverse events of lomitapide in the study of Cuchel et al. [16] were nausea, diarrhea, abdominal cramps and elevation in liver enzymes (34% and 14% had reversible AST and ALT levels, respectively, >3× and > 5× the upper limit of normal). The latter abnormalities were potentiated by alcohol consumption and use of medications that inhibit cytochrome P450 3A4. In the extension study [59], 21% presented with transitory elevations in AST/ALT >5× the upper limit of normal. Those alterations were managed by dose reduction or interruption of therapy.

Considering the reduction of VLDL export from the liver due to MTP inhibition there was increment in liver fat content detected by magnetic resonance spectroscopy (MRS). The MRS studies showed increments in the amount of liver fat occurring mostly within the first months of treatment that tended to stabilize with longer drug exposure. The mean baseline hepatic fat content was 1.0% (range 0–5%) and increased to 8.6% (range 0–33.6%) at week 26, 5.8% (range 0–16.5%) at week 56 and 8.3% (range 0–19%) at week 78 [16]. In the longer duration study of Blom et al. [59] the median hepatic fact content was 7.7% (95% CI, 5.7–14.6), 10.3% (95% CI, 6.5–14.2) and 10.2% (95% CI, 8.3–14.7) at weeks 126, 174 and 246, respectively.

Both gastrointestinal and liver fat adverse events accompany MTP inhibition in the gut and liver that lead, respectively, to steatorrhea and liver fat accumulation. A low-fat diet (<20% of calories as fat) and supplementation with essential fatty acids is recommended for those treated with lomitapide. It is important to note that the small number of subjects in those studies preclude robust conclusions about the longer-term hepatic safety of lomitapide. Indeed, one case of an individual with familial chylomicronemia syndrome with repetitive episodes of pancreatitis treated with lomitapide to reduce triglycerides and underwent repetitive liver biopsies showed not only steatosis and liver inflammation, but also fibrosis after 13 years of treatment [60].

In the USA, lomitapide is approved for adult HoFH and is available through a risk evaluation and mitigation strategy (REMS) program . Due to low-fat diet implementation to prevent steatorrhea, there is need for supplementation with vitamin E, linoleic acid, alpha-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) [61]. Also, it is important to pay attention to the potential pharmacological interactions with medications inhibiting the cytochrome P450 3A4 as well as patients treated with warfarin where changes in anticoagulation may occur [61]. Another limitation for lomitapide use is its extremely high cost, a fact that limits its access especially for developing regions of the world [62].

Use in Children and Adolescents

Lomitapide is approved for use in HoFH individuals older than 18 years old; however, there are case reports of "off label" use of this medication in children and adolescents with HoFH [38, 63]. Chacra et al. [63] and Ben-Omran et al. [38] report 11 pediatric HoFH patients (mean age 11.6 ± 1.1 years) who were treated with lomitapide (mean dose 24.5 ± 4.3 mg/day for a mean 20.0 ± 2.9 months). Lomitapide was started with a stepwise dose escalation from 2.5 mg or 5 mg/day and all subjects followed a low-fat diet and received lipid soluble vitamin supplements. The mean baseline LDL-C was 419 ± 75 mg/dL (10.7 ± 1.9 mmol/L) and was reduced by 58.4 ± 6.8% to 177 ± 46 mg/dL (4.5 ± 0.9 mmol/L). Six patients achieved LDL-C goals <135 mg/dL (3.4 mmol/L) and five had LA frequency reduced. Adverse events were mainly gastrointestinal in nature and similar to the ones of previous studies in adults [16]. There was no systematic evalua-

tion of liver fat in those studies. These data are encouraging but controlled studies are needed in children/adolescents with HoFH especially because one cannot wait for children to become adults to start more aggressive lipid-lowering therapy considering the exaggerated risk of early ASCVD and that LA is not widely available.

Apolipoprotein B Synthesis Inhibition (Mipomersen)

Mipomersen is a second-generation antisense oligonucleotide (ASO) against hepatic apoB100 messenger RNA (mRNA) [17, 56, 64, 65]. The oligonucleotide–mRNA complex is degraded by RNase-H and apoB synthesis, consequently reducing the production of different apoB-100 containing lipoproteins like VLDL, LDL, and Lp(a). Similar to lomitapide, mipomersen reduces LDL-C independently of the LDLR activity, however differently from the former it does not influence chylomicron production in the gut.

Mipomersen had been approved for HoFH only, however it was tested in different forms of severe hypercholesterolemia including heterozygous FH [17, 64, 66]. A recent published study level meta-analysis summarized the effects of mipomersen in these different populations from 13 randomized clinical trials [67]. Overall mipomersen reduced LDL-C, total cholesterol, and Lp(a) by approximately 26.4%, 21.4%, and 22.7% in comparison with placebo.

Specifically for HoFH, Raal et al. [17] evaluated mipomersen or placebo in 51 patients older than 12 years with LDL-C >135 mg/dL (3.4 mmol/L) despite use of maximum tolerated lipid-lowering therapy. No patients on LA were included in this trial. Mipomersen was administered at 200 mg weekly dose (160 mg if weight less than 50 kg) or placebo for 26 weeks. While all 17 patients randomized to placebo completed the treatment period, 6 of the 34 patients (18%) randomized to mipomersen dropped out from the study mainly due to adverse events. There was a mean 24.7% (95% CI –31.6 to −17.7) reduction in LDL-C from a baseline of 456 ± 144 mg/dL (11.7 ± 3.7 mmol/L).

In the long-term extension study that included either HoFH or heterozygous FH patients that were exposed for at least 1 year to mipomersen, Santos et al. [57] showed that the mean LDL-C reduction was 26 ± 18.7%. Of the 141 patients enrolled in this extension study 55% discontinued treatment within the first 2 years of treatment; 43% due to treatment emergent adverse events. The main adverse events associated with mipomersen are injection site reactions, flu-like symptoms, hepatic steatosis, and hepatic enzyme elevation [17, 64], with 75% experiencing injection site reactions versus 24% in the placebo group. Flu-like symptoms were reported in 29% and 24%, respectively, in those receiving mipomersen or placebo. In the longer-term extension study [64], magnetic resonance imaging studies performed only in those with >3× limit of normal elevations in ALT (n = 65) exposed for at least 1 year to mipomersen 25% had an increase of liver fat of more than 20% on at least one occasion. However, this regressed to normal at 24 weeks after drug cessation. Liver biopsies were performed in seven patients exposed to mipomersen (21–159 weeks) mainly due to elevation in liver enzymes [68]. In all patients only steatosis with no signs of inflammation or fibrosis was encountered. Indeed, hepatic steatosis was expected due mipomersen's mechanism of action (i.e., reduction in apoB synthesis and consequent reduction of triglyceride export from the liver in the form of VLDL) [56].

Injection site reactions can lead to skin pigmentation and rarely to skin necrosis [56]. In order to try reducing mipomersen side effects the FOCUS FH study tested an alternative regimen of this drug at 70 mg three times weekly versus the usual 200 mg once a week in severe FH patients [66]. Indeed, the former regimen showed less adverse events than the latter; however, there was also less efficacy in LDL-C lowering.

In late 2019 the mipomersen commercialization license was suspended after a demand from its producer Kastle Pharmaceuticals (FDA-2019-N-2040-0001). Despite this, the studies of this medication were highly impor-

tant and have opened a way for further research on more liver specific ASO drugs with the potential of less adverse events like the ones using selective GalNAc (N-acetyl galactosamine) formulations [69].

Lipoprotein Apheresis

Despite advances in pharmacological therapy there is still a huge unmet need for LDL-C control in HoFH. Selective LA techniques remove pro-atherogenic apoB100 containing lipoproteins (mainly LDL and Lp(a)) and, therefore, are an extremely useful therapy in severe FH, including both HeFH and HoFH forms [9]. LA also has pleiotropic effects such as reductions in inflammation and blood viscosity. Internationally there are different indications for LA, for instance in the USA the Food and Drug Administration approved LA for individuals who have HOFH with LDL >500 mg/dL (>12.8 mmol/L), heterozygotes with no known cardiovascular disease but LDL >300 mg/dL (>7.7 mmol/L), and HeFH with known cardiovascular disease and LDL >160 mg/dL (>4.1 mmol/L) [41]. A single LA procedure may reduce LDL-C by 65–70%. However, since LDL-C will raise again to pre-procedure levels in 1–2 weeks, weekly treatment is necessary to keep time-averaged LDL-C reductions >60% from baseline [9, 41].

LA can be performed in pregnant women and in children with HoFH [41], and it can be combined with classical lipid-lowering therapies as well novel ones like PCSK9 or MTP inhibitors without affecting effectiveness of the later treatments [19, 58]. Despite the absence of randomized clinical trials there is evidence that LDL-C lowering in regimens where LA is included prevent ASCVD events in severe forms of FH, including HoFH [25, 70].

LA use is associated with adverse events like mild to severe hypotension and nausea as well as problems with venous access [9]. Unfortunately, LA is expensive and is not widely available and requires a substantial weekly time commitment for patients.

Liver Transplantation

Liver transplantation (LT) has been seldom used as a treatment of HoFH. In their review, Ishigaki et al. describe 44 patients transplanted worldwide from 1985 until 2019 [71]. These numbers are probably underestimated, however. The rationale behind this procedure is that a liver graft with an adequate number of LDLR will restore the hepatic ability to remove circulating LDL particles and, therefore, reduce LDL-C. In some desperate circumstances liver and heart transplantations were performed due to concomitant severe cardiac disease. There is evidence that indeed LT may not only reduce elevated LDL-C but also regress xanthomas and atheroma burden [72]. Unfortunately, aortic valve disease may progress despite the intensive LDL-C lowering [72, 73]. Main complications of LT are those related to surgical procedures and the ones attributable to immunosuppression. Also, the shortage of donors is a barrier for more widespread procedures.

Future Treatments

Currently newer medications and genetic therapies are being tested in HoFH in order to reduce the unmet need to reduce LDL-C in this population.

Evinacumab

Evinacumab is a monoclonal antibody directed to Angiopoietin-like 3 (ANGPTL3) that was shown to reduce LDL-C by 49 ± 23% (range, 25–90) in 9 adults with HoFH after a month of treatment in an open label uncontrolled proof of concept study [74]. Evinacumab appears to reduce LDL-C by LDLR independent mechanisms not yet elucidated [75].

Recently the topline results of a double-blind randomized clinical trial that compared evinacumab with placebo (ELIPSE study: clinical trials.gov NCT03399786) were announced [76]. Sixty-five HoFH individuals (average age 42 years, range 12–75 years) were randomized to

receive a monthly injection of evinacumab 15 mg/kg administered intravenously every 4 weeks or placebo for 24 weeks. Among those who received evinacumab, 98% were treated with statins, 81% were receiving PCSK9 MAb therapy, 75% were on ezetimibe, 33% were on LA, and 26% were on lomitapide. In addition, 35% of evinacumab patients had null/null variants in the *LDR*. Evinacumab reduced LDL-C by a mean of 49% in comparison with placebo. Of importance, 47% individuals achieved LDL-C levels <100 mg/dL (2.5 mmol/L), compared to 23% for placebo. Similar reductions were achieved in null/null individuals. During treatment, 66% of evinacumab patients and 81% of placebo patients had adverse events. Flu-like symptoms and rhinorrhea were more common with evinacumab than with placebo at 11% and 7% versus 0%, respectively. All patients are being followed in an open label extension study. If results are confirmed in the long-term evinacumab will become a first line therapy for HoFH patients due to its intensive LDL-C lowering effect and apparent greater tolerability than lomitapide.

Gemcabene

Gemcabene is being investigated as lipid-modifying therapy and at the moment its mechanism of action is not well understood. Preclinical studies have shown that it may lower LDL-C by reducing cholesterol biosynthesis, lowering apolipoprotein C-III, and raising VLDL clearance among other hypothetical mechanisms [77]. In one pilot study testing different doses, Gemcabene reduced LDL-C by 30% on top of statins, ezetimibe, and PCSK9 inhibitors in eight individuals with severe familial hypercholesterolemia, including HoFH [78]. Further studies are necessary to evaluate the safety and efficacy of this medication in HoFH patients.

Genetic Therapy

Considering that in most situations HoFH is caused by variants in the *LDLR,* scientists have tried to restore LDLR function by means of genetic therapy. Currently studies are being developed using adenoviruses-mediated gene transfer specifically with AAV8 vectors [79]. The greatest challenges to genetic transfection are immunogenicity and persistence of transgene expression. A clinical trial with the AAV8 vector is being performed (clinical trials.gov NCT02651675) that is enrolling 12 HoFH patients and plans to follow them for 5 years. The development of genome editing techniques based on clustered-regularly-interspaced-short-palindromic-repeats/CRISPR-associated 9 (CRISPR/Cas9) has opened the possibility of repairing defects on the *LDLR* in HOFH [80]; however, many studies are still necessary in this area before it can be applied routinely.

Clinical Case

This is 32-year-old female patient who was evaluated for the first time at the age of 12 (2004) due to a total cholesterol of 484 mg/dL (12.4 mmol/L) and LDL-C 464 mg/dL (11.9 mmol/L), HDL-C 40 mg/dL (39 mmol/L), and triglycerides 160 mg/dL (1.8 mmol/L). Her mother noticed nodularities along the hand and elbow tendons, as well as cutaneous eruptions in the hands, elbows, and knees since very early in life. On physical examination she had tendinous xanthomas along her hands, elbows, and Achilles heel. She also had cutaneous xanthomas along the hands and buttocks, as well as bilateral corneal arcus. She had 2/6 systolic murmur at the aortic region of the chest wall with radiation into the neck. The clinically suspected diagnosis of HoFH was confirmed by a genetic test (homozygosity for Ala431Thr mutation in the exon9 of the *LDLR*). The echocardiogram showed double dysfunction of the aortic valve with systolic left ventricle/aorta gradient of 25 mm Hg and discrete reduction of the aortic lumen at the sinotubular region. She was treated with atorvastatin 80 mg, cholestyramine, and ezetimibe 10 mg; her LDL-C was still 228 mg/dL (5.8 mmol/L). In the meantime, she had a normal pregnancy with a healthy child diagnosed with heterozygous FH. During pregnancy, all medications were suspended.

In 2004, she underwent cardiac computed tomography angiography that showed a calcium score of 44 Agatston units (>99% percentile for age and gender) and nonobstructive plaque in the aorta, near the ostium of the left coronary artery and more distal segments of the right coronary artery, left anterior descending artery, and the diagonal branch. In 2006, when she was 24 years old she entered a clinical trial where she received mipomersen 200 mg weekly. With treatment her LDL-C was reduced to 175 mg/dL (4.5 mmol/L). During the study protocol she suffered a non-Q wave myocardial infarction at the age of 25 and underwent coronary artery bypass surgery due to sub-occlusion of the left main coronary artery. Also, the ascending aorta showed many areas of calcification. She continued mipomersen treatment for 4 years, complaining of flu-like symptoms and injection site reactions. During follow-up she developed moderate to severe aortic stenosis and a 50% obstruction in the right carotid artery diagnosed by Doppler ultrasound. Liver magnetic resonance imaging showed no signs of steatosis. In 2014 she discontinued mipomersen treatment and started lomitapide initially at 10 mg a day that was up titrated to 20 mg/day. Despite the recommendation of a low-fat diet, supplemented with essential fatty acids and liposoluble vitamins it was not possible to raise the lomitapide dose due to diarrhea. With that her last LDL-C was 189 mg/dL (4.8 mmol/L). Her liver enzymes remained within normal values and repeat ultrasound scans showed no hepatic steatosis. She showed no clinical signs of coronary heart disease with repetitive negative stress and myocardial scintigraphy tests. However, atherosclerotic plaques were diagnosed now in both carotid arteries and there was evolution of supra-aortic valve disease with a peak and mean left ventricle-aortic gradients of 110 and 49 mm Hg. The aortic valve showed cusp thickening, calcification, and discrete regurgitation. She is being followed every 6 months with serial echocardiograms in order to identify the adequate timing for aortic surgery that will involve not only valve but also ascending aorta procedures. The patient was not treated with LA due to its unavailability in Brazil. The case shows the severity of atherosclerosis, valve and supra-aortic valve disease, and the clear difficulties in managing HoFH patients.

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Lysosomal Acid Lipase Deficiency

21

Johnathon Seth Parham and James A. Underberg

Abbreviations

CAD	Coronary artery disease
CE	Cholesterol ester
CESD	Cholesteryl ester storage disease
CV	Cardiovascular risk
FCH	Familial combined hyperlipidemia
HDL-c	High-density lipoprotein cholesterol
HeFH	Heterozygous familial hypercholesterolemia
HMGCoA	3-hydroxy-3-methylglutayl-coenzyme A
Kg	Kilogram
LAL	Lysosomal acid lipase
LAL-D	Lysosomal acid lipase deficiency
LDL	Low-density lipoproteins
LDL-c	Low-density lipoprotein cholesterol
LDLR	Low-density lipoprotein receptor
LFTs	Liver function tests
LIPA	Lysosomal acid lipase A
Mg	Milligram
NAFLD	Nonalcoholic fatty liver disease
TG	Triglycerides
VLDL	Very low-density lipoprotein

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Introduction

Lysosomal acid lipase deficiency (LAL-D) is a rare, monogenic autosomal recessive lysosomal storage disorder is associated with significant morbidity and increased risk for premature mortality [1]. The disorder is characterized by marked and progressive accumulation of cholesterol esters (CE) and triglycerides (TG) in multiple organs, including the liver, spleen, and cardiovascular system [1–3]. Hypercholesterolemia and progressive liver disease are common clinical findings, and despite its severity and the potential for treatment, the disease remains underrecognized with many affected persons receiving no diagnosis or incorrect diagnoses [2].

Lysosomal acid lipase A (LIPA) is a critical enzyme in the hydrolysis of cholesterol esters and triglycerides into free cholesterol and fatty acids after endocytosis of low-density lipoproteins (LDL). Deficiency of lysosomal acid lipase (LAL) activity results in the accumulation of cholesterol esters and triglycerides in the lysosomes [4]. LAL-D represents a heterogeneous disorder that can affect all individuals, from infancy to adulthood. It presents along a clinical spectrum, with variable severity and rate of pro-

M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_21

gression hat is believed to be related to the degree of residual enzyme activity and the specific mutation involved [5, 6].

Etiology of Lysosomal Acid Lipase Deficiency

LAL-D results from mutations in the *LIPA* gene located on chromosome 10q23.2. The gene for LAL-D is comprised of 10 exons and is 45 kb in length [4]. To date, over 100 LIPA mutations have been identified [7]. Individuals are typically either homozygous or compound heterozygous for LIPA mutations, with more severe mutations generally seen in affected infants [8]. The most commonly found mutation in LIPA is E8SJM (c.894G > A), and estimates of prevalence of LIPA mutations vary widely in different studies, from one in 130,000 to one in 300,000 [2, 9, 10]. However, a genetic study of common LAL mutations showed that based on the number of individuals who carry these mutations the hypothetical prevalence of LAL-D should be higher than the actual reported prevalence, with calculated prevalence among Hispanic and Caucasian populations as 1 in 130,000 [9]. No formal studies of incidence have been performed in populations of European ancestry; however, one study demonstrated that Jewish infants of Iraqi or Iranian origin in a Los Angeles community appear to be most at risk of LAL-D, with an estimated incidence of 1 in 4200 [11]. LAL-D heterozygosity has not been extensively studied, but a study examining individuals heterozygous for the E8SJM mutation demonstrated altered lipid profiles similar to polygenic hypercholesterolemia [12].

Lysosomes are vesicular organelles with acidic interiors that contain hydrolytic enzymes (proteases, lipases) and are responsible for the digestion of both extracellular and intracellular substrates [13]. LDL particles enter cells through receptor-mediated endocytosis. The internalized endosome merges with lysosomes and LDL particles release CE and TG. The LAL enzyme hydrolyzes CE and TG to release free fatty acids (FFA) and free cholesterol (FC) that can migrate into the cytosol of hepatocytes, where they can be utilized for membrane building, energy transport and storage, and bile acid biosynthesis (Fig. 21.1) [2, 14]. When LAL activity is diminished, lipid metabolism is disrupted, and cholesteryl esters and triglycerides accumulate in lysosomes. Under normal physiological conditions, intracellular free cholesterol interacts with sterol regulatory element binding proteins (SREBPs), which leads to SREBP2-mediated downregulation of 3-hydroxy-3-methylglutayl-coenzyme A (HMGCoA) reductase activity and low-density lipoprotein receptor (LDLR) expression [2]. In LAL-D, due to the depletion of intracellular free cholesterol, HMGCoA reductase activity increases, causing increased intracellular synthesis of cholesterol as well as apolipoprotein-B. This results in increased production of very low-density lipoproteins (VLDL) [15, 16]. In addition, many cells of the body upregulate low-density lipoprotein receptor expression resulting in increased LDL-c uptake, notably hepatocytes, macrophages (affecting spleen, GI, vasculature), and the endothelial wall (Fig. 21.2) [1, 2].

High-density lipoprotein (HDL) cholesterol is decreased in LAL-D [1]. HDL particle formation is mediated by ATP-binding cassette transporter A1 (ABCA1) and lysosomal cholesterol acts as a source of cholesterol for ABCA1-mediated cholesterol efflux [17]. In fibroblasts from LAL-D patients, reduced HDL formation results from impaired upregulation and activation of ABCA1, with subsequent improvement in ABCA1-mediated efflux demonstrated upon the addition of recombinant LAL [18]. This results in a lipid profile in LAL-D of high total cholesterol, low-density lipoprotein cholesterol (LDL-c), triglycerides, and decreased high-density lipoprotein cholesterol (HDL-c) [1, 2]. Indeed, individual cases have demonstrated elevations of total cholesterol and low-density lipoprotein cholesterol similar to autosomal recessive hypercholesterolemia and homozygous familial hypercholesterolemia [19, 20].

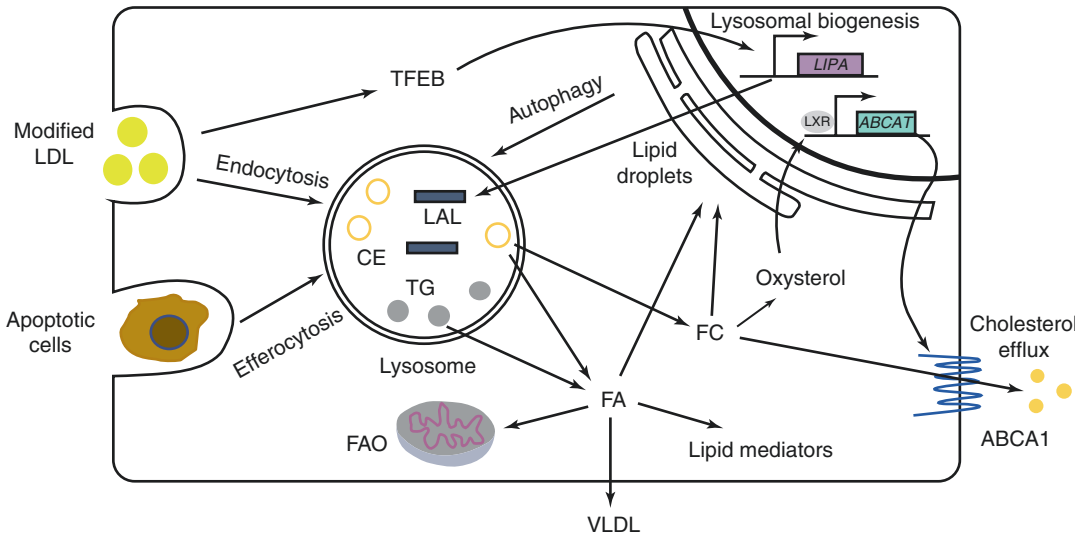

Fig. 21.1 Lysosomal acid lipase (LAL)–mediated lysosomal lipolysis links lipid metabolism to diverse cellular functions. LAL hydrolyzes cholesteryl ester (CE) and triglyceride (TG) in the lysosome to release fatty acids (FAs) and free cholesterol (FC). Modified low-density lipoprotein (LDL) internalized through scavenger receptor–mediated endocytosis is an important source of CE and TG for lysosomal hydrolysis. The hydrolyzed FAs and FC can be re-esterified and form lipid droplets in the endoplasmic reticulum (ER) for storage. Lipid droplets can be delivered to the lysosome for LAL-mediated hydrolysis via autophagy to provide energy supply and maintain cellular homeostasis. The engulfed apoptotic cells by macrophages through a process called efferocytosis also deliver neutral lipids to the lysosome, and LAL is essential for maintaining the efferocytosis capacity of macrophages. The lipolytic products of LAL have active biological roles. Hydrolyzed FAs are substrates for fatty acid oxidation (FAO) and synthesis of very low-density lipoprotein (VLDL) 0.32 CE-derived FAs also provide precursors for the synthesis of lipid mediators that have a broad spectrum of functional impact on inflammatory response and resolution. (Figure and legend reproduced with permission from Li and Zhang [68])

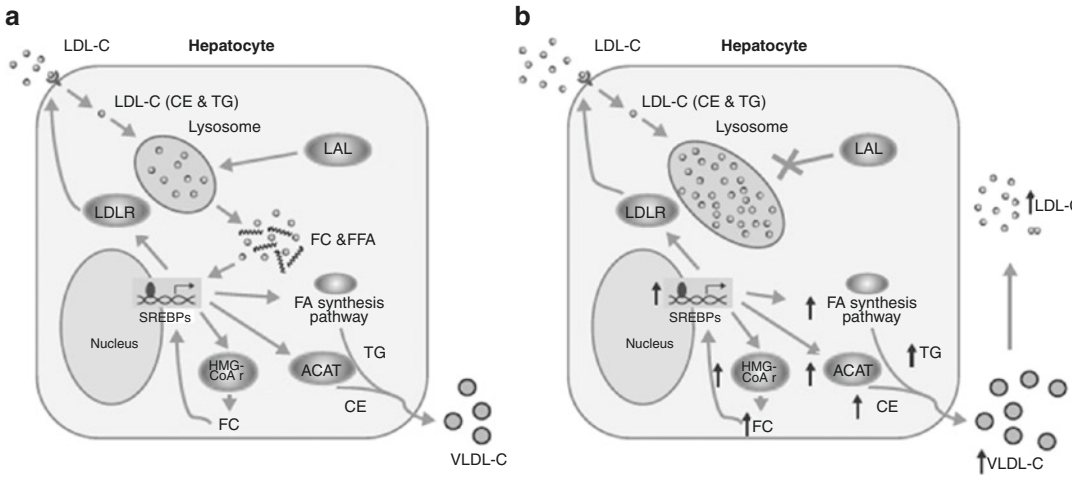

Fig. 21.2 Schematic view of cellular cholesterol homoeostasis in (**a**) healthy individuals and (**b**) patients with LAL-D. ACAT acyl-cholesterol acyltransferase, CE cholesteryl esters, FA fatty acid, FC free cholesterol, FFA free fatty acid, HMG-CoA r hydroxy-methyl-glutaryl-coenzyme A reductase, LAL lysosomal acid lipase, LAL-D LAL deficiency, LDL-C low-density lipoprotein cholesterol, LDLR low-density lipoprotein receptor, SREBPs sterol regulatory element binding proteins, TG triglyceride, VLDL-C very-low-density lipoprotein cholesterol. (Figure and legend reproduced with permission from Reiner et al. [2])

Clinical Manifestations of Lysosomal Acid Lipase Deficiency

Lysosomal accumulation of CE and TG has distinct physiological consequences across a wide range of affected organs and systems. The atherogenic dyslipidemia seen in LAL-D is associated with increased cardiovascular (CV) risk [1, 3, 21, 22]. One case report demonstrated an aortic plaque in a 9-year-old child with LAL-D who died [23]. In addition, genome-wide association studies in three case control cohorts identified LIPA as a susceptibility gene for coronary artery disease (CAD) [24–26]. CE and TG accumulation in hepatocytes and Kupffer cells (a specialized macrophage residing in the sinusoidal spaces of the liver) leads to elevated transaminases, hepatomegaly, microvesicular steatosis, and fatty liver, which over time progresses to fibrosis and cirrhosis [1, 2]. Hepatic manifestations of LAL-D can occur early; macrophage-derived foam cells containing lipids have been observed in the sinusoids and portal tracts of young patients, and death due to liver disease progression has occurred in patients as young as 7 years of age [1]. In a review of 135 cases of children and adults with LAL-D, 112 liver biopsies were performed with 64% demonstrating fibrosis and/or cirrhosis, including sinusoidal, portal/periportal, or septal fibrosis [1]. In the same group, of the 11 reported deaths, 73% were due to liver failure [1]. In one observational study of 49 children and adult patients, liver damage was evident in the majority of liver biopsies from patients with LAL-D, with 68% (17/25) of biopsied patients aged younger than 18 years exhibiting evidence of fibrosis and/or cirrhosis [27]. Splenomegaly, which can occur with portal hypertension [28], but may also be due in part to accumulation of macrophages laden with unprocessed CE [29], is another common manifestation [1]. The spleen can reach over 20 times its normal size by 2–3 months [30]and hypersplenism with anemia and thrombocytopenia can also occur [1, 31, 32]. The gastrointestinal (GI) system may also be affected with lipid accumulation in the intestinal mucosa. Clinical manifestations may include abdominal and epigastric pain, emesis, gallbladder dysfunction, diarrhea, GI bleeding, and malabsorption [1, 2]. Abnormal lipid deposition has also been described in lymph nodes, adrenal glands and skeletal muscle (Fig. 21.3) [33].

Clinical signs and symptoms	Hepatomegaly/hepatosplenomegaly
	Diarrhoea
	Abdominal and epigastric pain
	Vomiting
	Anaemia
	Malabsorption
	CHolestasis
	Cholestasis
	Steatorrhoea
	Poor growth
	Gallbladder dysfunction
	Coronary artery disase
	Aneurysm
	Stroke
	Adrenal calcification
	Oesophageal varics
Serum markers	Elevated total cholesterol
	Elevated low-density lipoptein cholesterol
	Decreased high-density lipoptein cholesterol
	Elevated serum transaminases
Liver biopsy findings	Bright yellow-orange in colour
	Enlarged lipid-laden hepatocytes and Kupffer cells
	Microvesicular steatosis (may be mixed with macrovesicular steatosis)
	Fibrosis
	Micronodular cirrhosis

Fig. 21.3 Summary illustrating range of clinical features, serum markers, and liver biopsy findings in children and adults with LAL-D. (Figure and legend reproduced with permission from Reiner et al. [2])

Infants are typically affected with the most severe and rapidly progressing form of LAL-D, historically called Wolman disease, likely due to most mutations affecting infants resulting in an absolute enzyme deficiency [34]. LAL-D presenting in infants is a rapidly progressive and fatal disease with median age at time of death being 3.7 months (range: 1.4–46.3 months) [35]. The median age of symptom onset is 1 month and approximately 50% of affected infants have adrenal calcifications [35, 36]. Failure to thrive with malabsorption and growth failure are often the first observed clinical manifestations and key contributors to premature mortality in affected infants [1].

Massive hepatomegaly results in profound abdominal distention and the disease rapidly progresses to fibrosis, cirrhosis, and hepatocellular failure (Figs. 21.4 and 21.5) [33]. In children and adults with LAL-D, residual LAL activity is often present, and the disease was historically called cholesteryl ester storage disease (CESD). However, the natural history of LAL-D in children and adults is less well defined and detection of disease is often incidental [37]. The severity of symptoms and clinical course in these LAL-D patients can be highly variable and patients may be diagnosed at any age, from the first few years of life through adulthood [2]. The median age of first reported manifestation of LAL-D is 5 years and 83% of symptom onset and/or diagnosis of LAL-D is by age 12 [1]. Hepatomegaly is nearly universal at diagnosis and complications, including CV disease, may occur as early as childhood, with progression to cirrhosis being common [1, 2, 37]. Systemic lysosomal lipid accumulation leads to progressive multisystem organ damage. In 135 children and adults with LAL-D, clinical manifestations were apparent in more than one organ system in 87% of cases, with the liver, cardiovascular system, and spleen being the most frequently affected [1].

Diagnosis of LAL-D

LAL-D shares similarities with other cardiovascular, liver, and metabolic diseases, making successful diagnosis a potential challenge. Based on its known prevalence, the LAL-D patient population is disproportionately younger than the general population, suggesting that many LAL-D patients may be missed [1, 2, 27]. Common misdiagnoses include cryptogenic cirrhosis, nonalcoholic fatty liver disease (NAFLD), heterozygous familial hypercholesterolemia (HeFH), familial combined hyperlipidemia (FCH), and polygenic hypercholesterolemia (Fig. 21.6) [16, 37–39]. A detailed family history will assist in determining autosomal recessive versus autosomal dominant patterns of

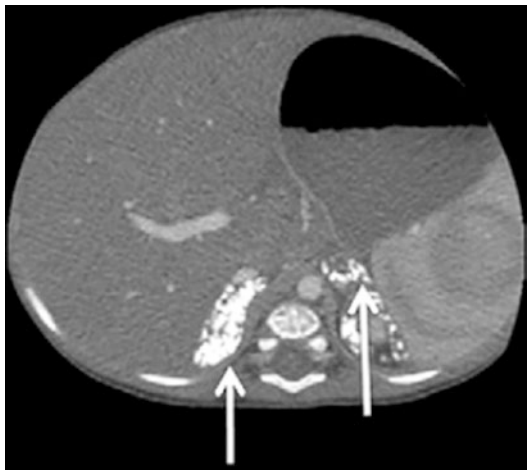

Fig. 21.4 Liver ultrasonography showing signs of adrenal calcification (white arrows). (Figure and legend reproduced with permission from Valayannopoulos et al. [69])

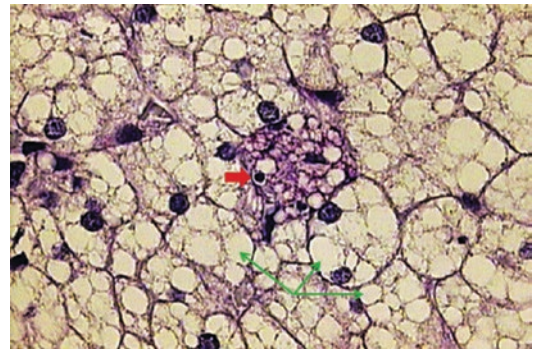

Fig. 21.5 Hepatocytes show microvesicular steatosis (green arrows). The foamy Kupffer cell (red block arrow) in the perivenular zone is PAS positive (PAS after diastase digestion, original magnification 630). PAS, periodic acid-Schiff. (Figure and legend reproduced with permission from Reiner et al. [2])

The hypercholersterolaemic phenotype in molecular diagnosed

		LIPA[a](*N*=21)	ApoB[b](*N*=43)	LDLR[b](*N*=2479)	PCK9-GOF[b](*N*=27)	ARH[b](*N*=38)
Sex	Male (%)	48	51	48	38	60
Age	mean (Years) [SD]	24[18]	38 [19]	26 [19]	36 [18]	25 [11]
	Range (Years)	3-68	7-75	1-84	7-68	4-47
TC	mean (mmol/l) [SD]	8.4 [2.0]	8.2 [1.7]	8.5 [2.3]	9.8 [2.3]	15.1 [3.0]
	Range (mmol/l)	4.9-12.9	4.5-11.4	4.3-19.1	6.7-16.3	8.2-25.9
LDLc	mean (mmol/l) [SD]	6.9 [1.7]	6.4 [1.5]	6.7 [2.2]	7.9 [2.5]	13.4 [3.0]
	Range (mmol/l)	3.5-10.3	3.1-9.8	3.0-16.5	4.9-15.0	6.52-24.3
HDLc	mean (mmol/l) [SD]	0.9 [0.4]	1.2 [0.3]	1.3 [0.3]	1.3 [0.5]	1.1 [0.3]
	Range (mmol/l)	0.4-1.9	0.8-1.9	0.4-3.3	0.45-2.6	0.6-1.9
TG	mean (mmol/l) [SD]	2.0 [0.8]	1.3 [0.7]	1.2 [0.8]	1.2 [0.8]	1.3 [0.7]
	Range (mmol/l)	1.0-3.8	0.3-3.4	0.1-7.6	0.4-4.1	0.5-4.4

Fig. 21.6 ApoB apolipoprotein B-100, ARH Autosomal recessive hypercholesterolemia, HDLc HDL-cholesterol, LDLc LDL-cholesterol, LIPA lipase A, LDLR LDL receptor, PCSK9 proprotein convertase subtilisin-like kexin type 9, TC total cholesterol, TG triglycerides. [a]Homozygous or compound heterozygous carriers extracted from literature [11, 12, 18–27]. [b]Heterozygous carriers extracted from Dutch ADH cohorts [28–31]. [c]Heterozygous carriers extracted from literature [32–37]. [d]Homozygous or compound heterozygous carriers extracted from literature [6, 38–45]. Carriers with reported baseline levels of TC, LDLc, HDLc, and TG were included. (Figure and legend reproduced with permission from Fouchier and Defesche [16])

inheritance. HDL-C levels are usually lower in LAL-D than in HeFH, although overlap can occur [40]. Liver abnormalities in LAL-D are well characterized and LAL-D should be considered in patients with signs of liver abnormalities; however, the rate of progression and presentation of symptoms may not be consistent between patients [21]. Serum transaminases may only be slightly elevated, and rarely, normal (Fig. 21.7) [1].

The 2012 American Association for the Study of Liver Diseases/American Gastroenterological Association/American College of Gastroenterology (AASLD/AGA/ACG) Guidelines recommend excluding competing etiologies for steatosis and coexisting frequent chronic liver diseases when evaluating a patient with suspected NAFLD [41]. A full viral/immunological profile should be carried out to exclude more common disorders such as viral hepatitis and autoimmune liver disease. Wilson disease shares many similarities with LAL-D; however, in the absence of central nervous system (CNS) involvement and Kayser–Fleischer rings (dark rings around the ocular iris), LAL-D should be considered [42]. Liver biopsy should be considered only after other causes of steatosis are ruled out by noninvasive means. In cases where microvesicular steatosis is observed on biopsy prior to diagnosis, LAL-D should be ruled out [41]. It is also recommended to test young children who are not overweight for monogenic causes of chronic liver disease, including lysosomal storage diseases [41]. The 2012 European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Guidelines notes that NAFLD does not typically occur in children younger than 3 and is rare in children younger than 10 [43]. Metabolic syndrome and NAFLD commonly present with persistently elevated liver function tests (LFTs) and dyslipidemia, though elevated LDL-c may not be seen. If persistently elevated LDL-c is demonstrated in this setting, or the patient is nonobese with fatty liver, consideration should be given to LAL-D [2]. Lastly, patients with FCH do not typically present with elevated ALT and LAL-D should be suspected [2].

Diagnosis of LAL-D can be accurately made via a blood test analyzing LAL enzyme activity using peripheral leukocytes or dried blood spots. Advances in dried blood spot techniques allow for rapid and reliable diagnosis with less than 10% mean normal enzymatic activity cited as diagnostic for LAL-D [44]. Supportive diagnostic measures for LAL-D include genetic testing measures and liver biopsy, though some patients may have mutations that can't be detected, and liver biopsy

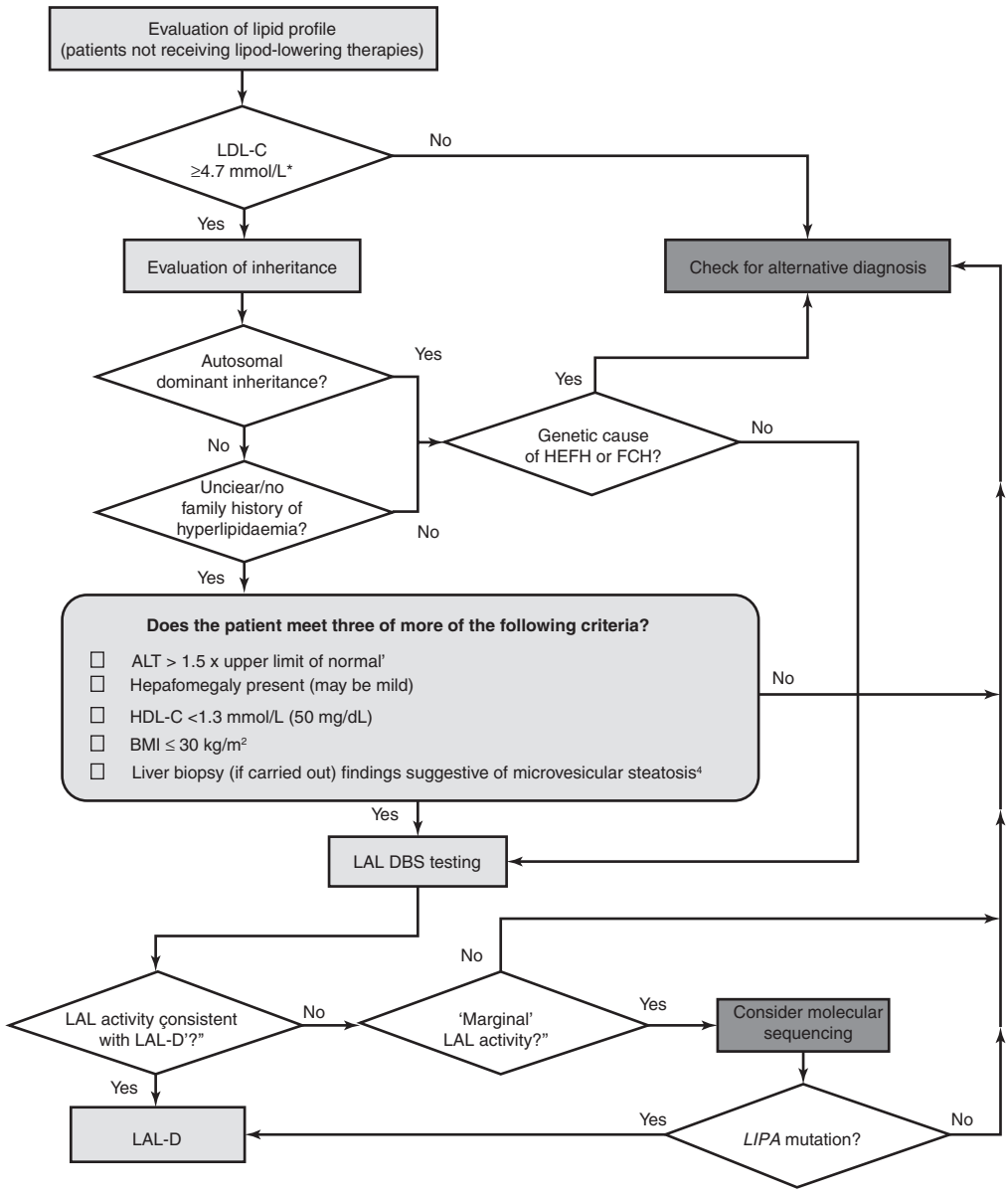

Fig. 21.7 Recommended screening criteria for LAL-D in patients at baseline assessment or not receiving lipid-lowering therapies. ALT alanine aminotransferase, BMI body mass index, DBS dried blood spot, FCH familial combined hyperlipidemia, HeFH heterozygous familial hypercholesterolemia, HDL-C high-density lipoprotein cholesterol, LAL lysosomal acid lipase, LAL-D lysosomal acid lipase deficiency, LDL-C low-density lipoprotein cholesterol, LAL gene. [a]Or below the 95th percentile for age and sex in children and adults. It should be noted that LDL-C might be lower in some patients with LAL-D, especially in those receiving statins. [b]Upper limit of normal for age and sex in healthy individuals, with no clear explanation (e.g., viral hepatitis, excessive alcohol consumption). It should be noted that, owing to periodic fluctuations in ALT levels, this sign may not always be detected. [c]Or above the 95th percentile for age and sex in children and adults. It should be noted, however, that BMI may be above 30 in some patients with LAL-D, depending on diet. [d]Please note that liver biopsy is not recommended as a diagnostic method for LAL-D. [e]When measured by DBS testing, mean LAL activity is approximately 1.00 nmol/punch/hour in healthy individuals. LAL activity less than or equal to 0.03 nmol/punch/hour (3% mean normal) is considered consistent with LAL-D. Marginal LAL activity is defined as a measurement between 0.03 nmol/punch/hour and 0.15 nmol/punch/hour (i.e., 3–15% mean normal). Patients with LAL activity in this range should be referred for molecular sequencing of the *LIPA* gene. Readers should note that reference ranges will vary between laboratories. (Figure and legend reproduced with permission from Reiner et al. [2])

is not diagnostic. The presence of microvesicular steatosis on biopsy may be suggestive of LAL-D but is not a feature unique to LAL-D [2, 45].

Clinical Management

Historical management of patients with LAL-D included low-fat diet, lipid-lowering therapies, and hematopoietic stem cell transplant and liver transplantation. Case reports of adult and pediatric patients with LAL-D treated with statins (HMGCoA reductase inhibitors) as monotherapy or in combination with other lipid-lowering therapies demonstrate reduction in LDL-c in most patients, though some patients had persistent elevations in LDL-c [16, 20, 22, 46–52]. Fibroblasts from some patients with LAL-D have demonstrated significant reduction in cholesterol synthesis upon treatment with statins, with one study showing a reduction in hepatic apolipoprotein-B production [46, 47]. However, one large observational study demonstrated persistence of LDL-c elevations in many LAL-D patients treated with statins [53]. In addition, there is evidence of progressive liver damage despite statin therapy in patients with LAL-D, with fibrosis progression occurring in all patients on long-term follow-up despite improvement in liver size in some patients [1, 47, 48, 52]. Ezetimibe, which inhibits gastrointestinal absorption of cholesterol by Niemann–Pick C1-like 1 protein has been used in patients with LAL-D. One study examined three LAL-D patients who were treated with ezetimibe for 9–10 years, with atorvastatin supplementation in two patients in the last 6 years [54]. All patients showed a reduction in LFTs, cholesterol, triglycerides, and no progression of liver fibrosis [54]. Tocopherol, which has vitamin E activity, promoted lysosomal exocytosis (i.e., excess lysosomal lipid was exported) and reduced lipid accumulation in fibroblasts from an infant with LAL-D [55]. Unfortunately, the unfavorable pharmacokinetics of tocopherol and rapid oxidation of vitamin E derivatives by the cytochrome P450 system make the therapeutic use of tocopherol in vivo a challenge [2]. A few infants with LAL-D have undergone hematopoietic stem cell transplantation [56–58]. However, high toxicity, problems with engraftment and failure to address the multisystem nature of the disease have limited success with stem cell transplantation as a viable therapeutic option [56–58]. Liver transplantation in patients with LAL-D has improved survival at 5 years post-transplant, but there is limited data on long-term outcomes [59, 60]. One case report of 18 LAL-D patients post liver transplant noted disease progression in 11 patients and death in 6. The progression was thought to be related to infiltration of the donor liver by host derived cells of monocyte-derived macrophages deficient in LAL enzyme activity [61].

Enzyme replacement strategies have been a successful strategy in treating many inborn errors of metabolism. Sebelipase alfa, a recombinant human LAL enzyme under the trade name Kanuma, has recently been made available. The medication binds to cell surface receptors via glycans and is internalized into lysosomes [62]. A phase I/2 open label study of sebelipase alfa was performed in eight adults which was extended to 52 weeks of treatment [63]. At week 52, the mean ALT and AST levels for the group were within normal limits; representing a mean change from baseline of −58% and − 40%, respectively. Patients had a mean reduction in liver volume on MRI by −12% and in hepatic proton density fat fraction by −55%. The mean changes for LDL cholesterol, total cholesterol, triglyceride, and HDL cholesterol were observed by −60%, −39%, −36%, and + 29%, respectively [64]. The treatment was well tolerated overall [64]. Another open label study examined sebelipase alfa in nine infants with LAL-D who demonstrated growth failure or other signs evidence of rapidly progressive disease with onset before 6 months of age [65]. The median age in this study was 3 months. Two deaths occurred due to advanced disease and an additional death occurred as a result of paracentesis-related complications. Survival to 12 months was noted in the remaining six participants. This was compared to zero survivors of a historical control cohort matched for age and clinical characteristics. These six infants showed improvements in weight-for-age and reductions in both hepatosplenomegaly and LFTs. Gastrointestinal symptoms improved as well [65]. Five of the children lived to ≥24 months with marked improvement in growth. One infant developed a serious adverse

event, but all other infusion related reactions were considered mild [65]. Lastly, there was a phase 3, multicenter, randomized, double-blind, placebo-controlled trial of sebelipase alfa called the ARISE trial [66]. This trial involved 66 patients with LAL-D and substantial baseline disease, 50 of whom were ≤ 18 years old and the age range was 4–58 years old. The placebo-controlled phase lasted 20 weeks. At 20 weeks the ALT level was normal in 11/36 (31%) treated patients, as opposed to 2/30 (7%) in the placebo group. MRI assessment of hepatic fat content demonstrated significant improvement in the treatment group compared to the placebo group. Lipid profiles were also significantly improved with treatment [66]. At 20 weeks in the sebelipase alfa group mean (SD) percentage change from baseline in the LDL particle number for patients using lipid-lowering drugs or not was −34.2% (−13.6) and − 21.7% (−20.6), respectively [66]. At 52 weeks, the changes in LDL particle number were − 43.0% (11.8) and − 43.8% (16.2), all of which was statistically significant compared to placebo [67]. The most common adverse reactions in the treatment group were headache, fever, nasopharyngitis, constipation, and nausea; however, rates were similar between treatment and placebo groups and most events were deemed by the investigators to be unrelated to the study drug [66].

Sebelipase alfa is given as an intravenous infusion, 1 milligram (mg) per kilogram (kg) every 2 weeks in adults. In rapidly progressive disease in infants 1 month or older 1 mg/kg every week is started and can be increased to 3 mg/kg every week. If disease is non-rapidly progressive, then infants 1 month or older are given 1 mg/kg every 2 weeks [62]. In the clinical studies infusion-related reactions were noted as consistent with anaphylaxis (in 3/106, 3%) or hypersensitivity (21/106, 20%), with events occurring in both infants and adults. The majority of the reactions occurred within 4 hours of finishing the treatment infusion and premedication and/or slower infusion rate allowed for continuation of subsequent treatments [62]. Anti-drug antibodies (ADA) did develop in some patients receiving sebelipase alfa, the majority developing ADA within the first 3 months of exposure. The impact of the development of ADA to sebelipase alfa on treatment or the occurrence of adverse events is unknown [62].

Conclusions

1. LAL-D remains a life-threatening and underdiagnosed autosomal recessive disease associated with significant morbidities and increased risk for premature mortality.
2. LAL-D results in progressive cholesterol ester and triglyceride accumulation, primarily in hepatocytes and macrophages, leading to hepatomegaly, microvesicular steatosis, cirrhosis, dyslipidemia, and accelerated atherosclerosis.
3. LAL-D is predominantly a pediatric disease with some affected children suffering early liver failure and requiring transplantation.
4. Diagnosis of LAL-D is performed with an easily available dry blood spot testing and measures degree of enzyme activity.
5. Individuals with less obvious signs and symptoms may remain undetected or misdiagnosed until a premature cardiovascular event or sudden death from liver failure. As such, early recognition and accurate diagnosis of LAL-D are critical, and unexplained liver and/or lipid abnormalities should raise suspicion of LAL-D. This is now even more imperative given the availability of enzyme replacement therapy.
6. The LAL Deficiency Registry is a global registry established to collect data from volunteers with LAL-D. The data will be used to help healthcare providers better understand the disease and its management. Health care providers (HCPs) can request to enroll online: www.laldeficiencyregistry.com.

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Lipodystrophies

22

Vinaya Simha and Abhimanyu Garg

Introduction

Lipodystrophies are a group of inherited and acquired disorders characterized by selective loss of adipose tissue [1–3]. The loss of body fat occurs in the absence of energy deficit, and results from a variety of genetic defects or acquired causes, which impair the development, survival, or function of adipocytes. The extent of body fat loss can also vary across the different lipodystrophy syndromes, ranging from localized loss confined to a small region; partial loss, affecting the extremities or upper body in patients with partial lipodystrophies; to near total loss of body fat in patients with generalized lipodystrophies. Lipodystrophies predispose patients to insulin resistance and other metabolic abnormalities including severe hypertriglyceridemia, hepatic steatosis, and diabetes mellitus. The severity of metabolic derangements seems to correlate positively with the extent of body fat loss. While metabolic complications do not occur in those with localized lipodystrophies, they are a common feature in others with partial or generalized lipodystrophies and this fact serves to highlight the critical role of adipocytes in the maintenance of glucose and lipid homeostasis. Understanding the pathophysiology of lipodystrophy syndromes will not only help care for these rare patients with complex lipid disorders, but will also provide invaluable insight into the pathogenesis of more common disorders characterized by excess adiposity and insulin resistance.

Classification

Lipodystrophies can be broadly classified as genetic or acquired varieties based on the etiology and as generalized or partial based on the extent of fat loss. Accordingly, four major categories of lipodystrophy syndromes have been traditionally recognized: Congenital Generalized Lipodystrophy (CGL), Familial Partial Lipodystrophy (FPL), Acquired Generalized Lipodystrophy (AGL), and Acquired Partial Lipodystrophy (APL). We will not discuss localized lipodystrophies in this chapter as they do not cause dyslipidemias. Another more common subtype is the High Active Anti-retroviral Therapy

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_22

(HAART)–induced Lipodystrophy in HIV-infected patients, which presents as mostly partial lipodystrophy. Much progress has been made in the last two decades in the identification of molecular basis of many genetic lipodystrophy syndromes, which has led to further subclassifications. Table 22.1 summarizes various genes, which have been implicated in patients with different subtypes of inherited lipodystrophy. Progress in understanding underlying reasons for acquired lipodystrophies, however, has been rather slow.

Table 22.1 Genetic causes of inherited lipodystrophy syndromes

Lipodystrophy syndrome	Inheritance	Gene	Putative gene function
Congenital generalized lipodystrophy (CGL)			
CGL1	AR	*AGPAT2*	Biosynthesis of triglycerides and phospholipids
CGL2	AR	*BSCL2*	Lipid droplet assembly and adipocyte differentiation
CGL3	AR	*CAV1*	Integral component of caveolae on adipocyte membrane which are involved in lipid transport
CGL4	AR	*CAVIN 1 (or PTRF)*	Regulation of caveolae formation
Familial partial lipodystrophy (FPLD)			
FPLD1	AD	Unknown	
FPLD2	AD	*LMNA*	Nuclear lamina protein: regulation of nuclear structure and function
FPLD3	AD	*PPARG*	Transcription factor regulating adipocyte differentiation
FPLD4	AD	*PLIN1*	Lipid droplet-associated protein
FPLD5	AR	*CIDEC*	Lipid droplet formation
FPLD6	AR	*LIPE*	Regulation of lipolysis
FPLD7	AD	*ADRA2A*	Regulation of neurotransmitter release in sympathetic ganglia
FPLD–other	AD	*AKT2*	Downstream insulin signaling
Other rare syndromes associated with lipodystrophy			
Mandibuloacral dysplasia (Type A, B)	AR	*LMNA, ZMPSTE24*	Nuclear lamina structure and function
Autoinflammatory syndromes (JMP, CANDLE)	AR	*PSMB8*	Regulation of proteasome function
SHORT	AD	*PIK3R1*	Regulation of growth signaling pathways
Wiedemann–Rautenstrauch syndrome (Types A,B,C)	AD, AD,AR	*FBN1*, *CAV1*, *POLR3A*	Constitutive element of extracellular microfibrils, Integral component of caveloae, DNA-directed RNA polymerase
Atypical progeroid syndrome	AD	*LMNA*	Nuclear lamina structure and function
MDP syndrome	AD	*POLD1*	DNA replication and repair

Abbreviations: *AD* autosomal dominant, *ADRA2A* Adrenoceptor alpha 2 a, *AGPAT2* 1-acylglycerol-3-phosphate O-acyltransferase 2, *AKT2* v-akt murine thymoma viral oncogene homolog 2, *AR* autosomal recessive, *BSCL2* Berardinelli–Seip congenital lipodystrophy 2, *CANDLE* chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature, *CAVIN1* Caveloae associated protein 1, *CIDEC* cell death–inducing DFFA-like effector c, *CGL* Congenital generalized lipodystrophy, *CAV1* caveolin 1, *FBN1* Fibrillin 1, *FPLD* Familial partial lipodystrophy, *JMP* joint contractures, muscle atrophy, microcytic anemia and panniculitis-induced lipodystrophy, *LIPE* lipase E, hormone sensitive type, *LMNA* lamin A/C, *MDP* mandibular hypoplasia, deafness, progeroid features, *PIK3R1* phosphoinositide-3-kinase regulatory subunit 1, *PLIN1* perilipin 1, *POLR3A* RNA Polymerase III Subunit A, *POLD1* DNA polymerase delta 1 catalytic subunit, *PPARG* peroxisome proliferator-activated receptor gamma, *PSMB8* proteasome subunit beta 8, *PTRF* polymerase I and transcript release factor, *SHORT* short stature, hyperextensibility or inguinal hernia, ocular depression, Rieger anomaly, and teething delay, *ZMPSTE24* zinc metallopeptidase STE24

Prevalence

Lipodystrophies are rare disorders, and while the prevalence of the different syndromes varies, they are uniformly low. The prevalence of CGL has been estimated to be 1 in 10 million with about 500 reported cases, mostly CGL1 and CGL2 subtypes [4, 5]. The prevalence of autosomal dominant FPL, Dunnigan variety (FPLD2) is estimated to be about 1 in 1 million with about a thousand reported cases [6]. However, it is very likely that many patients with partial lipodystrophy, especially men, and those with an atypical variety not related to *LMNA* mutation are often undiagnosed, and the true prevalence is probably higher. Further in some populations with a founder effect, like in Lebanon and Brazil for CGL, and in the New Brunswick region of Canada for FPLD, the prevalence is higher than current estimates. The two well-characterized forms of acquired lipodystrophies, partial and generalized, have been described in about 100 patients each [7, 8], but HAART-induced lipodystrophy in HIV-infected patients is more common. In the past, it was estimated that nearly 50% of patients on HIV-1 protease inhibitors-containing HAART for HIV infection developed lipodystrophy after approximately 2 years [9, 10], but the incidence has considerably decreased more recently, likely due to changing patterns of antiretroviral medication use.

Clinical Features of the Different Lipodystrophy Syndromes

Congenital Generalized Lipodystrophy (CGL)

CGL is an autosomal recessive disorder characterized by near-complete lack of body fat from birth or shortly thereafter during the neonatal period. Affected subjects also have muscular prominence, phlebomegaly, and acromegaloid appearance (Fig. 22.1a). Acanthosis nigricans, umbilical prominence, and hepatomegaly may also be noted. These physical features and metabolic abnormalities related to extreme insulin resistance are often evident from birth. Voracious appetite, likely due to low serum leptin levels exacerbates metabolic abnormalities. Female patients with CGL usually have hirsutism, clitoromegaly, oligomenorrhea, and polycystic ovaries. Genetic studies have helped identify different subtypes of CGL (Table 22.1). CGL types 1 and 2 are the most common with subtle differences in fat distribution between the two subtypes. Patients with CGL type 1 have preservation of mechanical adipose tissue over the scalp, in the orbits, in the palms and soles, and around joints [11]. Subtype recognition is useful as CGL2 is more often associated with mental retardation and cardiomyopathy [12]. Patients with CGL4 appear to be more prone for ventricular tachyarrhythmias and myopathy [13].

Familial Partial Lipodystrophy (FPLD)

Patients with FPLD have normal body fat distribution at birth, and lose variable amounts of subcutaneous fat, primarily from the extremities during childhood and adolescence. Autosomal dominant FPLD2, due to heterozygous mutations in *LMNA* is the most common subtype, called the Dunnigan variety, and is characterized by extreme paucity of subcutaneous fat from both the upper and lower extremities and anterior trunk including breasts (Fig. 22.1b). Fat around the face, neck, and upper back is preserved and often excessive. Intraabdominal fat is also preserved and often increased [14]. Muscular prominence and acanthosis nigricans are also noted. Physical and metabolic features usually manifest after puberty, but recent reports suggest that subtle fat loss and hyperlipidemia may be noted in prepubertal children as well [15]. Both physical features and metabolic disturbances are more prominent in female subjects [16]. It is also quite possible that the diagnosis of partial lipodystrophy is often overlooked in male subjects, especially those with FPLD1 where truncal fat is preserved. Similarly patients with FPLD3 due to *PPARG* mutations have fat loss confined to

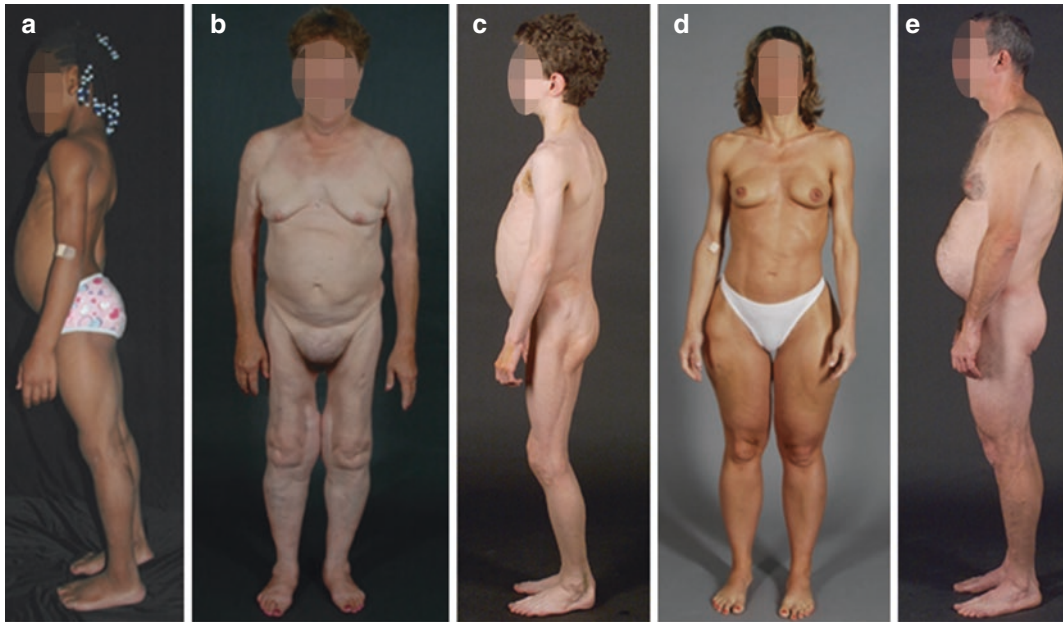

Fig. 22.1 Clinical features of different lipodystrophy syndromes. (**a**) Lateral view of an 8-year-old African American girl with congenital generalized lipodystrophy type 1 showing generalized loss of subcutaneous fat (from birth), acanthosis nigricans over the neck, prominent musculature, and acromegaloid features (enlarged mandible, hands, and feet). (**b**) Anterior view of a 65-year-old white woman with familial partial lipodystrophy of the Dunnigan variety showing loss of subcutaneous fat from the limbs and anterior truncal region (from puberty), atrophic breasts, and increased subcutaneous fat deposits in the face, anterior neck, suprapubic and vulvar region, and medial parts of the knees. (**c**) Lateral view of an 8-year-old German boy with acquired generalized lipodystrophy showing generalized loss of subcutaneous fat (from age 3 years) with marked acanthosis nigricans in the neck, axillae, and groin. (**d**) Anterior view of a 39-year-old white woman with acquired partial lipodystrophy (Barraquer–Simons syndrome) showing marked loss of subcutaneous fat from the face, neck, upper extremities, chest, and abdomen (from age 12 years), but increased subcutaneous fat deposition in the lower extremities. (**e**) Lateral view of a 39-year-old white man with HIV infection on protease inhibitor–containing highly active antiretroviral therapy showing features of partial lipodystrophy including marked loss of subcutaneous fat from the face and limbs, with increased subcutaneous fat deposition in the neck and abdomen. (From Hussain and Garg [2]; by permission of Oxford University Press)

extremities only. On the other hand, more widespread fat loss and autosomal recessive inheritance has also been rarely reported in some FPLD patients [17, 18].

Other Rare Syndromes with Lipodystrophy

Both partial and generalized loss of fat may accompany some rare genetic syndromes, many of which are also characterized by progeroid features. These include both neonatal progeroid syndrome (Wiedemann–Rautenstrauch Syndrome) and atypical progeroid syndrome where the progeroid manifestations start later in life. Fat loss in these patients is often accompanied by concomitant reduction in lean body and bone mass [19, 20]. Mandibuloacral dysplasia is characterized by distinct skeletal manifestations and either partial or generalized lipodystrophy [21]. SHORT syndrome (short stature, hyperextensibility and/or inguinal hernia, ocular depression, Rieger anomaly and teething delay) is associated with partial fat loss from the face and upper trunk, or localized fat loss from the hip region

[22, 23]. Autoinflammatory syndromes like JMP (joint contractures, muscle atrophy, microcytic anemia, and panniculitis-induced lipodystrophy) and CANDLE (chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature) are also extremely rare disorders due to proteasome dysfunction which are associated with panniculitis-induced lipodystrophy [24, 25].

Acquired Generalized Lipodystrophy (AGL)

AGL is also characterized by severe generalized fat loss (Fig. 22.1c) and metabolic abnormalities like in those with CGL but subjects have normal body fat distribution at birth. Fat loss usually occurs during childhood or adolescence and there is a marked female preponderance of 3:1 [7]. It is often seen in association with other autoimmune disorders such as juvenile dermatomyositis and systemic lupus erythematosus, and some patients have been reported to have anti-perilipin antibodies [26]. Focal inflammation of subcutaneous fat depots (panniculitis) may precede generalized fat loss in some patients, but no apparent cause can be identified in many patients (idiopathic AGL). About 100 patients with AGL have been reported in the literature.

Acquired Partial Lipodystrophy

Similar to AGL, APL (Barraquer–Simons syndrome) is also likely of autoimmune origin, but fat loss is confined to the face, upper extremities, and upper trunk. Lower extremities are spared from fat loss, and paradoxical increase in lower body fat may sometimes be seen (Fig. 22.1d). Metabolic abnormalities are less common compared to other lipodystrophy syndromes, but about 20–25% of patients develop membranoproliferative glomerulonephritis [8]. Like AGL, it is more common in female subjects and may be seen in association with other autoimmune diseases like dermatomyositis and systemic lupus erythematosus.

HAART-Induced Lipodystrophy in HIV-Infected Patients

This is the commonest form of acquired lipodystrophy, though its incidence is steadily decreasing likely due to reduced use of older nucleoside reverse transcriptase analogues like stavudine and HIV-1 protease inhibitors. Most patients develop a partial lipodystrophy similar to FPLD (Fig. 22.1e), but some also lose fat from the face [9, 27]. Excess accumulation of visceral adipose tissue is usually noted and metabolic abnormalities including diabetes and hypertriglyceridemia are common [28].

Metabolic Complications of Lipodystrophy

As discussed in the preceding sections, various subtypes of lipodystrophies have different clinical presentations, and unique genetic or acquired etiologies. However, they all share similar metabolic abnormalities including hypertriglyceridemia, low levels of high-density lipoprotein (HDL)-cholesterol, insulin resistance and diabetes mellitus, fatty liver disease, and features of polycystic ovary disease. Extreme insulin resistance is also accompanied by mild to severe acanthosis nigricans in the neck, axillae, groin and other regions of the body. The prevalence and severity of these metabolic complications may vary between the different lipodystrophy syndromes, and to a large extent appears to be influenced by the extent of fat loss. Severe hypertriglyceridemia is common in CGL patients of all subtypes with some case series reporting elevated triglycerides in about 70% of the patients [29]. Some patients even develop tuberous or eruptive xanthomas and acute pancreatitis. Lipid elevations may be mild during early childhood but become notable during later childhood or after puberty especially after the onset of hyper-

glycemia. Genetic lipodystrophies are clearly one of the major causes of monogenic hypertriglyceridemia besides type 1 hyperlipoproteinemia caused by deficiency of lipoprotein lipase and proteins or cofactors associated with its function. This includes genetic variants causing both CGL and FPLD. FPLD2 patients exhibit the most severe metabolic abnormalities among the different subtypes of partial lipodystrophy, especially female patients in whom serum triglycerides are two to three times higher than male patients [16]. Metabolic abnormalities are variable in the rare progeroid syndromes associated with lipodystrophy. Among the acquired lipodystrophy syndromes, AGL patients have marked hyperlipidemia similar to CGL patients, while APL is associated with only mild metabolic abnormalities if any. HAART-induced lipodystrophy in HIV-infected patients is also associated with moderate–severe hypertriglyceridemia, and may be influenced by both the extent of fat loss and concomitant medications.

The exact cause for hypertriglyceridemia in lipodystrophy is not clear, but most likely results from increased VLDL synthesis and secretion in the liver with concomitant delayed clearance of triglyceride-rich lipoproteins, including chylomicrons leading to Type V hyperlipidemia [30]. Absence of adipocytes or lack of functional adipocytes diverts dietary triglycerides to non-adipose tissue including the liver and muscle causing steatosis (Fig. 22.2). Fat loss also leads to

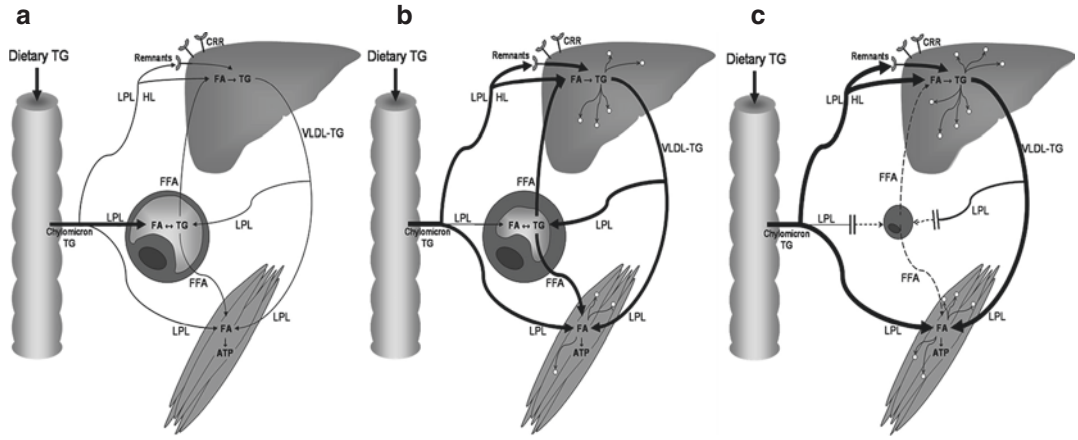

Fig. 22.2 Pathophysiology of hyperlipidemia in lipodystrophy. (**a**) In normal subjects, dietary triglycerides (TG) are transported from the intestines via chylomicrons and taken up by peripheral tissues, such as the liver, skeletal muscle, and adipose tissue after the hydrolysis of triglycerides into free fatty acids (FFAs) and glycerol by lipoprotein lipase (LPL) or hepatic lipase (HL). The FFAs are oxidized to yield energy in the skeletal muscle and liver, whereas in the adipocytes they are re-esterified to triglycerides for storage. The remaining triglycerides in the chylomicron remnants can be delivered to the liver via receptors for chylomicron remnants. Intra-adipocyte lipolysis also releases FFAs as needed to fulfill energy requirements. Delivery of triglycerides through chylomicron remnants as well as the delivery of FFAs can regulate hepatic triglyceride synthesis and secretion via very low-density lipoprotein (VLDL) particles. Note that in normal conditions, there is little or no triglyceride storage in the non-adipocytes. (**b**) and (**c**) The partial or near total lack of adipocytes in patients with lipodystrophies can interfere with this normal process in one of two ways. In partial lipodystrophies (**b**), overall, there could be decreased adipose tissue triglyceride uptake. Increased lipolysis may, however, occur in non-lipodystrophic adipose tissue, which tends to accumulate excess triglycerides. As a result, the net delivery of triglycerides and FFAs to the liver and skeletal muscles is increased. This leads to enhanced hepatic triglyceride synthesis and VLDL secretion. Furthermore, the capacity of liver and muscle (and perhaps other peripheral tissues) for fatty acid oxidation is overwhelmed by the excess FFA flux, leading to triglyceride deposition in these tissues. In generalized lipodystrophies (**c**), there is hardly any adipose tissue present, and thus there may be little contribution of the intra-adipocyte lipolysis to the FFA flux. Instead, most of the FFA or triglyceride flux may be contributed by the chylomicron metabolism. In the absence of adipocytes, the liver and skeletal muscles may be forced to serve a triglyceride storage function, which probably contributes to insulin resistance and dyslipidemia. (From Simha and Garg [30] with permission)

hypoleptinemia, and the resultant hyperphagia due to leptin deficiency further exacerbates dietary triglyceride load. Liver triglyceride synthesis increases due to both increased delivery of dietary fat and also due to increased de novo lipogenesis. Studies in congenital lipodystrophic mice deficient in the enzyme acylglycerol phosphate acyltransferase 2 (Agpat2) suggest upregulation of an alternate pathway involving monoacylglycerol acyltransferase 1(Mgat1) as a cause for increased de novo lipogenesis [31]. Accumulation of toxic lipid metabolites in muscle and liver cells interferes with post-receptor insulin signaling leading to selective resistance to insulin-mediated glucose uptake. Insulin resistance and uncontrolled diabetes further exacerbates dyslipidemia. The role of increased free fatty acids in the genesis of hyperlipidemia is not clear, and is unlikely to be a major determinant in patients with generalized lipodystrophy [31, 32].

Prevalence of Cardiovascular Disease in Lipodystrophy Syndromes

It is well recognized that many patients with both generalized and partial lipodystrophy develop severe hypertriglyceridemia, which sometimes can result in acute pancreatitis. However, the risk for atherosclerotic cardiovascular disease (ASCVD) is not very clear. Overall, there are very few reports of established coronary artery disease (CAD) in CGL patients, but it should be noted that most case series primarily comprise of children. In CGL patients who have been reported to have CAD, the ages ranged from 30 to 60 years [33]. Cardiomyopathy is much more common, especially in patients with CGL2 [12, 34, 35]. Patients with FPLD seem to have a much higher prevalence of ASCVD compared to controls and unaffected family members. This is particularly striking for female subjects with some case series reporting a three-fold risk elevation, while there was no increased risk in male subjects [36]. The risk is even higher when only subjects less than 55 years are considered. Nearly a quarter of FPLD subjects in this age group had evidence of ASCVD, while none of their unaffected relatives had premature ASCVD. They have a characteristic atherogenic lipid profile of elevated triglycerides and low HDL cholesterol without any elevation in low-density lipoprotein (LDL) cholesterol levels. ASCVD has been reported in about half a dozen patients with AGL, but not in APL patients. HAART-induced lipodystrophy in HIV-infected patients is also associated with increased cardiovascular risk.

Management of Dyslipidemia in Lipodystrophy

There are no known effective therapies to reverse or halt fat loss in lipodystrophy syndromes, and management has largely focused on correcting metabolic abnormalities. This includes medical nutrition therapy, traditional glucose and lipid-lowering therapies, and other novel and emerging therapies.

Diet

Diet is the cornerstone for management of metabolic complications of lipodystrophy. As discussed before, absence of adipose tissue results in diversion of dietary fat to non-adipose tissue which subsequently causes insulin resistance and dyslipidemia. Restriction of dietary fat and excess energy intake is therefore critical, and is particularly challenging as hypoleptinemia stimulates hyperphagia. Further, there is often a tendency of parents and caregivers to overfeed children with CGL, AGL, and APL to promote weight gain. Adequate education to avoid this is essential. When monitoring these children for optimal growth patterns, using body mass index (BMI) or weight for height are not appropriate, and as long as the child is gaining height appropriately, no effort at enhancing energy intake must be made. In fact it was shown nearly 50 years ago that use of appetite suppressants in CGL children helped improve metabolic parameters [37].

There is limited human data on optimal macronutrient composition for lipodystrophy

patients. Based on studies of lipodystrophic mice, it appears that low fat diet is beneficial [31]. Dietary fat restriction to less than 20–30 g/day is critical for patients with severe hypertriglyceridemia and chylomicronemia to prevent acute pancreatitis. In other patients, it would be reasonable to recommend 60% of total energy from complex carbohydrates, 10–15% from protein, and the rest from fat, with preference for *cis*-monounsaturated fat sources. Use of medium chain saturated fat sources may be advantageous in children requiring adequate fat energy for growth. For FPLD patients, low saturated fat, low cholesterol diet is advised in view of high risk of ASCVD. They should also attempt to lose weight and one marker of that can be reduction of fat deposition in non-lipodystrophic regions such as the chin, neck, and upper back [38].

Insulin and Other Glucose-Lowering Therapies

Metformin is traditionally considered to be the drug of choice for treatment of diabetes in patients with all forms of lipodystrophy. Limited data also show benefit of thiazolidinediones which decrease glucose levels and hepatic steatosis, and slightly improve hyperlipidemia [39, 40]. They do not however reverse fat loss, even in patients with lipodystrophy due to *PPARG* mutations, and may worsen excess fat deposition in non-lipodystrophic areas like the face, neck, pubis, labia major a, and elsewhere [41]. There are no studies of other relatively novel glucose-lowering medications like incretin-based therapies and sodium glucose cotransporter 2 (SGLT2) inhibitors in patients with lipodystrophy.

Insulin therapy is often necessary for satisfactory glucose control. The use of concentrated insulin preparations may ease administration of large quantities of insulin which may be necessary in these patients with extreme insulin resistance. Due to the absence of subcutaneous adipose tissue, insulin may be injected into the muscle which may affect its kinetics and duration of action. U-500 insulin administered three to four times a day may be employed in some patients with lipodystrophy. Improvement in glycemic control using large doses of insulin is key to improving dyslipidemia especially in those with chylomicronemia and recurrent attacks of acute pancreatitis.

Traditional Lipid-Lowering Therapy

Fibrates and long chain omega-3 polyunsaturated fatty acids from fish oils are commonly used to lower serum triglycerides, but many patients continue to have elevated levels despite their use. Statin therapy to lower cardiovascular risk has not been studied in lipodystrophy patients, but it seems prudent to use them in high-risk patients such as female patients with FPLD. Care should be taken to not exacerbate underlying myopathy, especially when using combination lipid-lowering therapy. Ezetimibe can be added to lower LDL cholesterol levels, if needed. Bile acid sequestrants should be avoided because they have the potential to increase serum triglycerides. The inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9), alirocumab and evolocumab, do not lower serum triglycerides and their use has not been reported in patients with lipodystrophy. The efficacy and safety of novel triglyceride lowering medications like Apolipoprotein CIII antisense oligonucleotide, which has been shown to significantly reduce triglycerides in other patients with severe hypertriglyceridemia, is being studied.

HIV-infected patients with HAART-induced lipodystrophy and dyslipidemia should be carefully treated with fibrates, statins, fish oil, and other lipid-lowering drugs, especially keeping in mind the drug interaction with HIV1- protease inhibitors.

Alcohol intake should be avoided in patients with lipodystrophies because of hepatotoxicity as well as exacerbation of hypertriglyceridemia. Patients should not smoke to reduce risk of ASCVD. Women should avoid oral estrogens for polycystic ovarian syndrome, contraception or post-menopausal hormone replacement therapy, as they can cause extreme hypertriglyceridemia and acute pancreatitis.

Leptin-Replacement Therapy

Marked hypoleptinemia is a feature of generalized lipodystrophy, both congenital and acquired, and restoration of normal serum leptin levels leads to significant improvement of metabolic abnormalities (Fig. 22.3). This was first noted in lipodystrophic mice in which serum glucose, lipid levels, and hepatic steatosis improved drastically with leptin administration or with transplantation of adipose tissue which could secrete leptin [42–44]. Subsequently, a 4-month open-label study in nine patients with severe hypoleptinemia and lipodystrophy (eight patients with generalized lipodystrophy and one with FPLD) showed a nearly 60% reduction in serum triglycerides and improvement in glucose control with a reduction in hemoglobin A1c by 1.6% [45]. Glucose and lipid-lowering medications were reduced or discontinued in many patients. Long-term studies in both smaller and larger cohorts of patients with generalized lipodystrophy, as also post-marketing studies, have confirmed these marked beneficial effects of leptin-replacement therapy [46–48]. Besides glucose and lipids, significant improvement in hepatic steatosis has been noted with reduction of intrahepatic fat content, and improvement in both liver function tests and histological features of steatohepatitis [49, 50]. Other benefits of leptin therapy include restoration of normal menstrual cycles in female patients, and reduction in proteinuria [51, 52]. There are currently over 200 patients with generalized lipodystrophy who have received human recombinant leptin therapy, metreleptin, some for over 15 years.

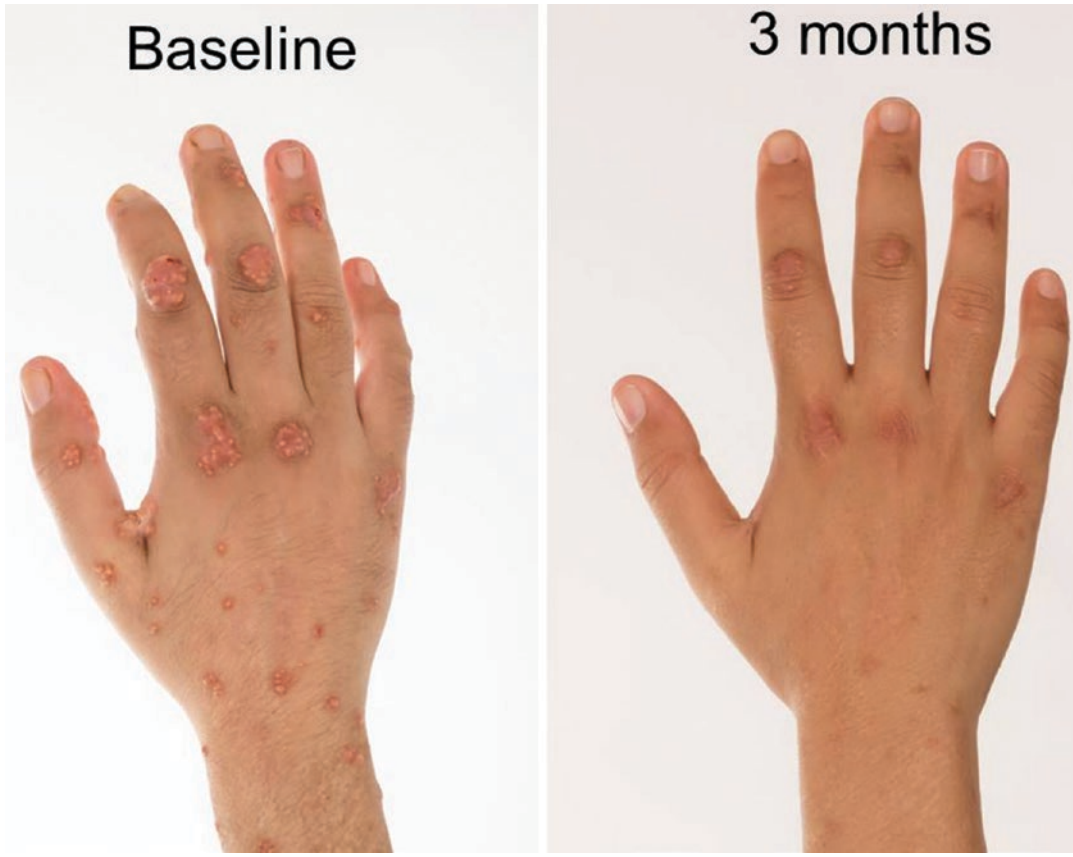

Fig. 22.3 Resolution of eruptive xanthomas from the right hand after 3 months of leptin-replacement therapy (5 mg subcutaneously daily) in a 19-year-old female patient with congenital generalized lipodystrophy and severe hypertriglyceridemia. Serum triglycerides decreased from 3675 mg/dL to 121 mg/dL during this period

Patients with FPLD also have variable degree of hypoleptinemia though some patients may even have normal or high serum leptin levels [53]. Leptin-replacement therapy in FPLD patients has also been shown to reduce plasma glucose and lipid levels, but to a lesser extent [54]. Leptin therapy lowered serum triglycerides by 30% in FPLD patients with hypoleptinemia, but without significant change in glucose control [55]. Further controlled-clinical trials are required to evaluate the efficacy of leptin therapy in FPLD patients, especially in those with steatohepatitis.

The precise mechanisms by which leptin-replacement therapy improves hyperlipidemia, hyperglycemia, and hepatic steatosis are not clear, but decreased food intake due to amelioration of hyperphagia is an important component. Leptin therapy has been shown to improve measures of hunger and satiety [56]. Even brief interruption of leptin therapy in a controlled environment without changes in energy intake has been noted to rapidly increase serum triglyceride levels [45]. Besides the well-known effect of leptin on central hypothalamic areas controlling hunger and satiety, it may also have a direct peripheral action on the liver and skeletal muscles to decrease steatosis. While this has been clearly demonstrated in animal studies [57, 58], it is harder to tease out the relative importance of central and peripheral effects of leptin administration in humans.

Leptin therapy has currently been approved by the United States Food and Drug Administration for treatment of patients with congenital and acquired generalized lipodystrophy. In Europe and Japan, it has been approved for patients with both generalized and partial lipodystrophy. Metreleptin, an analogue of human recombinant leptin, is available for subcutaneous injection after reconstitution with sterile water. It is administered daily at a starting dose of 5 mg in adult females and 2.5 mg in adult males. Weight-based dosing is recommended in children. Mild injection site reactions have been reported, but it is otherwise well tolerated with few side effects. Weight loss from loss of lean body mass can occur, and patients should be closely monitored for hypoglycemia if they are on glucose-lowering therapies which will need to be adjusted. Lymphomas have been reported in three patients with AGL while on leptin therapy, and it is not clear whether this was related to the underlying disease or therapy. Neutralizing antibodies to recombinant leptin can also develop during therapy, which could potentially blunt the response, but their clinical significance is not clear. Overall, it appears that leptin therapy is safe and efficacious for the treatment of metabolic complications of generalized lipodystrophy. It is important to inform patients at the outset that it does not however restore fat loss.

Surgical and Cosmetic Procedures

Fat loss, especially from the face, can lead to significant psychological distress in many patients. Cosmetic procedures including dermal fillers and auto fat transplantation should be offered to such patients. Liposuction to reduce excess fat accumulation around the chin and neck in some FPLD patients can be done for cosmetic purposes. There are also case reports of patients with partial lipodystrophy who benefit from gastric bypass surgery which may be a useful option in appropriately selected patients [59–61].

Conclusions

Lipodystrophies are rare inherited and acquired disorders characterized by selective loss of adipose tissues. Despite significant genotypic and phenotypic heterogeneity, they are all characterized by similar metabolic complications including severe hypertriglyceridemia, diabetes with marked insulin resistance, hepatic steatosis, and features of polycystic ovarian disease in female subjects. Traditional glucose and lipid-lowering therapies are often inadequate to restore good metabolic control. Leptin-replacement therapy appears safe and effective in patients with generalized lipodystrophy.

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HIV and Dyslipidemia

23

Carlos D. Malvestutto and Judith A. Aberg

Human Immunodeficiency Virus (HIV) Infection

In the last 30 years, HIV has evolved from a near certain "death sentence" to its current status as a chronic illness that can be managed with effective and well-tolerated antiretroviral therapy (ART). People with HIV (PWH) are living longer, and the life expectancy gap with the general population has progressively shortened [1, 2].

Although acquired immunodeficiency syndrome (AIDS) mortality continues to decrease, cardiovascular disease (CVD) and CVD-related mortality has increased in PWH. Compared to the general population, PWH have a higher relative risk of CVD [3]. Studies have reported a 1.5–2-fold increased relative risk of CVD compared to the general population [4–9]. The increased risk of CVD in the HIV-infected population has been linked to a higher prevalence of traditional risk factors such as smoking [10–12], substance use disorders [13], hypertension [14–16], and metabolic disorders including diabetes [17, 18] and dyslipidemia [19, 20].

HIV-Associated Immune Activation and CVD Risk

Adjusting for traditional CVD risk factors does not fully explain the excess CVD risk observed in PWH. HIV infection itself has a substantial impact on the development CVD, as shown by the association between lower CD4+ T cell and HIV viremia with increased incidence of CVD [6, 21–24]. Immune activation and chronic inflammation associated with HIV infection are believed to play a key role in the development of atherosclerosis in PWH. In the setting of chronic inflammation, atherosclerosis is accelerated [25, 26]. HIV infection induces both immune suppression and immune activation. CD4+ T regulatory cells in the gut mucosa are rapidly depleted enabling microbial translocation and activation of the innate and adaptive arms of the immune system [27]. The resulting persistent activation of monocytes and T cell subsets translates into a pro-inflammatory environment manifested by increased circulating inflammatory markers and cytokines such as IL-6, CRP, and sICAM-1 [28, 29]. Activated monocytes targeting the tunic intima of the affected coronary arteries transform to macrophages and then, upon internalization of oxidized low-density lipoprotein (LDL), to foam

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_23

cells which form the lipid core of the developing atheroma. HIV infection appears to directly affect macrophage cholesterol metabolism as HIV Nef protein causes the ATP-binding cassette transporter A1 to function abnormally, impairing cholesterol efflux from infected macrophages [30]. This results in accumulation of lipids and accelerates the transformation of macrophages into foam cells. Plaque macrophages also secrete cytokines and matrix metalloproteinases which can degrade the fibrous cap overlying the atheroma, ultimately resulting in plaque rupture [31–33]. In virally suppressed individuals on ART, immune activation is increased compared to HIV-uninfected persons, as pro-inflammatory monocytes are predominant and contribute to vascular inflammation and atherosclerosis [34, 35]. Similarly, activated T cells (CD38+ HLA- DR+) are overrepresented in PLWH compared to HIV-uninfected control subjects [21]. This is true even in the case of HIV elite controllers who have no measurable viremia when not taking ART. Although far less abundant than macrophages, activated T cells are also recruited into the endothelium and produce pro-atherogenic mediators that contribute to plaque growth and exacerbation of atherosclerosis [36] (see Fig. 23.1).

Characteristics of Atherosclerotic Plaque in PWH

Coronary computed tomography angiography (CCTA) and cardiac fluorodeoxyglucose-positron emission tomography (FDG-PET) studies have shown PWH to have predominantly non-calcified coronary atherosclerotic plaque [34], increased prevalence of high-risk morphology [35], and vascular inflammation [37, 38]. These high-risk, non-calcified plaques are more vulnerable to rupture, resulting in acute MI [39, 40]. This type of high-risk plaque has been observed even in PWH who are taking ART and have a low prevalence of traditional CVD risk factors [34, 35, 37].

Assessment of Atherosclerotic Cardiovascular Disease (ASCVD) Risk in PLWH

The increased risk of CVD in PWH is not accurately predicted with existing CVD risk calculators. The Framingham Risk Score (FRS) from the Framingham Heart Study [41] and the 2013 ACC/AHA Pooled Cohort Equations (PCE) [42] are the most widely used algorithms for the general population. The 2013 HIV Medical Association (HIVMA) of the Infectious Diseases Society of America (IDSA) Primary Care Guidelines [43] endorsed the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III Guidelines [44] which adopted the FRS as its preferred CVD risk prediction. The 2016 European Society of cardiology (ESC)/European Atherosclerosis Society (EAS) Guidelines [45] provide a cardiovascular risk predictor for European countries calculator called SCORE [46]. The 2016 ESC/EAS Guidelines list treatment for HIV as a SCORE risk factor modifier [45].

The Expert Panel of the National Lipid Association (NLA) recommended estimating risk as outlined in the NLA Recommendations for Patient-Centered Management of Dyslipidemia: Part 1 [47], which endorsed applying either FRS or PCE for ASCVD risk estimation. The NLA Expert Panel Part 2 consensus view was that it was reasonable to consider HIV as a risk factor for ASCVD in risk factor counting [48]. Subsequently in 2018, the ACC/AHA and NLA in collaboration with multiple professional societies endorsed chronic HIV as a risk-enhancing factor favoring statin therapy in patients at 10-year risk of 5–7.5% (borderline risk) [49]. Both FRS and PCE have been shown to underestimate ASCVD Risk in PLH [50–52]. A study by Law et al. (2006) compared the performance of FRS in predicting MIs among D:A:D Study participants and found that FRS over-predicted MI incidence in patients not taking ART and under-predicted MI rates in patients taking ART [53].

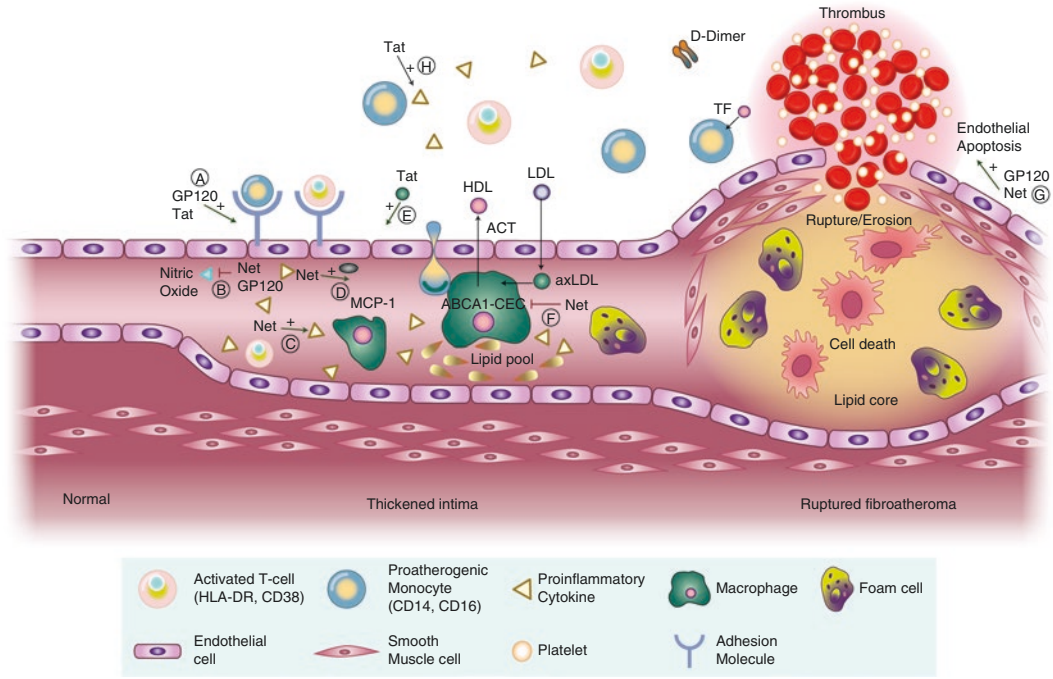

Fig. 23.1 Development of atherosclerosis in HIV. HIV promotes chronic inflammation and immune activation that accelerate the development of atherosclerosis. Persistent activation of monocytes and T cell subsets produces an inflammatory milieu that increases atherogenic monocytes, activated T cells (CD38 + HLA-DR+), and cytokines. These, in turn, increase monocyte migration into the vasculature. Activated monocytes transform to macrophages and, subsequently, into foam cells after internalization of oxidized low-density lipoprotein. HIV directly affects macrophage cholesterol metabolism by impairing cholesterol efflux from infected macrophages which results in accelerated transformation of macrophages into foam cells. Activated T cells are also recruited into the endothelium and produce pro-atherogenic mediators that contribute to the formation of plaque. HIV makes the plaque more vulnerable to rupture by reducing smooth muscle cell proliferation and promoting the apoptosis of foam and endothelial cells. (A) Tat and pg120 induce expression of adhesion molecules; (B) Nef and gp120 reduce endothelial NO production; (C) Nef induces inflammatory cytokine release from macrophages; (D) Nef promotes endothelial cell MCP-1 secretion; (E) Tat promotes monocyte transmigration; (F) Nef inhibits cholesterol efflux from macrophages; (G) Nef and gp120 induce endothelial apoptosis and promote fibroatheroma rupture, resulting in formation of acute thrombus. ABCA-1 ATP-binding cassette transporter-A1, CEC cholesterol efflux capacity, RCT reverse cholesterol transport, TF tissue factor, HDL high-density lipoprotein, LDL low-density lipoprotein, oxLDL oxidized LDL, MCP-1 monocyte chemoattractant protein-1. (Adapted from Nou et al. [25])

There is no validated 10-year risk score calculator specifically validated for PWH although two scoring systems have been proposed. The Data Collection on Adverse Effects of Anti-HIV Drugs (D:A:D) Study Group developed a 5-year risk calculator that includes traditional CVD risk factors and exposure to individual ART drugs [50]. The Veterans Aging Cohort Study (VACS) Index was developed to predict mortality and includes age, CD4 count, viral load, hemoglobin, aspartate and alanine transaminase, platelets, creatinine, and hepatitis C and has been used to predict coronary heart disease (CHD) but has not been validated for such use [54]. A study by Nery et al. (2013) in Brazilian PWH compared the FRS, D:A:D, Prospective Cardiovascular Munster (PROCAM) [55], and an expanded FRS that included a number of additional measures including elevated creatinine, elevated hs-CRP, elevated albuminuria, and family history of CVD and metabolic syndrome status [56]. The expanded FRS performed best in predicting CVD risk in this population of PWH. Table 23.1 summarizes the characteristics of the available CVD

Table 23.1 Risk scores and algorithms for assessing CV risk in the general population and among persons with HIV

	Framingham risk score	SCORE	PROCAM	REYNOLDS
Population	General population from one geographic area. Framingham MA (USA)	European	European men	Men and women from the USA, no known CVD (men were non-diabetic)
Age	30–74 years	19–80	35–65 years	Men 57–80 Women ≥45
Data collection	1968–1971 original Framingham cohort, 1971–1975 and 1984–1987 Offspring studies	1967–1992	Recruitment 1979–1985 and followed for 10 years	Men: 1995–2008, followed for median of 10.8 years Women: 1992–2004, followed for a median of 10.2 years
Years risk prediction	10-year risk of CHD events 30-year risk of CHD and stroke	10-year risk of CVD fatality	10-year fatal or nonfatal MI or sudden cardiac death	10-year risk for CVD
Variables	Sex, age, total-C, HDL-C, smoking status, systolic blood pressure (treated/not treated), diabetes	Sex, age, total-C or total-C/HDL-C, systolic blood pressure, smoking status	Age, LDL-C, HDL-C, triglycerides, smoking status, diabetes, family history of MI, systolic blood pressure	Sex, age, smoking status, total cholesterol, HDL-C, hs-CRP, parental history of MI <60 years of age, glycated hemoglobin (if diabetic)
Guidelines using score	NCEP ATP III [216] Canadian Cardiovascular Society [217] International Atherosclerosis Society [218] National Lipid Association Recommendations	European (ESC/EAS) [219]	None	None
Website	http://cvdrisk.nhlbi.nih.gov/	www.heartscore.org	http://www.chd-taskforce.de/procam	www.reynoldsriskscore.com
Discrimination and calibration in HIV+	c-statistic [50, 220, 221]: 0.65, 0.71, 0.77 O/E [50, 221]: 1.18, 1.51	c-statistic [221]: 0.57 O/E [221]: 1.20	Unknown	Unknown
Reference	D'Agostino RB. *Circulation.*2008;117:743 [41] Pencina MF. *Circulation.*2009;119:3078 [222]	Conroy RM. *European Heart Journal.*2003;24:987 [223]	Assmann G. *Circulation.*2002;105:310	Ridker, PM. *Circulation.* 2008;118:2243 Ridker PM. *JAMA.* 2007;297:611 [224]
Notes		Different versions applied to countries with low or high risk		Men were in the Physicians' Health Study and women in the Women's Health Study

Abbreviations: *ASCVD* atherosclerotic cardiovascular disease, *CHD* coronary heart disease, *D:A:D* Data Collection on Adverse Events of Anti-HIV Drugs, *eGFR* estimated glomerular filtration rate, *FIB-4* (years of age X aspartate transaminase) ÷ (platelets x $\sqrt{}$alanine transaminase), *hs-CRP* high sensitivity C-reactive protein, *MI* myocardial infarction, *NCEP ATP III* National Cholesterol Education Program Adult Treatment Panel III [216], *NHLBI* National Heart, Lung, and Blood Institute of the National Institutes of Health, *O/E* observed-to-predicted events ratio, *PROCAM* Prospective Cardiovascular Munster study, *SCORE* Systematic Coronary Risk Estimation, *VACS* Veterans Aging Cohort Study

	Pooled Cohort Equations	D:A:D	VACS
Population	Population-based cohort studies funded by NHLBI	D:A:D cohort of HIV+ men in Europe, Argentina, Australia, and the USA	HIV+ US veterans, men
Age		16–85	≥18
Data collection	Varied	1999–2008; followed for a median 4.8 years	2000–2007
Years risk prediction	10-year risk of ASCVD	5-year risk of CVD	5-year mortality
Variables	Sex, age, race (white or black), total-C, HDL-C, systolic blood pressure, treatment for high blood pressure (if systolic >120 mm hg), diabetes, smoking status	Number of years on indinavir, lopinavir, currently on indinavir, lopinavir, abacavir, sex, age, current cigarette smoker, previous cigarette smoker, diabetes, family history of CVD, systolic blood pressure, total-C, HDL-C	Age, CD4 count, HIV-1 RNA (viral load), hemoglobin, FIB-4, estimated GFR, hepatitis C infection status
Guidelines using score	2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults [225] National Lipid Association Recommendations [226]	None	None
Discrimination and calibration in HIV+	c-statistic [220, 221]: 0.65, 0.71 O/E [221]: 1.20; may be better than FRS at higher categories of predicted risk [220]	c-statistic [50, 221]: 0.72, 0.77 O/E [50, 221]: 1.33, 0.95	c-statistic [227] (for CHD death): 0.77 O/E: unknown
Website	http://tools.cardiosource.org/ASCVD-Risk-Estimator/	http://www.cphiv.dk/tools/dadriskequations	http://www.vacohort.org/welcome/vacsindexinfo.aspx
Reference	Goff DC Jr. *J Am Coll Cardiol.* 2014;63:2935 [42]	Friis-Moller N. *European Journal of Cardiovascular Prevention and Rehabilitation.* 2010.17;491 [50]	Tate JP. *AIDS.* 2013;27:563 [228]
Notes	Risk scores account for white and black race Eliminated targets for LDL-C [225]		Predicts mortality and CHD death for HIV+ patients who have been treated with ART for at least 1 year [54, 227, 229, 230]

From Jacobson et al. [48]

risk prediction calculators including available related to their discrimination and calibration in populations of PWH.

Lipid Profile in HIV Infection

During the natural history of HIV and progression to AIDS, a specific pattern of dyslipidemia is observed characterized by decreased plasma total, LDL, and high-density lipoprotein (HDL) cholesterol and increased plasma triglyceride (TG) levels [57, 58]. Plasma HDL cholesterol is likely reduced by impaired cholesterol efflux from macrophages and HIV-stimulated endothelial lipase and phospholipase A2 [58, 59]. Elevated plasma concentrations of interferon-alpha and tumor necrosis factor (TNF-alpha) in patients with AIDS may impair the clearance of TG-rich lipoproteins and alter free fatty acid metabolism and lipid oxidation [60, 61]. Wasting syndrome seen in advanced AIDS can also contribute to decreased HDL and LDL cholesterol levels. Elevated TG is caused by a combination of very-low-density lipoprotein (VLDL) overproduction and reduced TG clearance [62, 63]. With viral suppression on ART, LDL cholesterol often returns to baseline [64, 65]. Studies have reported that HIV/hepatitis C (HCV) coinfection is associated with an even higher risk of adverse CV outcomes compared with HIV-monoinfected persons [66, 67]. However, HCV infection alone is associated with lower TC and TG levels compared with uninfected controls [68], and HIV/HCV coinfection attenuates some of the atherogenic lipid changes observed with HIV infection alone [69]. Despite having lower LDL cholesterol, HIV/HCV-coinfected patients have increased proprotein convertase subtilisin/kexin 9 (PCSK9) compared to HIV-monoinfected individuals [70].

Effect of ART on Lipid Levels

While ART generally reduces CVD risk attributed to uncontrolled HIV infection, specific ART drugs are known to adversely affect lipid profiles in PWH. A meta-analysis of 15 clinical trials of first-line ART showed that the protease inhibitors (PIs) fosamprenavir and lopinavir, both boosted with ritonavir, and the non-nucleoside analogue reverse transcriptase inhibitors (NNRTI) efavirenz in combinations with nucleoside analogue reverse transcriptase inhibitors (NRTIs) other than tenofovir were associated with the largest increases in total cholesterol (TC) at 48 weeks [71]. PIs and NNRTIs can worsen dyslipidemia [72] although the effects of the drugs on lipids can vary widely between different drugs in the same class. For example, lopinavir is known to cause significant increases in LDL cholesterol [73] compared to second-generation PIs such as atazanavir [74] or darunavir. Ritonavir is a PI that was initially approved for the treatment of HIV infection at high doses but was then used in lower doses (100–200 mg per day) as a pharmacokinetic booster for other PIs due to its ability to inhibit cytochrome P450 3A (CYP3A). Even at boosting doses, it resulted in mean triglyceride (TG) concentration increases of 30 mg/dl and increases in other lipid parameters [75]. Cobicistat is a selective CYP3A inhibitor without inherent antiretroviral activity that has been developed to pharmacokinetically boost PIs and the integrase inhibitor elvitegravir [76]. For patients on ritonavir-boosted darunavir containing ART regimens, switching ritonavir to cobicistat resulted in improvements in all lipid parameters including HDL cholesterol. For patients with elevated TG at baseline, TG decreased significantly after switching ritonavir to cobicistat [77].

Efavirenz is an NNRTI that has been consistently associated with increased TC, LDL, and HDL cholesterol levels [78–81] via activation of pregnane X receptor (PXR) signaling in the liver which regulates the expression of several key hepatic lipogenic genes including fatty acid transporter CD36 and cholesterol biosynthesis enzyme squalene epoxidase (SQLE), leading to increased lipid uptake and cholesterol biosynthesis in hepatic cells [82]. On the other hand, doravirine appears to have a neutral effect on lipids with minimal decreases in LDL cholesterol and non-HDL cholesterol [83].

Nucleos(t)ide analogue reverse transcriptase inhibitors (NRTIs) are generally lipid neutral except for stavudine which causes marked increased in lipids [84]. The NRTI tenofovir disoproxil fumarate (TDF), however, has intrinsic lipid-lowering properties [85]. Tenofovir alafenamide (TAF), a prodrug of tenofovir, is gradually replacing TDF in ART regimens because it has less of an adverse impact on renal and bone mineral density due to its reduced plasma dose since it concentrates intracellularly in high levels. As a result of the reduced tenofovir plasma concentration, the lipid-reducing effect of TDF is not seen with TAF. Newer drug classes including integrase strand transfer inhibitors (INSTIs) have not been associated with negative effects on plasma lipid levels [86, 87]. The CCR5 inhibitor maraviroc does not increase lipids and may cause lipid reductions in dyslipidemic patients [88]. The new attachment inhibitor fostemsavir appears to be either lipid friendly or lipid neutral. Fostemsavir was evaluated in a trial of treatment-experienced participants who were randomized to receive either fostemsavir (in one of the four different dose arms) with raltegravir and TDF or atazanavir with raltegravir and TDF. Participants in the fostemsavir arms had decreases to very slight increases in total cholesterol (TC) and TG but had significant decreases in LDL cholesterol, while those randomized to the atazanavir arm experienced mean increases in TC and LDL cholesterol and a slight decrease in TG [89]. The fusion inhibitor enfuvirtide is considered lipid neutral [90] as is the monoclonal antibody ibalizumab. Table 23.2 summarizes the effects of PIs and NNRTIs on lipids.

Table 23.2 Lipid changes associated with NNRTIs and PIs

ART class	Lipids			
NNRTIs	**TC**	**HDL-C**	**LDL-C**	**TG**
Efavirenz	↑	↑	↑	↑
Etravirine	↔	↔	↔	↔
Nevirapine	↑	↑↑(larger than with efavirenz)	↑	↑ (lower than with efavirenz)
Rilpivirine	↑	**Not known**	↑ (lower than with efavirenz)	↑ (lower than with efavirenz)
Doravirine	↔	↔	↔	↔
PIs				
Atazanavir	↑	↔	↑ by 16%	↓ by 12%
Atazanavir + ritonavir and atazanavir/cobicistat	↔	↔	↑	↑
Darunavir + ritonavir and darunavir/cobicistat	↔	↔	↑	↑
Fosamprenavir + ritonavir	↑↑	↔	↑	↑↑
Lopinavir/ritonavir	↑↑ (additional increase over ritonavir alone)	↔	↑	↑↑ (no additional increase over ritonavir only)
Nelfinavir	↑	↔	↑	↑
Ritonavir (low dose for boosting)	↑ by 10%	↓ by 5%	↑ by 16%	↑↑ by 26%
Saquinavir + ritonavir	↑↑	↔	↑	↑
Tipranavir + ritonavir	↑↑	Not known	Not known	↑↑↑

Modified from Myerson et al. [231]
↑ = some increase, ↑↑ = moderate increase, ↑↑↑ = large increase, ↓ = some decrease, ↓↓ = moderate decrease, ↓↓↓ = large decrease, ↔ = no significant change, *ART* antiretroviral therapy, *NNRTI* non-nucleoside analogue reverse transcriptase inhibitor, *PI* protease inhibitor, *TC* total cholesterol, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

Clinical Guidelines for Lipid Management

The 2013 HIV Medical Association (HIVMA) of the Infectious Diseases Society of America (IDSA) Primary Care Guidelines [43] recommend managing dyslipidemia in PWH as in the general population according to the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III Guidelines [44].

The 2013 American College of Cardiology (ACC)/American Heart Association (AHA) Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults introduced the use of the PCE to determine the 10-year ASCVD risk and recommended moderate- to high-intensity statins for primary prevention in adults with a 10-year ASCVD risk score ≥ 7.5%. The guidelines also suggested using additional risk assessment tools, such as non-invasive atherosclerosis imaging, to help guide statin treatment decisions in special population such as PWH [91].

When carotid artery intima-media thickness (CIMT) was measured in 352 adult PWH without clinical ASCVD at baseline as marker of subclinical atherosclerotic plaque, the study found that among PWH, the 2013 ACC/AHA Guidelines would recommend statins for more individuals than the ATP III Guidelines. However, among those PWH with carotid plaque at baseline, more than two out of three were not identified for statin therapy using either guideline [92].

The 2016 European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) Guidelines recommend risk assessment using the country-specific SCORE risk calculator and consideration of statin treatment in all PWH with dyslipidemia to achieve an LDL cholesterol target of <100 mg/dL [45].

The Expert Panel of the National Lipid Association (NLA) recommended estimating risk as outlined in the 2015 NLA Recommendations For Patient-Centered Management of Dyslipidemia: Part 1 [47]. This includes determining the number of risk factors, the use of risk prediction tools such as the ATP III FRS or the ACC/AHA PCE if two risk factors are present, as well as clinical judgment [48].

The Expert Panel viewed as reasonable the consideration of HIV as a risk factor for ASCVD in risk factor counting. For patients with HIV infection plus two other major ASCVD risk factors, cholesterol level targets for non-HDL and LDL are <130 mg/dL and <100 mg/dL, respectively. Although the Expert Panel endorsed classifying HIV as a major ASCVD risk factor, there was not enough information available to determine if HIV should be considered an "ASCVD risk equivalent" similar to diabetes plus two major ASCVD risk factors. See Table 23.3 for a summary of the 2015 NLA Guidelines recommendations for HIV-infected persons.

When a cohort of 3312 adult PWH in Washington, D.C., was assessed for need for statins using ATP III, 2013 ACC/AHA, or 2015 NLA Guidelines, 45% were eligible for statins based on NLA Guidelines, 40% were eligible based on 2013 ACC/AHA Guidelines, and 30% were eligible based on ATP III Guidelines. However, only 49%, 56%, and 73% of PWH eligible for statins according to NLA, 2013 ACC/AHA, and ATP III, respectively, were actually prescribed statins in this cohort [93].

A study of the Women's Interagency HIV Study (WIHS) cohort of 3453 women showed that indications for statin use increased from 16% to 45% of the cohort, based on ATP III or 2013 AHA/ACC Guidelines, respectively. However, the statin prescription rates for this population were very low. For those women with a statin indication, only 38% of HIV-seropositive women and 30% of HIV-seronegative women were prescribed a statin within 5 years of the indication. There was no statistically significant difference in statin prescription rates based on HIV serostatus [94].

When a similar analysis was done for a cohort of men followed in the Multicenter AIDS Cohort Study (MACS), the proportion of patients with indications for statin therapy by ATP III who were not prescribed statins were much smaller (11.1% and 14.9% for those with HIV and without HIV, respectively) compared to those in the WIHS cohort [95].

Table 23.3 2015 NLA Guidelines recommendations for persons with HIV

Recommendations	Strength	Quality
Clinicians should be aware that patients with HIV are at increased risk for ASCVD. The association between HIV infection and ASCVD risk is independent of the risk associated with major established ASCVD risk factors	A	High
A fasting lipid panel should be obtained in all newly identified HIV-infected patients before and after starting ART	A	Moderate
For primary prevention of ASCVD, HIV infection may be counted as an additional ASCVD risk factor for risk stratification	B	Moderate
Risk stratification is based on the NLA Recommendations for the Patient-Centered Management of Dyslipidemia: Part 1 [48] with initial risk stratification based on the number of major ASCVD risk factors (with the caveat that the presence of HIV infection may be counted as an additional risk factor), the use of risk prediction tools, such as the ATP III Framingham risk score or the ACC/AHA Pooled Cohort Equations if two risk factors are present, and the use of other clinical indicators to help inform clinical judgment, if needed	B	Moderate
The non-HDL-C and LDL-C goals described in the NLA Part 1 Recommendations should be followed for HIV-infected patients [48]. Atherogenic cholesterol goals may not be attainable in all patients, but there is incremental benefit to lowering non-HDL-C and LDL-C to approach these goal levels	B	Moderate
Elevated TG ≥500 mg/dL that is refractory to lifestyle modification or changes in ART (if an option) should generally be treated with either a fibrate (fenofibrate preferred) or prescription omega-3 fatty acids. After TG is lowered (<500 mg/dL), non-HDL-C and LDL-C should be reassessed for appropriate management	B	Moderate
Statin therapy is first-line for elevated LDL-C and non-HDL-C; however, interactions between statins and antiretroviral agents and other medications must be considered prior to initiating lipid-lowering therapy. The NLA Expert Panel recommends using atorvastatin, rosuvastatin, or pitavastatin as the generally preferred agents in HIV-infected patients	A	Moderate

From Jacobson e al. [48]

A study by Mosepele et al. comparing 1394 PWH in the Partners HIV Cohort to HIV-uninfected controls followed in the Partners Health System also found that a larger proportion of PWH were eligible to be prescribed statins according to 2013 ACC/AHA (38.6%) vs. ATP III (20.1%). However, among participants who had a CVD event during follow-up, only 59% of PWH would have been eligible to be prescribed a statin prior to the CVD event per 2013 ACC/AHA Guidelines compared to 71.6% for HIV-uninfected subjects who had a CVD event, highlighting the residual ASCVD risk in PWH that is not taken into account by the PCE in risk estimation [96].

Data from the National Ambulatory Medical Care Survey/National Hospital Ambulatory Medical Care Survey between 2006 and 2013 for 1631 visits by PWH and 226,862 visits by HIV-uninfected patients suggests that PWH are less likely to be prescribed statins when they had diabetes mellitus, CVD, or dyslipidemia compared to HIV-uninfected persons (23.6% vs. 35.8%, respectively ($P < 0.01$)) [97]. One of the barriers to prescription of statins when they are indicated in PWH may be concerns from clinicians about possible significant drug-drug interactions between ART drugs and lipid-lowering medications (see next section).

The 2018 American Heart Association (AHA)/American College of Cardiology (ACC) Guideline on the Management of Blood Cholesterol recommends using the ASCVD Risk Estimator Plus to assign the appropriate statin treatment group in lower-risk primary prevention adults 40–75 years of age with LDL cholesterol ≥70 mg/dL without diabetes [49]. HIV is listed as a "risk-enhancing factor" that must be considered in the clinician-patient discussion before starting statin therapy. For adults 40 to 75 years of age without diabetes and with LDL cholesterol ≥70 mg/dL, at a 10-year risk of ≥7.5% to 19.9% (intermediate risk), risk-enhancing factors (including HIV) favor initiation of statin therapy. The guidelines recommend reassessment of 10-year ASCVD risk estimate after a 3- to

6-month trial of lifestyle improvements, including smoking cessation. If the patient's ASCVD risk estimate is >5% over 10 years, it is reasonable to begin moderate-intensity statin therapy. Furthermore, the guidelines endorse consideration of a coronary artery calcium (CAC) scan which may improve risk assessment if the patient or clinician remains uncertain about the need for statin therapy [49]. However, it is important to note that there are no outcome studies to date to support this hypothesized benefit to the use of statins among PWH who otherwise do not have a clinical indication for use of a statin. The United States Preventive Services Task Force (USPSTF) noted statin therapy was associated with reduced risk of all-cause and cardiovascular mortality and CVD events, with greater absolute benefits in patients at greater baseline risk [98]. The USPSTF found that statins have a 0.46% absolute benefit (NNT = 217) for nonfatal heart attacks and 0.32% benefit (NNT = 313) for nonfatal stroke. The benefit of statins in persons with lower risk remains controversial, and the NNT to reduce events among PWH has yet to be determined.

Treatment of Dyslipidemia

Clinical Case Study

A 55-year-old white man diagnosed with HIV in 1993 transfers his care to a new provider in 2015 for insurance reasons. HIV viral load has remained suppressed on a fixed-dose combination of tenofovir alafenamide (TAF), emtricitabine, elvitegravir, and cobicistat (TAF/FTC/EVGc). He has a history of prior bilateral total knee arthroplasty for severe osteoarthritis in 2014 and developed a fracture of the right patella after a mechanical fall in 2015. He was referred to cardiology for pre-operative clearance in light of a strong family history of ASCVD, and he was unable to complete an exercise stress test. The pre-operative recommendation included a coronary computed tomography angiography (CCTA) that was abnormal. This patient underwent cardiac catheterization and was found to have 60–70% occlusion of the ramus intermedius. He was placed on aspirin, metoprolol, isosorbide mononitrate, and atorvastatin 40 mg daily. He subsequently underwent a percutaneous coronary intervention (PCI). After 2 weeks, he complained of chest pain described as constant, aching, and without radiation. He also noted generalized myalgias. Given the concern for drug interactions, the atorvastatin dose was decreased to 20 mg daily, and myalgias and chest pain resolved. However, his LDL cholesterol was 152 mg/dL. His combination ART regimen was changed to fixed-dose combination of tenofovir disoproxil fumarate (TDF)/emtricitabine and dolutegravir to avoid drug interactions, and the atorvastatin dose was increased to 40 mg which he tolerated. The patient was then prescribed atorvastatin 80 mg, but again he developed myalgias and was once again placed on atorvastatin 40 mg daily. After 4 months, his LDL cholesterol remained elevated at 148 mg/dL. The patient was referred to cardiology for inclusion in a study evaluating an open-label study of a PCSK9 inhibitor. Within 2 months of starting the PCSK9 inhibitor, his LDL decreased to 49 mg/dL and has ranged from 35 to 65 mg/dL for the past 2 years. He remains symptom-free, maintains healthy diet and BMI, and has had no further cardiovascular complications as of 2019.

Discussion

This patient with documented coronary artery disease has a clear indication for a high-potency statin and a treatment goal of <100 mg/dL for non-HDL cholesterol and <70 mg/dL for LDL cholesterol according to the 2015 NLA Dyslipidemia Guidelines [47]. His ART regimen is working well to maintain viral suppression, but there is an important drug-drug interaction between the pharmacokinetic enhancer cobicistat and several statins including atorvastatin that results in an increase in the area under the curve (AUC) and maximum concentration (Cmax) by 2.6-fold and 2.3-fold, respectively. While these concentration increases are not as pronounced as when cobicistat is paired with the PIs darunavir or atazanavir, they can lead to severe adverse effects from atorvastatin toxicity such as myopathy and rhabdomyolysis. The manufacturer of the

fixed drug combination TAF/FTC/COBI/EVG recommends initiating atorvastatin with the lowest starting dose and titrating carefully while monitoring for safety (e.g., myopathy) without exceeding a maximum atorvastatin dose of 20 mg daily [99]. This interaction is most likely the cause of the patient's myalgias when the atorvastatin dose was increased. Although ezetimibe has been shown to be safe for PWH and does not have significant interactions with ART drug classes, addition of ezetimibe to atorvastatin is unlikely to result in lipid target achievement for this patient. Generally, ART switches are not recommended for lipid control alone, but in this situation, there are potent and effective ART options available that can help to maintain viral suppression and eliminate drug interaction that limit the use of statins and other medications. In this case, switching to a TDF-containing regimen from a TAF-containing regimen takes advantage of the full inherent lipid-lowering properties of tenofovir which is very limited in the TAF formulation. Furthermore, switching to the potent, second-generation INSTI dolutegravir eliminates the need for pharmacokinetic boosting with cobicistat, permitting the safe maximum dosing of atorvastatin, if needed. Although PCSK9 inhibitors are still being evaluated in clinical trials in PWH, the potential of these agents for patients with limited lipid-lowering options is illustrated in this case.

Drug-Drug Interactions (DDIs) Between ART Drugs and Statins

Statins are the cornerstone of treatment of dyslipidemia. The main effect of statins is to reduce elevated LDL cholesterol. They may also contribute to modest increases in HDL cholesterol and TG reductions.

A key challenge in the treatment of dyslipidemia in PWH is the selection of statins and other lipid-lowering agents that are potent, effective, and safe to use with concomitant ARVs and other agents such as antifungals. Most statins are metabolized by the hepatic cytochrome P450 3A4 (CYP 3A4) and have significant pharmacokinetic interactions with ARVs that are also metabolized by this enzyme complex, PIs and NNRTIs in particular. Lovastatin, simvastatin, and atorvastatin are extensively metabolized by CYP3A4, while rosuvastatin, pitavastatin, and pravastatin undergo minimal metabolism via CYP isoenzymes and are eliminated mostly unchanged in bile and urine [100]. Failure to recognize potentially serious drug-drug interactions (DDIs) between some statins and specific ARVs could lead to toxic increases in statin concentrations, resulting in myopathy or rhabdomyolysis.

DDIs Between PIs and Statins

The most serious DDIs between PIs and statins occur when PIs are coadministered with lovastatin or simvastatin. For example, the area under the curve (AUC) for simvastatin is increased 6-fold when coadministered with unboosted nelfinavir and 30-fold when coadministered with ritonavir-boosted saquinavir [101]. Rhabdomyolysis has been reported with the concomitant use of simvastatin and ritonavir-boosted indinavir [102]. As a result of this serious DDI, the coadministration of PIs with lovastatin or simvastatin is absolutely contraindicated. This contraindication also extends to any use of the pharmacokinetic booster cobicistat (a selective CYP3A4 inhibitor) in combination with any ARV. The 2015 NLA Guidelines recommend against using lovastatin and simvastatin in PWH despite being generic and generally inexpensive [48].

Atorvastatin AUC increases moderately when coadministered with PIs [101]. For example, atorvastatin 10 mg coadministered with darunavir 600 mg boosted with ritonavir 100 mg results in a 3.4-fold increase in the atorvastatin AUC [103]. Older PIs such as tipranavir and fosamprenavir resulted in a 4.4-fold [104] and 1.3–1.5-fold [105] increase in atorvastatin AUC, respectively. Therefore, the FDA recommends avoiding the use of atorvastatin with tipranavir, using atorvastatin at the lowest possible dose with lopinavir/ritonavir, and not exceeding a dose of 20 mg of atorvastatin when combined with ritonavir- or cobicistat-boosted saquinavir, fosamprenavir, or darunavir [106].

There is insufficient data on DDIs between ART drugs and fluvastatin. However, since fluvastatin is primarily metabolized via CYP2C9, DDIs are unlikely with most PIs. Ritonavir is a known CYP2C9 inducer and could possibly reduce the fluvastatin AUC when coadministered with PIs [100]. The NNRTI etravirine is a CYP2C9, and it may increase serum concentrations of fluvastatin [107]. Given the lack of DDI data between ARVs and fluvastatin, other better studied statin options would seem more appropriate for patients taking ART.

No significant inhibition of rosuvastatin has been seen on CYP1A2, 2C9, 2C19, 2D6, 2E1, and 3A4 activity, while the most potent inhibition was found on CYP2C9 (resulting in only a 10% reduction in enzyme activity) [63]. Rosuvastatin is a substrate of the organic anion-transporting polypeptide 1B1 (OATP1B1) and breast cancer resistance protein (BCRP). PI-induced inhibition of OATP1B1 can cause a decrease in hepatocyte uptake of rosuvastatin, while hepatobiliary excretion increases rosuvastatin absorption, resulting in possible significant DDIs with PIs. When ritonavir-boosted atazanavir is coadministered with rosuvastatin, the rosuvastatin AUC and Cmax increased by sevenfold and threefold [108]. Although the rosuvastatin AUC increased moderately 2-fold when coadministered with ritonavir-boosted lopinavir, the maximum concentration of drug in serum (Cmax) increased 4.7-fold [109]. FDA recommends a maximum dose of 10 mg daily when coadministered with boosted atazanavir and lopinavir [110] (Crestor® package insert).

Pravastatin is metabolized primarily by glucuronidation and only minimally by CYP3A4 and can be used safely with most PIs [111]. Pravastatin AUC is mostly unchanged when coadministered with lopinavir/ritonavir and is increased in a non-clinically significant fashion by 81% when coadministered with ritonavir-boosted darunavir twice daily. However, for patients with certain polymorphisms within the SLCO1B1 drug transporter gene, pravastatin levels may increase fivefold [112]. Therefore, the lowest possible dose of pravastatin should be used in patients taking darunavir.

Pitavastatin shares similar metabolism and transport characteristics with pravastatin: it is also metabolized primarily by glucuronidation and only minimally by CYP enzymes, and it is taken up into human hepatocytes by OATP1B1 [113]. In contrast to pravastatin, pitavastatin PK parameters do not seem to be considerably affected by coadministration of ritonavir-boosted PIs and require no dose adjustment [114–116]. A comprehensive listing of pharmacokinetic interactions of PIs with statins is provided in Table 23.4.

Table 23.4 Interactions between ART and statins

Statin	Antiretroviral therapy drug class	
	Protease inhibitor, including cobicistat	Non-nucleoside reverse transcriptase inhibitor
Atorvastatin	AUC ↑↑ Use lowest starting dose and titrate carefully Do not exceed 20 mg daily with DRV/r, FPV/r, SQV/r AUC ↑↑ 488% with LPV/r ↑↑↑ 836% with TPV/r and should not be coadministered Start with lowest recommended dose and titrate while monitoring for safety with all cobicistat-containing regimens	↔but C_{max}↓ 33% with doravirine AUC ↓ 43% with efavirenz ↔but C_{max}↓ 37% with etravirine No data for nevirapine May need higher starting dose with efavirenz and etravirine No dose adjustments for rilpivirine
Fluvastatin	Use not recommended with nelfinavir	AUC ↑with etravirine May require higher starting dose with etravirine
Lovastatin	Contraindicated with all PIs and cobicistat (AUC ↑↑↑)	AUC ↓↓with efavirenz. May require higher starting dose No adjustment needed for rilpivirine

Table 23.4 (continued)

Pitavastatin	Modest AUC ↑ with ATV/r (31%) Modest ↓ AUC with DRV/r (20–26%) and LPV/r (20%). No dose adjustment required No dose adjustment with cobicistat	↔ with efavirenz and no dose adjustment needed No dose adjustment needed for rilpivirine
Pravastatin	↓AUC of except with DRV/r and LPV/r which ↑ AUC by 81% and 33%, respectively. Patients with low functioning SLCO1B1 haplotypes may have ↑↑↑ AUC 5-fold. Use lowest possible starting dose for patients on darunavir	AUC ↓ 40% with efavirenz ↔ with etravirine. May need higher starting dose
Rosuvastatin	AUC ↑↑4.7-fold and C_{max}↑↑ 2.1-fold with LPV/r. Do not exceed 10 mg daily. With LPV/r, use lowest necessary dose AUC ↑↑↑↑ 7-fold and C_{max} ↑↑ 3-fold with ATV/r. Do not exceed 10 mg daily. With ATV/r, use lowest necessary dose AUC ↑ 48% and C_{max}↑ 139% with DRV/r. Do not exceed 10 mg daily. With DRV/r, use lowest necessary dose AUC ↔ and C_{max}↑ 123% with TPV/r ↔ with FPV/r. Titrate dose carefully with LPV/r or ATV/r AUC ↑ 38% and C_{max}↑ 89% with cobicistat	Allowed. ↔. No reported interactions
Simvastatin	Contraindicated with PIs and cobicistat. (AUC ↑↑↑)	AUC ↓58% with efavirenz and ↓with etravirine No data for nevirapine. May require higher starting dose

Table modified from Myerson et al. [231]

Abbreviations and symbols: ↑ = some increase, ↑↑ = moderate increase, ↑↑↑ = large increase, ↓ = some decrease, ↔ = no significant change, *ATV/r* atazanavir/ritonavir, *AUC* area under the concentration-time curve, C_{max} maximum drug concentration, *DRV/r* darunavir/ritonavir, *FPV/r* fosamprenavir/ritonavir, *LPV/r* lopinavir/ritonavir, *SQV/r* saquinavir/ritonavir

[a]Nucleoside reverse transcriptase inhibitors and integrase inhibitors, except when boosted with cobicistat, do not have any significant drug-drug interactions with statins

DDIs Between NNRTIs and Statins

NNRTIs can also have significant DDIs with statins. Nevirapine is a selective inducer of CYP3A, and efavirenz is a mixed inducer/inhibitor of CYP3A. Efavirenz decreases the AUC of simvastatin and atorvastatin by 58% and 40%, respectively. It also reduces pravastatin AUC by 40% even though pravastatin is only minimally metabolized by CYP3A4. Increases in the usual dose of these statins may be required to achieve lipid-lowering targets for patients on efavirenz. No dose adjustment is required for pitavastatin when coadministered with efavirenz [116].

Rilpivirine is a substrate and mild inducer of CYP3A4. There were no significant changes in AUC and Cmax of atorvastatin when coadministered with rilpivirine, and no dose adjustment is required. No dose adjustments would be expected for other statins when coadministered with rilpivirine [117]. No major DDIs are expected between etravirine and pravastatin, pitavastatin, or rosuvastatin. Since lovastatin and simvastatin are CYP3A4 substrates, coadministration of etravirine may result in reduced drug concentrations of these statins requiring dose increases to achieve lipid targets. Fluvastatin and pitavastatin are metabolized partly by CYP2C9, and coadministration of etravirine may result in increased concentrations of these statins [118]. A comprehensive listing of pharmacokinetic interactions of NNRTIs with statins is provided in Table 23.4.

The atorvastatin area under the curve from time zero to infinity was similar with and without doravirine (geometric mean ratio [GMR] for doravirine-atorvastatin/atorvastatin, 0.98; 90% confidence interval [CI], 0.90 to 1.06), while the maximum concentration decreased by 33% (GMR for doravirine-atorvastatin/atorvastatin, 0.67; 90% CI, 0.52 to 0.85) [119].

Interactions Between Integrase Inhibitors and Statins

Newer first-line ARVs such as the INSTIs have few significant DDIs with most statins. The INSTI raltegravir is metabolized mainly by UGT1A1 glucuronidation and is not known to have any significant interactions with statins. Elvitegravir is a modest inducer of CYP2C9 and may decrease plasma concentrations of CYP2C9 substrates such as pravastatin. However, elvitegravir is co-formulated with the pharmacokinetic booster cobicistat which is a potent inhibitor of CYP3A and CYP2D6. Therefore, coadministration of the single-tablet regimen containing elvitegravir with cobicistat with statins that are metabolized by CYP3A is expected to result in increased plasma concentrations of such statins. Coadministration of this single-tablet regimen with rosuvastatin 10 mg resulted in an increase in AUC and Cmax of 89% and 38%, respectively [120]. As in the case of cobicistat-boosted PIs, the manufacturer recommends initiating atorvastatin at the lowest dose and titrating carefully when elvitegravir/cobicistat/TDF/emtricitabine is coadministered. The second-generation INSTI dolutegravir is metabolized by UGT1A1 and is not considered an inducer or inhibitor of CYP3A isoenzymes and is not expected to major DDIs with statins. The INSTI bictegravir is a substrate of CYP3A and UGT1A1 [121]. No significant DDIs with statins have been reported, and no dose adjustments of statins are required when coadministered with bictegravir.

Lipid-Lowering Studies in PWH Taking ART

General population studies have demonstrated mean reductions in LDL cholesterol of 30 to 50% with moderate-intensity statins and >50% with high-intensity statins [122, 123]. Some studies have shown poor lipid control for PWH with dyslipidemia on statin therapy [124–127]. For example, in a retrospective cohort study of 706 PWH initiating statins between 2009 and 2013, only one-third of patients had an LDL cholesterol reduction ≥30%, and less than 10% of patients achieved an LDL cholesterol reduction ≥50%. Only 10% of patients had been started on high-intensity statins which may be related to clinician concerns about possible DDIs with antiretrovirals (ARVs) and use of lower-potency statins like pravastatin. Cost of statins and low medication adherence may have also contributed to less than optimal response to statin treatment in PWH [128]. Furthermore, the higher prevalence of mixed dyslipidemias in PWH may require the use of multiple pharmacologic agent combinations to achieve lipid goals [129].

However, retrospective cohort studies comparing lipid endpoints between PWH and HIV-uninfected persons have showed similar LDL cholesterol and TC [95, 130] or slightly lower LDL cholesterol [126] responses.

A systematic review of 19 clinical trials of statin use in PWH on ART showed that pravastatin, rosuvastatin, and pitavastatin were associated with the best safety profiles when coadministered with ARVs. Atorvastatin appeared safe at lower than maximal dose. Rosuvastatin and atorvastatin were most effective at decreasing TC and LDL cholesterol [131].

A meta-analysis of 12 randomized clinical trials (RCTs) conducted in the USA, the UK, France, Australia, Switzerland, Uganda, and Colombia (studies published between 2001 and 2015) with 697 HIV-infected participants showed significant reductions in LDL cholesterol, total cholesterol, and non-HDL cholesterol and elevations in HDL cholesterol, with weighted mean differences of −27.8 mg/dL, −39.8 mg/dL, −31.3 mg/dL, and + 2.8 mg/dL, respectively, following treatment with mostly moderate-intensity statins. No significant changes in TG were found [132].

With the use of newer first-line ART regimens that include drug classes with few if any DDIs with statins, such as INSTI, and the increased

availability and lower cost of high-potency statins, improved lipid control, coupled with increased knowledge and emphasis on the prevention of CVD in PWH, increased use of statins, and improved lipid target achievement, is expected in more recent studies.

Efficacy of Statins

Head-to-Head Comparisons Between Statins in PWH

There are few head-to-head trials comparing statins in PWH. In an open-label, randomized trial of PWH with hypercholesterolemia, participants were randomized to rosuvastatin 10 mg/day, pravastatin 20 mg/day, or atorvastatin 10 mg/day. Of the 85 participants who completed this trial, the mean decrease in TC was significantly larger with rosuvastatin (25.2%) than with atorvastatin (19.8%, $p = 0.03$) and pravastatin (17.6%, $p = 0.01$) at 12 months. The reduction in LDL cholesterol was also greater with rosuvastatin (26.3%) than with atorvastatin (20.3%, $p = 0.02$) and pravastatin (18.1%, $p = 0.04$). Differences in TG and mean HDL were not statistically different between statins [133]. In another head-to-head study, 88 PWH with dyslipidemia taking PI-containing ART were randomized to receive rosuvastatin 10 mg/day or pravastatin 40 mg/day. After 45 days rosuvastatin reduced LDL cholesterol by 37% vs. 19% for pravastatin ($p < 0.001$). TG was reduced by 19% and 7%, for rosuvastatin and pravastatin, respectively ($p = 0.035$). Changes in HDL cholesterol did not differ significantly between the two statins [134]. The INTREPID, a placebo-controlled trial, randomized 252 PWH with dyslipidemia taking ART to pitavastatin 4 mg daily or pravastatin 40 mg daily. Persons on darunavir were excluded given a significant potential for DDI with pravastatin. LDL cholesterol was reduced by 31.1% with pitavastatin and 20.9% with pravastatin ($p < 0.0001$) at 12 weeks with sustained reductions through week 52 [135]. Additionally, the differences between treatments were also in favor of pitavastatin for total cholesterol, apolipoprotein B-to-apolipoprotein A1 ratio, and total cholesterol-to-HDL cholesterol ratio. Fifty-two weeks of pitavastatin 4 mg daily (vs. pravastatin 40 mg daily) led to a greater reduction in select markers of immune activation and arterial inflammation (sCD14, oxLDL, and LpPLA2) [136].

Using a proteomics approach in samples from the INTREPID study found a significant reduction in the levels of TFPI, PON3, and LDLR and an increase in Gal-4 and IGFBP-2, key proteins involved in coagulation, redox signaling, oxidative stress, and glucose metabolism. Pitavastatin led to a greater reduction in TFPI than pravastatin [137].

Atorvastatin

Several studies evaluating atorvastatin therapy in PWH have been published [133, 138–140]. In a meta-analysis of statin RCTs in PWH by Banach et al., no significant differences were found among different statins in changes in LDL cholesterol, HDL cholesterol, or TG. However, atorvastatin was found to be more efficacious in reducing plasma TC concentrations compared to all other statins ($p < 0.001$) [132].

Fluvastatin

There is limited data available on studies evaluating fluvastatin for dyslipidemia in PWH [141, 142]. The 2018 European AIDS Clinical Society (EACS) Guidelines recommend consideration of higher doses if fluvastatin is coadministered with ART [143]. Due to the paucity of data and availability of alternative, more potent, and safe statins, some experts recommend avoiding use of fluvastatin in PWH [144].

Lovastatin

Lovastatin has similar pharmacokinetic properties as simvastatin, and similar DDIs are expected when coadministered with PIs and NNRTIs. Therefore, the use of lovastatin is also not recommended by the Expert Panel of the 2015 NLA Guidelines [48].

Pitavastatin

As previously discussed, pitavastatin has been shown not to have significant DDIs with any ARVs including PIs and NNRTIs. The INTREPID

trial comparing pitavastatin 4 mg daily to pravastatin 40 mg daily in PWH on ART with dyslipidemia showed that pitavastatin was superior to pravastatin in LDL cholesterol and TC reduction [135]. Pitavastatin has also been shown to have a neutral effect on blood glucose and HgbA1C level in PWH [145]. Other data from INTREPID are discussed above. The REPRIEVE trial is a randomized, double-blind, placebo-controlled, multicenter trial designed to assess the efficacy of pitavastatin as a primary prevention strategy for CVD in PWH who do not meet the 2013 ACC/AHA Guideline thresholds for recommended statin initiation. Enrollment of 7550 participants was completed in March of 2019, and total duration of study follow-up is expected for up to 96 months [146, 147].

Pravastatin

Pravastatin has been extensively used for dyslipidemia treatment in PWH earlier in the HIV epidemic due to its well-characterized DDI profile with PIs and NNRTIs. A large number of studies have evaluated pravastatin for the treatment of dyslipidemia in PWH [148–158]. Pravastatin also reduced ApoB and ApbB/A1 ratio in a study of 174 PWH randomized to pravastatin or fenofibrate. However, markers of inflammation and platelet activation such as plasminogen activator inhibitor (PAI)-1, P-selectin, and hs-CRP were not appreciably reduced [159]. Pravastatin has also been shown to reduce brachial flow-mediated dilation (a surrogate measure of endothelial function) compared to placebo in PWH with hyperlipidemia on a PI-containing ART regimen [154].

Rosuvastatin

Rosuvastatin lipid-lowering efficacy has been evaluated in several studies in PWH [133, 134, 160]. It is considered the most potent lipid-lowering statin and resulted in the largest decreases in LDL cholesterol and TC in head-to-head studies compared to atorvastatin [133] and pravastatin [133, 134]. Rosuvastatin has also been shown to reduce inflammation and atherosclerosis in PWH. Rosuvastatin 10 mg daily for 2 years was found to reduce CIMT (a surrogate measure of atherosclerosis) in PWH [161]. In another study, PWH randomized to receive rosuvastatin had significant reductions in the surrogate markers of inflammation and immune activation Lp-PLA2, scD14, and IP-10 compared to those on placebo at 48 weeks [162].

Simvastatin

Simvastatin was evaluated in a trial by Rahman et al. in PWH taking efavirenz-containing ART which demonstrated lower than expected lipid-lowering responses [163]. This was not unexpected given the PK study by the ACTG A5108 team demonstrated a significant reduction in simvastatin levels by 58% [164]. Due to significant DDIs and availability of more effective, safer statins, simvastatin is not recommended for use in PWH by the Expert Panel of the 2015 NLA Dyslipidemia Guidelines [48]. Table 23.5 provides a comprehensive summary of clinical trials of statins in PWH.

Non-statin Lipid-Lowering Therapies

Diet and Lifestyle Modifications

The 2015 NLA Guidelines recommend that PWH with dyslipidemia should be counseled about lifestyle interventions including smoking cessation, diet, and exercise as an initial step [48]. Studies of diet and exercise interventions have demonstrated improvements in lipodystrophy and dyslipidemia in PWH. For example, a dietary intervention that restricted dietary saturated fats in PWH at the time of ART initiation has been shown to significantly improve TC, LDL cholesterol, and TG and protected participants from developing dyslipidemia [165]. A 24-week trial of Thai PWH with dyslipidemia randomized 72 participants to receive individual counseling with a nutritionist for seven session or standard of care. After 24 weeks, participants who received nutritional counseling had greater decreases in TC and LDL cholesterol than those assigned to standard of care treatment [166].

Exercise intervention studies have also demonstrated considerable benefits in the treatment of dyslipidemia for PWH. Aerobic (cycling) and resistance training three times a week for

Table 23.5 Clinical trials of statins in PLWH

Trial	Number of patients	Primary outcome	Results	Comments
Atorvastatin				
Atorvastatin 10–20 mg/day in patients treated with PI-containing ART with severe dyslipidemia [138] {Murillas, 1999 #138} {Murillas, 1999 #138}	15	Efficacy of atorvastatin in the treatment of dyslipidemia for 12 weeks	TC: 25% decrease TG: 35% decrease	No cases of myopathy One patient experienced transaminitis that resolved within 3 months Decreased lipid values observed at 12 weeks and maintained through 15 months
Prospective study of atorvastatin 10 mg/day in patients receiving ART 12 weeks with TC 240 mg/dL, with or without increased TG despite therapeutic lifestyle changes [139] {Palacios, 2002 #139}	20	Efficacy of atorvastatin in the treatment of dyslipidemia for 24 weeks	TC: 27% decrease TG: 41% decrease LDL-C: 37% decrease HDL: 1.3 mg/dL increase	No cases of myalgia, myositis, or increased CK Significant decrease in TC and LDL-C with atorvastatin
Open-label, randomized, prospective study of atorvastatin 10 mg/day, pravastatin 20 mg/day, or rosuvastatin 10 mg/day in HIV-infected patients treated with PI-containing regimens 12 months and dyslipidemia >3 months despite therapeutic lifestyle changes [133]	85	Evaluation of different statins in the management of PI-associated dyslipidemia	Overall: TC: 21% decrease LDL-C: 24% decrease Atorvastatin: TC: 20% decrease Pravastatin: TC: 18% decrease Rosuvastatin: TC: 25% decrease	Favorable tolerability profile with significant efficacy among all statins Rosuvastatin more effective than atorvastatin or pravastatin in decreasing TC and LDL-C
Randomized, double-blind, placebo-controlled trial of HIV-infected patients on ART 6 months with subclinical coronary atherosclerosis, arterial inflammation in the aorta, and LDL-C <130 mg/dL treated with either atorvastatin 20–40 mg/day or placebo {Lo, 2015 #140}	40 (atorvastatin [n = 21] versus placebo [n = 19])	Efficacy of statin treatment to reduce arterial inflammation regression of coronary atherosclerosis for 12 months	TC: 48% decrease TG: unchanged LDL-C: 39% decrease HDL: 1% increase	Similar rates of myalgia, transaminitis, and CK elevation in atorvastatin group vs. placebo, 5 vs. 6, 2 vs. 3, and 0 vs. 0, respectively Significant decreases in TC and LDL-C with atorvastatin vs. placebo
Pravastatin				
Pilot study of HIV patients receiving PI-containing regimens treated with pravastatin [149]	19	Efficacy of pravastatin 20 mg at bedtime in the treatment of dyslipidemia for 16 weeks	TC: 19% decrease TG: 37% decrease	No adverse effects noted CD4 and HIV RNA has no significant change with pravastatin treatment

(continued)

Table 23.5 (continued)

Trial	Number of patients	Primary outcome	Results	Comments
Randomized, open-label comparative study of HIV patients receiving PI-containing regimens treated with pravastatin or diet only [150]	31	Efficacy of pravastatin 40 mg/day versus diet only in the treatment of dyslipidemia for 24 weeks	Pravastatin TC: 17% decrease LDL-C: 19% decrease Diet only TC: 4% increase LDL-C: 6% decrease	Despite numerical change, TC differences did not reach statistical significance There was no difference in HDL or TG All patients were male
Open-label, randomized, prospective study of HIV patients receiving PI-containing regimens treated with pravastatin, fluvastatin, or fibrates [151] {Calza, 2003 #151}	106 (pravastatin [$n = 19$] versus fluvastatin [$n = 18$] versus fibrate [$n = 69$])	Efficacy and safety of pravastatin, fluvastatin, or fibrates in the treatment of diet-resistant hypertriglyceridemia for 12 months	Statin (either) TC: 25% decrease TG: 35% decrease LDL-C: 26% decrease HDL: 24% increase Fibrates (any) TC: 22% decrease TG: 41% decrease LDL-C: 23% decrease HDL: 20% increase	Cholesterol changes versus baseline were significant, but no when compared to each other
Placebo-controlled, double-blind, crossover study of HIV patient receiving PI-containing regimens with pravastatin [152]	20	Efficacy of pravastatin 40 mg/day versus placebo in the treatment of dyslipidemia	TC: 18% decrease LDL-C: 21% decrease	Significant decreases in TC and LDL-C with pravastatin vs. placebo No significant difference in flow-mediated dilation
Randomized, crossover, double-blind placebo-controlled study of HIV-infected patients with dyslipidemia receiving PI-containing regimens with pravastatin [154]	29	Efficacy of pravastatin 40 mg/day versus placebo in the treatment of dyslipidemia for 8 weeks	Data only reported as median +/− interquartile ranges	Significant decreases in TC, LDL-C, and TG with pravastatin vs. placebo Significant increase in flow-mediated dilation with pravastatin
Randomized, open-label study of HIV-infected patients with dyslipidemia receiving PI-containing regimens with pravastatin or fibrates versus switching to NNRTI [153]	130 (pravastatin [$n = 36$] or bezafibrate [$n = 31$] versus nevirapine [$n = 29$] or efavirenz [$n = 34$] switch)	Efficacy of pravastatin or bezafibrate versus switching ART to NNRTI (NVP or EFV) in the treatment of mixed hyperlipidemia for 12 months	Nevirapine TG: 25% decrease LDL-C: 25% decrease Efavirenz TG: 9% decrease LDL-C: 9% decrease Pravastatin TG: 41% decrease LDL-C: 40% decrease Bezafibrate TG: 47% decrease LDL-C: 35% decrease	Significant decreases in TG and LDL-C with lipid medication versus switching to NNRTI Comparable viral efficacy Switching to NVP demonstrated greater TG reduction than switching to EFV

Randomized, open-label, study of HIV-infected patients with dyslipidemia receiving ART with pravastatin, fenofibrate, or both [205]	174 (pravastatin [*n* = 86] or fenofibrate [*n* = 88])	Efficacy of pravastatin or fenofibrate or both in the treatment of combined dyslipidemia for 48 weeks	Pravastatin (12 weeks) LDL-C: 20% decrease TG: 13% decrease Fenofibrate (12 weeks) LDL-C: 8% increase TG: 35% decrease HDL: 11% increase	Pravastatin significantly reduced LDL-C versus baseline and fenofibrate at 12 weeks Fenofibrate significantly reduced TG and increased HDL versus baseline and pravastatin at 12 weeks Over 75% of patients enrolled were initiated on dual therapy
Randomized, placebo-controlled study of HIV-infected patients with dyslipidemia receiving PI-containing regimens with pravastatin [155]	33 (pravastatin [*n* = 16] versus placebo [*n* = 17])	Efficacy of pravastatin 40 mg/day in the treatment of hypercholesterolemia for 12 weeks	Time-weighted change in TC decreased and subcutaneous fat increased with pravastatin	No change in TG versus placebo
Randomized, placebo-controlled study of HIV-infected patients with dyslipidemia receiving PI-containing regimens with pravastatin [148]	21 (pravastatin [*n* = 12] versus placebo [*n* = 9])	Efficacy of pravastatin 40 mg/day in the treatment of TC 213 mg/dL for 12 weeks	Data only reported as medians	TC and LDL-C decreased significantly with pravastatin No virological failure
Placebo-controlled, 2x2 factorial study of HIV patients receiving ART with pravastatin +/− lisinopril without compelling indication [156] {Baker, 2012 #156}	34 (pravastatin +/− lisinopril [*n* = 18] versus placebo +/− lisinopril [*n* = 16])	Efficacy of pravastatin 20 mg/day with or without lisinopril with no statin indication for 4 months	No change in TC, LDL-C, or inflammatory markers with pravastatin	No meaningful adverse effects Lisinopril reduced blood pressure
Randomized, open-label, crossover study of HIV patients on ART with pravastatin, +/− phytosterols [157]	36	Efficacy of pravastatin 40 mg/day +/− phytosterols 2 g/day in patients with LDL-C 130 mg/dL for 12 weeks including a 4 week washout	Pravastatin LDL-C: 29% Phytosterols LDL-C: 9% Both LDL-C: 27%	Adding phytosterols to pravastatin does not add any LDL-C benefit

(continued)

Table 23.5 (continued)

Trial	Number of patients	Primary outcome	Results	Comments
Randomized, prospective comparative study of HIV-infected patients with dyslipidemia receiving PI-containing regimens with pravastatin versus ezetimibe + fenofibrate [158] {Grandi, 2014 #158}	42	Efficacy of pravastatin 40 mg/day versus ezetimibe 10 mg/day + fenofibrate 200 mg/day in the treatment of dyslipidemia for 6 months	Pravastatin TC: 6% decrease LDL-C: 18% decrease Ezetimibe + fenofibrate TC: 11% decrease LDL-C: 17% decrease	Similar lipid parameter changes with combination non-statin therapy as with moderate-intensity statin Both arms were well tolerated
Rosuvastatin				
Rosuvastatin reduces vascular inflammation and T cell and monocyte activation in HIV-infected subjects on ART [162] {Funderburg, 2015 #162}	147 (rosuvastatin [n = 72] vs. placebo [n = 75])	Assess changes in baseline to 48 weeks in plasma inflammatory and coagulation indices and markers of lymphocyte and monocyte activation	LDL-C: 23.4% decrease HDL: 0.7% increase TG: 5.5% increase	Significant reduction in LDL-C with rosuvastatin vs. placebo No significant changes in HDL or TG Significant decrease in sCD14, Lp-PLA2, and markers of monocyte and lymphocyte activation in rosuvastatin vs. placebo
Rosuvastatin versus pravastatin in dyslipidemic HIV-1-infected patients receiving PIs: a randomized trial {Aslangul, 2010 #134} [134]	83 (rosuvastatin [n = 41] vs. pravastatin [n = 42])	Compare the efficacy of rosuvastatin and pravastatin on plasma lipid levels in HIV-1-infected patients on at least one PI after 45 days	Rosuvastatin: LDL-C: 37% decrease TG: 19% decrease HDL: 2.5% increase TC: 28% decrease Pravastatin: LDL-C: 19% decrease TG: 7% decrease HDL: no change TC: 14% decrease	LDL-C: 19% decrease TG: 7% decrease HDL: no change TC: 14% decrease Significant reduction in LDL-C, TG, and TC with rosuvastatin vs. pravastatin No difference in HDL No renal, hepatic, or muscular events in either group
Rosuvastatin for the treatment of hyperlipidemia in HIV-infected patients receiving protease inhibitors: a pilot study [160] {Calza, 2005 #160}	16	Evaluate rosuvastatin for the management of PI-related dyslipidemia in HIV-positive patients over 24 weeks	TC: 21.7% decrease TG: 30.1% decrease LDL-C: 22.4% decrease HDL: 28.5% increase	Significant decreases in TC, LDL-C, and TG and significant increase in HDL with rosuvastatin No significant clinical or laboratory adverse effects

Two-year treatment with rosuvastatin reduces carotid IMT in HIV type 1-infected patients receiving highly ART with asymptomatic atherosclerosis and moderate cardiovascular risk [161]	36	Assess changes in carotid IMT and evaluate effect on lipid parameters with rosuvastatin for 24 months	TC: 25.3% decrease LDL-C: 29.8% decrease HDL: 11.6% increase TG: 16.5% decrease Right internal carotid IMT: 23.7% decrease Left internal carotid IMT: 25.6% decrease Right carotid bifurcation IMT: 18.7% decrease Left carotid bifurcation IMT: 21.4% decrease	Significant reductions in TC, LDL-C, TG, IMT with rosuvastatin No serious adverse events reported
Other statins				
Fluvastatin				
Placebo-controlled, double-blind, randomized crossover study of HIV-infected patients with dyslipidemia receiving PI-containing regimens treated with fluvastatin [141]	16	Safety and efficacy of fluvastatin 40 mg/day in the management of dyslipidemia for 4 weeks	TC: 54% decrease TG: 18% decrease	Significant reduction in TC with fluvastatin vs. placebo
Prospective, non-randomized, open-label study of treatment-experienced HIV-infected patients on ART 2 years with hypercholesterolemia despite therapeutic lifestyle changes [142]	25 (fluvastatin [n = 12] vs. pravastatin [n = 13])	Compare the effectiveness fluvastatin and pravastatin for the treatment of hypercholesterolemia and potential interactions with ART for 12 weeks	Fluvastatin: TC: 19% decrease LDL-C: 30% decrease Pravastatin: TC: 14% decrease LDL-C: 14% decrease	Greater reductions in TC and LDL-C with fluvastatin compared to pravastatin No changes in ART serum concentrations
Pitavastatin				
After 52 weeks, pitavastatin is superior to pravastatin for LDL-C lowering in patients with HIV [135]	252 (pitavastatin [n = 126] vs. pravastatin (n = 126])* *99 vs. 91 patients completed 52 weeks	Evaluate long-term (52 weeks) safety and efficacy of pitavastatin vs. pravastatin in HIV-infected adults with dyslipidemia	Pitavastatin LDL-C: 29.7% decrease TC: 19.1% decrease TG: 2.0% decrease HDL: 8.9% increase Pravastatin LDL-C: 20.5% decrease TC: 13.7% decrease TG: 6.3% decrease HDL: 7.2% increase	Safety profiles were similar between agents Pitavastatin displayed a significantly greater decrease in LDL-C and TC at 52 weeks No difference in changes in TG or HDL

(continued)

Table 23.5 (continued)

Trial	Number of patients	Primary outcome	Results	Comments
Effects of pitavastatin on lipid profiles in HIV-infected patients with dyslipidemia and receiving ATV/RTV: a randomized, double-blind, crossover study {Wongprikorn, 2016 #142} [232]	24 (pitavastatin [n = 12] vs. placebo [n = 12])	Determine efficacy and safety of pitavastatin in HIV-infected patients with dyslipidemia who are receiving ATV/RTV at 12 weeks	TC: 13.7% decrease LDL-C: 21.8% decrease TG: 24.6% increase HDL: 5.3% increase	Significant decrease in TC and LDL-C at 12 weeks with pitavastatin vs. placebo No difference in TG or HDL No adverse events reported in pitavastatin group
Simvastatin				
Retrospective chart review of HIV-infected men receiving EFV-based ART and concomitant simvastatin 20 mg/day [163]	13	Evaluate the safety and efficacy of simvastatin for the treatment of dyslipidemia for up to 6 months	TC: 20% decrease TG: 21% decrease LDL-C: 36% decrease	No cases of myalgia, myositis, or increased CK Reduction in TC, TG, and LDL-C values with simvastatin

From Chastain et al. [144]

PI protease inhibitor, *ART* antiretroviral therapy, *TC* total cholesterol, *TG* triglycerides, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *CK* creatinine kinase, *HIV* human immunodeficiency virus, *NNRTI* non-nucleoside reverse transcriptase inhibitor, *NVP* nevirapine, *EFV* efavirenz, *sCD14* soluble CD14, *Lp-PLA2* lipoprotein-associated phospholipase A2, *carotid IMT* carotid intima-media thickness, *ATV/RTV* atazanavir/ritonavir

10 weeks led to a 25% reduction in TG levels and 18% reduction in TC levels [167]. A study of resistance exercise training for 1–1.5 hours per day for 4 days a week for 16 weeks resulted in 27% decrease in TG in PWH on ART [168]. In a 16-week study comparing aerobic exercise and resistance training in PWH, both types of exercise increased HDL-C and reduced inflammatory cytokines, but a significant decrease in TG was only seen with resistance training [169].

Ezetimibe

Ezetimibe reduces dietary and biliary cholesterol absorption at the intestinal brush border, resulting in a reduction of hepatic cholesterol stores and an increase in cholesterol clearance from blood. It has been shown to be effective in reducing LDL cholesterol levels for PWH who are unable to reach LDL cholesterol reduction targets with statin treatment alone. Unlike statins, ezetimibe does not interact with CYP3A isoenzymes and does not have significant DDIs with ARVs [170]. Its efficacy in reducing LDL cholesterol when added to a statin has been evaluated in several studies in PWH [170–174].

In the RCT ACTG A5209 trial, 44 PWH on stable ART and stable statin therapy with LDL cholesterol >130 mg/dL were randomized to receive ezetimibe or placebo for 12 weeks. The median percentage decrease in LDL cholesterol was 20.8% with ezetimibe and 0.7% with placebo. Reductions in TC, non-HDL cholesterol, and ApoB were also observed [172].

Adding ezetimibe to rosuvastatin has been shown to be possibly more effective at decreasing non-HDL cholesterol and TC for PWH on ART than increasing rosuvastatin dose. In a randomized, open-label trial of PWH who had ApoB levels >0.80 g/L despite taking rosuvastatin 10 mg daily for 12 weeks, participants were randomized to either ezetimibe 10 mg plus rosuvastatin 10 mg daily or to rosuvastatin 20 mg daily for 12 weeks. Improvements in ApoB were observed in both groups without significant differences between the groups. However, there were larger reductions in TC, TG, and non-HDL cholesterol in the ezetimibe plus rosuvastatin group compared to the rosuvastatin 20 mg/day group [173].

Switching ART to Manage Dyslipidemia

A strategy that may be considered in patients with significant dyslipidemia who are on ART that contribute adversely to their lipid profiles is to switch ART to ART with a more favorable lipid profile. With the increased availability of potent and well-tolerated ARV options, this may be possible for patients taking ART with poor lipid profiles. However, maintenance of virologic suppression should be paramount in any switches in ART while keeping in mind that there may be a risk of medication intolerance or possible loss of virologic control when ART regimens are changed, particularly in patients with archived viral resistance mutations. For example, in the SWITCHMRK 1 and 2 studies, patients on the PI lopinavir/ritonavir with undetectable viral load at baseline were randomized to switch to the INSTI raltegravir or remain on the same PI regimen. There were significant improvements in TC, non-HDL cholesterol, and TG at 24 weeks for those who switched to raltegravir compared to those remaining on lopinavir/ritonavir. However, patients who switched to raltegravir (which has a lower genetic barrier to viral resistance than lopinavir/ritonavir) had a higher proportion of virologic failure than patients who remained on lopinavir/ritonavir [175]. Furthermore, in patients with pre-existing dyslipidemia prior to ART initiation, lipids are unlikely to normalize by switching ART alone without concomitant lipid-lowering medications [129].

At the present time, ART regimens that contain the NRTIs stavudine, zidovudine, or didanosine should be replaced with other ARVs that are not associated with adverse effects. A significant improvement in TC, LDL cholesterol, and TG has been observed when these drugs are switched to either tenofovir or abacavir [176–178]. Due to its inherent lipid-lowering properties, adding tenofovir to an existing ART regimen has been shown to improve the lipid profile for a dyslipidemic patient [85]. Replacing abacavir with tenofovir has also been shown to improve lipids in dyslipidemic patients [179]. However, the TDF form of tenofovir is associated with bone mineral density loss and renal

dysfunction in some patients [180]. The TAF form of tenofovir has a decreased associated risk of these complications. However, due to its lower plasma concentration, the lipid-lowering effect of tenofovir is much more limited with TAF. In patients taking emtricitabine/TDF/elvitegravir/c who were switched to emtricitabine/TAF/elvitegravir/c, the lipid profile worsened significantly [181].

Replacement of ritonavir-boosted PIs is often an effective switching strategy in dyslipidemic patients on ART. The current PI can be switched to a more lipid-favorable PI such as ritonavir-boosted darunavir [182] or to boosted or unboosted atazanavir resulting in improved dyslipidemia with significant decreases in TC, LDL cholesterol, and TG [183–186].

Another strategy is to replace a PI with an NNRTI such as etravirine [187, 188], rilpivirine [189, 190], or possibly doravirine. In an open-label study of 31 PWH with ART-associated dyslipidemia with either a boosted PI or efavirenz, and a statin, were switched to etravirine. Twelve weeks after the switch, 56% of those who had switched to etravirine no longer required statins [188]. Switching to efavirenz is not recommended due to its poor lipid profile, association with central nervous system adverse effects, and low genetic barrier to resistance. Similarly, switching to nevirapine is not recommended due to its toxicity profile and low genetic barrier to resistance.

Studies have also demonstrated that switching from a PI to an INSTI is also a viable replacement strategy resulting in improvements in TC, TC/HDL cholesterol, and TG [190, 191]. Switching from a ritonavir-containing PI to a DTG regimen in virologically suppressed patients with high cardiovascular disease risk was non-inferior to remaining on the PI regimen and significantly improved lipid profiles [192]. In another phase 3, non-inferiority trial, participants who were virologically suppressed on boosted protease inhibitor regimens containing abacavir and lamivudine at baseline found that those who were switched to bictegravir, emtricitabine, and tenofovir alafenamide resulted in significant decreases in concentrations of fasting total cholesterol, LDL cholesterol, and triglycerides and in the total cholesterol-to-HDL ratio at week 48 compared with participants who remained on boosted protease inhibitor therapy, but no significant differences were found on those who received TAF and FTC as the background nucleosides [193].

It should be noted that replacing a PI for the INSTI elvitegravir/c may result in improvements in TC and TG, but such improvements may be limited by the presence of the pharmacokinetic booster cobicistat [194].

Switching from PIs or NNRTIs to the CCR5 inhibitor maraviroc improves TC and TG levels [195], but it may not be a practical option since it is mostly used as part of a salvage ART regimen for patients failing first- and second-line ART. Switch of NNRTIs from the pro-atherogenic efavirenz to other NNRTIs such as rilpivirine [190, 196] or etravirine [188] also results in improvements in improved lipid profiles and may be a viable switch option. Ritonavir is used in low doses as a pharmacokinetic booster for PIs. However, even in low doses, it is known to induce dyslipidemia. Switching pharmacokinetic boosters from ritonavir to cobicistat has been shown to result in significant improvements in TC, LDL cholesterol, and TG and even in HDL cholesterol levels in virally suppressed PLWH with dyslipidemia [77].

In contrast to ART switch strategies, studies have shown that adding a statin results in more substantial improvements in lipid profiles [74, 197, 198]. For example, 136 PWH with dyslipidemia taking a PI-containing ART regimen were either switched to the NNRTI nevirapine or the INSTI raltegravir or continued the same ART regimen but started rosuvastatin 10 mg daily. A greater decline in LDL cholesterol was achieved with rosuvastatin than with either ART switch, while a greater decline in TG was achieved by switching from the PI to either nevirapine or raltegravir [197].

In general, ART switch for lipid lowering only is not recommended. When considering ART switch, providers should weigh carefully the overall risks and benefits when deciding who may truly benefit from an ART switch. Providers

are advised to the current Department of Health and Human Services (DHHS) Guidelines best practices and considerations that should be applied before switching ART regimens [199].

PCSK9 Inhibitors

PCSK9 inhibitors (alirocumab and evolocumab) are a new class of monoclonal antibodies approved for use in combination with maximally tolerated statin therapy and diet in adults with familial hypercholesterolemia or with clinical ASCVD who need improved control of LDL cholesterol. In the general population, they reduce LDL cholesterol and non-HDL cholesterol by 60% [200, 201]. An RCT evaluating the efficacy of evolocumab in PWH with hyperlipidemia and/or mixed dyslipidemia is currently ongoing (NCT 02833844) [202]. The EPIC-HIV trial is evaluating the effect of PCSK9 inhibition with alirocumab on vascular inflammation, endothelial function, and non-calcified plaque (NCT03207945) [203].

Management of Elevated TG in PLWH

Elevated TG is the most common lipid derangement seen in PWH and may be associated to interferon-alpha-induced decrease in lipase activity and TG clearance [204]. According to the 2015 NLA Guideline Recommendations for HIV-Infected Persons, TG must be measured while fasting. Elevated TG ≥500 mg/dL that remains refractory to lifestyle modification or changes in ART should be treated with either a fibrate (preferred) or prescription omega-3 fatty acids [48]. However, these agents may raise LDL cholesterol.

Table 23.6 Main randomized and observational studies evaluating fibrate therapy in PWH

Author	Type of study	N	Criteria for entry	Fibrate treatment	Mean reductions from baseline
Calza (2005) [160] {Calza, 2005 #153}	RC	130	TC >250 mg/dL and TG >200 mg/dL	Bezafibrate (400 mg daily) for 12 months	TC = –38% TG = –47%
Calza (2003) [151]	RC	106	TG >300 mg/dL	Gemfibrozil (1200 mg daily), bezafibrate (400 mg daily), or fenofibrate (200 mg daily) for 12 months	TC = –22% TG = –41%
Bonnet (2004) {Bonnet, 2004 #143} [233]	PR	66	TC >213 mg/dL and/or TG >194 mg/dL	Several fibrates for 12 months	TC = –7% TG = –29%
Visnegarwala (2004) [125]	RE		Starting a lipid-lowering therapy	Statins for a median follow-up of 70 weeks	TC = –9% TG = –11%
Aberg (2005) [205]	RC	86	Combined dyslipidemia	Fenofibrate (200 mg daily) plus pravastatin (40 mg daily) for 12 months	LDL-C = from −8 to −14 mg/dL TG = –66 to −144 mg/dL
Palacios (2002) [139]	PR	20	TG >400 mg/dL	Fenofibrate (200 mg daily) for 24 weeks	TC = –14% TG = –54%
Badiou (2004) [234] {Badiou, 2004 #144}	RC	36	TG >177 mg/dL	Fenofibrate (200 mg daily) for 3 months	TC = –14% TG = –40%
Rao (2004) [235] {Rao, 2004 #145}	PR	55	High TG	Fenofibrate (54–162 mg daily) for 6 months	TC = –6% TG = –38%
Balasubramanyam (2011) [236]	RC	191	TG >150 mg/dL	Fenofibrate (145 mg daily) with diet and exercise for 24 weeks	TC = –9% TG = –37%

Modified from Calza et al. [63]
LDL-C low-density lipoprotein cholesterol, *RC* randomized controlled study, *PR* prospective or cohort study, *RE* retrospective study. *TC* total cholesterol, *TG* triglycerides

Fibrates

Table 23.6 summarizes the studies evaluating the efficacy of fibrates in PWH on ART.

The ACTG A5087 study showed optimal improvement in TG at 12 weeks for patients taking fenofibrate compared to those taking pravastatin (−35% vs. −13%, $p < 0.001$) [205]. However, there was no additional improvement in TG after pravastatin was added to fenofibrate after 12 weeks on fenofibrate alone [159].

Fish Oils

Fish oils that are long-chain omega-3 polyunsaturated fatty acids (PUFAs) lower TG and CVD in the general population. They are less effective than fibrates in lowering TG. An advantage of PUFAs for PWH on ART is that they lack DDIs with ART drugs. RCTs have been conducted to evaluate PUFAs in PWH with elevated TG on ART [206–210]. PLWH with high TG on ART experienced a mean decline in TG between 19 and 57% after 8–16 weeks of treatment [63]. ACTG A5186 randomized PLWH on ART with high TG to receive fish oil 6 g/day or fenofibrate 160 mg/day. A reduction in TG of 58% and 46% was achieved for fenofibrate and fish oil, respectively, at 8 weeks. Patients who failed to achieve TG goal <200 mg/dL were treated with both fish oil and fenofibrate. However, only 23% of study participants reached TG goal [209].

Niacin

Niacin can raise HDL-C by reducing lipid transfer from HDL to VLDL [211] and can decrease TG when used in higher doses in the general population [212]. It is generally the least well tolerated of drugs available to lower TG, and usually high doses are required. It should be reserved for patients who do not tolerate a fibrate or omega-3 fatty acid agent. In PWH with TG levels >200 mg/dL, extended-release niacin was administered in increasing doses for up to 44 weeks. At 48 weeks, median TG decreased by 38% [213]. A similar study of niacin given for only 14 weeks showed a median 34% decrease in TG at 18 weeks [214]. In a study of PWH on ART with low HDL cholesterol and TG >150 mg /dL, participants were randomized to receive extended-release niacin with aspirin or fenofibrate. Although HDL cholesterol improved modestly in both arms, neither intervention improved endothelial function or inflammatory markers [215].

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Cardiovascular Disease in Women: Focus on Lipid Management

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Cardiovascular Disease and Coronary Heart Disease Burden in Women

Accurate assessment of atherosclerotic cardiovascular disease (ASCVD) risk and prompt diagnosis and treatment of lipoprotein abnormalities are essential to reduce the large and growing health and economic burdens from cardiovascular disease (CVD) in women. Despite dramatic declines in total CVD mortality among women in the USA between 2000 and 2010 [1], CVD remains the #1 killer of women, causing almost one out of three female deaths in 2016 [2]. Worrisome trends and poorer outcomes have been observed in younger and middle-aged women compared to older women. Although the overall age-adjusted mortality rate from coronary heart disease (CHD) dropped steadily in the USA between 1979 and 2011, little to no decline was observed in women younger than age 65 years, despite declining death rates in older women [3]. Based on the National Health and Nutrition Examination Survey (NHANES) data, between 1988 and 2004, the prevalence of myocardial infarction (MI) increased in younger women, despite declines in men [4]. Data from the National Registry of Myocardial Infarction indicate that middle-aged women who suffer MIs have higher in-hospital mortality compared to their male counterparts, despite less obstructive disease, due to presumed sex-specific

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_24

differences in treatment and possibly biology [5, 6]. These data are consistent with under-recognition and undertreatment of coronary risk in women in the latter half of the twentieth century and emphasize the need for more intensified prevention efforts in younger and middle-aged women.

The prevalence of occult CHD detectable by coronary artery calcium testing also is high in women in the USA, ranging from 20.1% in those age 40 years or older in the Framingham Heart Study (FHS) to 35–45% among those age 45–84 years in the Multi-Ethnic Study of Atherosclerosis (MESA) [7]. These data are consistent with the high lifetime risk of manifest CHD in women in the USA, calculated to be one in three at age 40 based on prospective data from the FHS [8].

Prevalence of and Screening for Hypercholesterolemia in Women

Population-wide screening for lipid disorders is well-supported in women, in whom the prevalence of hypercholesterolemia is high and increases with age, in part due to menopausal hormone shifts. Although cholesterol levels have decreased steadily in women and men in the USA, data from the NHANES 2016 time period showed that 41.6 million or 40.4% of adult US women have a total cholesterol of ≥200 mg/dL and 16.1 million, or 12.4%, have a level of ≥240 mg/dL [2]. Applying NHANES data from 2005 to 2010 and criteria from the 2013 American College of Cardiology (ACC)/American Heart Association (AHA) Cholesterol Treatment Guideline, 53.6 million American women age 60–75 years meet criteria for lipid treatment [9]. The ACC/AHA recommend lipid screening of all women ≥20 years of age every 5 years, and the US Preventive Services Task Force (USPSTF) recommendations suggest screening of all adult women with elevated risk of CHD based on at least one other risk factor, also every 5 years. In the USA, obstetricians, gynecologists, family physicians, internists, cardiologists, endocrinologists, neurologists, lipid and prevention clinics, workplace wellness programs, and pharmacy-based clinics are well-positioned to undertake screening for lipoprotein disorders in women.

Evidence-Base for Lipid Treatment in Women: Observational Data

Evidence-based guidelines for lipid lowering in women have been supported by cohort data and randomized controlled trials that show a bidirectional causal association between abnormal lipoprotein levels and CHD risk in women. These data have been largely concordant and are now robust, but studies were limited initially by lower quality evidence that probably led to undertreatment of women. The first cohort evidence of a link between elevated blood cholesterol and CHD incidence in women was published by FHS authors in 1961 and was subsequently echoed in other North American, European, and Israeli cohorts by the 1980s [10]. A 1991 systematic review of 22 cohort studies in 90,349 women and 891,882 men showed that elevated low-density lipoprotein cholesterol (LDL-C) conferred a higher unadjusted relative risk (RR) of CHD death in women compared to men under age 65, with attenuation of this association in older women (1.98 vs. 1.16, respectively). Elevation of blood triglyceride levels also conferred a higher unadjusted RR of CHD death in women vs. men under age 65 and this relationship persisted in older women (RR 1.39). This evidence supports the atherogenicity of very low-density lipoprotein (VLDL) remnants in women. Low levels of high-density lipoprotein cholesterol (HDL-C) also conferred a 2.13-fold RR of CHD in younger women that persisted in older subsets [11]. Although these pooled data were unadjusted, they were the first large-scale evidence that abnormal lipid levels confer coronary risk in women, which previously had been debated. They were also concordant with later observational data from (1) the FHS that showed that an adjusted total cholesterol to HDL-C ratio of 7.5 or higher fully abolishes the female coronary disease advantage [12]; (2) the Atherosclerosis Research in the Communities Study that demon-

strated a graded relationship between LDL-C and CHD death in women after adjustment for age and race [13]; and (3) the Framingham Offspring Study [14] and the Women's Health Study [15] that showed that elevated levels of non-HDL-C, apolipoprotein (Apo) B, and high-sensitivity C-reactive protein (hs-CRP) independently predict CHD risk in women. Data are also concordant with a 25-year follow-up of 4019 mostly Caucasian women in the FHS that estimated the cumulative risk of fatal and nonfatal CHD events based on cholesterol "strata" and showed a lifetime risk from elevated total cholesterol (≥240 mg/dL) of ~33% at age 40 and ~39% at age 50 [16]. Finally, the strong association between blood cholesterol levels and vascular mortality in women also has been observed globally. In a 2007 meta-analysis of individual participant data from 61 prospective observational studies from Europe, the USA, Australia, Japan, and China that included 8667 female vascular deaths over a mean follow-up of 13 years, each 1 mmol/L (39 mg/dL) lower blood cholesterol level was associated with a lower risk of vascular death across age groups that was most pronounced in younger women age 40–49 (hazard ratio [HR] 0.43 for each 1 mmol/L lower cholesterol, 95% confidence interval [CI] 0.42–0.48) and became attenuated but remained significant even in older years (HR 0.73, 95% CI 0.68–0.78 at age 60–69; HR 0.92, 95% CI 0.86–0.97 at age 80–89) [17].

Statin Therapy and ASCVD Risk Reduction in Women

Primary Prevention

As discussed above, hypercholesterolemia is causal in the development of coronary atherosclerosis and incident atherothrombotic cardiovascular (CV) events in women as it is in men. Statins lower atherogenic lipoproteins to a similar degree in both sexes and clinical trials of statin therapy suggest that women benefit from lipid-lowering with reduction in ASCVD risk [18].

Controversy about the benefit of statins among women arises in the interpretation of individual clinical trials, since clinical trials are, by definition, powered for the overall trial population and tend to be underpowered to reliably assess benefit in smaller subgroups. Given that women make up a minority of trial participants in most statin trials, few trials are sufficiently powered to assess statin benefits in the subgroup of women. Furthermore, absolute event rates tend to be lower among women than men. Meta-analysis can be helpful in this situation by pooling data from multiple trials, but it can only take into account heterogeneity in participant characteristics if the meta-analysis pools individual patient data. This section will summarize selected trials of statin therapy that enrolled primary prevention cohorts or enrolled predominantly patients without preexisting ASCVD and that had reasonable power and duration to estimate benefits among women. The discussion of meta-analyses will then focus on the work of the Cholesterol Treatment Trialists' (CTT) Collaboration, which has pooled individual patient data from 27 statin trials according to a statistical protocol that was finalized before any of the trials had reported their results [19].

Review of Selected Trials That Included Individuals Without Prior ASCVD

The Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) primary prevention trial included 6801 women and 11,001 men with elevated hs-CRP and LDL-C of ≤130 mg/dL randomized to rosuvastatin 20 mg vs. placebo [20]. The composite primary endpoint included MI, stroke, hospitalization for unstable angina, arterial revascularization, or CV death. Women in this trial were older than men and had more CV risk factors than men, but absolute event rates were lower in women than in men. Rosuvastatin, compared to placebo, reduced the primary endpoint in women by 46%, in men by 42% (HR 0.54, 95% CI 0.37–0.80, $P = 0.002$ for women; HR 0.58, 95% CI 0.45–0.73, $P < 0.001$ for men). The extrapolated 5-year number needed to treat (NNT) values for the prevention of one primary

endpoint event was 22 for men and 36 for women. For the more restricted, "hard endpoint" of MI, stroke, or any death, the 5-year NNT was 23 for men and 52 for women [3].

The Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA) trial randomized 7832 Japanese primary prevention patients with total cholesterol levels between 5.7 mmol/L (221 mg/dL) and 7 mmol/L (271 mg/dL) to diet or diet plus pravastatin 20 mg daily and followed them for 5 years [21]. The study is unique in that 68.4% of participants (n = 5356) were women. Women were on average 4 years older than men; had higher prevalence of hypertension, but lower prevalence of diabetes; and were far less likely to smoke (6.2% vs. 51.7%) and to consume alcohol (12.4% vs. 68.5%) compared to their male counterparts. In the control group (diet intervention alone), women had lower CHD event rates than men, but higher stroke and mortality rates than men. Point estimates of benefit for CHD, combined CHD and cerebrovascular disease, stroke, and mortality were similar in women and men (all P-values for heterogeneity were > 0.42). Interestingly, the HR for mortality achieved statistical significance among women, but not among men.

The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) was a double-blind, placebo-controlled, primary prevention trial that enrolled 997 women and 5806 men without CVD and randomized them to diet with lovastatin vs. diet alone and followed them for an average of 5.2 years [22]. Women were on average 5 years older than men (>30% of women were ≥65 years old), were more likely to have hypertension and less likely to have an HDL-C level of <35 mg/dL. Among men, the 37% relative risk reduction in major cardiac events achieved statistical significance (RR 0.63, 95% CI 0.50–0.81); among women, the 46% relative risk reduction was not statistically significant (RR 0.54, 95% CI 0.22–1.35).

The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial–Lipid-Lowering Trial (ALLHAT-LLT) randomized 10,355 individuals aged 55 years or older to pravastatin 40 mg daily vs. usual care; 49% were women and 86% had no history of CHD [23]. The primary outcome was total mortality and did not achieve significance among women or men (RR for women 0.98, 95% CI 0.83–1.17; for men RR 0.99, 95% CI 0.86–1.14). The secondary outcome of CHD death and nonfatal MI also did not achieve benefit overall (RR 0.91, 95% CI 0.79–1.04), with sex-specific RR estimates of 1.02, 95% CI 0.81–1.28 among women and 0.84, 95% CI 0.71–1.00 among men without evidence of sex by treatment interaction. The authors attributed the overall lack of statistical benefit of this trial to the very modest 9.6% differential in total cholesterol and 16.7% difference in LDL-C between treatment groups.

The Anglo-Scandinavian Cardiac Outcomes Trial–Lipid Lowering Arm (ASCOT-LLA) randomized 10,305 hypertensive individuals (1942 women) to atorvastatin 10 mg vs. placebo [24]. Of note, about 10% of participants had prior stroke or transient ischemic attack, 5% had peripheral arterial disease, and 4% had other CV diseases. The study was stopped prematurely at 3.3 years of follow-up due to a 36% reduction in primary events in the atorvastatin arm. The unadjusted HR was 1.10, 95% CI 0.57–2.12 for women and 0.59, 0.44–0.77 for men. Favorable long-term follow-up results extending to 16 years have since been published but do not provide sex-specific estimates.

The Heart Protection Study (HPS) randomized 20,536 individuals (5082 women) to simvastatin vs. placebo. Among men, 46% had prior coronary, cerebrovascular, or peripheral vascular disease; among women, 40% had prior evidence of these manifestations of ASCVD [25]. Simvastatin allocation was associated with a statistically significant 19% reduction of first major vascular event among women and a statistically significant 22% reduction among men. Sex-specific estimates among primary prevention patients in this study are not available.

The Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) randomized 5804 individuals (3000 women) to 40 mg of pravastatin vs. placebo and followed them for an average of 3.2 years [26]. Almost half of the population had prior vascular disease. There was

a statistically significant 15% overall reduction in the primary endpoint (CHD death or nonfatal MI or fatal or nonfatal stroke) with HR 0.85, 95% CI 0.74–0.97 and 19% reduction in CHD death and nonfatal MI with HR 0.81, 95% CI 0.69–0.94, but no impact on fatal or nonfatal stroke. Sex-specific estimates are only available for the primary endpoint: for women, the HR was 0.96, 95% CI 0.79–1.18, for men 0.77, 95% CI 0.65–0.92 with a P-value for interaction of 0.13.

Meta-Analysis: The CTT Collaboration

In 2015, the CTT Collaboration published a meta-analysis of individual data from 174,149 participants (27% women) in 27 primary and secondary prevention randomized trials to compare efficacy and safety of LDL-C-lowering therapy among men and women [21]. These included 22 trials that compared statin to control and 5 trials that compared more to less intensive statin therapy. Outcomes included major vascular events, major coronary events (defined as nonfatal MI or coronary death), coronary revascularization (percutaneous revascularization or coronary artery bypass grafting), stroke stratified by type, site-specific cancers, and cause-specific mortality. Overall, women were on average 3 years older than men, had more hypertension, more diabetes, and less prior vascular disease. In an analysis among individuals without prior vascular disease that was adjusted for differences in baseline characteristics among women and men, there was a 25% reduction in major vascular events per 1 mmol/L reduction in LDL-C overall, 28% among men (RR 0.72, 99% CI 0.66–0.80), and 15% among women (RR 0.85, 99% CI 0.72–1.00). An additional analysis that stratified by sex and baseline CV risk similarly showed that there was statistically significant benefit per 1 mmol/L LDL-C reduction in both women and men at all levels of 5-year baseline risk (<10%, 10–<20%, 20–<30%, and 30% or greater). This meta-analysis thus demonstrates that women and men at similar risk of major vascular events achieve similar proportional and absolute benefits from statin therapy even in primary prevention and among individuals at low baseline risk.

Risk Stratification and Current Guidelines for Primary Prevention in Women

Many risk prediction instruments are available to estimate ASCVD risk including risk of MI, CHD death and nonfatal MI, and CHD and stroke. In the past, equations based on data from the Framingham study were recommended. In 2013, a new equation, the Pooled Cohort Equation, was published as part of the 2013 ACC/AHA Cholesterol-Lowering Guideline revision [27, 28]. This equation represented an improvement over prior iterations by pooling data from 5 cohorts instead of drawing inferences from a single population-based study, by taking into account ethnicity with different estimators of risk for non-Hispanic whites and blacks, and by combining coronary risk and stroke risk. The latter is especially important among women because stroke constitutes a greater proportion of ASCVD in women than in men and often occurs earlier in life in women compared to men. The Pooled Cohort Equation has been validated in the REasons for Geographic and Racial Differences in Stroke (REGARDS) study [29], a community-based US population, and in the Women's Health Initiative, a multiethnic cohort of postmenopausal women [30]. For individuals who have undergone coronary calcium scoring, a risk calculator, derived from MESA and recently validated in the Dallas Heart Study and the Heinz Nixdorf Recall Study, may provide further refinement in risk estimation [31].

Not captured in current risk calculators are conditions specific to women that are associated with increased CV risk. These include premature menopause below age 40 and history of pregnancy-associated disorders, such as pregnancy-induced hypertension, preeclampsia, gestational diabetes mellitus, small for gestational age infants, and preterm deliveries [32]. Development of hypertension and diabetes during pregnancy is primarily indicative of increased risk of future hypertension and diabetes and should prompt lifestyle counseling. It is less clear whether these factors convey incremental risk beyond those of hypertension and diabetes once they become established in middle age and beyond. The 2018

AHA/ACC Cholesterol Guideline includes sex-specific conditions in women as risk-enhancing factors for clinical decision-making for use of statin therapy [30]. Risk enhancers are defined as factors that can be used to favor initiation or intensification of statin therapy when the treatment decision is uncertain. A detailed pregnancy complication history should be included in the health record and should be considered by those providing primary or disease-related health care to reproductive age and postmenopausal women. Other risk factors for women include polycystic ovarian syndrome, functional hypothalamic amenorrhea, breast cancer treatment, and/or autoimmune diseases.

Inflammatory risk is captured in the Reynolds Risk Score by inclusion of hs-CRP in the risk prediction algorithm [33]. This score was derived from the Women's Health Study, a nationwide cohort of healthy female health professionals who were recruited into a factorial clinical trial of low-dose aspirin and vitamin E starting in 1992 and who were followed through 2004 with a mean follow-up of 10.2 years [34]. In the Women's Health Study, 95% of participants were Caucasian, approximately a quarter each had hypercholesterolemia and hypertension, 13% smoked, but only 2.6% had diabetes and only 18% had a body mass index (BMI) of ≥30 kg/m^2. Using the Framingham score, 85% of participants had <5% 10-year risk of CHD.

The impact of hs-CRP measurement on CHD risk reclassification was studied in 19,080 participants without diabetes (11,003 women; 45% black) not using lipid-lowering medications from the REGARDS study (age >45 years, without vascular diagnoses, and living dispersed across the USA) [35]. Participants were classified into four risk categories based on the Framingham vascular disease risk score. Participants with hs-CRP <1 mg/L were reclassified to the next lower risk group and those with hs-CRP >3 mg/L to the next higher risk group. Authors also assessed reclassification of risk based on the Reynolds Risk Score, incorporating hs-CRP and family history. Among women at 5–20% Framingham vascular predicted risk, hs-CRP data led to reclassification of 48% to a higher risk group and 19% to a lower risk group. For men, these percentages were 24% and 40%, respectively. Blacks were more often reclassified to a higher risk group than whites. Reynolds Risk Score data led to reclassification of 85% of women and 67% of men, almost exclusively to a lower risk group, than the Framingham vascular score. CV outcomes were not reported, and follow-up of this cohort is needed to determine the significance of these findings. Given the prevalence of obesity, hypertension, and diabetes in more contemporary cohorts of women and changing ASCVD event rates, the applicability of the Reynolds Risk Score to contemporary practice is unclear [36]. The current 2018 AHA/ACC Cholesterol Guideline considers elevated hs-CRP as an ASCVD risk enhancer but stops short of recommending routine hs-CRP measurement [30]. Inflammatory diseases, including rheumatoid arthritis, psoriasis, and human immunodeficiency virus, are similarly considered as ASCVD risk enhancers.

Risk estimation should not be used among women with primary hypercholesterolemia (i.e., LDL-C levels ≥190 mg/dL) [15]. Both women and men with primary hypercholesterolemia are at sufficient lifetime risk that high-intensity statin therapy is recommended for both. Given concerns about the safety of statins for the developing fetus, special considerations apply among women of childbearing potential. These are discussed in detail in a subsequent section.

Decisions about statin therapy in primary prevention among individuals without severe primary hypercholesterolemia and without diabetes are based on underlying risk, not sex [15]. Among middle-aged individuals (age 40–75 years), low risk is defined as a 10-year Pooled Cohort Equation-based ASCVD risk <5%, borderline risk as 5% to <7.5%, intermediate risk as ≥7.5% to <20%, and high risk as ≥20% in both sexes. Statin therapy is a Class I indication for intermediate and high risk individuals and a Class IIb indication for borderline risk individuals, while lifestyle measures alone are recommended in low risk individuals. For younger individuals, statin therapy can be considered among those with a family history of premature ASCVD and LDL-C levels of ≥160 mg/dL. It is important to remem-

ber that a detailed clinician-patient risk discussion should precede any treatment decisions in primary prevention, regardless of risk level, and is particularly important among individuals above age 75 years for whom randomized clinical trial evidence of statin benefit is more limited.

Diabetes

Diabetes mellitus increases ASCVD risk in both sexes. Investigators from Framingham suggested in 1986 that diabetes disproportionately affects ASCVD risk in women and eliminates the "female advantage" (compared to men) for CV morbidity and mortality [37]. A meta-analysis of 37 prospective, multinational studies in 2006 corroborated these results, concluding that the risk of death from CHD associated with type 2 diabetes (after adjustment for major coronary risk factors) was about 50% greater in women than in men [38]. This disproportionate impact was again confirmed in contemporary data from the UK Biobank that suggested a sex ratio (women/men) of relative risks of 2.91 for type 1diabetes and 1.49 for type 2 diabetes [39].

Sex-specific benefits of statin therapy among patients with diabetes have been reported by the CTT Collaboration, which summarized results from 14 trials (63% of participants without prior vascular disease) [40]. The RR for women comparing statin therapy vs. no statin therapy was 0.81, 99% CI 0.67–0.97 and for men 0.78, 99% CI 0.71–0.86. Of note, point estimates for benefit of statin therapy were identical for individuals with type 1 and type 2 diabetes mellitus.

CV risk among individuals with diabetes is not uniform. For most adults 40–75 years of age with diabetes, current guidelines recommend moderate intensity statin therapy [15]. Risk stratification with the Pooled Cohort Equation may identify individuals at higher risk, generally men >50 years of age and women >60 years of age or those with risk-enhancing factors. High-intensity statin therapy is considered reasonable among these high-risk individuals. Among adults older than 75 years with diabetes mellitus, continuation of statin therapy carries a Class IIa recommendation and initiation of statin therapy has a Class IIb recommendation, both independent of the patient's sex.

Secondary Prevention

Among patients with ASCVD, there are no sex differences in response to statins. The 2015 CTT meta-analysis cited above found a RR of 0.79, 99% CI 0.76–0.82 among men and a RR of 0.84, 99% CI 0.77–0.91 among women per 1 mmol/L (39 mg/dL) reduction in LDL-C [16]. There was also no heterogeneity in benefit by sex when individuals at high risk (≥20 to <30%) and very high risk (≥30%) were analyzed separately. Reductions in major coronary events, coronary revascularization, and stroke showed no heterogeneity by sex, although the point estimate for stroke did not achieve statistical significance among women. Importantly, all-cause death was reduced among women (RR 0.91 per 1 mmol/L LDL-C reduction, 99% CI 0.84–0.99 and for men 0.90, 99% CI 0.86–0.95). There were no increases in deaths from non-vascular causes or unknown causes.

Among the five trials that compared higher with lower intensity statin treatment, only 14% of participants were women [41]. The proportional reductions per 1 mmol/L reduction in LDL-C in women (RR 0.75, 99% CI 0.58–0.97) were similar to those among men (RR 0.71, 99% CI 0.63–0.80). A patient level pooled analysis of three intravascular ultrasound studies, including Reversal of Atherosclerosis with Aggressive Lipid Lowering Therapy (REVERSAL), A Study to Evaluate the Effect of Rosuvastatin on Intravascular Ultrasound-Derived Coronary Atheroma Burden (ASTEROID), and Study of Coronary Atheroma by Intravascular Ultrasound: Effect of Rosuvastatin Versus Atorvastatin (SATURN), concluded that women achieved greater degrees of coronary plaque regression compared to men among participants who achieved LDL-C levels below the median (64 mg/dL) [8]. This sex difference was not apparent among individuals with on-treatment LDL-C levels above the median. Both women and men had greater atheroma regression with greater LDL-C changes from baseline, but the association between change in LDL-C and change in atheroma volume was stronger among women than among men [8].

Consistent with these data, the 2018 AHA/ACC Cholesterol Guideline recommends high-intensity statin therapy for men and women with clinical ASCVD (acute coronary syndrome, history of MI, stable or unstable angina, coronary or other arterial revascularization, stroke, transient ischemic attach, and peripheral arterial disease including aortic aneurysm) [8]. For patients who cannot tolerate high-intensity statin therapy, maximally tolerated statin therapy is recommended. Addition of non-statin therapy for those who do not achieve a ≥50% reduction in LDL-C or for those at very high risk of future ASCVD events is discussed later in this chapter.

Non-Statin Therapies in Women: What Is the Evidence and What Is the Role?

The evidence for prevention of ASCVD in women with the use of non-statin drugs is limited, as no large randomized controlled trials have been adequately powered to evaluate sex differences in response to non-statin therapies. The available clinical trial outcomes data and gaps in evidence are summarized in this section.

Bile Acid Sequestrants

The Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) is currently the only CV outcomes trial of a bile acid sequestrant [42, 43]. LRC-CPPT was a multicenter, randomized, double-blind study that evaluated the efficacy of cholestyramine in reducing the risk of CHD events. This trial was conducted in 3806 asymptomatic men and did not include women.

Cholesterol Absorption Inhibitor

In the Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT), 18,144 patients with acute coronary syndrome and LDL-C 50–125 mg/dL were randomized to placebo/simvastatin 40 mg or ezetimibe/simvastatin 10/40 mg [44]. The trial included 4416 (24%) women. Participants were followed up for a median of 6 years for the primary composite endpoint of CV death, MI, hospitalization for unstable angina, coronary revascularization (≥30 days after randomization), or stroke. At 12 months, the addition of ezetimibe to simvastatin resulted in similar statistically significant reductions in LDL-C from baseline compared with simvastatin monotherapy in both men and women (absolute reduction, 16.7 mg/dL in men and 16.4 mg/dL in women) [45]. Women receiving ezetimibe/simvastatin had a 12% risk reduction compared to those receiving placebo/simvastatin for the primary composite endpoint (HR 0.88, 95% CI 0.79–0.99) compared with a 5% reduction for men (HR 0.95, 95% CI 0.90–1.01); however, the P-value of 0.26 for interaction was not significant. When the total number of primary events was considered, women had an 18% reduction with the addition of ezetimibe (HR 0.81, 95% CI 0.71–0.94), and men had a 6% reduction (HR 0.94, 95% CI 0.87–1.02), though the difference in effect between men and women did not achieve statistical significance ($P = 0.08$ for interaction).

Niacin

The Coronary Drug Project was the earliest trial of niacin among 3908 patients with prior history of MI randomized to niacin or placebo but did not include women [46]. The HDL-Atherosclerosis Treatment Study (HATS) was an angiographic trial of simvastatin plus niacin, antioxidants, simvastatin-niacin plus antioxidants, or placebo [47]. HATS was a small trial that included 160 patients, of whom only 21 were women. The primary endpoints were angiographic evidence of a change in coronary stenosis and occurrence of first CV event (death, MI, stroke, or revascularization), but the trial was underpowered to detect a difference in clinical outcomes. Due to the small number of women in the trial, no sex-specific outcomes were reported.

The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High

Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) study included 3414 patients (14.8% women) with established ASCVD [48]. All patients were treated with moderate intensity statin therapy (simvastatin 40–80 mg daily) plus ezetimibe 10 mg daily and were then randomized to extended-release niacin 1500–2000 mg daily or matching placebo. Following 36-month follow-up, the trial was terminated early due to lack of benefit for the primary composite endpoint of CHD death, MI, ischemic stroke, hospitalization for acute coronary syndrome, or revascularization. Due to the small number of women, CIs were wide and there was no heterogeneity in the primary outcome by sex ($P = 0.75$).

There were 4444 women (17.3%) in the Heart Protection Study 2–Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE), which evaluated the benefit of extended-release niacin plus laropiprant or placebo among 25,763 patients with history of MI, cerebrovascular disease, peripheral arterial disease, or diabetes mellitus with evidence of symptomatic coronary disease [49]. Participants received simvastatin at a dose of 40 mg daily or simvastatin plus ezetimibe to achieve a total cholesterol level <135 mg/dL. The primary outcome was first major vascular event (nonfatal MI, CHD death, stroke, or arterial revascularization). Among women randomized to niacin plus laropiprant, there was a trend toward harm compared to those randomized to placebo. There was a trend toward benefit among men randomized to niacin plus laropiprant compared to placebo, but this sex difference did not achieve statistical significance ($P = 0.07$).

Fibrates

Trials of gemfibrozil for both primary and secondary prevention of CHD events have included only men. Current guidelines recommend fenofibrate as the preferred fibrate for patients with hypertriglyceridemia, so the lack of evidence is likely not clinically relevant.

There are two large randomized controlled trials of fenofibrate that have included both men and women. Approximately 37.3% of the 9795 patients in the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study were women [50]. Participants were 50–75 years of age with type 2 diabetes not on baseline statin therapy, and 22% were secondary prevention with established ASCVD. Patients were randomized to micronized fenofibrate 200 mg daily or placebo. Over the course of the 5-year follow-up period, more patients allocated to placebo (17%) than fenofibrate (8%; $P < 0.0001$) initiated combination therapy, primarily with statins. The primary endpoint was the composite of CHD death or nonfatal MI. The outcome for prespecified subgroup analyses, including sex, was total CV events (CV death, MI, stroke, coronary or carotid revascularization). Reductions in total cholesterol and LDL-C with fenofibrate therapy were greater in women compared to men ($P < 0.001$): for total cholesterol, 14.0% (0.84 mmol/L) for women vs. 9.9% (0.49 mmol/L) for men at 4 months, and 9.5% (0.48 mmol/L) vs. 5.2% (0.25 mmol/L) at study close; and for LDL-C, 16.5% (0.53 mmol/L) for women vs. 9.4% (0.31 mmol/L) for men at 4 months and 9.8% (0.29 mmol/L) vs. 3.3% (0.10 mmol/L) at study close [51]. Men and women experienced similar reductions in triglycerides and increases in HDL-C. There was an 11% relative reduction in the primary endpoint in the total study population, but this difference was not statistically significant (HR 0.89, 95% CI 0.75–1.05, $P = 0.16$). There was no significant treatment by sex interaction for the primary endpoint (P for interaction = 0.3). Though the study was not adequately powered for sex-specific analyses and CIs were wide, women had a statistically significant reduction in the prespecified secondary endpoint of total CV events (9.5% for placebo vs. 7.7% for fenofibrate, $P = 0.04$). When adjusted for on-trial statin drop-in and other covariates, allocation to fenofibrate reduced total CV events in women by 30% (95% CI 8–46%), but there was no statistical evidence of heterogeneity of effect by sex (P for interaction = 0.17).

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial was a smaller trial than FIELD (1684 women, 3824 men) and

included primary and secondary prevention patients with type 2 diabetes, LDL-C 60–180 mg/dL, HDL-C <55 mg/dL for women and blacks, HDL-C <50 mg/dL for other patient groups, and triglycerides <750 mg/dL if patients were not on lipid-lowering therapy or <400 mg/dL if they were on therapy [52]. Participants were randomized to simvastatin plus fenofibrate or placebo and followed for 4.7 years for the primary composite outcome of MI, stroke, and CV death and 5.0 years for all-cause death. There were no statistically significant differences between treatment groups in the primary outcome ($P = 0.32$) or other prespecified secondary outcomes. There was a possible interaction by lipid subgroup, with a trend toward benefit among patients with high baseline triglycerides and low HDL-C (P for interaction = 0.057). There was a suggestion of heterogeneity of treatment effect by sex, with a benefit for men and possible harm for women (P for interaction = 0.01); however, this subgroup analysis is of limited value as the trial did not meet its primary endpoint. In addition, no sex-specific signal for harm was noted in the larger FIELD trial, adding to the uncertain significance of the treatment by sex interaction in ACCORD.

Omega-3 Fatty Acids/Fish Oil

The Japan EPA Lipid Intervention Study (JELIS) included 18,645 Japanese primary and secondary prevention patients, of whom the majority were women (68%) [53]. Patients were randomly assigned to receive either 1800 mg of eicosapentaenoic acid (EPA) daily with low-intensity statin or statin monotherapy with a 5-year follow-up. The primary endpoint was major coronary events (sudden cardiac death, fatal and nonfatal MI, and other nonfatal events including unstable angina pectoris, angioplasty, stenting, or coronary artery bypass grafting). At the mean follow-up of 4.6 years, there was a 19% relative reduction in risk in the primary endpoint (2.8% in EPA group, 3.5% in no EPA group; $P = 0.0.11$). In primary prevention patients, there was no statistically significant difference in major coronary events ($P = 0.132$). Women had a lower absolute risk of events compared to men, but there was no heterogeneity by sex for relative reduction in the primary endpoint, and the trial was not adequately powered to detect sex-specific differences.

The Vitamin D and Omega-3 Trial (VITAL) was a randomized, double-blind, placebo-controlled trial, with a two-by-two factorial design, to evaluate the effects of vitamin D3 2000 IU per day and omega-3 fatty acids (1 g per day omega-3 acid ethyl esters capsule containing 460 mg of EPA and 380 mg of docosahexaenoic acid [DHA]) for primary prevention of CVD and cancer among men 50 years of age or older and women 55 years of age or older in the USA [54]. Of the total 25,871 participants, 13,085 (50.6%) were women. The primary CV endpoint was a composite of MI, stroke, and CV death. Secondary CV endpoints were major CV events plus coronary revascularization (percutaneous coronary intervention or coronary-artery bypass grafting) and individual components of the primary endpoint. During the median follow-up of 5.3 years, there were events in 386 participants in the omega-3 group and in 419 in the placebo group (HR 0.92, 95% CI 0.80–1.06, $P = 0.24$). The HRs for prespecified secondary CV endpoints were as follows: for total MI, 0.72, 95% CI 0.59–0.90; for CV death, 0.96, 95% CI 0.76–1.21; for total stroke, 1.04, 95% CI 0.83–1.31; and for the expanded composite endpoint of CV events, 0.93, 95% CI 0.82–1.04. Additional CV endpoints included percutaneous coronary intervention (HR 0.78, 95% CI 0.63–0.95), coronary artery bypass grafting (HR 0.99, 95% CI 0.73–1.33), fatal MI (HR 0.50, 95% CI 0.26–0.97), and total CHD (HR 0.83, 95% CI 0.71–0.97). For both men and women, the CIs were wide and crossed the line of unity. The P-value for interaction by sex was not statistically significant ($P = 0.88$).

The Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial (REDUCE-IT) included 8179 individuals (2357 women, 28.8%) who were 45 years of age or older and had established CVD (70.7%) or were 50 years of age or older and had diabetes mellitus and at least one additional risk factor (29.3%) [55]. Patients had a fasting triglyceride level of 150–499 mg/dL

(1.69–5.63 mmol/L) and an LDL-C level of 41–100 mg/dL (1.06–2.59 mmol/L) and were on stable statin therapy. Participants were randomly assigned to icosapent ethyl 2 g twice daily or placebo that contained mineral oil to mimic the color and consistency of icosapent ethyl. The primary endpoint was a composite of CV death, MI, stroke, coronary revascularization, or unstable angina. The key secondary endpoint was a composite of CV death, MI, or stroke. The median duration of follow-up was 4.9 years. The median change in triglyceride level from baseline to 1 year was a decrease of 18.3% (−39.0 mg/dL) in the icosapent ethyl group compared to an increase of 2.2% (4.5 mg/dL) in the placebo group. The median change in LDL-C level from baseline was an increase of 3.1% (2.0 mg/dL) in the icosapent ethyl group compared to an increase of 10.2% (7.0 mg/dL) in the placebo group—a 6.6% lower increase with icosapent ethyl than with placebo ($P < 0.001$). The primary composite endpoint event occurred in 17.2% of the patients in the icosapent ethyl group, as compared with 22.0% of the patients in the placebo group (HR 0.75, 95% CI 0.68–0.83, $P < 0.001$), an absolute between-group difference of 4.8 percentage points (95% CI 3.1–6.5). A key secondary efficacy endpoint of CV death, MI, or stroke occurred in 11.2% of the patients in the icosapent ethyl group, as compared with 14.8% of the patients in the placebo group (HR 0.74, 95% CI 0.65–0.83, $P < 0.001$), corresponding to an absolute between-group difference of 3.6 percentage points (95% CI 2.1–5.0). The CIs for the primary endpoint were wide for women compared to men with fewer events and lower absolute risk of events. The P-value for interaction by sex was not statistically significant ($P = 0.33$). The rates of adverse events by sex were not reported.

Proprotein Convertase Subtilisin/Kexin-Type 9 (PCSK9) Inhibitors

The monoclonal antibodies that inhibit PCSK9 have emerged as a new class of drugs that effectively lower LDL-C levels; two agents are currently approved for use in the USA, alirocumab and evolocumab [56]. Both PCSK9 inhibitors have been demonstrated to improve CV outcomes in high-risk patients treated with baseline statin therapy with or without ezetimibe.

The Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) trial was a randomized, double-blind, placebo-controlled, multinational clinical trial in patients 40–85 years of age with clinically evident ASCVD, defined as a history of MI, nonhemorrhagic stroke, or symptomatic peripheral artery disease, as well as additional characteristics that placed them at higher CV risk [57]. Patients were randomly assigned to receive subcutaneous injections of evolocumab (either 140 mg every 2 weeks or 420 mg every month, according to patient preference) or matching placebo. The primary efficacy endpoint was major CV events (composite of CV death, MI, stroke, hospitalization for unstable angina, or coronary revascularization). The key secondary efficacy endpoint was the composite of CV death, MI, or stroke. A total of 27,564 patients (24.5% women) underwent randomization to either the evolocumab group or the placebo group. After a median follow-up of 26 months, evolocumab significantly reduced the risk of the primary composite endpoint (HR 0.85, 95% CI 0.79–0.92, $P < 0.001$). Likewise, evolocumab significantly reduced the risk of the key secondary composite endpoint of CV death, MI, or stroke (HR 0.80, 95% CI 0.73–0.88, $P < 0.001$). The magnitude of the risk reduction with regard to the primary and secondary endpoints tended to increase over time. There was a small number of women ($n = 6769$) compared to men ($n = 20{,}795$), but the CIs were relatively narrow for both and the P-value for interaction by sex was not statistically significant ($P = 0.48$). Adverse effects by sex were not reported.

The ODYSSEY OUTCOMES trial was a multicenter, randomized, double-blind, placebo-controlled trial that enrolled 18,924 participants (25.2% women) 40 years of age or older who had been hospitalized with an acute coronary syndrome (MI or unstable angina) 1–12 months before randomization and had an LDL-C level of at least 70 mg/dL, non-HDL-C level of at least

100 mg/dL, or Apo B of at least 80 mg/dL [58]. Participants on high-intensity or maximally tolerated statin therapy, with or without ezetimibe, were randomized to receive alirocumab subcutaneously at a dose of 75 mg or matching placebo. The dose of alirocumab was adjusted under blinded conditions to target LDL-C level of 25–50 mg/dL. The primary endpoint was a composite of CHD death, MI, ischemic stroke, or unstable angina requiring hospitalization. After a median follow-up of 2.8 years, the composite primary endpoint event occurred in 9.5% of patients in the alirocumab group and 11.1% of patients in the placebo group (HR 0.85, 95% CI 0.78–0.93, $P < 0.001$). The effect of alirocumab on the risk of the composite primary endpoint did not differ significantly according to any of the prespecified subgroup variables. The CIs were wide for women (0.77, 1.08) compared to men (0.74, 0.92) and the *P* for interaction by sex was not statistically significant ($P = 0.35$). Adverse effects of alirocumab were not reported by sex.

Recommendations for Use of Non-Statin Therapies

The current recommendations of the 2018 AHA/ACC Cholesterol Guideline for the use of non-statin therapies for management of dyslipidemia for women are the same as those for men [59].

Sex Differences in Adverse Effects of Lipid-Lowering Therapies

Similar to the available sex-specific randomized, controlled trial evidence for CV outcomes benefits of lipid-lowering therapy, data are limited for evaluation of sex differences in adverse events during treatment with the broad range of drug classes for management of dyslipidemia. Certainly, the strongest evidence base is for statin therapy in women, due to the large number of statin trials. CTT meta-analyses have shown no increase in non-CV mortality and no increase in rates of malignancy with statin therapy in women [60–62].

The Understanding Statin Use in America and Gaps in Patient Education (USAGE) survey was a self-administered, internet-based questionnaire, designed to evaluate reported side effects associated with statin use, clinician and patient interactions, as well as general attitudes and preferences regarding statin use; it also assessed whether women differ from men with regard to these characteristics [63]. A total of 10,138 adults participated in the USAGE survey between September 21, 2011, and October 17, 2011. Of the total respondents, 6146 (61%) were women. Women were more likely to report symptoms of depression, gastroesophageal reflux, and arthritis. Women were more likely to be dissatisfied with their statin medication than men (10% of women were dissatisfied compared with 6% of men; $P < 0.0001$). Among current statin users, 3% of women were dissatisfied with their statin medication compared with 2% for men ($P < 0.006$) and among former statin users, 57% of women were dissatisfied with their statin medication compared with 44% for men ($P < 0.01$). Among former statin users, women were more likely to have tried three or more statins (28% vs. 22%; $P < 0.05$) compared with men. Among current statin users, more women compared with men reported not filling a prescription or missing a dose of statin (35% vs. 31%; $P = 0.02$), and women were less likely to report taking their statin medication as prescribed compared with men (67% vs. 71%; $P = 0.007$). Although nonadherence rates were low, women were more likely to report being nonadherent with their statin compared with men (5% vs. 4%; $P < 0.05$).

In the JUPITER study of primary prevention patients with elevated hs-CRP, women were significantly older, were more likely to have hypertension and metabolic syndrome, had higher mean BMI and lower mean estimated glomerular filtration rates compared to men (all differences $P < 0.0001$) [64]. Although both women and men treated with rosuvastatin had higher glycated hemoglobin at 12 months, a higher incidence of physician-reported diabetes was observed in women treated with rosuvastatin vs. placebo (1.53 vs. 1.03 per 100 person-years, respectively; HR 1.49, 95% CI 1.11–2.01, $P = 0.008$) compared

with men (1.36 vs. 1.20 per 100 person-years, respectively; HR 1.14, 95% CI 0.91–1.43, $P = 0.24$). The test for heterogeneity of diabetes mellitus by sex was not significant (P for heterogeneity = 0.16). There were no sex-specific differences in any serious adverse events, including rhabdomyolysis, death from cancer, transaminase elevation >3 times upper limit of normal, or hemorrhagic stroke [65]. The rates of muscle-related adverse effects were similar among women and men in both treatment groups.

The Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trial included 21.9% ($n = 5911$) women and demonstrated no statistically significant sex differences in premature discontinuation of statin therapy, increases in hepatic transaminases, elevated creatine kinase levels, or myalgias/myositis [66].

The association of on-study LDL-C and CV events by sex and the impact of sex on adverse events was evaluated in six studies of atorvastatin [Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) trial (atorvastatin 80 mg vs. simvastatin 20–40 mg), the Treating to New Targets (TNT) trial (atorvastatin 80 vs. 10 mg), the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial (atorvastatin 80 mg vs. placebo), the Collaborative Atorvastatin Diabetes Study (CARDS), the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT), and the Atorvastatin Study for Prevention of Coronary Heart Disease Endpoints in Non-Insulin-Dependent Diabetes Mellitus (ASPEN)] [67]. In four of the six trials, there were higher discontinuation rates due to adverse events among women compared to men. Only the IDEAL trial reported a statistically significant interaction by sex (discontinuation rates for women: study drug 15.1% and comparator drug 4.6%; discontinuation rates for men: study drug 8.3% and comparator drug 4.1%; P for interaction = 0.010). Overall, myalgias were more frequently reported among women than men in both the atorvastatin and placebo groups (women, atorvastatin 11.3%, placebo 6.8%; men, atorvastatin 9.4%, placebo 4.6%).

There were no sex-specific differences in safety events with the addition of ezetimibe to simvastatin therapy in IMPROVE-IT [27]. In the FIELD trial, there were no sex-specific differences in adverse events of treatment with fenofibrate [49]. Sex-specific differences in adverse events have not been reported for the PCSK9 inhibitors or icosapent ethyl.

Clinically significant adverse drug effects and self-reported drug allergies have been reported to occur more frequently among women than men for a number of drug classes [68–71]. Factors that may play a role in the increased frequencies include sex differences in body fat, muscle mass, quantities of cytochrome P450 metabolic enzymes, coenzyme Q10 levels, pain perception and reporting of adverse reactions, and underlying genetic factors in drug metabolism [67, 72]. Women with clinically significant ASCVD or at high risk tend to be older with increased prevalence of comorbidities, and polypharmacy is very common in elderly women and men. With the ever-increasing options for pharmacotherapy for management of dyslipidemia and ASCVD risk reduction, clinicians should be aware of potential sex differences in adverse events [73]. Other issues in pharmacology of statin therapies and management of statin intolerance are considered in more depth in previous chapters.

Considerations in Lipid Management Across the Lifespan: From Pregnancy, Menopause, and Beyond

Conception and Pregnancy

Population screening for dyslipidemia for all women before or during reproductive ages is the best way to detect and reduce dyslipidemia and its deleterious consequences during pregnancy [74]. Even when a pregnancy is planned, patients or their physicians may not have recognized and/or addressed CVD risk factors or discussed their importance in complications of pregnancy and consequent residual vascular disease. Diagnosing and controlling lipids can reduce life-threatening

complications during pregnancy for the mother and affect the offspring's future risk of CVD.

The most common high-risk obstetrical condition is obesity, which is commonly associated with dyslipidemia, impaired glucose metabolism, and/or hypertension [75]. Preeclampsia, gestational diabetes, and/or hypertension, which are recognized risk factors for subsequent CVD, can ensue [76–79]. Even modest increases in maternal BMI are associated with an increased risk of fetal death, stillbirth, and neonatal, perinatal, and infant death. Weight loss prior to pregnancy can reduce obstetrical complications [80]. Optimal weight before pregnancy and maintained during pregnancy carries lower risk for fetal death, stillbirth, and/or infant death [69].

Pregnancy

Management of dyslipidemia depends upon a clear understanding of normal values during each trimester of pregnancy. Lipid levels change as pregnancy progresses [81, 82]. Levels fall slightly during the first trimester, then steadily rise to peak near term. In an uncomplicated pregnancy, total cholesterol and triglyceride levels do not exceed 250 mg/dL at any time.

Hypertriglyceridemia in Pregnancy

Among women with genetic dyslipidemias, hypertension, diabetes, or preeclampsia, triglyceride levels may rise substantially higher, and obstetrical complications are more likely. It is recommended to monitor for pregnancy-related hypertriglyceridemia in those women with prepregnancy fasting triglyceride level greater than 355 mg/dL (4 mmol/L) and to institute therapy when levels reach 880 mg/dL (10 mmol/L) [83].

Maternal metabolism undergoes several transformations due to hormonal changes and nutritional demands to satisfy maternal and fetal nutritional needs for fetal development. The initial phase of fatty acids accumulation results in triglyceride deposition in the maternal adipose tissue. In the late second and third trimesters of pregnancy, there is accelerated adipose tissue catabolism and increased availability of fatty acids and glycerol in the circulation, which lead to increases in production of triglycerides and VLDL [84]. Lipoprotein lipase (LPL) and hepatic lipase levels are reduced and LDL-C levels rise [85, 86]. Estrogen also increases hepatic VLDL synthesis and plays an important role in triglyceride elevation during pregnancy. These changes result in an atherogenic phenotype with increased circulating small dense LDL particles, as triglyceride level increases, and reduced HDL-C levels [79].

Patients with underlying genetic disorders may have severe hypertriglyceridemia during pregnancy. Triglyceride levels can be 2–3-fold higher than usual in pregnancy, and, rarely, there may be extremely high triglyceride and chylomicron levels. Genetic disorders ranging from homozygous LPL deficiency (familial chylomicronemia syndrome) to heterozygous missense LPL deficiency or polygenic causes can result in severe hypertriglyceridemia >1000 mg/dL (11.3 mmol/L) and may be associated with pancreatitis. Fortunately, acute pancreatitis during pregnancy is rare (1/1060–1/4449) [87, 88]. Common causes of pancreatitis in pregnancy are gallstones (66%), alcohol abuse (12%), idiopathic (17%), hyperlipidemia (4%), hyperparathyroidism, trauma, medication, and fatty liver of pregnancy.

Lifestyle interventions before pregnancy may help prevent complications of severe dyslipidemia and reduce adverse birth outcomes [89]. Ultralow-fat diets, omega-3 fatty acids, and insulin-sensitizing therapies may help, depending on residual LPL activity. The goal is to maintain plasma triglyceride concentration <1000 mg/dL [90]. Restriction of dietary fat to ≤20 g/day is usually sufficient to keep those with familial LPL deficiency free of symptoms between pregnancies [90].

Agents known to increase endogenous triglycerides (alcohol, oral estrogens, diuretics, isotretinoin, glucocorticoids, selective serotonin reuptake inhibitors, beta-adrenergic blocking agents, and fish oil supplements or medications) are all contraindicated to avoid higher chylomicron levels. For pregnant women with LPL deficiency, extreme dietary fat restriction to <2 g/day during the second and third trimesters of preg-

nancy with close monitoring of plasma triglyceride concentration is recommended. The lipid-lowering drugs used to treat other disorders of lipid metabolism are not effective in individuals with familial LPL deficiency.

Pregnancy and Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is underdiagnosed and the prevalence among reproductive women is unknown [91]. Better treatment from an early age will make pregnancy in FH less hazardous. Genetic testing of the partner is desirable, especially in cultures where consanguineous marriages are more common. Though genetic testing in pregnancy is available, it is not routinely used.

During pregnancy, women with FH have relative changes in plasma lipid levels that are similar to those in healthy women; however, the absolute increases are higher [92–94]. Women with FH have an increased risk of premature ASCVD, and the changes in lipoproteins during pregnancy may exacerbate this risk. Hyperlipidemia during pregnancy may induce atherosclerosis in the uteroplacental spiral arteries in combination with hypercoagulation and may result in thrombosis and placental infarctions, leading to placental insufficiency and fetal compromise [86]. Data from several studies do not support an association between maternal lipid levels and adverse pregnancy outcomes in FH, including preterm delivery, low birth weight, or congenital malformations compared to women without FH [95]. Evaluation of risk for the presence of CAD as well as aortic valve stenosis and supravalvular aortic stenosis should be considered prior to pregnancy [96].

Women with FH should receive prepregnancy counseling including information on ASCVD risk reduction; therapy during preconception, pregnancy, childbirth, and lactation; and contraception advice. With early identification and proper planning, most women with FH can have healthy pregnancies and healthy children. The decision to have children and the duration of breastfeeding are personal choices, and recommendations should be individualized.

Role of Medications in Pregnancy

All women of reproductive age should be counseled regarding the need for contraception when statins are prescribed. The preferred contraceptive methods are low-dose estrogen oral agents, intrauterine devices, and barrier techniques. In women older than 35 years, the latter two methods are preferable [97]. When a woman desires to attempt conception, current guidelines recommend discontinuation of statins 1–3 months prior to cessation of contraception [75].

Statins are labeled contraindicated in pregnancy based on a series of small animal studies in the late 1980s and the early 1990s that demonstrated skeletal malformations in rats and low weights in rats and rabbits. The statin doses used in the animal studies were much larger than doses used in pregnant women, and, since then, multiple case series, cohort studies, and one randomized controlled trial have examined the safety of statin use in pregnancy. Though there is limited information on adverse outcomes with statin therapy, women with FH should initiate statin therapy early after the diagnosis of FH and withhold statin therapy during attempts at conception and during pregnancy and lactation.

Bile acid sequestrants are safe, provided that the woman takes folate supplements, but effectiveness is uncertain in FH. Challenges are associated constipation and palatability for pregnant women suffering from nausea, particularly in the first trimester. The US Food and Drug Administration has mandated pregnancy registries for women who use the PCSK9 inhibitors during pregnancy. Ezetimibe, niacin, and fibrates have likewise been associated with teratogenicity and are not recommended during pregnancy. Lomitapide is not recommended at this time because of risk of potential embryo-fetal toxicity.

Women who accidentally become pregnant while on statins should stop taking them and undergo fetal assessment with a maternal-fetal specialist, but they can be reassured that the likelihood is low for fetal complications. The patient and her physician should discuss the risk of delaying cholesterol therapy while breastfeeding against the length of time she plans to breastfeed.

Role of LDL Apheresis

Lipoprotein apheresis is approved for use during pregnancy and considered safe for very high-risk women with known significant ASCVD or homozygous FH [98]. Women should be offered twice-weekly LDL apheresis, if practical, when pretreatment LDL-C is particularly high, if LDL-C lowering is inadequate, or there is evidence of progression of CVD.

Polycystic Ovarian Syndrome

Polycystic ovarian syndrome (PCOS) is the most common endocrine disorder of reproductive aged women and most often presents in childhood, adolescence, or earlier reproductive age. Current Rotterdam criteria for PCOS diagnosis include: (1) androgen excess (clinically or measured in blood), (2) oligo-ovulation/amenorrhea, and/or (3) polycystic ovaries, visualized most commonly by vaginal ultrasound. Women with PCOS have dyslipidemia, insulin resistance, hyperandrogenism, and metabolic and reproductive dysfunction. Higher rates of central adiposity strongly influence the phenotypic severity of PCOS [99]. Patients have higher triglyceride and non-HDL-C levels, lower HDL-C levels, and higher ratios of Apo CIII to Apo CII, even if not obese. Obesity aggravates the atherogenic lipoprotein phenotype (elevated levels of triglycerides and small, dense LDL particles and low HDL-C) that often accompanies PCOS.

Dyslipidemia is present very early and is clearly worse in obese PCOS adolescents compared to obese adolescents without PCOS [100]. All patients with PCOS should undergo initial lipid and diabetes screening and more frequent follow-up even if baseline levels are normal. Cascade screening is especially important. Mothers, sisters, brothers, and other family members often show prevalent diabetes, hypertension, dyslipidemia, and premature CVD [101].

Even nonobese women with PCOS have higher LDL-C and triglycerides during each trimester of pregnancy. Women with PCOS are prone to pregnancy complications, and gestational diabetes is the most common [102]. There are greater odds of preeclampsia, gestational hypertension, and having a child small for gestational age, large for gestational age, or with macrosomia. Dyslipidemia may be less responsive to lifestyle modifications and/or lipid-lowering medication if diabetes is not well-controlled. Women with PCOS are at increased risk of premature CVD, often as early as in their mid-30s [103].

Management of PCOS should focus on diet, exercise, and lipid-lowering pharmacotherapy as indicated.

Contraceptive Therapy and Considerations in Dyslipidemia

Contraceptive therapy should be individualized based upon shared decision-making. Combined oral contraceptives (COC) may have many positive attributes that may appeal to women with dyslipidemia. Depending upon the content in the pill, they are very effective for most patients in reducing acne and improving hirsutism. Generally, they are convenient and have continued efficacy for reduction of androgen excess symptoms for months after discontinuation. COC reduce the risk for endometrial and ovarian cancer and may reduce anemia by improving abnormal uterine bleeding. Of important concern, COC preparations increase the risk of venous thromboembolism [104].

The estrogen component of COC may increase triglycerides and HDL-C and lower LDL-C, and the effect is greater at higher levels of estrogen. Transdermal or vaginal preparations are less likely to worsen dyslipidemia but are still associated with increased thrombotic risk [105–107]. Androgenic progestins (norgestrel and levonorgestrel) can raise LDL-C and lower HDL-C. Other progestins are lipid neutral. Desogestrel raises HDL-C and lowers LDL-C.

Age, comorbidities, type, and severity of the dyslipidemia coupled with an understanding of compliance all enter into making an optimum contraceptive choice. In some cases, permanent sterilization of the female or male partner may be a preferred choice. The reader is encouraged to consult the Centers for Disease Control and Prevention website as an excellent reference to assist in complex decision-making: https://www.cdc.gov/reproductivehealth/unintendedpreg-

nancy/pdf/legal_summary-chart_english_final_tag508.pdf. This convenient resource is also available as a downloadable application and provides the most current information that may be useful for individualizing choice based on a well-recognized assessment of the quality of the evidence available.

Postmenopausal Hormone Replacement Therapy and Dyslipidemia

There is currently no high-quality evidence that hormone therapy protects against CVD [108]. The primary indication for use is for menopausal symptoms. Although hormone therapy lowers LDL-C and lipoprotein (a) and raises HDL-C, it has adverse effects on triglyceride levels, lipoprotein composition, and inflammatory and hemostatic markers. Baseline metabolic syndrome and high LDL-C increase CHD risk with hormone therapy use. If triglycerides are elevated as part of the dyslipidemia, transdermal or vaginal delivery may avoid aggravation of the dyslipidemia.

Summary

CVD remains the #1 killer of women, and clinicians must focus on early preventive strategies in women of all ages. Women and men at similar risk of major vascular events benefit from statin therapy even in primary prevention and among individuals at low baseline risk. The evidence for prevention of ASCVD in women with the use of non-statin drugs is limited; however, current guidelines for the use of non-statin therapies for management of dyslipidemia for women are the same as those for men. Clinically significant adverse drug effects and self-reported drug allergies have been reported to occur more frequently among women than men for a number of drug classes, and clinicians should be aware of potential sex differences in adverse events. There are important considerations in management of dyslipidemia in women across the lifespan, particularly during conception, pregnancy, and lactation.

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Management of Dyslipidaemia in the Elderly

25

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Abbreviations

ASCVD	Atherosclerotic cardiovascular disease
CVD	Cardiovascular disease
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
Lp(a)	Apolipoprotein-a
MACE	Major adverse cardiovascular events
MI	Myocardial infarction
PCSK9	Proprotein convertase subtilisin/kexin type 9
TC	Total cholesterol
TG	Triglycerides

Who Are the Elderly?

> To me old age is always fifteen years older than I am. [1]

Previous cardiovascular and lipid-lowering studies have traditionally defined the "elderly" as those aged 65 years or more. Our concept of "Who are the elderly?", however, may need to be revised as the proportion of older individuals and life expectancy increase.

Life expectancy has continued to increase worldwide and is now over 80 years in many Western countries [2, 3]. For example, in Australia, life expectancy for those reaching 65 years is a further 19 years for men and 22 years for women. For men and women aged 85 years, further life expectancy is 5.9 and 7.1 years, respectively [2, 3]. In 2017, 57% of the population were aged 65–74 years, 30% were aged 75–84 and 13% were aged 85+. Women comprised 51% of people aged 65–74 years and 54% of those aged 75–84 years. This rose to 63% for people aged 85 and over. The proportion of people aged 65+ in Western countries is similar – 15% in the USA, 18% in the UK and 15% in Australia. This proportion is expected to increase in 2020 by around 1.2% in Australia, 2% in the USA and 0.9% in the UK [4, 5].

In view of these data, it may be appropriate to classify the elderly into three age groups, 65–74 years, 75–84 years and 85+ years, and use an appropriate terminology for each age group, e.g. older (65–74), elderly (75–84) and very elderly (85+).

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_25

Dyslipidaemia

A patient with dyslipidaemia may be broadly defined by having one or more of the following: abnormal TC, abnormal LDL-C, abnormal HDL-C, abnormal TG, raised Lp(a) and/or taking lipid-lowering medication.

Dyslipidaemia also refers to patients with the "atherogenic triad" or "atherogenic lipoprotein profile": high TG, low HDL-C and increased small, dense lipoproteins as occurs in states of insulin resistance (e.g. diabetes and the metabolic syndrome). Isolated low HDL-C syndromes may also be classified as dyslipidaemias.

There are few trials in the elderly in which lipid-lowering therapy (LLT) focusses on either high TG or low HDL-C levels. This review will therefore be principally concerned with statin therapy for ASCVD prevention through reducing levels of LDL-C.

Why Is Treatment of Dyslipidaemia in the Elderly Important?

With increasing age, the overall risk of ASCVD increases because of a concomitant increase in the prevalence of risk factors (lack of physical exercise, obesity, diabetes, hypercholesterolemia and hypertension), and age itself is a potent risk factor for ASCVD events. Using ASCVD risk factor tools to calculate risk, the majority of patients aged >65 years are at either intermediate (10–15%) or high (>15%) ASCVD risk over 5 years.

Most ASCVD events occur in the elderly, and measures to prevent ASCVD should be prioritised. Figure 25.1 shows ASCVD prevalence according to age in Australia for 2014–2015. Those aged 75 years or more have severalfold higher rates of ASCVD than those aged <50 years, the relationship between age and ASCVD risk being semi-exponential. The prevalence of ASCVD is higher in men than women at all ages (Fig. 25.1).

Gradients of risk in the elderly depend on the number of risk factors present, as for younger age groups. Data from the Dubbo study for men (A) and women (B) aged 75 years are shown in Fig. 25.2. The reference group comprised not taking antihypertensive medication, systolic pressure 140 mmHg, non-smoking, high-density lipoprotein level 1.10 mmol/L no diabetes.

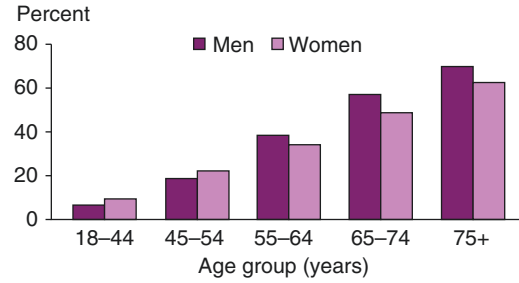

Fig. 25.1 ASCVD prevalence in Australian adults aged 18+ years, according to age and sex, 2014–2015. (Adapted from Refs. [5, 6])

The majority of elderly patients may qualify for consideration of LLT, particularly with statins, because of increasing ASCVD risk with increasing age. Dyslipidaemia is increasingly prevalent with increasing age and may also require LLT independently. Table 25.1 shows the proportion of Australians with dyslipidaemia by age in 2011–2012 [8]. This increased progressively from 44.3% (aged 18–34) to 81% (aged 65–74), with 77.7% of those aged 75+. The proportions who were not receiving LLT and had abnormal lipid levels varied from 43.3% (aged 18–34) to 61.1% (aged 45–54) [8].

Table 25.2 shows the prevalence of abnormal lipid levels by lipid type and sex, 2011–2012**.** The highest prevalence was in levels of TC and LDL-C (31.6–33.2%) and the lowest 9% for high TG in women [8].

Problems Associated with Treatment of Dyslipidaemia in the Elderly

Until recently, relatively few data supported treatment of dyslipidaemia in the elderly, with the possible exception of secondary prevention. For many physicians, the perception of benefit in the elderly has been uncertain, as too few elderly patients were included in individual statin trials.

As a result, statin therapy is likely to have been underused in the elderly.

Other considerations may also reduce initiation or continuation of statin therapy in the elderly, including patient preference, concern about adverse effects and drug interactions (especially as the number of drugs taken increases with ageing) and co-morbidities affecting life expectancy. In spite of this, statins are now taken by 44% of Australians aged 65+ [9].

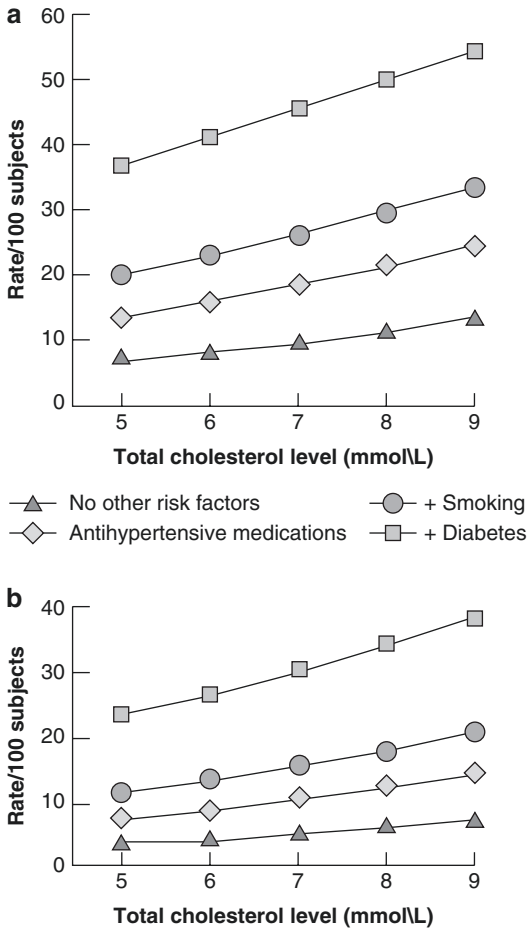

Fig. 25.2 Calculated 5-year cardiovascular disease risk for a 75-year-old man (**a**) and woman (**b**) according to cholesterol level and the presence of other risk factors. (Adapted from Simons et al. [7])

Current Focus of Lipid-Lowering Therapy

The focus of current LLT is to reduce levels of LDL-C. This is based on the well-documented role of LDL-C in initiating and promoting atherosclerosis and the benefits of LDL-C reduction in reducing cardiovascular disease (ASCVD) events, as shown in numerous clinical trials with statin therapy across many risk groups, ages and both genders.

Trends in Statin Use

Between 2002 and 2013, statin use in the US general population aged 40+ years increased by 79.8% from 17.9% to 27.8%. For secondary prevention, statin use increased from 49.8% to 58.1%, with less than one third high-intensity statin. Across all subgroups, statin use was 19% lower in women. Generic statin use increased from 8.4% to 81.8%, and the mean annual cost for patients decreased from US$348 to US$94. Total costs adjusted for GDP decreased from US$17.2 billion to US$16.9 billion [10].

These findings illustrate the recent use of statins in many Western countries: higher uptake and reduced costs due to the use of generic statins, with total expenditure being the largest for any class of drugs.

Table 25.1 Prevalence of medicated and unmedicated dyslipidaemia by age, 2011–2012 [8]

Age group	% with dyslipidaemia	No medication with abnormal lipid levels	Medication with abnormal lipid levels	Medication and normal lipid levels
18–34	44.3	43.3	1.0	0.0
35–44	59.2	54.5	3.0	1.7
45–54	70.9	61.1	6.2	3.7
55–64	78.9	55.1	13.4	10.4
65–74	81.0	42.9	19.0	19.0
75+	77.7	30.3	17.7	29.8

Table 25.2 Prevalence of abnormal lipid levels by lipid type and sex, 2011–2012 [8]

Sex	Total cholesterol	HDL-C	LDL-C	TG
Men, %	32.4	18.9	35.0	19.0
Women, %	33.2	27.2	31.6	9.0

A recent study of National Pharmacy claims data in Australia examined statin use in those aged 65+ years over a 10-year period from 2007 to 2016 [9]. The prevalence of statin use increased consistently each year from 34.2% in 2007 to 44.1% in 2016. The 1-year incidence of statin use declined from 68.5/1000 in 2007 to 59.0/1000 in 2016. Women were 18% less likely to initiate statins at all time periods. The highest incidence in statin initiations occurred in those aged 65–74 years, 15% and 45% higher than in those aged 75–84 and 85+ years, respectively. Atorvastatin was the most commonly prescribed statin, and use of high-intensity therapy increased consistently from 23.6% in 2007 to 30.5% in 2016 [9].

Of 852 patients aged ≥80 years, 359 (42%) were taking a statin on admission to a general medical unit, of whom 24% were treated for primary prevention and 63% for secondary prevention. The most commonly used statins were atorvastatin (53.5%; 40 mg/day), simvastatin (22.8%; 40 mg/day median dose) and rosuvastatin (19.2%; 10 mg/day median dose). Statin withdrawal occurred in 57 (15.9%), the majority [25] in the setting of palliation and 14 with musculoskeletal side effects. The authors cautioned against the use of high doses of high-potency statins in the elderly. Co-prescription of interacting drugs occurred frequently, 34.8% of patients having moderate potential for increased risk of statin toxicity, most commonly with proton-pump inhibitors, and 3.1% of patients having drugs which potentially reduced the efficacy of statin therapy [9].

These data reflect changing prescribing habits in the elderly, particularly greater use of high-intensity statins and increased prevalence of statin use. These trends were similar to those described in the USA and the UK, where a reduction in incidence of statin use was also observed and attributed to changes in clinical guidelines, increasing reports of side effects, particularly in the media, and increasing use of non-statin therapy [10].

Adherence and Persistence of Statin Therapy in the Elderly

While the prevalence of statin use in the elderly has increased, this does not necessarily reflect increased uptake, as compliance and discontinuation rates were not assessed [9]. A recent meta-analysis of adherence and persistence of statin therapy in those aged 65 years or more showed 1-year adherence among more than three million older patients was 59.7% (primary prevention 47.9% and secondary prevention 62.3%) [11].

In contrast, self-reported adherence in 190 subjects was 85.5%. Adherence at 2, 3, 4, 5 and ≥10 years was 59.6%, 55.3%, 35.9%, 35.7% and 28.4%, respectively. An arbitrary 80% adherence rate is regarded as the minimum level to achieve a satisfactory clinical effect of statin therapy. Persistence data were obtained from studies reporting statin discontinuation rates. At 1 year, persistence was 76.7% (primary prevention 76.0% and secondary prevention 82.6%). Among new statin users, 23.9% discontinued treatment after 1 year. Median proportion of persistent users at 2, 3 and 4 years was 68.1%, 63.3% and 61.2%, respectively [11].

As in younger age groups, theoretical benefits of statin therapy can only occur in those who are compliant. Worse clinical outcomes have been consistently associated with statin discontinuation. For example, post-MI rates of death were 3× higher in those discontinuing statins and fatal stroke 7× higher in patients who were non-adherent to statins and antihypertensive therapy. Post-MI low antihypertensive adherence was associated with 62% higher statin non-adherence [11].

Discontinuation was reported as due to statin side effects in a minority (2–4%) of patients, so that other factors may be responsible such as physician-initiated discontinuation, adverse media reports and lack of patient conviction that statins are necessary.

Strategies to improve adherence and reduce discontinuation would seem to be equally, if not more, important to strategies for initiating statins in the elderly. Several potential strategies are available to improve compliance. These include simplified drug regimens with less polypharmacy, use of combination single-tablet ezetimibe-statin co-therapy, patient education and counselling, reminders and reinforcement, providing extended-care ancillary health workers, reducing gap payments and emphasising the potential benefits of statin therapy with regard to ASCVD events (especially stroke) and longer-term improved lifestyle resulting from lower rates of ASCVD. Careful assessment of side effects is also necessary as many patients can be reassured that their symptoms are not related to statin therapy.

Outcomes of LLT: Large-Scale Meta-analyses of Statin Trials

The initial large-scale meta-analysis of statin trials was published in 2005 by the Cholesterol Collaboration Trialists' Collaborators (CTTC) [12]. This showed statin therapy (statin vs. placebo and higher vs. lower statin doses) to have the following outcomes for both primary and secondary prevention:

- Reduction in ASCVD events in proportion to LDL-C lowering and duration of therapy so that 1 mmol/L reduction in LDL-C is associated with about 22% relative risk reduction (RRR) in ASCVD over a 5-year period.
- Similar RRR for both genders, all age groups studied, and different levels of baseline risk.
- Absolute risk reduction (ARR), however, is greater with higher baseline risk. Elderly patients, with higher baseline risk compared with younger patients, would be expected to have greater ARR.
- Numbers needed to treat to prevent one ASCVD event depend on ARR and therefore baseline risk and would be expected to be lower in elderly compared with younger patients.
- No increase in serious adverse events, particularly cancer and overall mortality.
- Most of the benefit from statin therapy is due to reduction in acute coronary syndromes and cardiac deaths, with lesser benefits in reducing stroke and heart failure.
- Improved ASCVD incidence is expected to result in improved quality of life.

A subsequent CTTC meta-analysis in 2012 supported the above conclusions in patients with low levels of LDL-C [13].

A 2013 meta-analysis of 18 statin primary prevention RCTs, with mean patient age 57 years of whom 60% were men, showed the following NNTs to prevent 1 event over 5 years: 49 (total ASCVD events), 88 (total CHD events), 96 (revascularisation), 138 (all-cause mortality) and 155 (total stroke events). All were statistically significant [14].

The Heart Outcomes Prevention Evaluation-3 (HOPE-3) and Justification for the Use of Statins in Prevention (JUPITER) meta-analyses of primary prevention in the elderly were published in 2017 [15]. These trials involved rosuvastatin therapy in patients with increased ASCVD risk. In JUPITER, rosuvastatin 20 mg was compared with placebo in 17,802 patients with LDL-C <130 mg/dL and high-sensitivity C-reactive protein ≥2 mg/L. The HOPE-3 trial treated 12,705 patients at intermediate ASCVD risk with rosuvastatin 10 mg vs. placebo.

Reduction in ASCVD events with rosuvastatin therapy was consistent in all age groups including those aged 70+ years (see Fig. 25.3).

Pooled estimate event reductions of 25%, 49% and 26% were observed for the age groups <65 years, 65–<70 years and 70+ years, respectively. There was no statistically significant heterogeneity by age. Some benefit was considered

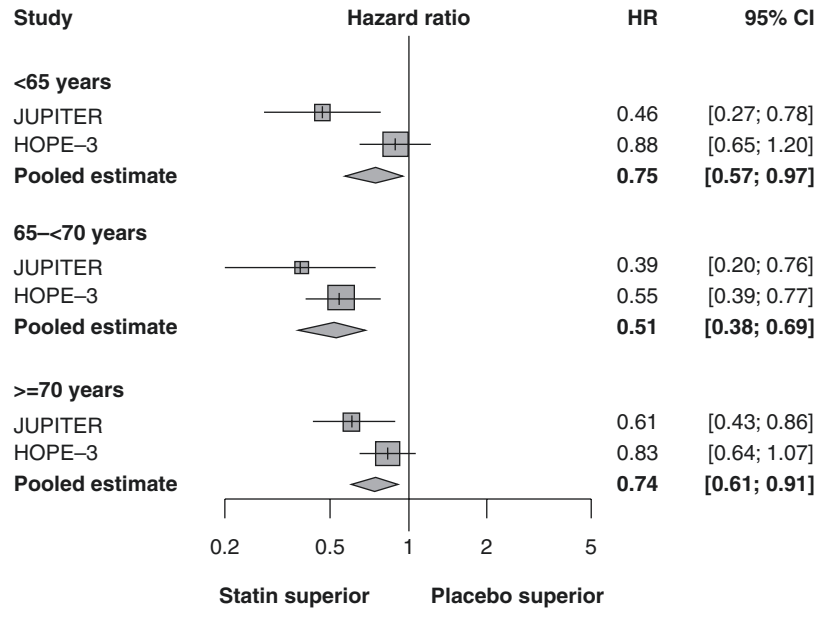

Fig. 25.3 Meta-analyses within age subgroups on the composite end point of nonfatal MI, nonfatal stroke or ASCVD death in JUPITER and HOPE-3 trials. (Adapted from Ridker et al. [15])

likely for those aged >80 or more years. Uncertainty remained with regard to haemorrhagic stroke, cognitive function, drug interactions, adherence, quality of life and cost-effectiveness [15].

A meta-analysis of data for patients with low LDL-C levels published in 2018 also supported the earlier CTTC conclusions [16]. In addition, the latest CTTC meta-analyses of 28 previous statin RCTs focussed on the elderly aged >75 years, who comprised 8% (14,483) of the total 186,854 patients. It was estimated that about 1/3 of patients in this age group are being treated with statins in the UK [17]. Individual data from 22 trials and summary data from 1 trial of statin therapy vs. control were analysed, as were individual data from 5 trials of more vs. less intensive statin therapy. Median follow-up in all trials was 4.9 years. Subjects were divided into the age groups 55 years or younger, 56–60 years, 61–65 years, 66–70 years, 71–75 years and >75 years. Effects of statins on major adverse cardiovascular events (MACE), cause-specific mortality and cancer incidence were estimated and compared in the different age groups.

The conclusions reinforced those of the earlier meta-analysis and showed benefit with statin therapy in the elderly for CHD, MI, revascularisation and stroke. Less clear-cut benefit was observed for overall mortality.

For MACE, similar significant reduction in incidence occurred in all age groups, with a non-significant trend towards less proportional benefit with increasing age. Overall, statin therapy or a more intensive statin regimen produced a 21% proportional reduction in MACE per 1.0 mmol/L reduction in LDL-C. For major coronary events, similar results were observed, with 24% RRR per 1.0 mmol/L reduction in LDL-C and a significant trend for less benefit with increasing age. For coronary revascularisation, there was a 25% RRR per 1.0 mmol/L reduction in LDL-C, but no trend towards less proportional benefit with increasing age. For stroke, there was a 16% RRR per 1.0 mmol/L reduction in LDL-C, but no trend towards less proportional benefit with increasing age. For secondary prevention in patients with previous ASCVD, the reduction in MACE was similar, irrespective of age, but appeared smaller among older than younger individuals not known to have vascular disease. For vascular mortality there was a 12% proportional reduction per 1.0 mmol/L reduction in LDL cholesterol, with a trend towards smaller proportional reductions with older age, but this trend did not persist after exclusion of the heart failure or dialysis trials.

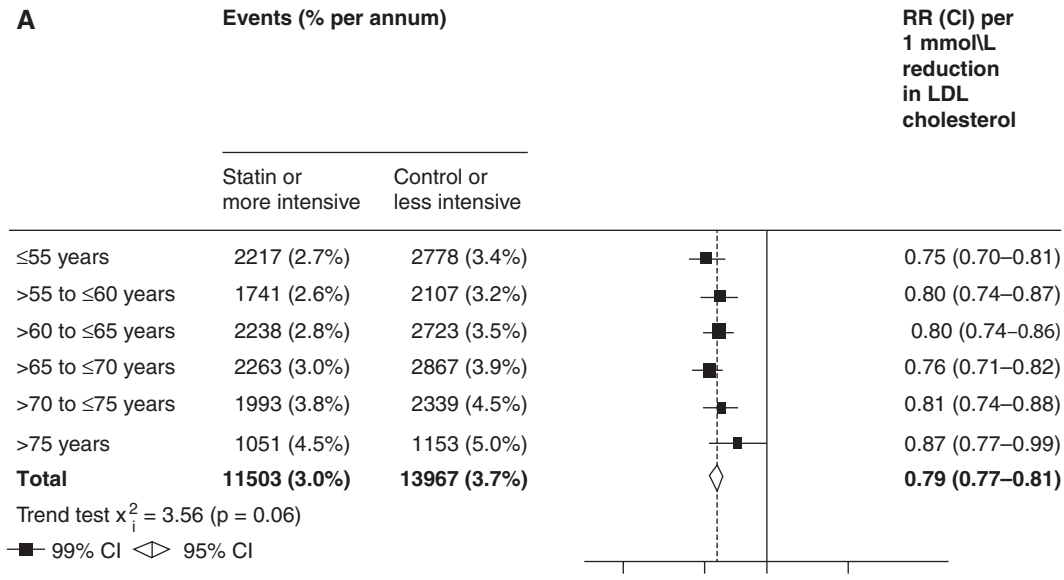

Fig. 25.4 Effects on major vascular events per mmol/L reduction in LDL-C according to age and statin therapy [17]

Statin therapy had no effect at any age on nonvascular mortality, cancer death or cancer incidence. Figure 25.4 shows the effects on MACE per mmol/L reduction in LDL-C according to age and statin therapy [17].

Statistically significant reductions in MACE occurred in each age group from ≤55 years to >75 years, with small differences in RRR per mmol/L LDL-C reduction between age groups (varying from 25% in the youngest age group to 13% in the oldest age group, with 21% overall). This trend for reduced RRR with increasing age was of borderline statistical significance.

Absolute ASCVD risk reduction (ARR) increased progressively with age from 2.7% to 4.5% in the statin or more intensive group and from 3.4% to 5.0% in the control or less intensive group. Numbers needed to treat to prevent one event (100/ARR%) varied with increasing age from 143 in the youngest age group to 167, 167, 111, 167 and 200, respectively, in progressively older groups. Overall NNT was 167.

An example was given for primary prevention of two persons aged 63 and 78 years with identical risk factors and predicted MACE rates of 2.5% vs. 4.0% per year, respectively. Reducing risk by 20% with 1.0 mmol/L LDL-C reduction would prevent 50 and 80 first MACE, respectively, per 10,000 treated over a period of 1 year.

An editorial accompanying the CTTC meta-analysis interpreted the data to indicate 0.5% annual ARR in major ASCVD events per 1 mmol/l reduction in LDL-C for patients aged >75 years [18].

Guidelines for Lipid-Lowering Therapy in the Elderly

The UK National Institute for Health and Care Excellence (NICE) recommendations were confined to suggesting that people aged over 85 years be considered for statin therapy, because of increased absolute ASCVD risk compared with younger people and a greater likelihood of benefit with statins. NICE recommended that other factors are taken into consideration such as shorter life expectancy, polypharmacy, impaired renal function, other co-morbidities and frailty. It was suggested benefit may only be reduction in nonfatal MI [19].

Only two studies were identified in adults >65 aged years: PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) and Studies

Assessing Goals in the Elderly (SAGE). The mixed primary/secondary prevention PROSPER trial included 5804 patients with a mean age of 75 years, of whom 43.2–45.2% had a history of vascular disease. After a median follow-up of 3.2 years, the greatest benefit (hazard ratio 0.64, 95% CI 0.52–0.80) occurred in patients with low baseline HDL-C (<1.11 mmol/L). Statistically significant benefits occurred in men (HR 0.77, CI 0.65–0.92) and those with previous vascular disease (HR 0.78, CI 0.66–0.93). Non-significant changes occurred in women (HR 0.96, CI 0.79–1.18) and in those without previous vascular disease (HR 0.94, CI 0.77–1.15) [20].

The SAGE trial included 893 patients with stable CAD aged 65–83 years who had 1+ episode(s) of myocardial ischemia on 48 h ambulatory ECG [21]. They were randomised to atorvastatin 80 mg or pravastatin 40 mg for 12 months, when the total duration of ischemia was significantly reduced in both groups to the same degree. In the atorvastatin group, in whom lower LDL-C levels were achieved, there were non-significant reductions in major ASCVD events (−29%) and total mortality (−67%).

In the European Society of Cardiology/European Atherosclerosis Society (ESC/EAS), 2016 guidelines for the management of dyslipidaemias were updated in 2019 [22, 23]. Treatment with statins is recommended for older adults with established ASCVD in the same way as for younger patients. Since older people often have co-morbidities and have altered pharmacokinetics, lipid-lowering medication should be started at a lower dose and then titrated with caution to achieve target lipid levels that are the same as in younger subjects. Statin therapy is recommended for primary prevention in adults aged 75 years or less according to the level of risk. For primary prevention in those aged >75 years, statin therapy may be considered for those at high risk or above [23].

With regard to primary prevention, a meta-analysis of 8 RCTs with 24,674 patients aged >65 years showed that statin treatment reduced myocardial infarction (MI) and stroke risk with relative risks (RR) of 0.61 and 0.76, respectively. All-cause mortality was not reduced significantly (RR 0.94) [24]. Two other primary prevention trials in the elderly include the AFCAPS-TEX-CAP (Air Force/Texas Coronary Atherosclerosis Prevention) study and the JUPITER trial. Risk reduction in AFCAPS-TEX-CAP was similar above and below the median age (57 years for men and 62 years for women). Risk reduction in JUPITER was similar for patients older and younger than 70 years, with NNT (number needed to treat for 4 years to prevent one event) of 24 and 36 in older and younger groups, respectively [25, 26].

Subgroup analyses of other secondary prevention statin trials have shown similar RRR in younger vs. older patients. These include the Heart Protection Study (HPS), Scandinavian Simvastatin Survival Study (4S), Long-Term Intervention with Pravastatin in Ischaemic Heart Disease (LIPID) study, Cholesterol and Recurrent Events (CARE) and Treating to New Targets (TNT) trials.

The HPS included 20,536 patients with CHD aged 40–80, who were randomised to either placebo or simvastatin 40 mg for 5 years. The RRR for CAD events and CAD mortality were similar for the age groups <65, 65–70 and >70 years [27]. All-cause mortality was significantly reduced from 14.7% to 12.9% due to 18% reduction in CHD mortality, marginally significant reduction in other vascular deaths from 2.2% to 1.9% and a non-significant reduction in non-vascular deaths from 5.6% to 5.3%. Nonfatal MI/CHD death and nonfatal/fatal stroke and revascularisation were reduced significantly. The reduction in ASCVD event rates was similar (and significant) in each subcategory of participant studied, including those aged either under or over 70 years at entry.

In the 4S study, 1021/4444 patients with established CHD were aged 65+ years, and ASCVD events were compared with younger patients after 5.4 years of treatment with simvastatin 20–40 mg daily vs. placebo [28]. In patients aged 65+ years in the simvastatin group, relative risks (95% confidence intervals) for clinical events were as follows: all-cause mortality, 0.66 (0.48–0.90); CHD mortality, 0.57 (0.39–0.83); major coronary events, 0.66 (0.52–0.84); any

atherosclerosis-related event, 0.67 (0.56–0.81); and revascularisation procedures, 0.59 (0.41–0.84). Statin therapy resulted in similar reductions in ASCVD events in elderly (65+ years of age) compared with younger patients. Mortality rates increased substantially with age, and the ARR for both total and CHD mortality in statin-treated subjects was about twofold greater in older patients.

In the CARE trial, 1283 patients aged 65–75 years with previous MI, total cholesterol <6.2 mmol/L (240 mg/dL) and LDL-C 3.0–4.5 mmol/L (115–174 mg/dL) were randomised to pravastatin 40 mg or placebo for 5 years [29].

There was 32% RRR in major coronary events with pravastatin therapy ($P < 0.001$), 45% RRR in coronary death ($P = 0.004$) and 40% RRR in stroke incidence ($P = 0.03$). The 5-year NNT were 11 (CI, 8–24) to prevent a major coronary event and 22 (CI, 15–53) to prevent a coronary death. For every 1000 older patients treated, 225 cardiovascular hospitalisations would be prevented compared with 121 hospitalisations in 1000 younger patients.

In the Treating to New Targets (TNT) study, the effect of treatment with atorvastatin 80 versus 10 mg was determined in the period after the occurrence of a first ASCVD event. 10,001 patients with stable CHD were treated with either atorvastatin 80 or 10 mg for 4.9 years. In patients receiving atorvastatin 80 mg, the relative risk of a first recurrent event was significantly decreased compared to those receiving atorvastatin 10 mg. Significant benefit with the 80 mg dose was also observed for second, third, fourth and fifth recurrent events. Similar findings were recorded in 3809 patients aged 65+ years of age compared to younger patients [30].

The LIPID study, treating 1000 patients for 6 years would prevent 45 deaths and 47 MACE in the older age group (65–75 years) vs. 22 and 32, respectively, in younger patients (31–64 years). In the older age group, pravastatin 40 mg reduced mortality by 21% (CI, 7% to 32%), CHD death by 24% (CI, 7% to 38%), CHD death or nonfatal MI by 22% (CI, 9% to 34%), MI by 26% (CI, 9% to 34%) and stroke by 12% (CI, −15% to 32%) [31].

These data support the contention that treatment of older patients, who are at highest ASCVD risk, is likely to result in greater reduction in absolute numbers of ASCVD events compared with younger patients. Data for patients aged >80–85 years, however, were limited, and ESC/EAS recommendations were for clinical judgement to be used in these patients.

The ESC/EAS recommendations point out that prevention of ASCVD in the elderly can result from ASCVD risk factor control from an early age as possible, with attention paid to prevention of cigarette smoking, blood pressure control, appropriate dietary habits, regular exercise and avoiding overweight.

The 2015 National Lipid Association (NLA) recommendations for the management of dyslipidaemia advocated the view that elderly patients aged 65–80 years should receive a high-intensity statin for secondary prevention after special consideration for the potential risks and benefits. For those aged over 80, a moderate-intensity statin for secondary prevention was recommended. For primary prevention in patients aged 65–79 years, a moderate- or high-intensity statin was recommended for patients at "very high risk" or "high risk", and a moderate-intensity statin was recommended for those at "moderate risk". For those aged over 80, a moderate- or a low-intensity statin was recommended, if appropriate for patient frailty, co-morbidities and co-therapy [32, 33]. These recommendations were updated in 2018 with NLA endorsement of the AHA/ACC/Multi-society guidelines [34].

In 2017, the American Association of Clinical Endocrinologist (AACE) guidelines for the management of dyslipidaemia recommended that patients aged >65 years be screened for dyslipidaemia. Those with multiple risk factors, other than age, should be considered for lipid-lowering therapy. It recommended using LDL-C targets instead of treating uniformly with high-intensity statin therapy [35].

The 2013 American College of Cardiology/American Heart Association (ACC/AHA) controversially disbanded LDL-C targets and instead classified patients into groups for whom

different intensity statins were appropriate. Moderate-intensity statin was recommended for secondary prevention in those aged >75 years. High-intensity statin was recommended for those aged 40–75 using the Pooled Cohort Risk Equation. For primary prevention in those aged over 75, no specific recommendation was made [36].

In 2018, the AHA/ACC/Multi-society guidelines (henceforth termed AHA/ACC 2018 guidelines) included recommendations for lipid management in the elderly [34].

For secondary prevention, initiation or continuation of high-dose statin therapy was recommended for those ≤75 years, aiming for ≥50% reduction in LDL-C levels. For those >75 years, high-intensity statin therapy was considered reasonable if tolerated, considering co-morbidities, potential adverse effects, drug-drug interaction, patient frailty and patient preferences. Initiation of either high- or moderate-dose statin therapy was also considered reasonable. An upper age cut-off for moderate-intensity statin therapy was not identified.

For primary prevention, it was considered reasonable to initiate moderate-intensity statins for patients aged >75 years with LDL-C 1.7–4.8 mmol/L (70–189 mmol/L). For those aged 76–80, with LDL-C levels as above, coronary artery calcium (CAC) measurement could be used to reclassify patients and avoid statin therapy in those with zero scores (see section below on CAC). Discontinuation of statin therapy may also be reasonable for those aged 75+ with physical or cognitive decline, multi-morbidity, frailty or reduced life expectancy.

For people with diabetes aged >75 years, statin therapy should be continued if tolerated and initiated after a clinician-patient discussion of potential benefits and risks. For those with diabetes aged 40–75 years, moderate-intensity statin therapy is indicated regardless of ASCVD risk, other than those with multiple ASCVD risk factors for whom high-intensity statin therapy is reasonable and those with ASCVD risk ≥20% for whom it is reasonable to add ezetimibe to maximally tolerated statin therapy. The aim of therapy in these latter groups is to reduce LDL-C levels by at least 50%. The guidelines suggest statin therapy is recommended for primary prevention in those with ≥7.5% ASCVD risk over 10 years. Because ASCVD risk increases with age, this level of risk applies to most patients aged >75 years [34].

Ongoing Primary Prevention Trial

The STAREE study involves treatment with atorvastatin 40 mg vs. placebo in patients aged >70 years of age without ASCVD and is due for completion in 2022 [37].

The purpose of the trial is to assess improvement in either quality of life or some other composite measure that demonstrates that the benefit outweighs other factors.

The primary end point is the time from randomisation either to death, development of dementia or disability, or to a major fatal or nonfatal cardiovascular event. Secondary end points include ASCVD death, myocardial infarction, hospitalisation, new-onset diabetes, fatal and nonfatal cancer (excluding non-melanoma skin cancer), cognitive decline, quality of life, cost-effectiveness of statin, stroke, need for permanent residential care, all-cause dementia and frailty/disability.

Adverse Outcomes Associated with Statin Therapy in the Elderly

With recent media reports of adverse outcomes with statin therapy, the general public as well as health professionals have become more concerned with the potential for side effects.

Potential ADR with statins include:

- Musculoskeletal system (MSS) effects manifest by muscle aches and pains, muscle tenderness, muscle stiffness and/or cramping with or without elevation of creatine kinase (CK) levels (including myalgia, myopathy, myositis and rhabdomyolysis).
- Nervous system effects (e.g. memory loss, intellectual impairment, peripheral neuropathy).

- Hepatic effects (increased transaminases and impaired liver function).
- Renal effects (impaired renal function).
- Metabolic effects, especially impaired glucose tolerance and increased new-onset diabetes mellitus (NODM).
- Effects on other systems (e.g. allergy, skin reactions, gastrointestinal disturbance).

Of most practical importance are MSS effects and NODM. MSS symptoms are often experienced in the elderly most commonly as a result of osteoarthritis, but statin-related symptoms occur more frequently in older than in younger patients [38, 39].

MSS effects in RCTs of statin therapy are uncommon, generally being reported in clinical trials with frequencies <1% for myalgia, <0.1% for myopathy and myositis and <0.01% for rhabdomyolysis. Indicate ADR may occur (*up to* 10–15% or higher in some studies). To summarise statin-related MSS side effects:

- MSS effects are more frequent with increasing statin doses, particularly with high-intensity statin regimens that lower LDL-C most effectively.
- MSS ADR are more frequent with increasing age, possibly related to a number of factors which may increase the toxicity of statins; these include:
  - Impaired renal and/or hepatic function with impaired statin clearance, higher blood levels and increased MSS toxicity
  - Reduced muscle mass and altered muscle metabolism with increased sensitivity to statin ADR
  - Polypharmacy with increased likelihood of drug/statin interactions, resulting in higher statin blood levels (e.g. CYP40 inhibitors such as calcium channel blockers)
  - More frequent vitamin D deficiency, which has been hypothesised to aggravate MSS toxicity
- Therefore statins should be used more cautiously in elderly patients, considering the use of:
  - Lower doses of less potent stains
  - Statins with the lowest potential for drug interactions
  - Modified co-therapy to minimise drug interactions
  - More frequent monitoring of symptoms and biochemical indices (CK, transaminases)
  - Vitamin D supplements if indicated

An increased incidence of NODM with statin therapy has been recently described, manifest by hyperglycaemia and increased insulin levels, without evidence of increased target organ damage. A recent Japanese study showed an increased incidence of NODM in statin users of 124.6 per 1000 person-years compared with 22.6 per 1000 person-years in non-users. After adjusting for confounding factors, the HR was 1.91 (95% CI, 1.38–2.64) for low-potency statins and 2.61 (2.11–3.23) for high-potency statins [40].

The general consensus is that NODM poses less of a risk to ASCVD outcomes than if statins were not continued, i.e. the protective effects of statin therapy outweigh the potential for harm in those who develop NODM. A meta-analysis of 13 statin RCTs (involving 91,140 patients) showed that treating 255 patients with statins for 4 years led to 1 extra case of diabetes mellitus, whereas 5.4 cardiovascular events were prevented [41].

Predisposing factors to NODM include central obesity, hyperinsulinaemia and impaired fasting glucose. Patients with the metabolic syndrome should therefore be monitored more closely for NODM and preventive measures undertaken as required (weight loss, exercise and reduced intake of refined carbohydrates).

Coronary Artery Calcium Scoring in the Elderly

Coronary artery calcium scoring (CAC) may be used in the elderly (as in younger age groups) to improve the precision of risk assessment and increase the appropriateness of statin prescribing. Elderly patients with zero CAC may have statins withdrawn or the dose reduced, as their actuarial MACE rate is low; an 84-year-old male with

CAC = 0 has a lower event rate than a 50-year-old male with CAC >400 [42].

The latest 2018 ACC/AHA guidelines on LLT suggest that CAC helps clarify which patients are most likely to benefit from statin therapy and in whom therapy may be safely deferred [34]. As for younger age groups, CAC predicts rates of ASCVD in the elderly, as well as all-cause mortality. In 3570 patients aged 70+ years, 2.1% died after a mean 5.8 years of follow-up, and increasing CAC was associated with reduced survival [42].

Mortensen et al. followed 5805 patients (mean age 69 years) for mean 2.7 years, during which 138 and 91 patients experienced ASCVD and CHD events, respectively. Eighty-six percent qualified for ACC/AHA 2013 guidelines for statin therapy with ASCVD 10-year risk ≥7.5% [43]. They down-classified patients from statin-eligible to statin-ineligible if CAC or carotid plaque burden (cPB) were absent (32% and 23%, respectively) and up-classified patients from optional to eligible if CAC ≥100 or cPB ≥300 mm^2. They suggested that withholding of statins could be considered in patients without CAC or cPB, who had low ASCVD rates [43].

For those with CAC 0 and no risk factors, CAC can be repeated in 5 years and therapy reassessed. Patients who might also benefit from CAC included older patients aged 55–80 (males) and 60–80 (females) with estimated ASCVD risk qualifying for LLT, but who question the benefit of statin therapy. In this group, CAC can be a helpful arbitrator for prescription and motivator for adherence to LLT. In summary, CAC is a useful tool to improve precision of ASCVD risk and the need for statin therapy in elderly patients [44].

Non-statin Therapy

Trials of non-statin therapy have not been found to reduce ASCVD events nor mortality in the elderly population.

Ezetimibe

Ezetimibe use increased by an annual average of 19% in Australia between 2006 and 2015, possibly due to publication of IMProved Reduction of Outcomes: Vytorin Efficacy International (IMPROVE-IT) trial and the additional LDL-C-lowering benefit of ezetimibe with statin co-therapy [45].

The IMPROVE-IT trial treated ACS patients (age > 50 years, mean age 64 years) with ezetimibe 10 mg plus simvastatin 40 mg vs. simvastatin 40 mg alone. The primary composite outcome (CV mortality + major CV events + nonfatal stroke) was reduced significantly with combination therapy. Results were not extrapolated to the elderly population because of small numbers in the trial [46].

A recent post hoc analysis of IMPROVE-IT showed 8.7% absolute risk reduction in the primary end point for 2798 patients aged 75 years or more (HR 0.8, CI 0.7–0.9) compared with ARR of 0.8% (HR 0.96, CI 0.87–1.06) and 0.97% (HR 0.9%, CI 0.9–1.05) for those aged 65–74 years and <65 years, respectively [47]. The incidence of adverse drug reactions did not differ between age groups. The 6-year NNT to prevent 1 CVD event was 11 in those aged 75+ years and 125 in those aged <75 years.

PCSK-9 Inhibitors

The PCSK-9 inhibitors show great promise for those whose LDL-C levels are uncontrolled with maximum-tolerated doses of statins, but their use in the elderly has not been defined as yet by RCT subgroup analyses.

PCSK-9 inhibitors have been shown to lower LDL-C by about 60% and to reduce ASCVD events in the Open-label Study of Long-term Evaluation against LDL Cholesterol (OSLER) and the Tolerability of Alirocumab in High Cardiovascular Risk Patients with Hypercholesterolemia Not Adequately Controlled with Their Lipid Modifying Therapy (ODYSSEY LONG TERM) study [48, 49].

In the Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease (FOURIER) study, patients aged 40–85 years (mean age 63 years) were treated with evolocumab + statin vs. statin alone. Evolocumab lowered major

ASCVD events by 15%. The proportion of elderly patients was not specified [50].

PCSK-9 inhibitors are especially useful for LDL-C control in patients with familial hypercholesterolaemia (FH), usually in combination with ezetimibe and maximum-tolerated statin therapy. It is no longer unusual to manage elderly patients with FH, some of whom have survived episodes of revascularisation, while maintaining optimal LDL-C-lowering therapy over decades.

Nicotinic Acid

In the Atherothrombosis Intervention in Metabolic Syndrome with low HDL/HIGH triglycerides (AIM-HIGH) trial of ASCVD patients previously on statins, approximately 46% were aged >65 years. Niacin in addition to simvastatin 40–80 mg did not lower the risk of ASCVD events [51].

The Heart Protection Study 2 – Treatment of HDL to reduce the Incidence of Vascular Events (HPS2-THRIVE) study enrolled patients with ASCVD aged 50–80 years, and there was no reduction in ASCVD incidence adding niacin to statin therapy [52].

In view of these findings, in addition to impaired glucose tolerance and other side effects, niacin is not routinely used for treatment of dyslipidaemia.

Bile Acid Sequestrants

No data are available for the use of resins in the elderly.

Fibrates

No RCTs with fibrates (gemfibrozil, fenofibrate and clofibrate) have been studied in the elderly. Fibrates, however, are routinely used to control high TG levels and to prevent pancreatitis at all ages. Levels of HDL-C can be increased with fibrate therapy, but there is no evidence that this will result in reduced ASCVD events. Fenofibrate is preferred to gemfibrozil with statin co-therapy as the risk of MSS ADR is lower.

Omega-3 Supplements

An early study of omega-3 supplementation in 563 Norwegian men, aged 64–76 years, of whom 72% had no overt ASCVD, showed adjusted hazard ratios of 0.53 (95% CI, 0.27–1.04, $P = 0.063$) and 0.89 (CI, 0.55–1.45, $P = 0.64$) for all-cause mortality and ASCVD events, respectively, after 3 years [53].

Subsequent meta-analysis of omega-3 supplementation in 10 RCTs involving 47,803 subjects with prior CHD and mean age 64 years showed no significant associations with ASCVD events after 4.4 years of treatment [54].

The REDUCE-IT trial showed reduction of CVD events with high-dose omega-3 therapy in 8179 statin-treated patients aged 45+ years with established CVD or diabetes and other risk factors [55]. Patients had high TG levels (135–499 mg/dL−1.52–5.63 mmol/L) and LDL-C 41–100 mg/dL (1.06–2.59 mmol/L); 45% of patients were aged 65 years or more. Treatment with icosapent ethyl (eicosapentaenoic acid ethyl ester, EPA) 2 g twice daily was compared with placebo over 4.9 years. Overall, there was a 4.8% ARR in the primary end point (HR 0.75, CI 0.68–0.83; $P < 0.001$). For those aged 65+ years, the ARR was 1.5% (HR 0.87, CI 0.76–1.00). For those aged <65 years, the ARR was 5.7% (HR 0.65, CI 0.56–0.75).

Summary and Conclusions

Previous statin trials strongly support secondary ASCVD prevention in all age groups. There is least controversy, therefore, in the need to treat elderly patients with manifest ASCVD. The usual cautions in treating the elderly remain, however, but areas of controversy include whether to use lower doses of less potent statins to avoid ADR and whether LDL-C targets should be different if less potent statins were to be used routinely.

With regard to primary ASCVD prevention in the elderly, statin therapy is recommended for patients at high ASCVD risk, with variation in the degree of risk with different guidelines. An important consideration is the increase with ASCVD risk associated with ageing, partially dependent on other ASCVD risk factors.

One way to ameliorate less effective LDL-C lowering with less potent statins is to consider co-therapy with ezetimibe, which is generally well-tolerated and results in about 20% further LDL-C lowering. Targets may therefore be achieved more readily, at the expense of one additional drug and further polypharmacy.

Considering the above guidelines, LDL-C lowering therapy in the elderly is likely to be beneficial in terms of reducing ASCVD events, improving quality of life and possibly increasing life expectancy [56]. When considering initiating or continuing statin therapy, perhaps one should ask: "Why should this patient not be on statin therapy?" as opposed to "Should this patient be on statin therapy?" When either the physician or patient is in doubt, CAC scoring may be useful to improve precision of risk prediction, arbitrate the need for LLT and motivate adherence to therapy.

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Management of Dyslipidemia in Children

26

Julie A. Brothers and Stephen R. Daniels

Background

The leading cause of mortality in the United States is cardiovascular disease (CVD) [1–5]. The precursors to atherosclerosis start in childhood with damage to the arterial endothelium noted as early as the first decade of life. Lipid abnormalities are present in approximately 20% of children and adolescents in the United States [6] and represent a combination of lifestyle-related dyslipidemia and genetic causes of dyslipidemia. Screening for lipid disorders identifies both types of dyslipidemia and should serve as an impetus for implementing lifestyle modifications. In this chapter, we will review the definition of dyslipidemia in childhood, screening for dyslipidemia, and treatment recommendations regarding diet and lifestyle and provide guidance for starting medication when necessary. We will present two clinical cases to highlight management of different types of dyslipidemias.

Dyslipidemia and Normal Lipid Values in Children

Dyslipidemia occurs due to abnormal lipoprotein metabolism which leads to elevated levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and/or triglycerides (TG) and/or low levels of high-density lipoprotein cholesterol (HDL-C). Non-HDL-C, which is calculated as TC minus HDL-C, can also be elevated. The non-HDL-C measures cholesterol carried by all of the atherogenic apolipoprotein B (apo B)-containing lipoproteins in the bloodstream. Cholesterol is essential in our body and is used to make hormones and helps to make up cell membranes, fat-soluble vitamins, and bile. Triglycerides are needed to help store and supply energy to the body. Lipids are hydrophobic and travel attached to hydrophilic lipoproteins in the bloodstream; apolipoproteins are proteins that play an important role in the metabolism of lipoproteins and are located along the polar outer layer of plasma lipoproteins [7].

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_26

Progression of Atherosclerosis

Subclinical atherosclerosis, as indicated by increased carotid intima-media thickness, is commonly found in children with heterozygous familial hypercholesterolemia (HeFH) and obesity-related dyslipidemia [8]. The first lesion visible to the naked eye is the arterial fatty streak and has been found in children and adolescents with dyslipidemia. While these are generally reversible, dyslipidemia commonly tracks into adulthood, and if the lipid levels remain abnormal, the fatty streaks can progress to fibrous plaques, the latter of which are irreversible [9].

Normal Versus Abnormal Lipoprotein Values

Normal and abnormal lipoprotein levels are shown in Table 26.1a and 26.1b [10, 11]. The "borderline" or "high" TC, LDL-C, and non-HDL-C levels correspond to greater than the 75th and 95th percentiles, respectively [11, 12]. For TG, borderline levels correspond to greater than the 75th percentile, whereas high levels are greater than the 90th percentile. For HDL-C, "optimal" levels are greater than the 50th percentile, "borderline" is the 50th percentile and below, and "low" is less than the 25th percentile [13–15].

Table 26.1a Cholesterol level classification for children and adolescents (in mg/dL) [10–15]

Lipid category	Optimal	Borderline-high	High	Low
TC	<170	170–199	≥200	
LDL-C	<110	110–129	≥130	
Non-HDL-C	<120	123–144	≥145	
TG				
0–9 yr	<75	75–99	≥100	
10–19 yr	<90	90–129	≥130	
HDL-C	>45	40–45		<40

TC total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *non-HDL-C* non-high-density lipoprotein cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol

Table 26.1b Cholesterol level classification for young adults (20–24 yrs., in mg/dL) [10–15]

Lipid category	Optimal	Borderline-high	High	Low
TC	<190	190–224	≥225	
LDL-C	<120	120–159	≥160	
Non-HDL-C	<150	150–189	≥190	
TG	<115	115–149	≥150	
HDL-C	>45	40–45		<40

TC total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *non-HDL-C* non-high-density lipoprotein cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol

Screening for Dyslipidemia

The lipid screening guidelines in children and adolescents are shown in Table 26.2.

Universal Screening

In 2011, the integrated screening guidelines from the National Heart, Lung, and Blood Institute expert panel were released. In it, the panel recommended that all children without additional cardiovascular risk factors should be screened for dyslipidemia at least once between the ages of 9 and 11 years and again between 17 and 21 years of age [14]. This recommendation was confirmed in the 2018 American Heart Association/American College of Cardiology's Guideline on the Management of Blood Cholesterol [15]. These recommendations were made in order to help identify children and adolescents who may not have been screened using the previous guidelines [13, 16, 17]. Selective screening alone can miss a large number of children with dyslipidemia for many reasons, including: parents who may not be aware of their cholesterol levels, family history is unknown, and/or parents who are too young to have developed early atherosclerosis [16, 18–22]. The universal screening ages were selected in order to not miss a true dyslipidemia during puberty, as LDL-C levels can decrease up to 20% during this time. Also, identifying youth with either severe hypercholesterolemia or multifactorial dyslipidemia can help them get started with adequate therapy, including lifestyle modifi-

Table 26.2 Cholesterol screening recommendations by age [14]

Age	Screening recommendation	Labs
<2 years	No screening	
2–8 years	No routine screening	
	Screening if: Family history of early heart disease Parent with TC ≥ 240 mg/dL Family history is unknown Moderate or high risk condition (see Table 26.3)	FLP × 2*
9–11 years	Universal screening	Non-FLP
	If non-HDL-c ≥ 145 or HDL-c < 40	
	Then obtain	FLP × 2*
	If LDL-c ≥ 130 or non-HDL-c ≥ 145 or	FLP
	HDL-c < 40 or	
	TG > 100 (age < 10 yr) or > 130 (age ≥ 10 yr)	
	Then obtain	Repeat FLP*
12–16 years	No routine screening	
	Screening if:	
	New knowledge of CVD risk, same as 2–8 yr. FLP × 2*	
17–21 years	Universal screening	
	Ages 17–19:	Non-FLP
	If non-HDL-c ≥ 145 or HDL-c < 40	
	Then obtain	FLP × 2*
	If LDL-c ≥ 130 or non-HDL-c ≥ 145 or	FLP
	HDL-c < 40 or	
	TG > 100 (age < 10 yr) or > 130 (age ≥ 10 yr)	
	Then obtain	Repeat FLP*
	Ages 19–21:	Non-FLP
	If non-HDL-c ≥ 190 or HDL-c < 40	
	Then obtain	FLP × 2*
	If LDL-c ≥ 160 or non-HDL-c ≥ 190 or	FLP
	HDL-c < 40 or TG ≥ 150	
	Then obtain	Repeat FLP*

CVD cardiovascular disease, *FLP* fasting lipid profile, *non-FLP* non-fasting lipid profile, *LDL-c* low-density lipoprotein cholesterol, *HDL-c* high-density lipoprotein cholesterol, *non-HDL-c* non-high-density lipoprotein cholesterol (total cholesterol – high density lipoprotein cholesterol), *TC* total cholesterol, *TG* triglycerides,* 2nd lipid profile should be obtained 2 weeks-3 months after the first lipid profile

cations and medication, if necessary. Another benefit of universal screening is reverse cascade screening, which can help identify other family members, including parents, grandparents, aunts, uncles, and siblings, with dyslipidemia. Relatives of children with hypercholesterolemia are at an increased risk of early CVD [23, 24].

For universal screening, either a non-fasting lipid profile (non-FLP) or fasting lipid profile (FLP) can be used. If a non-FLP is performed, then the non-HDL-C can be calculated from the TC and HDL-C. The FLP includes the TC, HDL-C, TG, and a calculated LDL-C. The LDL-C can usually be calculated indirectly: LDL-C = TC − (HDL-C + TG/5). When there is significant hypertriglyceridemia (i.e., >400 mg/dL), one can use the non-HDL-C value to determine if further evaluation is necessary. If there are any abnormal lipid values on the non-FLP (see Table 26.2), then an FLP should be obtained twice more, approximately 2 weeks to 3 months apart, and the average of two FLPs should be used to determine management. If the first screening used is an FLP and there are abnormal lipid values, then a repeat FLP should be performed, 2 weeks to 3 months after the first, and the results of the two tests averaged (see Table 26.2).

Selective Screening

For children ages 2–8 years and 12–16 years, selective screening based on family and/or personal health history is recommended, as shown in Table 26.3. If the first FLP is abnormal (see Table 26.1), then a repeat FLP should be performed

Table 26.3 Risk factors and special risk conditions for screening children for dyslipidemia, ages 2–8 and 12–16 years

Positive family history: a first- or second-degree relative with documented CVD (e.g., angina pectoris, peripheral, or cerebral vascular disease, myocardial infarction, coronary artery disease, or sudden death) by age < 55 years for a male and < 65 years for females
High risk factor/condition
Systemic hypertension requiring drug therapy
Cigarette smoking
Severe obesity (BMI ≥ 97th percentile)
Diabetes (type I and type 2)
Chronic/end-stage kidney disease/post-renal transplant
Post-orthotopic heart transplantation
Kawasaki disease, currently with aneurysm
Moderate risk factor/condition
Systemic hypertension (blood pressure > 95th percentile for gender and age) not requiring drug therapy
Obesity (BMI ≥ 95th percentile but < 97th percentile)
HDL-C < 40 mg/dL
Kawasaki disease with regressed aneurysm
Chronic inflammatory disease
HIV infection
Nephrotic syndrome

Adapted from Ref. [14]
CVD cardiovascular disease, *BMI* body mass index

ideally 2 weeks to 3 months after the first one, and the results for treatment recommendations should be based on the average of these two FLPs.

Evaluation for Secondary Causes of Dyslipidemia

A more thorough health evaluation should be initiated once a child has been confirmed with dyslipidemia. This should start with a complete family history identifying first- and second-degree relatives who have a history of hypercholesterolemia, premature CVD (age ≤ 55 years for male, ≤ 65 years for female), diabetes mellitus, overweight, and hypertension. If any first- and/or second-degree relatives have not had lipid screening, then they should be checked (reverse cascade screening). For children and adolescents with severe hypercholesterolemia, a primary or genetic cause of dyslipidemia should be considered, and the most common will be reviewed later in this chapter.

Along with a thorough family history, secondary causes of dyslipidemia should be investigated (Table 26.4) [25]. Most secondary causes can be identified by performing a complete medical history, review of systems, and physical examination. Laboratory studies that should be obtained to assess for certain disease states that can cause or be associated with dyslipidemia should include hepatic function tests for non-alcoholic fatty liver disease or obstructive liver disease; fasting blood sugar and hemoglobin A1c levels for diabetes or glucose intolerance in obese patients; thyroid function tests for hypo- or hyperthyroid; a urinalysis; and blood urea nitrogen and creatinine levels for renal disease.

Lifestyle-Related Dyslipidemia

Clinical Case 1

A 10-year-old female, A.C., presents to the lipid clinic for evaluation of dyslipidemia. A.C. is 10 years old and had a non-FLP checked as part of routine screening by her pediatrician. At that time, the HDL-C was 38 mg/dL and TC 164 mg/dL, giving her a non-HDL-C of 126 mg/dL. Because her HDL-C was < 40 mg/dL, an FLP was performed. This showed (in mg/dL) TC 172, HDL-C 39, TG 258, and LDL-C 82 mg/dL. She was referred for further evaluation.

Mom reports that A.C. started gaining more weight than usual around age 5 years, when she started school. Family history was unremarkable for early heart disease or dyslipidemia in first- or second-degree relatives, although her mom did not know if her dad had ever had his cholesterol levels checked. A.C. was not involved in organized athletics but was interested in soccer and participates in gym class three times per week and recess. Her sedentary time is 2–3 h on weekdays and up to 6 h on the weekends.

A 3-day dietary history was obtained. A.C. would commonly skip breakfast or just drink

Table 26.4 Secondary causes of dyslipidemia: the four "Ds"

Diet	High saturated/trans fat
	Excessive carbohydrate
	Excessive alcohol intake
	Anorexia nervosa
Disease:	Cardiac Heart transplantation Congenital heart disease Hepatic Intrahepatic cholestasis Chronic liver disease Primary biliary cirrhosis Hepatitis (acute or chronic) Biliary atresia Alagille syndrome
	Renal Chronic renal failure Nephrotic syndrome Hemolytic-uremic syndrome
	Rheumatic Systemic lupus erythematosus Rheumatoid arthritis
	Storage Gaucher disease Glycogen storage disease Tay-Sachs disease Niemann-Pick disease
	Other Post-cancer therapy Klinefelter syndrome Progeria Burns
Drugs:	Oral estrogens and progestins Oral contraceptives Anabolic steroids Corticosteroids Thiazide diuretics Beta-blockers Bile-acid binding resins Glucocorticoids Protease inhibitors (most) Retinoic acid derivatives Anticonvulsants
Dysmetabolism:	Diabetes, type I and type II
	Obesity
	Insulin resistance
	Acute intermittent porphyria
	Hypopituitarism

8–12 ounce of juice or chocolate milk; for lunch she would eat a school lunch with a fruit, occasional vegetable, and chocolate milk or fruit juice; snacks consisted of cookies, chips, yogurt with Oreos, and fruit; dinners were mainly a lean meat, pasta or rice, vegetables, and 8–12 ounces of juice; and for dessert, she would eat cookies or ice cream 3–4 times/week.

On physical examination, her height was at the 47th percentile, weight at the 95th percentile, and BMI at the 98th percentile, placing her in the obese category. Her blood pressure was 102/58. Notable examination findings included faint abdominal striae on her abdomen and faint acanthosis nigricans behind her neck. Additional laboratory studies revealed normal thyroid studies; ALT 52 IU/L (upper limit normal 42 IU/L) and AST 20 IU/L; and glucose 98 mg/dL and hemoglobin a1c 5.5% (normal < 5.7%).

Combined Dyslipidemia of Obesity

Studies have shown that 75% of overweight adolescents become obese adults [8, 26]. Lipid abnormalities in this patient population are quite common with more than 1/3 having some form of dyslipidemia, most commonly high TG and low HDL-C [6]. In this population, increased adiposity leads to an atherogenic lipid phenotype, which is called combined dyslipidemia of obesity (CDO). This is characterized by elevated TG, low HDL-C, elevated non-HDL-C, and normal or mildly elevated LDL-C levels. Also, the LDL-C particles (LDL-P) tend to be the small, dense subtype.

Additionally, patients with CDO commonly have elevated insulin levels and central fat deposition [26]. Having the CDO lipid profile may also signal increased risk of future premature CVD. In the Framingham Offspring Study, CDO was one predictor of early clinical cardiovascular events, such as myocardial infarction and death from CVD [27]. Also, those who had elevated TG levels and TG/HDL-C ratios at 12 years of age and who continued to have a similar lipid phenotype in adulthood were also at increased risk for experiencing premature CVD as an adult [28].

In Clinical Case 1, A.C.'s lipid profile and body habitus are most consistent with CDO.

Diet and Lifestyle Intervention for Combined Dyslipidemia of Obesity

Dietary Intervention

The most recent integrated cardiovascular risk reduction guidelines recommend a stepped approach to dietary intervention for dyslipidemia in children (see Table 26.5). The first step is the Cardiovascular Health Integrated Lifestyle Diet (CHILD-1). After a trial of CHILD-1, a more intensive lipid-lowering dietary regimen can be undertaken, if necessary, focusing more on lowering LDL (CHILD-2-LDL) or triglycerides (CHILD-2-TG), depending on the patient's lipid phenotype [14].

Table 26.5 Cardiovascular Health Integrated Lifestyle Diet (CHILD-1) for children and adolescents at increased cardiovascular risk

Age	Diet and lifestyle recommendation
0–6 months	Exclusively breastfeed through 6 months. If not possible, then expressed breast milk should be offered. If not possible, then iron-fortified infant formula
6–12 months	Gradually add solids while breastfeeding until at least 12 months
	If weaning from breastfeeding, provide iron-fortified formula until 12 months
	No dietary fat restriction
12–24 months	Transition to lower fat (2%, 1%, fat-free) unflavored cow's milk, if not continuing to breastfeed. This should be decided on by parents and the health care provider using child's variables, including: appetite, growth, dietary intake of other nutrient-dense foods and intake of other sources of fat, and family history of obesity and early CVD
	Transition to table foods with total fat ≤30% of daily calories, low in saturated fats and avoiding trans fats transition to table foods with total fat ≤30% of daily calories, low in saturated fats and avoiding trans fats
	No more than 4 ounces/day of fruit juice, served in a cup
	Limit sodium intake
	Encourage diet rich in fruits, vegetables, whole grains, low-fat and fat-free milk and milk products, and products lower in sugar
2–10 years	Main beverage should be fat-free unflavored milk
	Encourage water consumption, limit sugar-sweetened beverages
	Dietary fat intake should be 25–35% of total daily calories with limited saturated fats and no trans fats.
	Diet high in fiber, limit simple/refined carbohydrates
	Fiber goal: age + 5 grams/day
	No more than 4 ounces/day of naturally sweetened juice
	Limit sodium intake
	Encourage diet rich in fruits, vegetables, whole grains, low-fat and fat-free milk and milk products, and products lower in sugar
	Support healthy eating habits: daily breakfast, family meals, limit fast-food intake
11–21 years	Main beverage should be fat-free unflavored milk
	Encourage water consumption, limit sugar-sweetened beverages
	Dietary fat intake should be 25%–35% of total daily calories with limited saturated fats and no trans fats
	Diet high in fiber, limit simple/refined carbohydrates
	Fiber goal: 14 grams/day for every 1000 calories consumed
	No more than 4 ounces/day of naturally sweetened juice
	Limit sodium intake
	Encourage diet rich in fruits, vegetables, whole grains, low-fat and fat-free milk and milk products, and products lower in sugar
	Support healthy eating habits: daily breakfast, family meals, limit fast-food intake

Adapted from Ref. [14] Table 5.1

Cardiovascular Health Integrated Lifestyle Diet-1 (CHILD-1)

The CHILD-1 diet is a diet that is applicable to all individuals age 2 and above across the population. It consists of a low-saturated fat (< 10% of total calories), moderate-total fat (≤ 30% of total calories), and low-cholesterol diet (< 300 mg/day) with minimal trans fats [10, 14]. There should be zero sugar-sweetened beverages and limited amounts of high-sodium, high-sugar, and highly processed and fried foods. In addition to diet, physical activity is also important. Sedentary time should be less than 2 h daily, physical activity should be at least 60 min daily with 3–4 of those days being moderate to vigorous activity, and weight loss/weight stabilization should be encouraged. The CHILD-1 approach is shown in Table 26.5. After 3 months of CHILD-1 and lifestyle changes, a repeat FLP should be obtained, and any changes to diet and lifestyle can be further implemented.

For overweight and obese children and adolescents with CDO, a trial of the CHILD-1 regimen should be implemented. The whole family should be included in the discussion and implementation of the dietary plan. They should meet with a registered dietitian/nutritionist to ensure they are making the correct dietary changes in a healthy manner. When obtaining the dietary history in a patient with CDO, always inquire about not just the food and amount that the child is eating but also what and how much the child drinks throughout the day. Eliminating sweetened beverages (e.g., soda, iced tea, lemonade, flavored milk, sports drinks) will both have a TG-lowering effect and improve weight management. Additionally, there should be an increase in complex carbohydrates and fiber from foods such as whole grains, nuts, seeds, fruits, vegetables, and legumes and, ideally, consumption of low-mercury, oily fish at least twice weekly.

Lifestyle Changes

Along with dietary changes, increasing exercise and reducing sedentary time are very important. The most recent guidelines from the US Department of Health and Human Services [29] recommend that preschool-age children (3–5 years) should be physically active throughout the day with a reasonable target of at least 3 h. School-age and adolescent children (6–17 years) should participate in 60 min of moderate to vigorous physical activity daily, with muscle- and bone-strengthening activities 3 days/week each. In those who do not participate in sports, focus should be on increasing physical activity in daily activities (e.g., taking stairs instead of elevators, walking to/from school, walking the dog) and encouraging the patient to try new activities, such as classes at a local gym. Concomitantly, weight loss in those who are done growing or weight stabilization/weight loss for those who are still growing should be stressed.

Medication Management

Currently, no medication is indicated in the treatment of CDO. Studies in adults with CDO using statin medications have shown improvement in arterial stiffness [30–32] and reduced cardiovascular effects. No studies have been published on patients with CDO and statin medication. There is a current study evaluating the use of statins for pre-teens and adolescents and its effect on vascular health. Rarely, youth with CDO are prescribed omega-3 fatty acid medication if their TGs remain > 400 mg/dL despite adequate attempts at diet and lifestyle changes.

Clinical Case 1 Follow-Up

Four months after the initial visit, A.C. returned to clinic. She decreased her sugar-sweetened drinks to one daily serving, changed to skim from 2% milk, and increased her fruit and vegetable intake. She joined the lacrosse team and lost 5 lb while growing ½ inch. Her lipid panel had significantly improved and had nearly normalized (in mg/dL): TC 158, HDL-C 42, LDL-C 93, TG 115, and non-HDL-C 116. Her ALT also normalized at 38 IU/L, fasting glucose was 93 mg/dL, and hemoglobin A1c was 5.5%.

Genetic Causes of Hypercholesterolemia in Childhood

Clinical Case 2

A 12-year-old male, T.S., presents to the lipid clinic for further evaluation of hypercholesterolemia. His fasting lipid panel was checked due to his father recently having a myocardial infarction requiring three coronary stents at the age of 41 years. Further history reveals that his paternal grandfather had a myocardial infarction at age 50 years. He has a 14-year-old brother with normal lipid levels. T.S. participates in both the football and baseball teams at school. His sedentary time is 2 h daily on the weekdays and 2–3 h on the weekends. His family follows a low-saturated fat diet at home. For breakfast, T.S. generally has low-sugar cereal with skim milk or a bagel. T.S. buys lunch at school, which may include cheeseburgers, chicken nuggets, pizza, and cheesesteaks, accompanied by French fries and cookies. He snacks on pretzels, fruit, or a bowl of cereal. He drinks water with breakfast and dinner and either chocolate milk or soda at school.

On physical examination, his height is at the 60th percentile, and his weight is at the 90th percentile; his BMI is at the 91st percentile, placing him in the overweight category. His blood pressure is 116/74. The rest of his examination is benign. There are no tendon xanthomas. He is Tanner stage 2. An average of two fasting lipid panels reveal (in mg/dL) TC 342, HDL-C 54, LDL-C 266, and TG 112. Non-HDL-C is 288 mg/dL. Thyroid function, urinalysis, glucose, insulin, AST/ALT, and CK levels were all within normal limits.

Heterozygous Familial Hypercholesterolemia

Heterozygous familial hypercholesterolemia (HeFH) is an autosomal codominant disorder and is one of the most common causes of significant hypercholesterolemia in childhood. It occurs in approximately 1:200–1:250 people in the United States [33]. HeFH is caused by at least 1 of the 500 LDL receptor gene defects that lead to either a defective or a diminished number of LDL receptors. This results in decreased LDL clearance from the circulation by the liver, which leads to a greater accumulation of LDL particles in the blood. Children and adolescents with HeFH may develop subclinical atherosclerosis with evidence of greater carotid intima-media thickness (CIMT) and abnormal brachial artery reactivity, indicating endothelial dysfunction. While youth with HeFH generally do not manifest clinically apparent coronary artery disease until adulthood, if left untreated, they have a high risk of developing premature atherosclerosis. Familial defective apolipoprotein B100 is a rarer condition (approximately 1:1000 people) that presents with a similar phenotype but is treated in the same manner as HeFH. Genetic testing for HeFH is now available and can be considered for children and adolescents and, if positive, can support screening their family members as well [34].

In Clinical Case 2, given the family history and his lipid levels, T.S. appears to have HeFH. In children and adolescents with HeFH, both TC and LDL-C levels will be > 95th percentile for age/sex and can be seen as young as age 2 years. Commonly, these children have normal TGs and normal or low HDL-C. If the patient is overweight or obese, there may also be elevated TGs. When there is concern for HeFH, a thorough family history should be obtained, focusing on the patient's first- and second-degree relatives. Generally, the patient has one parent with significantly elevated TC and LDL-C and a history of tendon xanthomas (Fig. 26.1) or a family history of premature CVD in first- and/or second-degree relatives. Tendon xanthomas are not seen in children and adolescents with HeFH as they normally develop in adulthood but may be palpable on the parent, notably if the parent was untreated until adulthood.

Familial Combined Hyperlipidemia

Another common inherited cause of hypercholesterolemia is familial combined hyperlipidemia (FCHL), which is an autosomal dominant

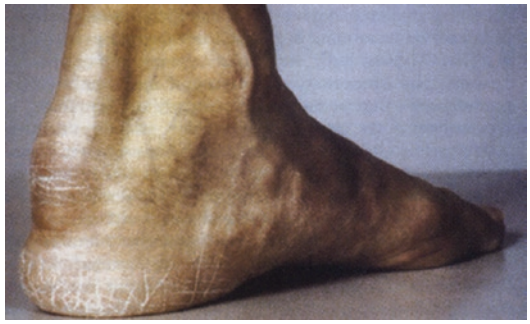


Fig. 26.1 Tendon xanthoma in an adult patient with heterozygous familial hypercholesterolemia. (From Durrington [62], with permission from Elsevier)

lipid disorder, with similar prevalence in the United States as HeFH. A suspicion for FCHL should be raised when the FLP demonstrates a mixed dyslipidemia consisting of elevated TGs (200–800 mg/dL), low HDL-C (< 40 mg/dL), and mildly to moderately elevated TC levels (200–400 mg/dL) along with a family history of hypercholesterolemia or premature CVD. If apolipoprotein B levels are checked, they should be elevated relative to the LDL-C level. Patients with FCHL do not have tendon xanthomas on physical examination. While FCHL should be on the differential for T.S. in Clinical Case 1, the absence of hypertriglyceridemia and a normal HDL-C makes it more likely that he has HeFH.

Therapy for Pure Hypercholesterolemia

Dietary and Lifestyle Intervention

Similar to as described above, a 3–6-month trial of CHILD-1 diet should be implemented (see Table 26.5). If the LDL-C remains ≥ 130 mg/dL, a more intensive lipid-lowering dietary regimen should focus on lowering LDL-C further (CHILD-2-LDL). The family should also meet with a medical dietitian who is experienced with treating dyslipidemia to ensure the child is meeting the requirements for protein, carbohydrates, and vitamins for adequate growth and development [14].

Cardiovascular Health Integrated Lifestyle Diet-2-Low-Density Lipoprotein (CHILD-2-LDL)

The CHILD-2-LDL diet recommends dietary fat intake of 25–30% of total calories, but with reducing saturated fat to nor more than 7% of daily calories and monounsaturated fat no more than 10% of daily calories and avoidance of trans fats [14, 16]. After at least 3 months on CHILD-2-LDL, a repeat FLP should be performed. Because this is a more restrictive diet and more complicated to implement, it is important for a dietitian to assist the family with diet changes.

Adjunctive Dietary Therapies

Phytosterols

Phytosterols (plant sterols and stanols) are natural products that have been concentrated into pills, spreads, and food products. A meta-analysis found that doses of ~1–3 grams/day are effective in lowering LDL-C by 5–10% in adults and in children with HeFH [35–37]. Phytosterols may also be useful in lowering TGs, with a modest effect of up to ~28%, with a greater response in those with TGs ≥ 150 mg/dL [38]. Use of plant sterols can lower beta-carotene levels, which can be helped by eating more fruits and vegetables [39].

Dietary Fiber

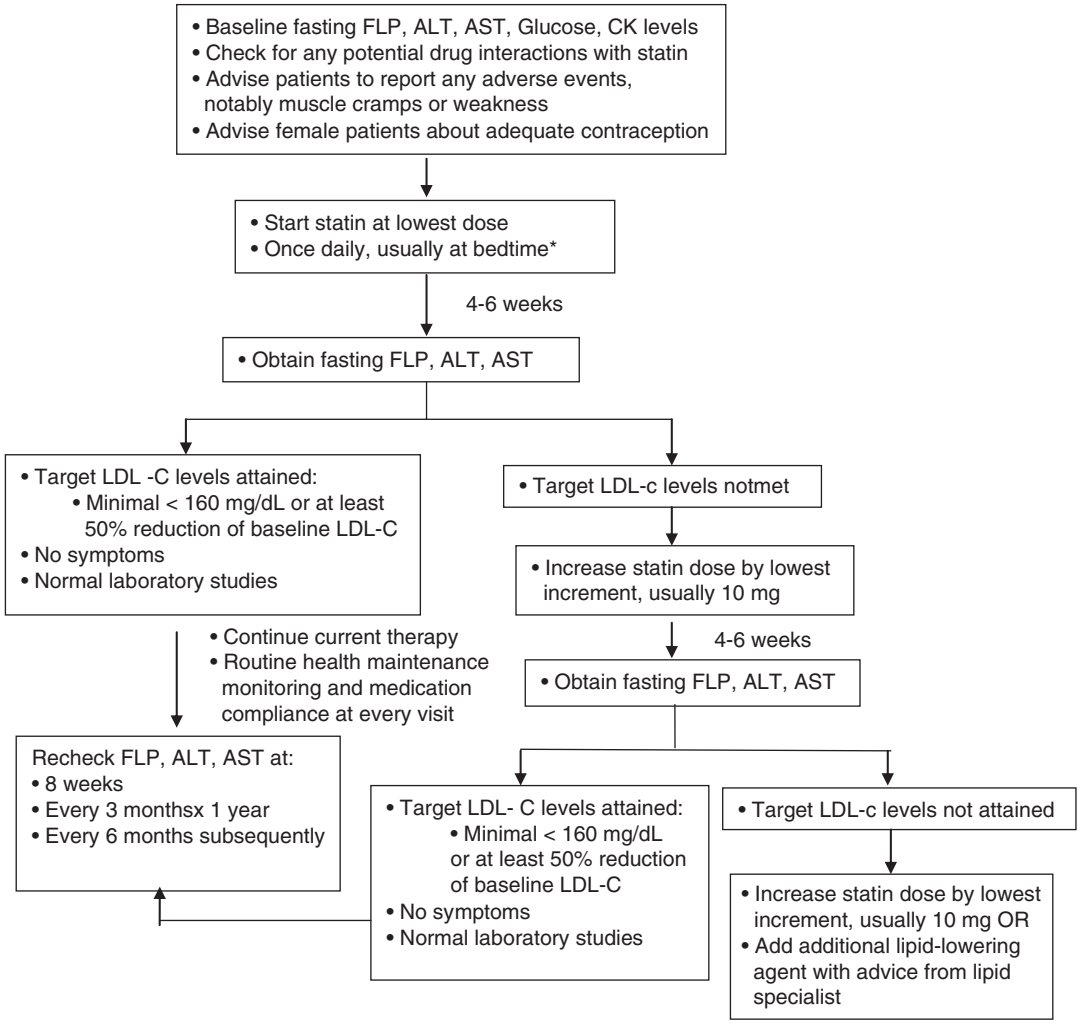
Soluble, viscous fibers form a gel in the gastrointestinal tract and can reduce TC, LDL-C, and non-HDL-C. Common foods that are high in viscous fibers include oats, barley, legumes, some fruits, and vegetables (e.g., legumes, apples, pears, prunes), as well as supplements containing psyllium husk or methylcellulose [40, 41]. Recommendations for children include up to 6 grams/day of supplemental psyllium fiber for ages < 12 years and up to 12 grams/day for children 12 years and older, making sure they are drinking plenty of water while taking fiber.

Medication for Elevated Low-Density Lipoprotein Cholesterol

Because of the severity and genetic nature of HeFH and FCHL, most children and adolescents are unable to attain their LDL-C and/or non-HDL-C targets with diet, over the counter supplements, and lifestyle changes alone.

Medication Initiation

In children 10 years and older with LDL-C over their target levels based on risk factor and/or risk conditions (see Table 26.3), a statin medication should be initiated. The LDL-C cut points for consideration of starting a statin medication are outlined in Fig. 26.2. Children younger than age 10 years usually are not started on LDL-lowering



FLP=fasting lipid profile; ALT=alanine aminotransferase; AST=aspartate aminot ransferase; CK=creatine kinase

*A torvastatin and rosuvastatin can be taken morning or evening due to long half -life

Fig. 26.2 Dietary therapy targets for high LDL cholesterol [14]

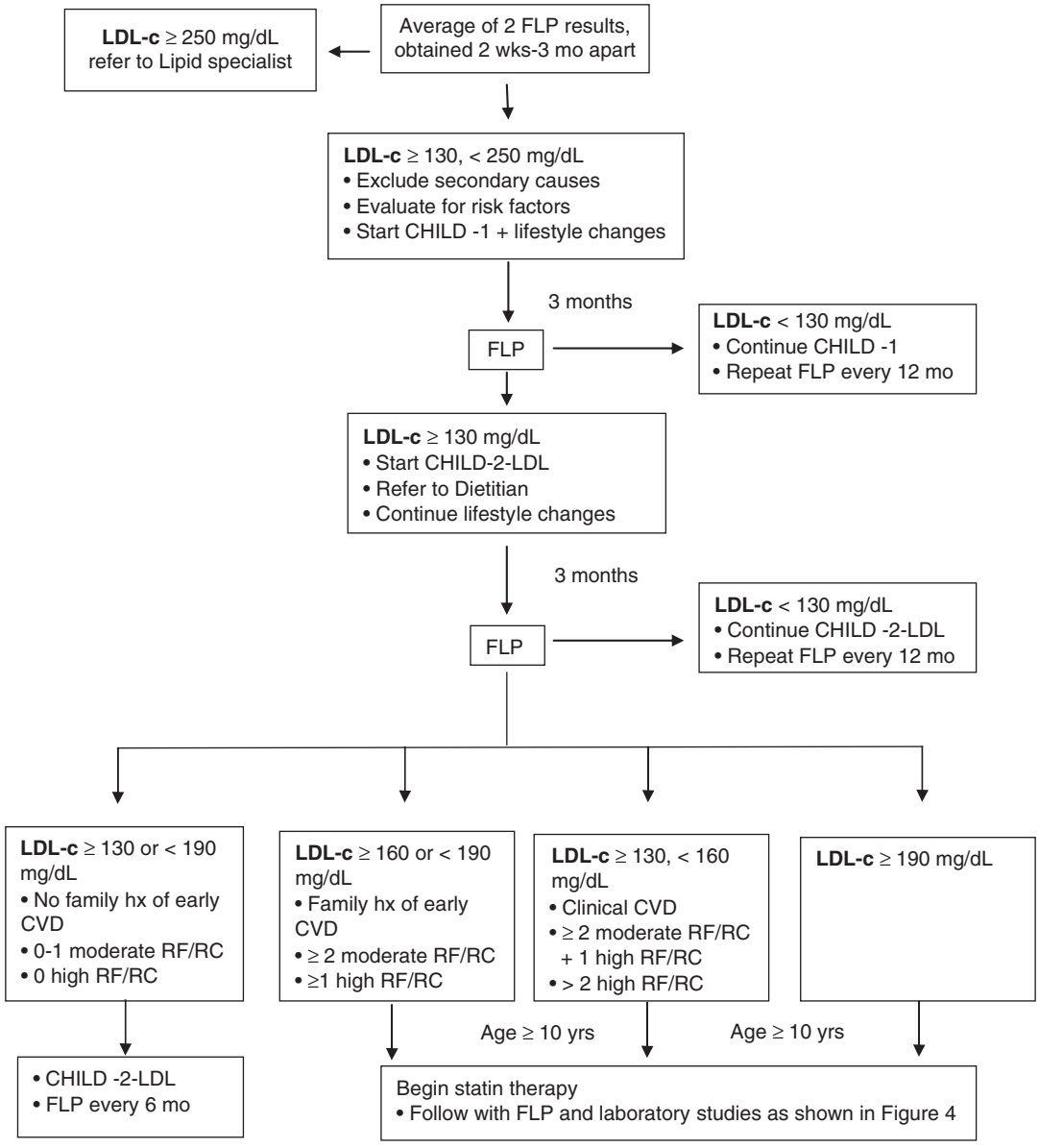


Fig. 26.3 How to initiate, titrate, and monitor children taking statin medication [14]

medication; however, in those children who are at significantly increased risk of developing premature CVD, then a statin should be considered at age 8 years. These include children who consistently have LDL-C levels >190 mg/dL in addition to multiple first- and second-degree relatives with early CVD, at least one high-risk factor/condition, and/or at least two moderate-risk factors/conditions (see Table 26.3 and Fig. 26.2). These patients may ultimately need additional lipid-lowering medication and should be referred to a lipid specialist for further evaluation and treatment.

How to Initiate, Titrate, and Monitor Children on Statin Medication

Figure 26.3 outlines how to initiate, titrate, and monitor children and adolescents on a statin

medication. The lowest possible dose should be started and should be taken once daily, ideally at bedtime. Rosuvastatin and atorvastatin can be taken in the morning due to their long half-lives. Patients should not "double up" if they forget their medication; they should just take it the next day at their usual time.

After approximately 4–8 weeks of daily statin use, an FLP along with hepatic enzymes should be obtained. If LDL-C levels are at target, then continue the statin at that current dose, and re-check the FLP in 3 months and every 6 months thereafter. If normal, liver enzymes should be measured annually if no dose adjustments have been made. However, if the LDL-C remains above target levels despite good compliance, increase the statin by 10 mg increment, and a repeat FLP should be obtained 4–8 weeks later. If target LDL-C is still not obtained and the patient is compliant, then increasing by another increment is reasonable or referral can be made to a lipid specialist for consideration of adding a second lipid-lowering medication [14, 42].

The most common concerns for patients and their families regarding adverse effects are liver toxicity and muscle pains. Before starting a statin, one should assess for contraindications to use, such as current liver disease. Also, a baseline creatinine kinase (CK) level should be checked but does not need to be checked again unless the patient has symptoms. Girls should be counseled that statins may be teratogenic, and this discussion should be documented. It should be noted that the female patient is not pregnant before starting a statin; if she is sexually active, she needs to be counseled about using adequate birth control. Current medications should also be reviewed to assess for potential drug interactions, notably with cyclosporine, niacin, fibrates, erythromycin, azole antifungals, nefazodone, and many HIV protease inhibitors. Grapefruit juice consumption should be limited if the patient is taking atorvastatin, simvastatin, or lovastatin due to inactivation of intestinal CYP3A4, which leads to increased statin blood levels [43].

Muscle side effects, notably pain, cramps, weakness, or any other muscle-based symptoms, should be evaluated in the context of recent physical activity. If concerns for myotoxicity are present, then the medication should be stopped, and the CK level checked. Statin-induced myotoxicity is uncommon and may include asymptomatic elevation in CK levels, myalgia (muscle weakness or pain without CK elevation), myositis (same as myalgia with CK elevation >10 times upper limit normal), and rhabdomyolysis (widespread destruction of muscles with CK > 10 times upper limit normal and decreased renal function/failure). True myotoxicity in children is extremely rare. In fact, a meta-analysis of statin use in the pediatric population found the risk of statin-induced myotoxicity was the same for those taking a statin versus placebo [44]. Nevertheless, if muscle toxicity is suspected, discontinue the statin medication due to the rare possibility of rhabdomyolysis, which can be life-threatening. If CK levels are normal and muscle symptoms resolve with 1–2 days, then restart the statin 1–2 weeks later at the next lowest increment dose (e.g., if on 20 mg, then start at 10 mg), and assess for symptoms. Another alternative is a trial of a different statin.

Minor transaminase level elevations may occur (< 3 times the upper limit normal) after starting a statin. There is no need to discontinue the statin if this occurs as these fluctuations are often transient. If the liver transaminase levels are > 3 times the upper limit of normal, stop the medication, and re-check transaminase levels in 2 weeks. Once levels have normalized, the statin can be resumed, but start at the next lowest increment dose, and re-check levels in 4–6 weeks [42].

While on a statin medication, assess the growth and development at every visit. Each visit should also be a time to discuss a heart healthy diet, compliance with medication, and ways to continue to reduce cardiovascular risk, such as daily exercise, no smoking, and maintaining a healthy weight. Adolescent girls should be reminded about the abstinence from sexual activity or use of appropriate birth control [42].

Table 26.6 Medications for reducing elevated LDL-c in children and adolescents [42]

Medication class	Medications and starting doses	Effect on lipids	Potential side effects
HMG-CoA reductase inhibitors (statins)	Atorvastatin[a]: 10 mg QHS or QD Lovastatin[a]: 10 mg QHS Pravastatin[b]: 10 mg QHS (8–13 Y) 10 mg QHS (14–18 Y) Simvastatin[a]: 5 mg QHS Rosuvastatin[a]: 5 mg QHS or QD	Decrease LDL-C Increase HDL-C Decrease TG	Elevated liver enzymes, CK levels, hemoglobin a1c; muscle cramps and weakness; myopathy; rhabdomyolysis
Bile acid sequestrants	Cholestyramine[c]: 2 gm daily divided 2–4 times per day Colestipol granules[c]: 5gm QD Colesevelam HCl for Oral suspension[a]: 1 packet (3.75 gm packet) QD or 1 (1.875 gm packet) BID mixed with liquid	Decrease LDL-C Increase HDL-c Increase TG	Gastrointestinal symptoms, including: bloating, gas, abdominal cramping
Cholesterol absorption inhibitors	Ezetimibe[a]: 10 mg QD	Decrease LDL-C Increase HDL-c Decrease TG	Elevated liver enzymes; gastrointestinal upset, myopathy

BID twice daily, *CK* creatine kinase, *gm* grams, *QHS* every night, *QD* every day, *Y* years
[a]FDA approved for children 10 years and older
[b]Approved for pre-pubescent children (age 8 years and older)
[c]Not FDA approved for children

Therapy Goals

After statin initiation, the therapy goal is LDL-C ≤ 160 mg/dL or 50% of the starting LDL-C. Once that is achieved, a goal of ≤ 130 mg/dL can be considered, with an ideal goal of ≤ 110 mg/dL. There needs to be a balance between achieving desired LDL-C level and increasing medication dosage, as increasing doses leads to smaller LDL-C-lowering effects but with the increased risk of more side effects.

Statins (Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitors)

Statin medications or hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors are the first-line therapy to reduce LDL-C and non-HDL-C in the pediatric population [13, 34, 42]. Statins reduce the amount of intracellular cholesterol by inhibiting the rate-limiting enzyme in endogenous cholesterol biosynthesis, HMG-CoA. This leads to upregulation of hepatic LDL-C receptors, thereby increasing hepatic clearance of LDL-C from the circulation. Statins also decrease VLDL and intermediate-density remnants, which results in modest TG lowering, which is generally larger in those with an elevated TG level (≥ 150 mg/dL). They may also slightly increase HDL-C levels.

The FDA-approved statins for use in the pediatric population are listed on Table 26.6 for ages 10 years and older (≥8 years for pravastatin). The safety and efficacy of statin use in the pediatric population has been demonstrated in several clinical trials, including through the pubertal years [45–48]. Also, there are no differences in numbers of adverse events in children compared to the adult population [44]. Studies in children have also shown that statins can slow the progression of atherosclerosis, thereby decreasing the future risk of developing CVD if started at a younger age [45, 48].

Bile Acid Sequestrants

Bile acid sequestrants used to be the initial medication of choice for children as they were felt to be safer due to their lack of absorption [10].

However, their poor palatability and resulting poor compliance along with the well-documented safety and efficacy of statin medications have made bile acid sequestrants second- or third-line therapy for lipid lowering in children [14]. Bile acid sequestrants work by binding bile salts in the intestinal lumen, which decreases bile salt absorption, leading to decreased levels of bile salts in the liver. Less hepatic bile salts signals the liver to convert cholesterol to bile salts, thus depleting hepatic intracellular bile salts, which then signals the upregulation of LDL-C receptors to increase clearance of LDL-C from the circulation. Bile acid sequestrants usually lower LDL-C by 10–20%. They can raise HDL-C slightly but may also moderately increase TG levels [49–51]. Bile acid sequestrants may be used along with a statin medication in those children who do not meet their LDL-C target with statins alone with no increase in adverse events [51].

Cholesterol Absorption Inhibitors

Ezetimibe should be considered as a second medication for those children who do not meet LDL-C targets while on a statin alone [14] and is dosed at 10 mg daily. It inhibits cholesterol absorption at the level of the intestinal villi, decreasing the absorption of dietary cholesterol, upregulating LDL-C receptors, and improving LDL-C clearance from the circulation. The addition of ezetimibe can lead to an additional 20% of LDL-C lowering when used with a statin without significantly increased adverse events [52–54].

Clinical Case 2 Follow-Up

T.S. returned to clinic after 6 months of dietary and lifestyle changes. He packed his lunch for school, joined the soccer team, and lost 5 pounds. However, his cholesterol did not change significantly. He was started on atorvastatin 10 mg and was up-titrated to 20 mg without any side effects. He was compliant with his medication. His most recent laboratory studies showed (in mg/dL) TC 198, HDL-C 58, LDL-C 140, and TG 72.

Other Common Genetic Dyslipidemias

Familial Hypertriglyceridemia

Familial hypertriglyceridemia (FHTG) is an autosomal dominant lipid disorder occurring in approximately 1 in 500 people in the general population. Certain lifestyle factors or medications can increase VLDL production and worsen hypertriglyceridemia. These may include diets high in simple carbohydrates, being overweight or obese, having insulin resistance, drinking alcohol, or receiving estrogen therapy. This should be discussed with these patients routinely.

FHTG is caused by increased VLDL production and/or decreased VLDL catabolism. The lipid profile is notable for significantly elevated TGs (250–1000 mg/dL), normal or mildly increased TC (usually < 250 mg/dL), and low HDL-C. LDL-C levels are generally low or normal, and apolipoprotein B levels are normal. The ratio of TC/TG tends to be lower in FHTG than in FCHL. A more severe form of FHTG is due to increased production of both VLDL and chylomicrons. These patients generally have TG levels ≥ 1000 mg/dL and are at risk for developing pancreatitis, notably when the TG levels increase to > 2000 mg/dL. The presence of eruptive xanthomas (see Fig. 26.4) may be seen in patients with TG levels ≥ 1000 mg/dL.

Therapy for Hypertriglyceridemia

Dietary Therapy Goals

With focused attention to dietary changes and increased physical activity, a majority of pediatric patients see either normalization or improvement in the triglycerides. After 6 months of the CHILD-1 and then CHILD-2-TG diet, which emphasizes decreased carbohydrate intake, along with weight loss or stabilization if needed, if the TGs are ≤ 100 mg/dL (age < 10 years) or <130 mg/dL (age ≥ 10 years), the child should continue with the CHILD-2-TG diet and check an FLP annually. If the TGs

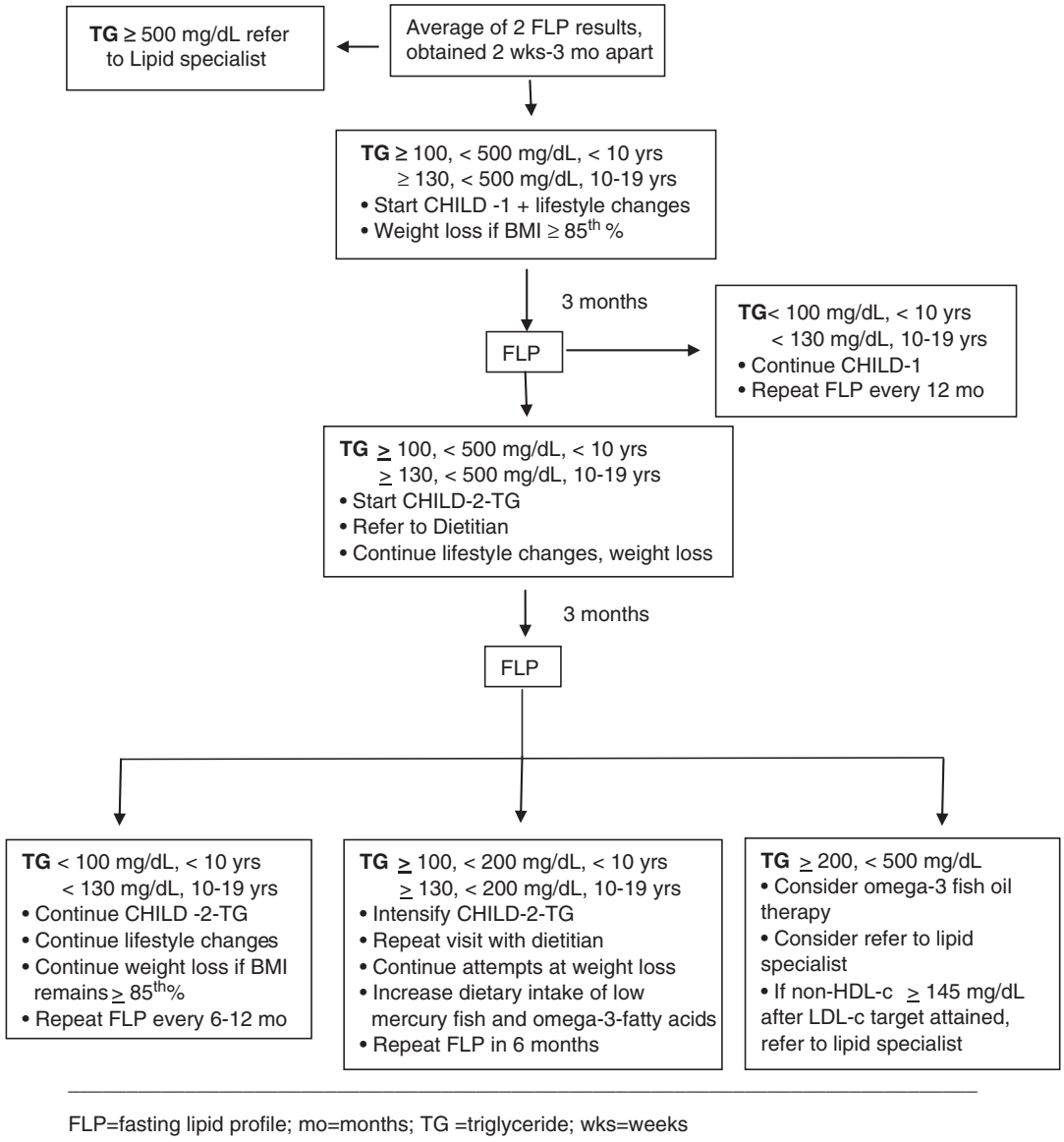


Fig. 26.4 Eruptive xanthoma in an adult patient with triglyceride levels > 1000 mg/dL. (From Leaf [63], with permission from Elsevier)

remain ≥100 mg/dL but < 200 mg/dL (age < 10 years) or > 130 mg/dL but <200 mg/dL (age ≥ 10 years), then the child should continue or intensify CHILD-2-TG and should continue with weight loss/stabilization if needed and increase dietary omega-3 intake and repeat the FLP in 6 months. If the TGs remain ≥ 200 mg/dL, referral to a lipid specialist is advised. Also, if the average of two fasting TG levels is at least 500 mg/dL or a single fasting TG level is 1000 mg/dL or higher, this is likely due to a genetic hypertriglyceridemia, and the patient should be referred to a lipid specialist.

Pharmacologic Therapy

Medication is rarely needed to treat hypertriglyceridemia in children and adolescents. Dietary changes and increasing physical activity, along

with weight loss when needed, are usually enough to lower TG levels.

Long-Chain Omega-3 Fatty Acids

Omega-3 fatty acids (or fish oil) decrease the release of VLDL-C by reducing the synthesis of hepatic fatty acids and TG and also increase fatty acid degradation Higher doses of 2–4 grams/day have been shown to be safe and effective in adults and can lower TG by 30–45% and increase HDL-C levels by 6–17%; however, some formulations (containing docosahexaenoic acid) may increase LDL-C levels up to 31% [55]. There have been mixed results in the adult literature regarding primary CVD reduction with TG lowering from omega-3 fatty acids [56, 57], but those with elevated TGs and low HDL-C levels appear to be a subgroup in which CVD risk reduction may be seen [58]. Indeed, the use of 4 grams daily of icosapent ethyl, a purified eicosapentaenoic acid ethyl ester, in adults with continued hypertriglyceridemia while on a statin, was associated with a significantly lower (25%) risk of ischemic events in those taking the medication compared to placebo [59]. In children, 1 small randomized trial of 25 patients with hypertriglyceridemia taking 4 grams of prescription fish oil (Lovaza) did not show significant difference in the treatment versus the placebo groups [60]. There have been no reports of adverse effects on muscle, liver, or glucose levels [55]. Dosing for TG-lowering effect should be 2000–4000 mg daily of the eicosapentaenoic acid (*EPA*) and docosahexaenoic acid (*DHA*) *components of the fish oil capsule.*

Fibric Acid Derivatives

Fibric acid derivatives, or fibrates, increase clearance of VLDL-C and TGs and decrease hepatic synthesis of TG, which serves to lower TGs and increase HDL-C levels. While not FDA-approved for children, a small study in children demonstrated efficacy in lowering TG levels [61]. Fibrates should only be prescribed in the pediatric population under the guidance of a lipid specialist.

Therapy Goals

Triglyceride goals should be < 200 mg/dL, ideally < 130 mg/dL for children ages 10–19 years and < 100 mg/dL for children age 10 years and younger. Also, a decrease in BMI to < 85th percentile should be targeted as reducing BMI helps with decreasing TG levels.

Conclusion

Dyslipidemia in childhood places youth at risk for premature atherosclerosis in adulthood. After identification, the first step to help improve lipids is nearly always dietary changes and increase in physical activity. Even small changes can impact lipids, weight, and cardiovascular risk and often will obviate the need for lipid-lowering medication. In those with genetic dyslipidemias, while diet and lifestyle are helpful, these patients commonly will need prescription medication. Statins are the medication of choice for LDL-C reduction in the pediatric population. Genetic testing should be offered to those with suspected HeFH, along with their family members. In suspected HeFH, referral to a lipid specialist is beneficial. Omega-3 fatty acids may be used for continued significantly elevated TGs, if there has been diet and lifestyle modification but no appreciable decrease in TG levels. It is imperative that those who care for children and adolescents identify patients at risk for early CVD and treat or refer them appropriately. Only by identifying and treating young patients with dyslipidemia will we have the potential to reduce cardiovascular risk and hope to improve the lives of these at-risk children.

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Lipoprotein Subfractions in Clinical Practice

27

Jeffrey W. Meeusen

Introduction

In clinical discussions of cardiovascular risk, the term "lipids" has become synonymous with cholesterol. The focus on cholesterol can be traced back to a report on the residents of Framingham, MA [1]. The study found a strong association between blood cholesterol concentrations and risk for coronary artery disease. Subsequent studies identified that the association between blood cholesterol and cardiovascular disease varied by the concentrations of cholesterol carried by different lipoproteins.

The necessity to distinguish serum cholesterol according to density and electrophoretic migration was emphasized in a series of articles in continuous issues of the *New England Journal of Medicine*, which codified the Fredrickson phenotypes [2–6]. The cholesterol fractions were empirically defined according to methods available at the time. Blood cholesterol with a density between 1.019 and 1.063 g/L and an electrophoretic migration in the "beta" region eventually became known as low-density lipoprotein (LDL) cholesterol [7]. Cholesterol migrating in the "alpha" region with a density >1.063 became known as high-density lipoprotein (HDL) cholesterol.

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These early lipoprotein fractions allowed distinction between cholesterol measures that were associated with increased or decreased atherogenic burden. As continued technological advances have allowed for lipoprotein subfractionation with increasing granularity, the prevailing hypothesis is that these separations will further isolate the most atherogenic measures of cholesterol and lipoproteins. The objectives of this chapter are to describe the physiological basis of lipoprotein heterogeneity, distinguish between available clinical methods for lipoprotein subfraction measurement, and evaluate the clinical benefits of lipoprotein subfraction measurements in patient treatment decisions.

Lipoproteins

Lipoproteins are spherical particles of lipids formed around proteins. Lipoproteins enable transport of insoluble lipids, such as cholesterol, through the aqueous blood. Solubility is maintained by locating polar portions of apolipoproteins and polar lipids (e.g., phospholipids) at the outer surface of the particle. The interior or lipoprotein "core" contains completely nonpolar lipids (also known as neutral lipids), such as triglycerides and cholesteryl esters. The physiological function of lipoproteins is redistribution of lipids between organs and tissues via the bloodstream.

M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_27

Plasma lipoproteins are continuously remodeled while circulating throughout the body. Lipid metabolizing enzymes and transfer proteins enable lipid redistribution and have a major impact on the size and density of lipoproteins measured in blood plasma. The metabolic pathways of lipoproteins are covered in detail elsewhere in this book. However, a brief review is relevant emphasizing the relationship between lipid transport and lipoprotein size and density.

Lipoprotein Subclass Heterogeneity as a Function of Triglyceride Transport

Triglyceride redistribution is accomplished by apolipoprotein B (apoB)-lipoproteins. Hepatocytes synthesize apoB, which acts as the scaffold of very-low-density lipoprotein (VLDL). Enterocytes synthesize a truncated apoB isoform (apoB-48), which forms chylomicrons. ApoB is the primary structural component around which the lipid particle is formed in both VLDL and chylomicrons.

Once in the blood circulation, VLDL and chylomicrons interact with lipoprotein lipase (LPL). LPL is expressed on the capillary endothelium in peripheral tissues and hydrolyzes triglycerides into free fatty acids. Triglycerides are neutral lipids stored in the core of the lipoprotein. As LPL breaks down the triglycerides, the free fatty acids generated move to the lipoprotein surface and eventually out of the lipoprotein and into the surrounding tissue. The net result of lipase activity is that triglycerides are lost. The loss of triglycerides causes a restructuring of the lipoprotein to accommodate the smaller core. The triglyceride-depleted VLDL are smaller lipoproteins referred to as intermediate-density lipoproteins (IDL). The lipolysis products formed from chylomicrons are referred to as chylomicron remnant lipoproteins (CRLP).

Hepatic lipases (HL) further reduce the triglyceride content of VLDL, chylomicrons, IDL, and RLP. Under healthy conditions, IDL and CRLP are rapidly removed from the blood circulation via two hepatic receptor proteins: the low-density lipoprotein receptor (LDLR) and LDLR-related protein (LRP). Both LDLR and LRP have a high affinity for apoE, while LDLR also has some weak affinity for apoB.

In normal physiology, apoE-facilitated clearance prevents significant accumulation of IDL or chylomicron remnants. Unfortunately, apoE-mediated clearance is not completely efficient. As the IDL and remnant lipoproteins continue to shrink due to the removal of core triglycerides, excess surface components (phospholipids, non-esterified cholesterol, and apoE) are lost and find their way to the HDL pool (more on this in the next section) [8]. When apoB-lipoproteins are too small to carry any apoE, the result is LDL.

LDL is enriched with cholesterol and contains no apolipoproteins other than apoB. Clearance of LDL relies on the low-affinity interaction between the LDLR and a single apoB ligand per particle. Consequently, the in vivo half-life of LDL is significantly longer than other apoB-lipoproteins.

Lipase activity and triglyceride redistribution lay the foundation of apoB-lipoprotein size and density distribution. This pathway of triglyceride transport is why circulating apoB-lipoproteins can, and do, exist in a continuous size and density distribution from VLDL with diameter >80 nm and density <1.006 g/L to small LDL with diameters ~20 nm and density as high as 1.063 g/L.

Lipoprotein Subclass Heterogeneity as a Function of Cholesterol Transport

ApoA-I is the structural protein of HDL. In lipid metabolism, a key role of apoA-I (and HDL) is removal of excess cholesterol from blood plasma and peripheral tissues. This process is referred to as reverse cholesterol transport.

Like apoB, apoA-I is synthesized by both hepatocytes and enterocytes. However, while apoB-lipoproteins are fully assembled prior to secretion, apoA-I is released directly into the circulation. This lipid-poor apoA-I is referred to as nascent HDL (also called preß-HDL due to its electrophoretic migration pattern) [9].

The nascent HDL is converted to a mature HDL by the activity of lecithin-cholesterol

acyltransferase (LCAT). LCAT is a plasma enzyme that combines cholesterol and free fatty acids into cholesteryl esters. These cholesteryl esters are transferred to the nascent HDL where they add to the core of the growing HDL particle [10].

The mature HDL particle is larger and more spherical than the disc-shaped nascent HDL. This shape leads to a conformation change exposing the apoA-I binding site specific for the scavenger receptor class B, type I (SR-BI) [11]. SR-BI is expressed on adrenal glands and hepatocytes and facilitates rapid cellular internalization of HDL (and the cholesterol it carries). This is the underlying reason that HDL particles also exist in a continuous distribution of size and density ranging from 7.2 nm to 12.9 nm and 1.063 g/L to 1.170 g/L [12].

Finally, cholesteryl ester transfer protein (CETP) is an important enzyme implicated in both the cholesterol and triglyceride transport pathways of apoB and apoA-I. CETP enables the exchange of a triglyceride in one lipoprotein for a cholesteryl ester in another lipoprotein [13]. CETP binds to VLDL, IDL, LDL, and HDL with equal affinity; however, >80% of CETP is associated with HDL. This is consistent with the observation that the molar concentration of HDL is an order of magnitude larger than VLDL, IDL, and LDL combined [14].

It is hypothesized that CETP activity is a primary mechanism in the formation of small-dense LDL and large buoyant HDL [15]. The net activity of CETP is transfer of triglycerides from VLDL to HDL. This forms triglyceride-enriched HDL which can potentially bind apoE and therefore increase hepatic clearance via LDLR and LRP [8].

The mechanism of CETP-mediated small-dense LDL formation is less clear. It has been theorized that lipase activity on triglyceride-depleted and cholesterol-enriched apoB-lipoproteins creates a small-dense LDL. Analyses of plasma from subjects taking CETP inhibitors are potentially contradictory to this theory. In two studies of different CETP inhibitors, subjects on treatment had a relative increase in LDL size compared to placebo [16, 17].

LDL Subfractionation Methods

Density-based subfractionation by analytical ultracentrifugation is considered the benchmark in lipoprotein subfractionation [18]. However, there is no gold-standard method for lipoprotein subclassification or consensus nomenclature defining small versus large lipoproteins. Despite these limitations, several methods are routinely used by clinicians to determine treatment strategies. While certainly not an exhaustive summary, the most common methods currently in clinical use are discussed below.

Gel Electrophoresis

Separation of apoB-lipoprotein by polyacrylamide gradient gel electrophoresis was one of the original methods to identify small versus large LDL cholesterol [19]. Electrophoresis separates primarily on size (and, to a lesser extent, charge), which deviates from the benchmark ultracentrifugation method that separates on density. However, gel electrophoresis can be easily implemented in clinical laboratories and the technique allowed for commercialization of kit-based systems.

Gel electrophoresis techniques claim to distinguish up to seven unique size categories for LDL [20]. Modeling the distribution of LDL cholesterol staining patterns led to a binary classification system. According to the seminal work by Austin and colleagues in 1986, subjects with a majority of LDL cholesterol migrating at bands ≥25.5 nm were considered pattern A. Subjects with smaller LDL cholesterol were considered pattern B [21].

A systematic review of commercial gel electrophoresis systems found that the classification pattern of a given patient varied significantly based on the LDL subfractionation method used [22]. Classification according to pattern A or B was discrepant in as many as 76% of cases. A 2012 report on commercially available gel electrophoresis systems found that subjects were classified with >80% concordance despite the fact that the methods

compared used different cutoffs for small LDL (25.5 nm and 26.8 nm) [23].

Density Gradient Ultracentrifugation

Density gradient ultracentrifugation performed by adjusting the solution density and sequentially separating the supernatant is, in fact, the benchmark method. However, a commercial method known as the vertical auto-profile (VAP) uses a single-step gradient ultracentrifugation method [24]. The VAP test measures cholesterol as does gel electrophoresis. However, VAP separates cholesterol fractions based on density as in analytical ultracentrifugation. The concordance of LDL pattern classification between VAP and gel electrophoresis methods is reported to be between 11% and 73%. The majority of discrepant cases are comparing pattern B by VAP and pattern A by gel electrophoresis [22, 25, 26].

Nuclear Magnetic Resonance

Measuring lipoprotein particles by ^{1}H nuclear magnetic resonance (NMR) spectroscopy was first described by Otvos and colleagues [27]. Several commercially available NMR-based methods are now in routine clinical use [28–31]. NMR is distinct from previous methods in two major aspects. First, NMR subfractional analysis does not require separation of fractions. NMR measures the nuclear resonance of protons within lipoproteins. These protons resonate with differing frequency depending on the size and density of the lipoprotein. Lipoprotein particle sizes are derived from the relative amplitude of the proton NMR signal for each subclass.

The second major difference is that NMR directly measures the number of lipoprotein particles. This is unique in that all other methods measure the concentration of cholesterol carried within a given lipoprotein. Despite this fundamental difference in the molecular moiety measured, NMR has a high degree of concordance with VAP and gel electrophoresis [25, 26, 32, 33].

Ion Mobility

The ion mobility (IM) subfraction method works on the principle that particles of a given size and charge behave in a predictable manner when carried in a laminar flow of air and subjected to an electric field. An ultracentrifugation step is required to remove albumin, and the concentration for particles between 1.7 nm and 54 nm is measured [34]. Comparisons between IM, gel electrophoresis, VAP, and NMR have reported 70–90% concordance in the classification of LDL phenotype patterns [26].

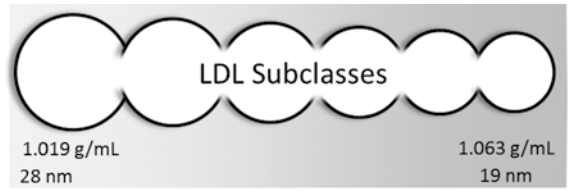
Direct Measurement of Small, Dense LDL Cholesterol

Direct measurement of the small, dense LDL cholesterol subfraction without any separation of other lipoproteins was first reported in 1998 [35]. The method uses various surfactants and modified cholesterol esterase enzymes and is able to be used on standard health system clinical chemistry analyzers. The method compares reasonably well to preparative ultracentrifugation and other subfraction methods [36, 37] (Fig. 27.1).

Clinical Utility of LDL Subfractions

No medical society has endorsed the use of lipoprotein subfractions. The National Lipid Association, the National Academy of Clinical Biochemistry, the American College of Cardiology, and the American Heart Association (AHA) have all published guidelines specifically recommending *against* the use of LDL subfractions [38–40]. The guidelines cite a lack of sufficient evidence to support LDL subfraction measurement for initial clinical assessment or on-treatment management decisions.

None of the LDL subfraction methods in clinical use have sought Food and Drug Administration (FDA) clearance for subfractionation of LDL. The commercial interests associated with the VAP test sought and received FDA clearance based on substantial equivalence to traditional methods for mea-



Method	Measure	Separation	Phenotype Cutoff
Gradient Gel Electrophoresis	Cholesterol	Size	Pattern A 26.5 nm Pattern B
VAP	Cholesterol	Density	Pattern A 1.04 g/mL Pattern B
NMR	Methyl-protons (particles)	Size	Pattern A 20.5 nm Pattern B
Ion Mobility	Particles	Size	Pattern A 21.6 nm Pattern B
Direct sdLDL-C	Cholesterol	Density	Pattern A 1.045 g/mL Pattern B

Fig. 27.1 Comparison of clinical LDL subfractionation methods

suring VLDL-C, LDL-C, and HDL-C but made no claims regarding LDL subfractions [41]. Similarly, the interests associated with the NMR test sought and received FDA clearance based on substantial equivalence between traditional enzymatic methods for LDL-C, HDL-C, and triglycerides and NMR-measured total LDL particle number (concentration), HDL-C, and triglycerides [42]. Many other methods remain as laboratory developed tests that have not been reviewed by the FDA.

The most recent method to be cleared by the FDA was the direct homogeneous method for small-dense LDL cholesterol [43]. As for other methods, it was cleared based on its substantial equivalence to LDL cholesterol and made no mention of lipoprotein subfractions. Unlike most FDA clearance documents for LDL subfractions, the direct homogeneous method included clinical data with cardiovascular outcomes from a large epidemiological study. The study was of the Atherosclerosis Risk in Communities (ARIC) cohort and was subsequently published [44]. In the published report, the authors conceded that "small dense LDL-C was not significantly associated with risk for incident coronary heart disease after further adjustment for other lipid risk factors, such as LDL-C, apo B, or total cholesterol."

The lack of independent predictive information has been repeatedly cited in many opinion papers and consensus documents [20, 45, 46]. In the years since all the relevant medical societies issued guidelines recommending against LDL subfractions [38–40], new investigations from several large epidemiological studies with cardiovascular outcomes have been published. In most studies, the association between LDL subfractions and outcomes was not adjusted for LDL cholesterol or apoB [45, 47]. In every instance where adjustments for total LDL cholesterol, total LDL particles, or apoB were reported, LDL cholesterol subfractions were no longer significantly associated with outcomes [44, 48–52].

The conclusion, after decades of research, is that small, dense LDL is highly correlated with total LDL and does not provide independent predictive value. This is the first of four criteria for novel risk markers published by the AHA, which LDL subfraction testing does not meet [53]. The AHA recommends that novel risk markers should (1) add predictive information to established risk markers, (2) predict future outcomes in prospective studies, (3) improve clinical outcomes, and (4) be determined as cost-effective when compared to established risk markers. No prospective studies using LDL subfractions to guide treat-

ment have been reported; therefore, no data on the use LDL subfractions to improve clinical outcomes have been reported. Finally, while LDL subfraction methods are relatively low cost (compared with imaging or genetic analyses), it is difficult to claim a non-informative marker is cost-effective at any price.

In the absence of clinical data demonstrating independent utility of LDL subfractional analysis, proponents often refer to the associations between LDL subfractions and atherosclerosis pathophysiology. Small, dense LDL particles are considered to have greater susceptibility to oxidation [54], augment inflammation [55], have stronger binding capacity to the endothelium [56], and greater infiltration into the subendothelial space and interactivity with subendothelial proteoglycans [57]. While there may be merit to these scientific findings, these considerations alone do not justify the use of LDL subfractions in clinical practice.

HDL Subfractions

The inverse association between HDL cholesterol and cardiovascular risk is well established. Furthermore, there is much evidence to support HDL functions as antioxidative, anti-inflammatory, antithrombotic, and anti-atherogenic. However, several clinical trials and experiments that successfully increased HDL cholesterol failed to improve outcomes [58–62]. Studies of genetic variants that raise or lower the level of HDL cholesterol have also not shown associations with cardiovascular disease risk, which contrasts to results for studies of variants that raise or lower LDL cholesterol and VLDL cholesterol (triglyceride-rich lipoprotein cholesterol). The inconsistent findings for HDL cholesterol have led to a general appreciation that HDL functionality is the preferred measure and that it is much more complex than HDL cholesterol concentration [63, 64].

The variety of HDL physiological functions is made possible by the heterogeneity of circulating HDL particles. Direct measures of HDL activity are available but remain limited to research applications at present. Reverse cholesterol transport studies have demonstrated that the size and composition of HDL can influence the mechanism and capacity for cholesterol efflux and other potential protective activities [65]. Thus, measurement of HDL size and composition is hypothesized to be a reasonable surrogate for protective HDL activity.

As in apoB-lipoproteins, HDL exists in a polydisperse range of sizes and densities. Moreover, the phospholipid, cholesterol, triglyceride, and protein composition of HDL subfractions are much more heterogeneous in comparison to LDL subclasses. Recent reports suggest that the functional activity of HDL may stem from the protein and lipid composition of HDL [66] and that the functionality of a given subfraction can be altered without a change in size [67].

As in the case of LDL subfractions, there is no standard method or definitions for HDL subfractions. A proposed nomenclature was published by authors representing the various commercial laboratories that offer HDL subfraction testing [12]. Unfortunately, the nomenclature was not adopted and no commercially available testing follows the guidelines (Fig. 27.2).

Comparisons of HDL subfractionation methods have not been published as extensively as comparisons of LDL subfraction methods. One study compared a gradient gel electrophoresis method, VAP, and NMR. The authors normalized the relative concentrations of large, medium, and small HDL reported to the total HDL measured. In this way, correlation and bias between the various methods were quantifiable [68]. Unsurprisingly, the study found a large amount of disagreement across methods.

In the case of HDL, the approach of normalization does not adequately address some confounders. As mentioned before, the definitions of large, medium, and small HDL are not consistent, even among methods that separate according to the same parameter [12]. In addition, the various methods measure a different component of HDL (i.e., apoA-I, cholesterol, or particle number). We cannot assume that changing the relative amount of one HDL component should require an equal change in another component. There is no evidence to support this assumption and some which contradicts this view [66].

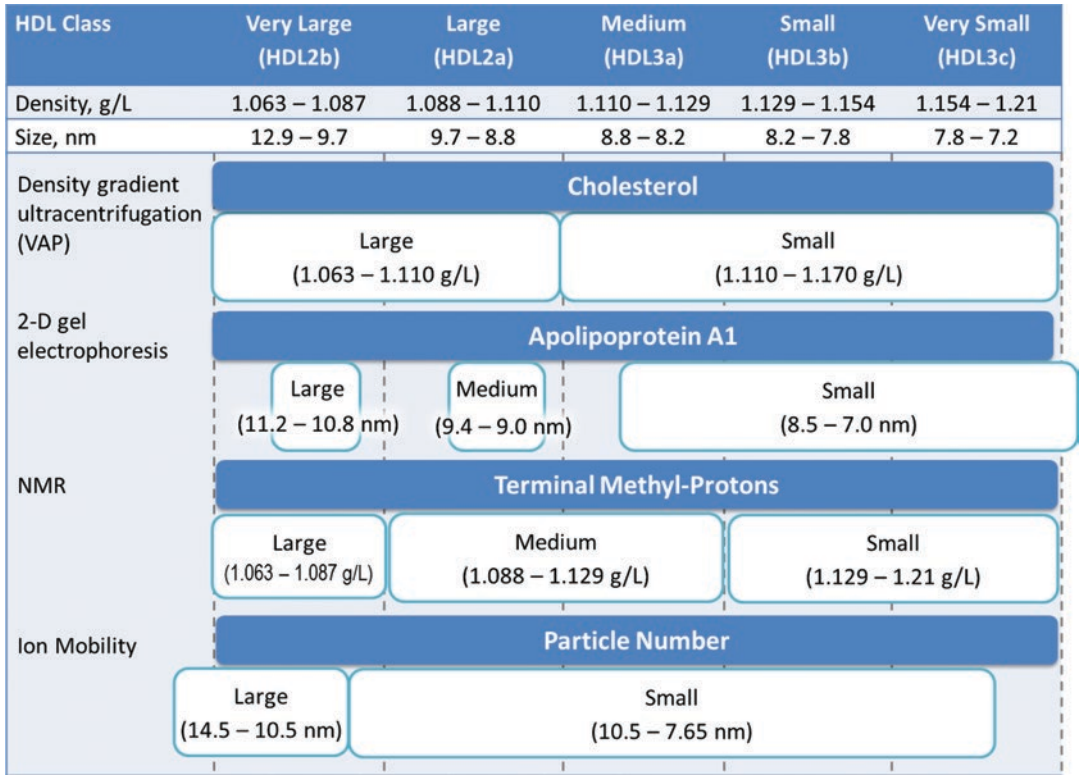


Fig. 27.2 HDL subfractionation methods separate by different techniques, measure different lipoprotein components, and define large, medium, and small by different criteria

As an example, we can consider the relationship between apoA-I and HDL particle concentration. ApoA-I is the primary apolipoprotein of HDL. Unlike apoB, which always exists in a 1:1 ratio per lipoprotein particle, the stoichiometry for apoA-I varies. Studies have shown that a given HDL may contain between one and five copies of apoA-I. There are some steric constraints that dictate in order for the number of apoA-I copies per each HDL particle to increase, HDL size must also increase. However, a larger HDL does not necessarily require more copies of apoA-I [69].

Several clinical studies using HDL subfractions have also been reported. Data regarding the association of specific HDL subfractions with cardiovascular outcomes are conflicting. Increases in small HDL (α3 and pre-ß1 HDL) measured by 2D electrophoresis were positively associated with coronary heart disease [70]. However, a VAP study found that small HDL cholesterol was inversely associated with myocardial infarction and mortality [71]. Furthering the confusion are data that suggest that medium and large, but not small, HDL particle concentrations correlate with cholesterol efflux capacity [72].

The clinical outcome findings, as in the analytical comparison of HDL subfraction methods, are confounded by the fact that different methods measure different HDL components. HDL cholesterol is primarily esterified and stored in the lipoprotein core [66]. Simple math for spherical surface area and volume confirms that minimal increases in concentration of larger HDL particles allow for exponential increases in large HDL cholesterol concentration. Thus, we cannot expect similar relationships between methods when we are measuring particles versus cholesterol content in subfractions separated by different means and defined by different limits [73].

Lipoprotein Subfractions and the Practicing Clinician

In summary, there are many commercially available methods for lipoprotein subfractionation. None of the subfraction methods are approved by the FDA. No medical society endorses the use of lipoprotein subfractions for patient care. The most relevant medical societies in lipid and cardiovascular care all recommend against the use of subfractions (including the AHA and the National Lipid Association). The lipoprotein component measured, the means of subfractionation, and the definition of large, medium, and small are not standardized. Clinical studies investigating the utility of lipoprotein subfractions report no benefit over standard lipid measures. In the case of HDL, there are conflicting reports regarding whether small or large lipoproteins are associated with cardiovascular outcomes. These methods provide interesting academic findings and may eventually lead to improved patient care.

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Ion Mobility Lipoprotein Analysis

28

Sarah M. King and Ronald M. Krauss

Plasma Lipoproteins, Lipoprotein Heterogeneity, and Cardiovascular Disease

The relationships between lipoprotein concentrations and cardiovascular disease (CVD) risk have been recognized since the 1950s [1]. Standard lipid tests measure low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides and are a cornerstone of cardiovascular disease prediction and management. Though the clinical utility of standard lipid tests is well established, a significant proportion of cardiovascular disease risk is not addressed by these tests.

Each of the major plasma lipoprotein classes – HDL, LDL, intermediate-density lipoproteins (IDL), and very-low-density lipoproteins (VLDL) – consists of multiple subclasses of lipoprotein particles with differing size, composition, and pathophysiologic properties [2, 3]. Because of their atherogenic nature, LDL particles are major targets in CVD prevention and management. Both the identification of at-risk patients and the goals for therapeutic intervention have been based on LDL-C values, but this measurement does not consider the role of LDL particles and their heterogeneity. In particular, multiple distinct subclasses of LDL particles can be discriminated on the basis of particle diameter [4, 5]. At least eight subspecies of LDL particles have been identified and grouped into four categories based on size as well as density: LDL-1 (large and buoyant), LDL-2 (medium size and density), LDL-3 (small and dense), and LDL-4 (very small and dense) [4–8]. Moreover, peak LDL diameter displays a bimodal distribution in humans, with a predominance of large LDL designated as "pattern A" and predominance of small LDL as "pattern B" [5]. The small LDL phenotype is positively correlated with plasma triglyceride and inversely related to plasma HDL-C; this cluster of traits defines an "atherogenic lipoprotein phenotype" [9], which has also been designated "atherogenic dyslipidemia".

As discussed further below, high levels of small, dense LDL particles (sdLDL) are associated with increased CVD risk [10–15]. Recently, elevated sdLDL cholesterol has been shown to predict the development of coronary heart disease (CHD) in two large cohort studies, independent of standard lipids, including total LDL cholesterol, whereas there was no relation of CHD to levels of large, buoyant LDL-C [16, 17]. It has been

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_28

suggested that these risk relationships may be attributed, at least in part, to greater atherogenic properties of sdLDL than larger, more buoyant LDL due to lower LDL receptor affinity, and prolonged plasma residence time greater binding to arterial wall proteoglycans, and increased oxidative susceptibility [5, 18]. Despite this evidence, it has been surmised that large and small LDL particles have similar atherogenicity, principally based on analyses in which levels of both subclasses were included in models assessing their relationships to CHD risk [19]. However, this analytic approach fails to consider that the existence of LDL subclass phenotypes based on the dichotomous distribution of LDL particle diameters precludes the application of simple linear statistical models that jointly incorporate both large and small LDL levels, and that principal component analysis, as discussed below, is more appropriate for this purpose.

The desire to improve CVD risk prediction has advanced the development of several technologies to measure features of lipoprotein particles that are not captured by their cholesterol levels. The evidence that elevated plasma concentration of apolipoprotein B (apoB), which exists in a ratio of one molecule per VLDL, IDL, and LDL particle, was a more accurate predictor of CVD risk than LDL-C [20] helped reinforce the importance of the lipoprotein particles themselves, not just their lipid content, in the pathogenesis of atherosclerosis. ApoB can be considered a proxy for LDL particle number, so it is not surprising that LDL particle concentration (LDL-P) has also been found to have a better predictive value than LDL-C, particularly in the subset of patients in whom there is discordance between LDL-C and LDL-P levels [21].

There are now several methods for lipoprotein subfraction analysis, including nuclear magnetic resonance spectroscopy (NMR), Vertical Auto Profile (VAP-II), gel electrophoresis (LipoPrint), and aerosol ion mobility (IM). However, currently, there is no standardized method for measuring concentrations and distributions of lipoprotein particle subclasses, making direct comparisons between studies that use different platforms challenging and confounding the interpretation of clinical studies [22]. This chapter will focus on IM lipoprotein analysis.

Ion Mobility Methodology

IM analysis of lipoprotein particles is based on the principle that particles of a given size and charge behave predictably when carried in a laminar air flow and subjected to an electric field [23, 24]. The sample preparation for ion mobility involves mixing small volumes of plasma or serum (0.03 mL) with a fibrin removal solution and then precipitating and binding the lipoprotein particles to a magnetic bead using dextran sulfate. The plasma proteins can then be rinsed away, and the lipoprotein particles are released from the beads using a slightly alkaline solution [25]. Sample preparation in the first version of the procedure involved a 135 min ultracentrifugation step. However, the newer magnetic bead procedure allows for the preparation of 96 samples in under 90 min, with quantitative recovery of lipoprotein particles.

The measurement process occurs on the IM instrument (Fig. 28.1) and begins when the plasma lipoproteins are aerosolized using an electrospray generator and introduced into a flow of air containing approximately 5% CO_2. In the electrospray chamber, a polonium α-particle emitter nearly neutralizes the particles. The proportion of singly charged particles emerging from the electrospray chamber can be calculated using Fuchs charge distribution. The airflow carries these particles to the differential mobility analyzer (DMA), where they are confined in a thin flow stream by a laminar concurrent flow of air called a sheath flow. The particle-free sheath flow recirculates through the DMA at a constant flow rate. As the particles are carried through the DMA, an electric potential across the sheath flow causes the particles to drift toward a collection slit. Ramping the applied potential causes particles of different diameters to pass through the slit, thus allowing particles between 17.5 and 542.0 Å to be sampled; the size range can be extended to include larger particles if desired. At any given electrical potential, particles of predict-

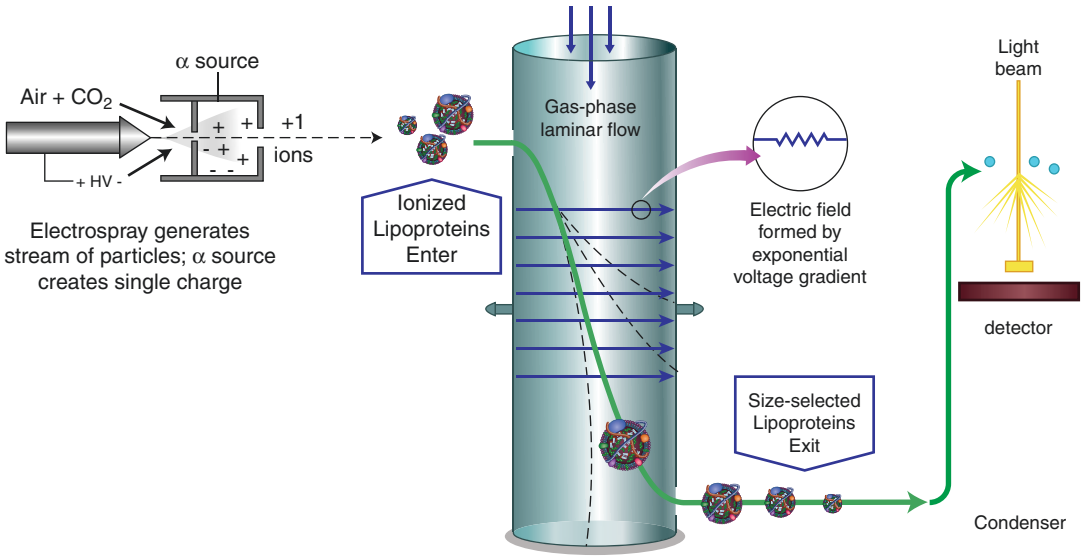

Fig. 28.1 Schematic depiction of the aerosol ion mobility analyzer. The electrospray generator on the left generates singly charged particles in the gas phase. The particles are carried by airflow to the differential mobility analyzer (center) where ramping of an electric potential across the sheath flow causes the particles to drift toward a collection slit in a predictable manner according to their diameter. Finally, the particles are enlarged by vapor condensation and detected by light scatter in the condensation particle counter (right). (Adapted from Caulfield et al. [23])

able size pass through the collection slit and enter a separate air stream that carries them to a particle counter. The particle counter first enlarges the particles by condensing a vapor onto each particle and then detects the droplets, now several microns in diameter, via light scatter. Knowledge of the electrical potential applied to the DMA, the dimensions of the DMA, and the flow rate of air passing through the DMA permits accurate calculation of particle diameter and the number of particles in a discrete size range. This method is high throughput, uses a 96-well format, and has a scan time of about 2 min per sample.

IM quantifies lipoprotein fractions across the entire lipoprotein spectrum, including HDL, LDL, IDL, and VLDL, and has the potential for measuring even larger particles, such as chylomicrons. The data are reported as plasma particle concentrations in nmol/L in size intervals corresponding to defined lipoprotein subclasses, as well as an overall lipoprotein particle profile [23, 26]. An example of an IM profile is shown in Fig. 28.2. The boundaries used to describe major subfraction intervals were ascertained from visual inspection of individual profiles, conformity with previous size intervals determined by gradient gel electrophoresis, and ion mobility analysis of subfractions isolated by density gradient ultracentrifugation. These intervals include, from smallest to largest diameters, HDL small (equivalent to HDL3 + 2a, 7.7–10.5 nm), HDL large (equivalent to HDL2b, 10.5–14.5 nm), LDL very small (LDL IIIa + LDL IVa–c, 18.0–20.8 nm), LDL small (LDL IIIa, 20.8–21.4 nm), LDL medium (LDL IIb, 21.4–22.0 nm), LDL large (LDL I + LDL IIa, 22.0–23.3 nm), IDL small (IDL2, 23.3–25.0 nm), IDL large (IDL1, 25.0–29.6 nm), VLDL small (29.6–33.5 nm), VLDL medium (33.5–42.4 nm), and VLDL large (42.4–52.0). The clinical laboratory report includes the diameter and phenotype (pattern A or pattern B) of the major LDL species. Because the particles are binned in somewhat arbitrary size intervals, it is possible that small but significant features of the lipoprotein particle spectrum could be obscured by binning the data. However, since the complete, unbinned scan data, comprising 1200 size intervals, are available for each IM analysis, techniques such as peak fitting and curve deconvolution can be used to uncover additional regions of interest.

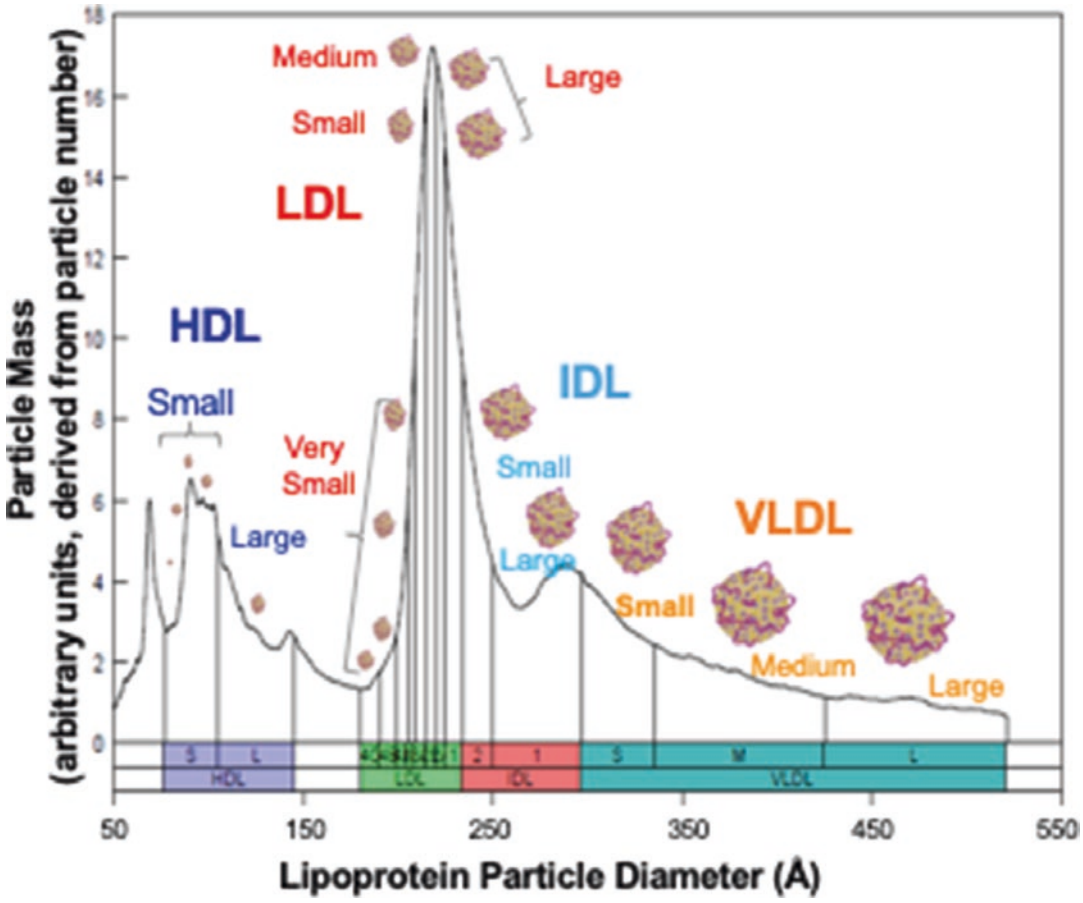

Fig. 28.2 Ion mobility lipoprotein profile. Particle numbers are converted to arbitrary mass units to generate a lipoprotein profile. The size intervals correspond to the indicated lipoprotein subclasses, and concentrations (in nmol/L) for each subclass are reported in addition to the profile

Ion Mobility Subfractions and Cardiovascular Disease

IM is a relatively new technology, but several studies provide evidence that levels of lipoprotein subfractions determined by this method deliver additional information beyond standard lipid measurements to improve CVD risk prediction. In a follow-up to the HDL-Atherosclerosis Treatment Study, investigators found an independent, positive association between very small LDL IIIb measured by IM and progression of coronary artery stenosis [27]. Importantly, the angiographic measurement of coronary stenosis may better reflect the atherogenic properties of lipoproteins than measurement of cardiovascular events, which reflect both atherogenesis and factors that promote plaque rupture and thrombosis [28]. These findings suggest that LDL IIIb could be used as a marker of coronary disease progression.

One population most likely to benefit from improved cardiovascular risk characterization are individuals with moderate risk who have low to normal LDL cholesterol levels. In 4594 initially healthy individuals in the population-based Malmö Diet and Cancer Study Cardiovascular Cohort, levels of total LDL-P and small- and medium-sized LDL subclasses measured by IM at baseline were predictive of cardiovascular events (myocardial infarction, stroke, coronary heart disease death) after a mean follow-up

of 12.2 years, while large HDL particles were inversely associated with risk [26]. Principal component analysis revealed a major component consisting of an inverse correlation between small and medium LDL and large HDL particles that was predictive of cardiovascular outcomes, consistent with the atherogenic dyslipidemia characteristic of type 2 diabetes, insulin resistance, and metabolic syndrome [9].

IM lipoprotein measurements were also made in samples from the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial [25]. This was a primary CVD prevention trial in individuals considered to be at intermediate risk due to elevated levels of hsCRP but without elevated LDL cholesterol. In 5600 participants in the placebo arm of JUPITER, baseline LDL cholesterol was not predictive of CVD events, whereas levels of medium and small LDL particles were associated with incident CVD independent of standard risk factors including plasma lipid levels. Levels of very small LDL were also predictive of CVD outcome, and notably the smallest LDL species, LDL IVc, was associated with the category of CVD plus all-cause death independent of plasma lipid concentrations.

Further analysis of IM lipoprotein subfractions was performed in 1919 participants from the Malmö Diet and Cancer Study Cardiovascular Cohort who would not have been classified into one of the statin benefit groups identified by the 2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol [29] on the basis of their risk factor levels at study entry [30]. Total LDL-P as well as small and medium LDL subclasses were associated with incident CVD after adjustment for traditional risk factors. In a separate analysis focused on incident CVD in a population without elevated LDL-C, as in JUPITER, small and very small LDL and individual subfractions within very small LDL (LDL IVa, LDL IVb, and LDL IVc) were associated with CVD events independent of standard lipids [31]. Taken together, these findings indicate that consideration of a patient's LDL particle subfraction levels, in particular medium, small, and very small LDL, could aid in the decision to treat when traditional risk factors are ambiguous.

Ion Mobility Analyses in Other Clinical Settings

Lipoprotein subfraction measurements may have clinical utility in diagnosis and management of CVD risk in other chronic disease states. Conventional CVD risk factors do not accurately predict mortality among maintenance hemodialysis patients, in whom ~50% of total mortality is attributable to CVD [32]. However, in a cohort of 235 hemodialysis patients who were followed for up to 6 years, larger LDL particle diameter or higher large LDL particle concentrations measured by IM were predictive of greater survival, whereas higher levels of very small LDL particle concentration were associated with higher all-cause mortality [33].

Recent studies have implemented IM analysis in pediatric populations. Despite normal lipid levels in a group of healthy pubertal and prepubertal children, IM analysis showed significant differences in LDL and HDL subfraction levels between those who were lean and obese [34]. In a clinical trial of low-dose atorvastatin in children with type 1 diabetes, insulin sensitivity was found to be strongly inversely correlated with apoB and small LDL IIIa concentrations at randomization and throughout the study in both treatment and placebo groups, suggesting that the relationship between LDL IIIa and insulin sensitivity merits further study [35].

While LDL has been a predominant clinical focus of lipoprotein subfractionation, this technology may also prove useful in elucidating the potential cardioprotective effects of HDL. Like LDL, HDL also consists of a heterogeneous spectrum of particles that are not adequately captured by the HDL-C measurement. In an analysis of 1380 post-menopausal women from the Multi-Ethnic Study of Atherosclerosis, total HDL-particle concentration (HDL-P) measured by IM, but not HDL-C, was inversely associated with carotid intima-media thickness (cIMT) after adjustment for covariates, whereas interestingly HDL-C, and not HDL-P, was positively associated with the presence of carotid plaque [36]. In addition, higher large HDL-P was associated with higher cIMT close to menopause but with

lower cIMT later in life, suggesting that changes in HDL distribution and functionality may affect CVD risk during the menopause transition.

Future Directions and Summary

A very recent analysis explored generating a functional risk score from IM lipoprotein profiles to consider the complete size-specific particle abundance from each sample and avoid loss of data due to binning. This procedure was applied to a case-control subset from the Malmö Cohort described above [37]. In this population the functional risk score was positively associated with CVD risk, even after adjustment for traditional risk factors. An advantage of this approach is that it incorporates risk for the entire range of lipoprotein particle diameters and accounts for correlations between the different size regions.

Lipoprotein (a) [Lp(a)] is a lipoprotein particle which consists of an LDL-like particle covalently attached to a large glycoprotein, apo(a), by a disulfide bridge [38]. Lp(a) is an independent risk factor for CVD, and genetic studies have pointed to its causal role [39]. Polymorphisms of the apo(a) gene generate molecular isoforms of Lp(a) that vary from 300 to 800 kDa, presenting a challenge for measurement and interpretation of Lp(a) levels in plasma [40]. It has been suggested that Lp(a) particle concentration may be a better metric for estimating risk of atherosclerosis related to Lp(a) [41]. During IM analysis, Lp(a) particles remain in the sample preparation and thus contribute to the overall lipoprotein profile. Current studies are in progress to develop criteria for identifying and measuring Lp(a) particles in plasma using IM.

In summary, IM measurement of lipoprotein particle subclasses has shown evidence for potential clinical utility, especially in identifying at-risk patients when interpretation of traditional risk factors is ambiguous, and in monitoring the effectiveness of lipid-lowering therapies. Moreover, detailed measurement of individual lipoprotein subspecies has the potential to reveal biological pathways that underlie the atherogenic role of apoB-containing lipoproteins and the atheroprotective role of HDL and its subspecies.

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29 How ApoB Measurements Could Improve Prevention of Cardiovascular Disease

Allan D. Sniderman

Introduction

Trapping of apolipoprotein B (apoB) particles within the arterial wall is the fundamental cause of atherosclerosis [1], and the damage to the arterial wall due to trapping of apoB particles is more directly related to the number of apoB particles in plasma than to the mass of cholesterol within them. This is why apoB is a more accurate marker of cardiovascular risk and a more reliable guide to the adequacy of therapy to reduce cardiovascular risk than low-density lipoprotein cholesterol (LDL-C) and non-high-density lipoprotein cholesterol (non-HDL-C). Nevertheless, apoB has not been recommended as the primary measure of the atherogenic lipoproteins by the major guidelines primarily because apoB does not significantly improve the short-term 10-year prediction of cardiovascular risk. This has been seen as decisive because, except for profound elevation of LDL-C or diabetes, selection of subjects for primary prevention of cardiovascular disease (CVD) with statins is based on the 10-year risk of a CVD event as calculated by an approved algorithm such as the AHA/ACC Multisociety Guidelines [2]. If apoB does not improve prediction, how could apoB improve prevention?

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However, the problem is not with apoB or with the risk algorithm; the problem is with our interpretation of the risk algorithm. Risk algorithms accurately measure risk, but do not accurately assign responsibility for risk. Thus, the causes of CVD only modestly influence the calculation of the risk of a clinical event in the standard 10-year risk algorithms [3]. Accordingly, which marker of the atherogenic lipoproteins – LDL-C, non-HDL-C, apoB, or even total cholesterol (TC) – is used matters little in the estimation of short-term risk. Understanding why the causes of atherosclerosis count for so little in the estimation of 10-year risk is key to improving the prevention of CVD. Therefore, this issue will be dealt with in detail. Indeed, the central argument of this chapter is that we must place more emphasis on the causes of CVD disease and less on the short-term risk of a clinical event.

The argument has also been made that apoB is too complex for physicians to understand. Nonsense. Each atherogenic particle contains one molecule of apoB. Therefore, apoB equals the total number of atherogenic particles. What could be less complex? It is the conventional lipid panel, which is complex and cumbersome, contradictory, and confusing. The conventional lipid panel consists of TC, triglycerides (TG), LDL-C, non-HDL-C, and HDL-C, five numbers, of which, in reality, only one – LDL-C – drives everyday clinical decisions. Yet not only is LDL-C inferior to apoB and non-HDL-C as a

M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_29

measure of risk – as AHA/ACC explicitly acknowledges [2], the measurement of LDL-C is problematic. Should LDL-C be calculated or measured? And if calculated, by which method [4, 5]? And if measured, by which assay, since none are standardized and none have been shown to be a more accurate measure of risk than calculated LDL-C? TG may point to increased risk, but no guideline states directly how clinical decisions should be altered based on TG. If TG does point to increased risk, is it the TG or is it the cholesterol within the very-low-density lipoprotein (VLDL) particles that matters? Are VLDL particles as atherogenic as low-density lipoprotein (LDL) particles? Non-HDL-C may be superior to LDL-C, but non-HDL-C is listed by AHA/ACC only as an alternate to LDL-C. Why does the superior not displace the inferior? Could it be because it is so hard for so many to understand a non-number? If TC is inferior to LDL-C and non-HDL-C, why is TC used to calculate risk? In countless epidemiological studies, HDL-C is inversely related to risk, but increasing HDL-C does not reduce risk. Moreover, there is serious doubt as to whether HDL-C is causally related to atherosclerosis. So why do we measure HDL-C routinely other than to calculate non-HDL-C? If we do not use non-HDL-C, why should we measure HDL-C? In summary, the conventional lipid model is confusing, complex, cumbersome, and contradictory, but to make matters worse, it is also incomplete and inaccurate.

Here, in a nutshell, is the argument that will be presented in this chapter. ApoB integrates and extends the information in a conventional lipid panel and therefore simplifies and improves the process of cardiovascular care. ApoB measures the number of atherogenic particles in plasma, and the number of atherogenic particles in plasma is the principal driver of the rate at which the complex atherosclerotic lesions that can suddenly injure or kill us form, develop, and mature within the walls of our arteries [6, 7]. The cholesterol within apoB particles that are trapped within the arterial wall does injure the arterial wall. But cholesterol only enters the arterial wall within apoB particles, and cholesterol is not the only component of the apoB particle that can injure the arterial wall. Oxidized phospholipid and oxidized apoB are potent triggers of the inflammatory response [8–10]. Moreover, the mass of cholesterol within the apoB particle can vary substantially. Therefore, neither LDL-C nor non-HDL-C is as accurate measure of the risk posed by the atherogenic lipoprotein particles as apoB.

Furthermore, apoB clarifies the relation of VLDL particles and (LDL) particles – and therefore, cholesterol and TG – to cardiovascular risk. Each VLDL particle contains one molecule of apoB [11]. Each LDL particle contains one molecule of apoB. Evidence from Mendelian randomization analyses demonstrate that VLDL and LDL particles are, more or less, equally atherogenic [12]. Therefore, apoB integrates the information from VLDL apoB and LDL apoB and extends the information from TG, LDL-C, and non-HDL-C. In addition, accurate diagnosis of the atherogenic apoB dyslipoproteinemias is not possible with a conventional lipid panel but is possible with the apoB algorithms based on TC, TG, and apoB [13]. Nevertheless, for routine clinical follow-up, apoB is all that needs to be measured (Fig. 29.1). ApoB is not too complex for physicians and patients to understand. It is the conventional lipid system that is complex, confusing, and contradictory. ApoB clarifies, unifies, and simplifies clinical care.

Limitations of the Risk Model of Atherosclerosis

Every major guideline group has adopted the 10-year Risk Model of cardiovascular prevention as their primary strategy to select subjects for pharmacological prevention of CVD. Restricting

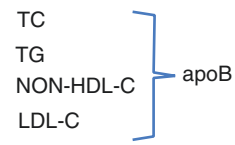

Fig. 29.1 This figure illustrates how for routine clinical care apoB could replace total cholesterol (TC), triglycerides (TG), LDL cholesterol (LDL-C), and non-HDL cholesterol (non-HDL-C)

pharmacological therapy with its costs and risks to those most likely to suffer a CVD event seems just simple, common sense. Moreover, CVD is multifactorial in origin [14]. Accordingly, no approach to prevention can be built on only one factor such as blood pressure or smoking or serum lipoproteins or blood sugar. The effects of all must be integrated, which is, of course, the purpose and strength of a risk algorithm.

Because the bottom line is the integrated effect of all the variables, the critical test of whether a new variable should be added to the algorithm became whether inclusion of that variable significantly improved prediction. Of the metrics by which this can be estimated, the most used has been the c-statistic. ApoB has not been shown to substantially increase the c-statistic in any prospective observational study. This has been taken as a "drop-dead" argument against the value of apoB. However, as is so often the case, a conclusion that seems incontestable evaporates instantly when examined with examined in a fresh light. The problem that has been overlooked with the c-statistic argument is that the non-modifiable factors that increase risk – age and sex – dominate the 10-year calculation of risk [3]. Removing blood pressure and blood lipids from any risk algorithm changes the c-statistic only marginally, notwithstanding that treating blood pressure and blood lipids reduces cardiovascular risk substantially [3, 15].

Unfortunately, this profound prevention/prediction paradox has generally been ignored. How can what matters so much for outcome after treatment matter so little for prediction of the event without treatment? The answer, in large part, is that age is treated as an independent variable in the risk prediction algorithms, whereas age is the period of time during which our arteries are exposed to the malign influences of blood pressure and the blood lipids [16, 17]. The adverse effects of age on the arterial wall are not independent of the adverse effects of the causes of disease. Age is the period of time over which the causes of disease cause disease. We may enter age as independent statistical variable in a mathematical model, but that does not make age an independent biological variable. The answer to the paradox is banal: the product of the equation – risk – is correct. It is the interpretation of the result that is wrong. Causes do matter even if the c-statistic says they do not.

This is not the only limitation of the Risk Model. Atherosclerosis is a tangled web of proliferative and destructive processes that occur within the arterial wall in reaction to the trapping of apoB particles, which over time transform the normal arterial wall from a series of thin and orderly layers into a twisted mass of calcified scar interspersed with pools of cholesterol through which fragile, newly formed vessels stretching from the adventitia course toward what was formerly the media [18]. A tissue that was supple and elastic, covered with an antithrombotic endothelium, resistant to the entry of apoB particles, becomes, over time, a tissue that is stiff, distorted, and brittle tissue with an endothelium that admits apoB particles much more easily than normal and no longer resists interaction with platelets. Within the diseased wall lie pools of cholesterol surrounded by bands of fibrous tissue, which can thin and suddenly rupture, exposing the cholesterol within the wall to the blood within the lumen, provoking an acute thrombosis, which can, abruptly and disastrously, halt all nutrient flow to the organ it supplies. Alternatively, within the fragile network of newly formed nutrient vessels within the arterial wall, one segment may rupture, producing an acute intramural hematoma. Yet again, a patch of endothelium may abruptly erode, provoking the formation of an acute platelet thrombosis. The atherosclerotic arterial wall is, in other words, loaded with a series of bombs that can go off at any time without any advance notice [19].

Why paint such a lurid picture in such purple prose? Because it is accurate and because once advanced disease is present, the normal architecture of the wall can never be restored. Much healing is possible, but complete resolution of all the structural abnormalities with the therapy now available is not. Therefore, risk of a clinical event remains after the most intense LDL-lowering therapy. Accordingly, when possible, preventing the anatomic devastation within the arterial wall induced by the trapping of apoB particles should

be the true objective of prevention. Unfortunately, this is not possible so long as a risk model with a time horizon of 10 years remains the primary tool to select subjects for preventive therapy.

Why? Because clinical events can only occur when complex lesions are present. Accordingly, only individuals with complex lesions can be at risk for a clinical event. Figure 29.2 illustrates the challenge for prevention based on 10-year risk. Panel A illustrates the coronaries of 20 men, age 20; none of them have complex lesions. Ten-year risk in the group is zero. By age 40, 5 have complex lesions: 10-year risk for the group is also low because only 5 out of 20 are at risk. Nevertheless, there is a finite likelihood of an event for the five with diseased arteries. By age 60, ten have lesions, and risk for the group is now high because the proportion of the group with advanced disease is so high.

Consider what this sequence means for prevention. Presently, the guidelines select subjects for pharmacological prevention of CVD based almost exclusively on risk. With a threshold level of risk set at 7.5%, this means that prevention will not involve large numbers until age 60, the age at which, in this example, half already have advanced disease [20]. They may be asymptomatic, but their arteries are not normal. Treatment will substantially diminish the chances of an acute catastrophic transformative change in an artery. But it will not eliminate it. Too much damage has already been done. The Risk Model is, therefore, a delayed model of prevention. Delay costs dearly. More dearly than is generally appreciated. First, the benefits of prevention are limited because advanced disease is already present in so many. Second, many events have already occurred during the "low-risk" period. Indeed, almost half of all infarcts and strokes occur before age 60 [21]. How can so many events occur when risk is low?

The words we use trick us all the time because what we think they mean is not what they actually mean. The word risk is a particularly pernicious example. In this instance, we think we are calculating the risk of an individual, but, in reality, the risk we calculate is the risk of a group of individuals, and we can never be sure whether this averaged risk applies to the individual in front of us [22]. In fact, the risk for the group is determined by the minority with advanced disease, and the risk for the individuals with advanced disease is necessarily much higher than the risk for the group. The trouble does not end there. Risk is a fraction: the number of events over a unit time per standard number of subjects. Risk may be low in those who are younger, but there are many more who are younger than who are older, particularly at the ages when risk becomes extraordinarily high. This is why of the total number of CVD events, so many occur before the age at which risk begins to rise so dramatically [21]. Based on their 10-year risk, most of those with early events would never have qualified for prevention of the events, which injured or killed them. For all these reasons, we risk too

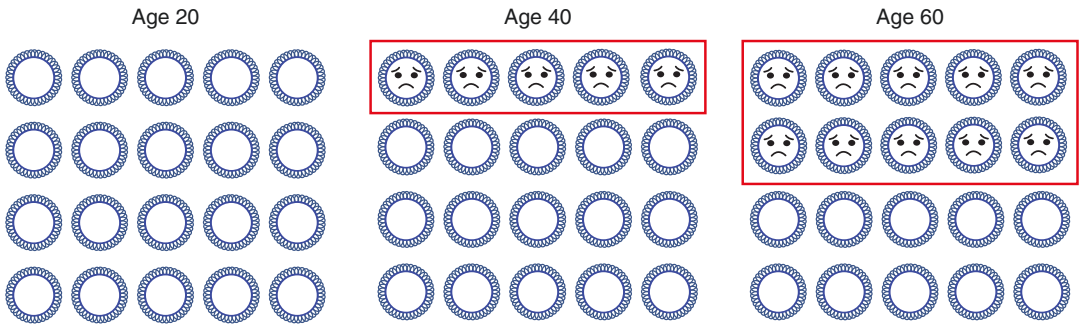

Fig. 29.2 This figure illustrates the relation between age and risk in terms of the proportion of those with disease that is sufficiently advanced at any age to cause a clinical event versus the proportion of those without advanced disease. The risk for any group is determined by this proportion. The risk for the individual who is affected is, obviously, substantially higher

much if we risk everything on the Risk Model of prevention of CVD.

The ApoB Causal Exposure Model of Atherosclerosis

The ApoB Causal Exposure model of atherosclerosis states that trapping of apoB particles within the arterial wall over time is the primary cause of atherosclerosis and atherosclerosis causes clinical events [23]. The number of apoB particles in the lumen of an artery is the primary determinant of the number of apoB particles that will enter and be trapped within the artery. Therefore, the number of apoB particles in plasma is a primary determinant of the risk of a clinical event. From this, it follows that lowering plasma apoB should be one of our principal strategies to lower the risk of a cardiovascular event.

ApoB Particles Versus ApoB Mass

ApoB is present in its full-length form as apoB100. ApoB100 is an integral structural component of VLDL particles, LDL particles, and Lp(a) particles. ApoB100 particles are secreted by the liver [24]. ApoB48 is the truncated form of apoB100 [25], and apoB48 is an integral structural component of chylomicron and chylomicron remnant particles, which are secreted by the intestines. Because each apoB48 particle contains one molecule of apoB48 and because each apoB100 particle contains one molecule of apoB100 and because all the immunoassays to measure apoB recognize both apoB48 and apoB100, plasma apoB equals the total number of apoB48 and apoB100 particles. Moreover, except for intact chylomicrons, all the other apoB particles are small enough to enter the arterial wall and, once there, initiate and promote the atherosclerotic process. Therefore, apoB equals atherogenic particle number. However, as will be detailed beneath, with the exception of type III hyperlipoproteinemia, even postprandially, the number of apoB100 particles is 50–100-fold greater than the number of apoB48 particles [26]. Accordingly, effectively, plasma apoB is the number of apoB100 particles, i.e., the sum of VLDL and LDL particles, and explains why fasting is not necessary to measure apoB.

Another point: the word apoB can be used in two senses. The sense in which I will use it – the sense I urge the reader to adopt – is the physiological sense: apoB equals the number of apoB particles in plasma. The higher the apoB, the more apoB particles there are in plasma. The lower the apoB, the fewer apoB particles in plasma. ApoB particles are bad. The more apoB particles we have, the worse off our arterial walls will be; the fewer we have, the better off our arterial walls will be.

We need to distinguish this sense of apoB from the other, which is the measure of the mass of apoB protein in plasma. This definition is technically true, but it misses the physiological point. Lipids – TC, LDL-C, TG, and high-density lipoprotein cholesterol (HDL-C) – as well as apoB are all reported by our laboratories as mass per unit volume of plasma. LDL-C, for example, is reported as mg/dl or mmol/L. So is apoB, which is quantitated in mg/dl or g/L. We are so used to this notation that we do not recognize how limiting it is. ApoB seems to be just one more in a list of independent variables, whereas it is the number of packages or particles that contain cholesterol and TG.

Cholesterol and TG, unlike sodium and potassium, are not soluble in water and, therefore, can only be transported in plasma within lipoprotein particles [24]. Within the apoB lipoprotein particles, the mass of cholesterol per apoB particle may vary. This is the critical fact that determines all that follows. The mass of cholesterol within LDL particles is not the same as the number of LDL particles. Take two patients, each with an LDL-C of 110 mg/dl. The concentration of LDL-C, expressed as the mass of cholesterol per volume of plasma, is the same in both. Nevertheless, as illustrated in Fig. 29.3, the number of LDL apoB particles in which this cholesterol is contained is not. The first patient has twice as many apoB particles as the second. The apoB particles of the first patient are smaller and contain less cholesterol than the apoB particles of the second.

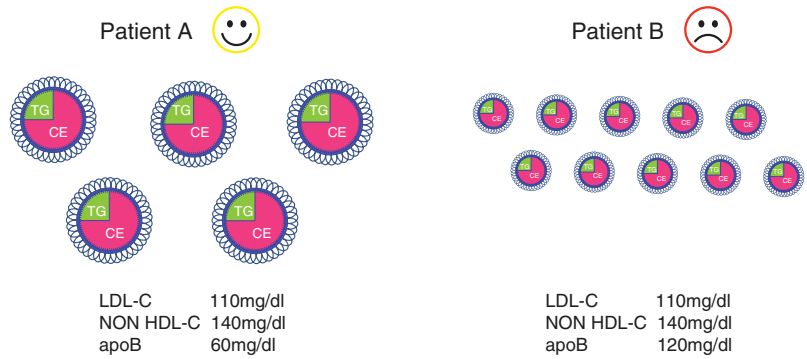

Fig. 29.3 This figure illustrates, schematically, two patients. Both have the same levels of LDL-C and non-HDL-C. However, patient A has a small number of cholesterol-enriched apoB particles, whereas patient B has a large number of cholesterol-depleted apoB particles. Patient B is at increased cardiovascular risk due to atherogenic dyslipoproteinemia, whereas patient A is not

His/her apoB particles are fewer in number and larger because they contain more cholesterol.

Please remember that particles are three-dimensional spheres, whereas the figures in this chapter are only two dimensional, and so far as the mass of cholesterol within it, it is the volume of the particle, not its area, that matters. Volume is a function of radius to the third power, whereas area is a function of radius to the second. This means that small changes in the radius of a particle are associated with large changes in its volume. Bottom line: the two patients, whose LDL-C is the same, are not the same so far as their respective numbers of LDL particles are concerned. The objective of this chapter is to review the evidence that their cardiovascular risk will be determined by the number of apoB particles, not the mass of cholesterol within them. Accordingly, patient A has a low risk of CVD attributable to atherogenic lipoproteins, whereas patient B has a high risk of CVD attributable to atherogenic lipoproteins, notwithstanding their levels of LDL-C and non-HDL-C are the same.

ApoB Particles and Plasma ApoB

All apoB particles, except intact chylomicrons and the largest VLDL particles, are small enough to enter the arterial wall, and, therefore, all apoB particles, except intact chylomicrons and the largest VLDL particles, are proatherogenic [24]. However, their relative importance as drivers of risk is determined by the numbers of apoB particles in one class versus the numbers in another. As illustrated in Fig. 29.4, in normotriglyceridemic patients, there are usually nine times as many LDL particles as VLDL particles in fasting plasma, while in postprandial period, there are usually nine times or more VLDL particles than chylomicron and chylomicron particles [26]. These proportions are not fixed in stone. In mild to moderately hypertriglyceridemic subjects (TG 1.5–<3.0 mmol/L), the ratio of LDL to VLDL particles drops to 8 to 1, while in moderately to severely hypertriglyceridemic subjects, the ratio can decrease to as low as 4 to 1 [26].

Nevertheless, with one exception, type III hyperlipoproteinemia, which will be discussed beneath, there are always many more LDL particles than any other class of apoB particles. Because most of the cholesterol in plasma is present in LDL particles, this explains why LDL-C (and indeed non-HDL-C, which is made up principally of LDL-C) is a more accurate marker of cardiovascular risk than TG, notwithstanding that hypertriglyceridemia is more common than hypercholesterolemia in patients with vascular disease and in those subgroups at high risk for vascular disease such as diabetes and abdominal obesity [26].

Remember the distinction between particle number and mass. For those who think in terms

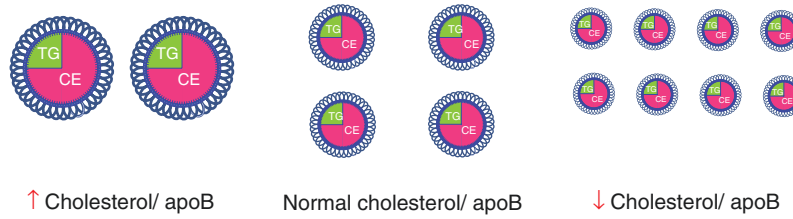

Fig. 29.4 This figure illustrates schematically the numeric relation between the number of VLDL apoB particles and the number of LDL apoB particles

of mass, if TG is 200 mg/dl and LDL-C is 120 mg/dl, values that would be typical in patients with type 2 diabetes or vascular disease, since TG are the dominant lipid in VLDL whereas cholesterol is the dominant lipid in LDL, there must be the sense that VLDL is more important than LDL in producing increased cardiovascular risk in this patient because the level of TG expressed as mass is higher than the level of LDL-C expressed as mass. This is the illusion that mass matters most. The particle paradigm instantly dispels this illusion: risk is driven in this patient by LDL not by VLDL because the number of LDL particles in this patient is so much greater than the number of VLDL particles. Risk due to the atherogenic lipoproteins, in fact, is generally just the sum of the number of VLDL and LDL particles. A VLDL apoB particle equals an LDL apoB particle in atherogenic potential. Therefore, it is total apoB, not VLDL or LDL apoB, that counts.

This great simplification – this dissolution of the endless does TG matter debate – is the great advance that Ference and his colleagues provided with their landmark Mendelian randomization study, which will be reviewed in detail beneath [12]. They have transformed the management of dyslipoproteinemia: if the question originally was whether apoB added significantly to the conventional lipid panel, the question, now, is whether the conventional lipid panel adds to apoB? The answer is that it does not. With only TG, TC, and apoB, all the atherogenic dyslipoproteinemias can be accurately diagnosed and, with the exception of hypertriglyceridemia severe enough to cause pancreatitis, type III hyperlipoproteinemia, and Lp(a), apoB is all that needs to be known to assess the adequacy of the response to therapy. In effect, for routine clinical care, one number can replace five numbers.

ApoB Particle Number as a Marker of Cardiovascular Risk

Background

TC was the first index of cardiovascular risk due to the plasma lipoproteins. TC was simple to measure, accurately and inexpensively, in large numbers of subjects. However, the strong inverse relationship between HDL-C and cardiovascular risk [14] roiled the lipid world and resulted in LDL-C supplanting TC as the principal measure of risk, a position which it has occupied until today. Greater precision was gained but at the cost of greater complexity and greater cost. Nevertheless, even from the outset, limitations in the precision with which LDL-C could be calculated were appreciated [27]. In response to these limitations and the challenge of apoB, non-HDL-C was eventually introduced as a more accurate marker of risk than LDL-C [28].

Non-HDL-C includes the cholesterol in VLDL particles as well as in any apoB48 and Lp(a) particles that may be present. Non-HDL-C is the inclusive atherogenic cholesterol index just as apoB is the inclusive atherogenic particle index. The singular advantage of non-HDL-C over apoB has always been stated to be that no additional cost was involved to calculate non-HDL-C whereas there was an additional cost to measure apoB. But non-HDL-C is not free. To calculate non-HDL-C, a conventional lipid panel must be measured. This does not come for free. The value and the cost of measuring/calculating non-HDL-C should be measured against the value and the cost of measuring apoB. If apoB is superior to non-HDL-C, there is no reason to measure/calculate non-HDL-C and no reason to pay for the measurement/calculation of non-HDL-C.

The Evidence

By this point, there have been multiple generations of studies comparing apoB to the cholesterol markers. The designs of the studies to compare the markers and the epidemiological methods to analyze their results have varied, starting from the very simple, to the more sophisticated, to the even more sophisticated. Yet, the results have remained very much the same: apoB is superior to LDL-C/non-HDL-C as a marker of risk and an index of the adequacy of therapy.

Case-Control Studies

The first generation of studies were simple, cross-sectional, case-control comparisons. The first two reports established the pattern of observations that were confirmed in most, but not all, studies of this period. Pietro Avogaro and his colleagues demonstrated that apoB and the apoB/apoA1 ratio were higher in 218 patients with MI compared to 160 controls [29]. Sniderman and his colleagues studied 90 subjects who had undergone coronary angiography, of whom 31 had normal coronary arteries whereas 59 had significant coronary lesions. TG, TC, and LDL-C were higher in the coronary group, but LDL apoB most clearly separated the groups [30]. Both studies supported the previous observations that LDL-C and TG were higher in subjects with CVD, but total and LDL apoB made these differences more apparent. The most prominent early negative study was by Vega et al., who reported that measuring apoB added little to diagnostic accuracy [31]. In this and many of her subsequent studies, apoB was measured by chemical methods rather than by immunoassay. As it turns out, the chemical method to measure apoB is not as accurate or precise as the immunochemical approach, and this, I suspect, accounts for the differences between their results and so many others.

Prospective Observational Studies Analyzed by Conventional Methods

An extensive series of prospective observational studies comparing apoB or LDL particle number (LDL PN) as markers of cardiovascular risk have been reported. The great majority demonstrated apoB or LDL PN was a more accurate marker of cardiovascular risk than LDL-C. Only a small number of such studies did not support this conclusion [32]. By contrast, there was no clear outcome for the TC/HDL-C ratio versus the apoB/apoA1 ratio with some studies, such as the Framingham Heart Study, strongly favoring the former [33] whereas others, such as the AMORIS study, just as strongly supporting the latter [34]. Many also demonstrated that apoB was superior to non-HDL-C, although a significant number did not [32]. Of the latter, the Emerging Risk Factor Collaboration (ERFC) gained the most attention [35]. ERFC did report that non-HDL-C was equally predictive to apoB – the finding that was highlighted in multiple reviews – but it also reported that TC was equal in predictive power to LDL-C, non-HDL-C, and apoB, a finding not supported by other studies and not noted in these reviews. Moreover, a meta-analysis of prospective observational studies demonstrated a hierarchy of predictive powers with apoB superior to non-HDL-C, which was superior to LDL-C [36]. On balance, therefore, the evidence demonstrated unequivocally that apoB was superior to LDL-C and strongly suggested that apoB was also superior to non-HDL-C.

Discordance Analyses

Discordance analysis has settled the issue as to whether apoB is superior to non-HDL-C as well as LDL-C. Cholesterol is a major component of all apoB particles. This is why the plasma levels of TC, LDL-C, non-HDL-C, and apoB are all highly intercorrelated. When one changes, the others change. Unfortunately, conventional epidemiological methods were not designed to deal with variables that are highly intercorrelated. If the mass of cholesterol per apoB particle were constant, apoB and the cholesterol markers would, necessarily, predict risk identically. Indeed, if all the components of apoB particles were present in the same proportion, any component of the particle would predict risk just as accurately as any other component. However, while cholesterol and apoB are highly intercorrelated, they are not perfectly correlated because

the mass of cholesterol per apoB particle varies substantially, both within individuals and, more importantly, among individuals [24]. These differences are driven by cholesteryl ester transfer protein (CETP)-mediated core lipid exchanges [26]. The result is that apoB particles may be cholesterol-enriched, be cholesterol-depleted, or contain an average mass of cholesterol. For those in the middle, those whose apoB particles contain an average mass of cholesterol, LDL-C, non-HDL-C, and apoB will predict cardiovascular risk equally well (Fig. 29.5).

Discordance analysis was designed to compare the markers in the other two groups [37]. For those with cholesterol-rich particles, the level of LDL-C/non-HDL-C, relative to the population, will be greater than the level of apoB. If risk is more closely related to LDL-C/non-HDL-C than apoB and if LDL-C/non-HDL-C are high but apoB is normal or low, observed cardiovascular risk should be high. However, if risk is more closely related to apoB (the number of particles) rather than to the mass of cholesterol within them, risk should be low, not high. Conversely, if cholesterol-depleted apoB particles are present and apoB is high, but LDL-C/non-HDL-C are normal or low, if apoB is correct, risk should be high, whereas if LDL-C/non-HDL-C are correct, risk should be normal or low. Discordance analysis ensures the two classes of markers must make diametrically opposite predictions. Therefore, one must be right and the other wrong.

Eight discordance analyses have been published [12, 37–44]. These include the Framingham Offspring Study [38], the Women's Health Study [39], the INTERHEART study [40], and the CARDIA study [41]. While the definitions of discordance have varied – and therefore the size of the discordant groups have varied from 20% to 66% of the total – the major findings have been the same. In all eight, apoB or LDL particle number predicted risk correctly, whereas LDL-C did not [7]. Four studies directly compared non-HDL-C and apoB [38–41]. In all four, apoB predicted risk correctly, whereas non-HDL-C did not. The conclusion that follows, as relentlessly as the night follows the day and the day follows the night, is that CVD risk relates more directly to the number of apoB particles within the lumen of the artery than to the mass of cholesterol within them. Just as the number of apoB particles within the lumen of the artery is the primary determinant of the number of apoB particles that are trapped within the arterial wall, the number of apoB particles trapped within the arterial wall is the fundamental driver of the atherosclerotic process within the arterial wall. Not to accept this – to continue to argue that non-HDL-C and apoB are equivalent markers of cardiovascular risk – is not to accept that our conclusions should be evidence-based and that our practice should change as the evidence changes.

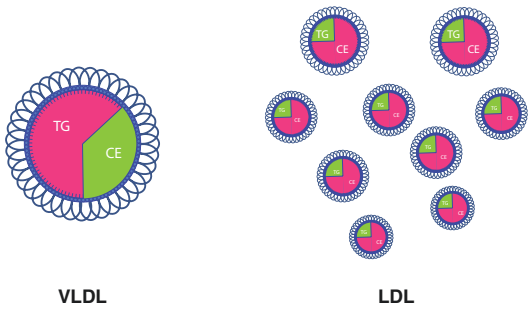

Fig. 29.5 This figure illustrates the principles on which discordance analysis is based. Three groups are created based on the physiologically-based differences in cholesterol mass per apoB particles: one is characterized by cholesterol-enriched particles, one by apoB particles containing a normal mass of cholesterol, and one by cholesterol-depleted apoB particles

Are All ApoB Particles Equally Atherogenic?

VLDL

Almost from the beginning, the lipid world has been divided into a cholesterol camp and a TG/HDL-C camp. The epidemiological results favoring LDL-C over TG as a risk factor for CVD were multiplied by the randomized clinical trials demonstrating almost uniformly that statins, which are seen primarily as LDL cholesterol-lowering agents, substantially reduced cardiovascular risk whereas fibrates, which are seen primarily as TG-lowering agents, only influenced outcome marginally and

inconsistently [26]. More recently, more fuel has been added to the TG fire by the series of Mendelian randomization analyses that appeared to demonstrate that TG increased risk even after LDL-C had been accounted for [45–47]. At the same time, among the multiple TG-rich lipoproteins, increasing emphasis was put on the potential importance of remnants in atherogenesis. Unfortunately, the definition of remnants was highly variable. The conventional definition had been the relatively cholesterol-rich, TG-poor particles generated during the normal metabolism of chylomicrons and VLDL. This restricted definition was expanded into a generic definition of all the cholesterol in the TG-rich lipoprotein: that is, as VLDL-C plus chylomicron-C [46]. Nevertheless, it remains undecided whether it is only the cholesterol in these particles or the TG or both that create the atherogenic risk. Even so, the emphasis on TG has generated a whole new series of targets for intervention such as apoCIII, ANGPTL3, and ANGLPTL4, which, based on genetic studies, were associated with reduced clinical risk [26].

The thrust of all this research is the claim that there is a unique atherogenic risk associated with VLDL particles. However, using Mendelian randomization, Ference and his colleagues have shown that the benefit of lowering VLDL, particle for particle, is equivalent to the benefit of lowering LDL, particle for particle [12]. They did so by creating genetic equivalents of drugs that act either by activating lipoprotein lipase (LPL), such as a fibrate, or the LDL receptor, such as a statin. The LPL equivalent agent resulted in substantial lowering of TG, but modest lowering of LDL-C and apoB, whereas the statin equivalent resulted in substantial lowering of LDL-C and apoB, but modest lowering of TG. Thus, the effects of lipids and apoB differed dramatically. Nevertheless, the clinical benefit of the TG-lowering LPL agent, expressed per 10 mg/dl lower of apoB, was the same as the clinical benefit of the cholesterol-lowering LDL receptor-activating agent per 10 mg/dl lower of apoB. The straightforward interpretation is that VLDL and LDL particles are equally atherogenic. Therefore, total apoB is all one needs to know.

LDL

It has been recognized for decades that LDL particles are heterogeneous in composition [48–50]. With the understanding that shifts in the core lipids – TG and cholesterol ester (CE) – mediated by CETP could produce differences in the size and composition of LDL particles [51] with small, cholesterol-depleted LDL particles becoming the dominant phenotype in patients with hyperTG, the question was raised as to whether these smaller, denser, cholesterol-depleted LDL particles were, particle for particle, more atherogenic than larger, more buoyant, cholesterol-enriched LDL particles. Indeed, considerable in vitro evidence quickly accumulated suggesting this might indeed be the case. Thus, smaller LDL particles bound more easily to the glycosaminoglycans of the arterial wall [52, 53] and were more prone to oxidation than larger LDL particles [54, 55]. Prospective observational studies appeared to support these findings [56, 57]. However, these studies did not correct for particle number, and, when this was done, there were no discernible differences in atherogenic risk associated with the different LDL particles [58]. What adverse properties were less in the larger particles compared to the smaller ones were presumably compensated for by the larger mass of cholesterol within them. Whatever the explanation, the operative conclusion is that there is no gain in subclassifying LDL particles to gain greater information as to atherogenic risk. There is no basis, therefore, to argue to measure small dense LDL cholesterol or VLDL or LDL particle number separately. Once again, total apoB is all one needs to know.

LDL-C and ApoB to Guide Treatment

The 2013 AHA/ACC Guidelines created a considerable stir by declaring that no target levels for therapy would be set because no trial had been specifically designed so that all participants achieved one predesignated level of LDL-C versus another [59]. While technically correct, this caused considerable discomfiture among clini-

cians since the ongoing results of the RCTs were all consistent with the hypothesis that a lower LDL-C is a better LDL-C. At the same time, PCSK9 inhibitors were being introduced into care so that even lower levels of LDL-C would be easily achievable [60, 61]. The 2018 AHA/ACC multisociety guidelines squared this circle by introducing the concept of a 50% reduction in LDL-C as denoting an adequate response to therapy [2]. Since the objective of therapy is to achieve an adequate response to therapy, a 50% reduction is logically equivalent to a goal (or target) of therapy. While this may have settled the controversy about goals – one man or woman's target could be another man or woman's goal – it does not deal with how well (or rather how poorly) LDL-C can be measured and the existential issue as to whether LDL-C should be the primary target of therapy. The body of evidence supporting apoB as the primary target of lipid-lowering therapy is robust and resolves the dilemmas of whether LDL-C, non-HDL-C, and TG as targets or goals of lipid-lowering therapy.

Statins Differentially Affect LDL-C, Non-HDL-C, and ApoB

Statins reduce LDL-C, non-HDL-C, and apoB by similar, but not identical, amounts [62]. Statins reduce LDL-C more than they reduce non-HDL-C and they reduce non-HDL-C more than they reduce apoB. Thus, if statins reduced LDL-C by 42.1%, non-HDL-C is reduced by 39.6%, whereas apoB is reduced by 33.1%. Put differently, the reduction in non-HDL-C is 94% of the reduction in LDL-C, while the reduction in apoB is 79% of the reduction in LDL-C. Since the absolute benefit of any intervention, whatever it may be, must be the same for all three markers, this means that benefit per mg/dl lowering of apoB must be greater than the benefit per mg/dl lowering of LDL-C or non-HDL-C. This is one reason apoB is a more effective tool in your clinical toolbox than LDL-C and non-HDL-C to determine the adequacy of lipid lowering.

Indeed, a meta-analysis of RCTs confirmed that change in apoB was more closely related to the benefit of therapy than LDL-C than non-HDL-C [63]. These differences are large. Thus, a 40% reduction in non-HDL-C would result in 200,000 fewer cardiovascular events over 10 years than a 40% reduction in LDL-C, whereas a 40% reduction in apoB would result in 500,000 fewer events. Accordingly, targeting apoB results in a gain of 500,000 events that would not occur compared to targeting LDL-C versus a gain of only 200,000 for non-HDL-C versus LDL-C [63]. Given these results, why would we continue to use non-HDL-C or LDL-C?

Evidence from RCTs

There are two ways to look at markers and clinical outcomes: one is which marker best predicts residual risk, whereas the other is which marker best predicts the benefit of therapy? A participant level meta-analysis of eight major statin trials demonstrated that on-treatment non-HDL-C was marginally more closely related to the risk of a second event than apoB [64]. The precision of the comparison is limited because at low levels, the atherogenic lipoproteins likely explain less of residual risk and the absolute decreases possible are limited. Moreover, there is no reason to believe that the rank order of the markers in predicting risk will differ between those who have not had an event versus those who have, and this evidence definitively supports apoB.

As to benefit – that is to say, the decrease in events per unit lowering of a marker – a frequentist meta-analysis of seven major statin RCT trials demonstrated that the benefit of statin therapy was more closely related to the decrease in apoB than to the decreases in either LDL-C or non-HDL-C [63]. A Bayesian meta-analysis of the same data also favored apoB compared with LDL-C or non-HDL-C [63]. Moreover, a previous Bayesian meta-analysis by Robinson et al. had also shown benefit from statin therapy correlated more closely with apoB than with the other two markers [65]. However, the differences were not as clear as in the study by Thanassoulis [63]. Nevertheless, differences in statistical methodology likely explain much of this. For

example, in the Thanassoulis analysis, changes in levels of a marker before and after therapy were compared by a paired t test [63], whereas in the Robinson analysis, they were compared by an unpaired t test [65]. Since values before and after therapy in the same patients are being compared, the more powerful paired method seems to be the more reasonable choice.

In these analyses, apoB is not simply statistically superior to LDL-C and non-HDL-C; apoB is clinically superior. Thus, if equivalent levels of non-HDL-C and apoB are selected to match an LDL-C of 70 mg/dl, there would be a 26% further reduction in clinical events if the equivalent non-HDL-C target of 90 mg/dl was employed, whereas there would be a 58% reduction beyond that achieved with a LDL-C of 70 mg/dl if the equivalent level of apoB was used [63]. These are differences that are large enough to matter.

Moreover, these differences exist in the real world: in six trials of statin therapy that achieved an average LDL-C of 70 mg/dl (1.8 mmol/L), the 10th percentile of the American population, the average apoB was 80 mg/dl, a value that corresponds to approximately the 35th percentile of the American population, demonstrating that a substantial portion of those meeting the very-high-risk target for LDL-C have levels of apoB that could contribute meaningfully to residual risk [66]. Sathiyakumar and his colleagues extended these results in a detailed analysis in a survey of subjects within the NHANES dataset. They found that in those who meet their LDL-C and non-HDL-C targets of 70 mg/dl and 100 mg/dl, respectively, 31–34% of all and 40–50% of high-risk subjects do not meet the population equivalent apoB target of 65 mg/dl [67]. Failure to measure apoB, therefore, can commonly result in undertreatment. Undertreatment means that events that could have been prevented will not be prevented.

These results should not be surprising. Cholesterol-diminished apoB particles are much commoner in all the groups that are at high risk of CVD [26]. These include men, those with abdominal obesity, hypertriglyceridemia, or diabetes; and South Asian subjects. The significance of this is illustrated in Fig. 29.6. In any individual with apoB particles with an average cholesterol content, the levels of apoB and LDL-C/non-HDL-C will be equivalent in terms of population percentile. In those with cholesterol-enriched apoB particles, the level of LDL-C/non-HDL-C in terms of population percentile will be high relative to the level of apoB. However, in those with cholesterol-diminished apoB particles, the level of apoB relative to the population will be higher. This means that post-therapy, apoB relative to the other two markers, LDL-C and non-HDL-C, will be higher. This explains the results just reviewed from the six statin trials, in which LDL-C and non-HDL-C were at treatment targets whereas apoB was still at the 35th percentile [66], as well as the results of Sathiyakumar and his colleagues [67].

CTT demonstrated that the relative benefit of statin therapy was reasonably constant: for every mmol/L (38.g mg/dl) reduction in LDL-C, the risk of a CVD was reduced by about 20% [68]. It follows that the absolute benefit of therapy depends on the baseline level of LDL-C: the higher the baseline level, the greater the absolute reduction that is possible and therefore the greater the absolute benefit achievable with therapy. Simply put, the higher the baseline LDL-C, the greater the benefit possible. For apoB, the corresponding figure is that for every reduction of 28 mg/dl in apoB, events are reduced by 24% [36].

Evidence from Mendelian Randomization Studies

The critical issue is which index – LDL-C or apoB – should be chosen when LDL-C/non-HDL-C and apoB are discordant, that is, when one is relatively higher relative to the other? As noted above, all the discordance analyses have demonstrated that when apoB is discordant from LDL-C/non-HDL-C, apoB predicts CVD risk correctly whereas LDL-C/non-HDL-C do not. Cholesterol ester transfer protein inhibitor (CETPI) agents were developed to test the hypothesis that raising HDL-C would reduce CV events. However, as a consequence of raising

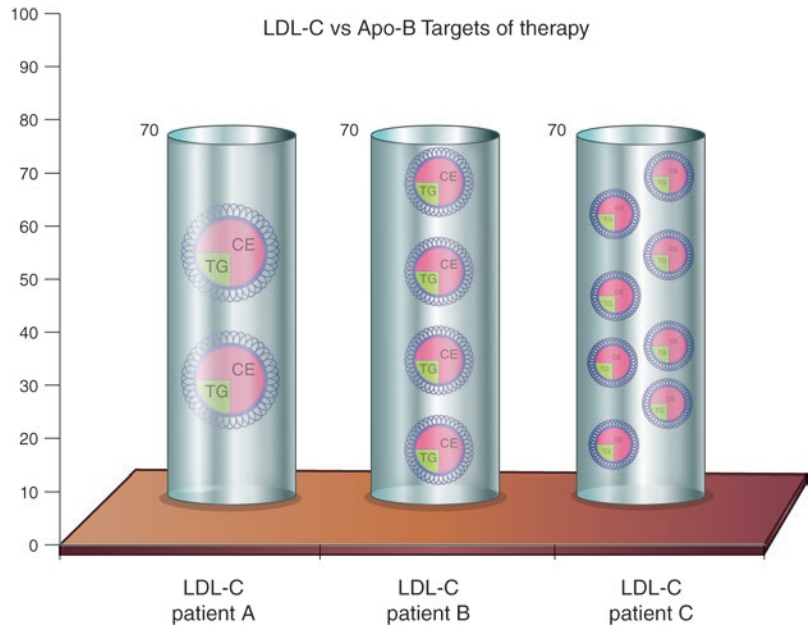

Fig. 29.6 This figure illustrates apoB particles in a patient with type III dyslipoproteinemia. Note the massively increased number of abnormal remnant particles relative to the number of LDL particles

HDL-C by reducing transfer of CE to apoB lipoprotein particles, LDL-C was also reduced substantially. Notwithstanding these favorable lipid changes, trials of statin-CETPI combination therapy, such as the ACCELERATE trial [69], which demonstrated a large decrease in LDL-C, did not result in significant clinical benefit, a result which challenged the causal role of LDL-C in CVD [70].

To resolve the dilemma, Ference et al. [70] created genetic equivalents for combination statin-CETPI therapy by identifying all alleles within 100 kb of either side of the *HMGCoAR* gene or the *CETP* gene that were associated with lower levels of LDL-C. A score incorporating these alleles was created for each gene. A *CETP* score at or above the median was associated with higher levels of HDL-C, lower levels of LDL-C and apoB, and lower levels of cardiovascular risk. An *HMGCoAR* score at or above the median was not associated with significant changes in HDL-C but was associated with lower levels of LDL-C, apoB, and cardiovascular risk. For participants with both scores above the median, the reduction in LDL-C was additive, but the reduction in apoB was attenuated. The attenuated reduction in apoB was associated with a nonsignificant decrease in cardiovascular risk, thus explaining the otherwise paradoxical finding of a significant decrease in LDL-C with combination statin-CETPI therapy without clinical benefit. These results demonstrate that benefit was associated with the decrease in apoB, not the decrease in LDL-C.

Other analyses in this study included genome-wide association studies, which compared independent variants associated with lesser reductions in LDL-C vs apoB to variants producing similar reductions in LDL-C and apoB [70]. Thus, in the first group, the reductions in LDL-C and apoB were discordant, whereas in the second, they were concordant. Benefit related to apoB in both groups but to LDL-C only in the group with concordant reduction, demonstrating again that benefit related more directly to the decrease in the number of apoB particles than to the mass of cholesterol within them. The findings from this study predated, but predicted precisely, the borderline positive results of the REVEAL (Randomized Evaluation of the Effects of Anacetrapib Through Lipid Modification) study [71]. Thus, Mendelian randomization confirms that the primary mechanism of benefit from lowering LDL-C relates to the lowering of the number of LDL particles – that is, to the lowering of apoB.

Laboratory Measurement of ApoB and Lipids

ApoB can be measured accurately and inexpensively using standardized immunoassay methods in standard clinical laboratories. This method to measure apoB has been approved by consensus statements from both American [72] and European clinical chemists [73]. Indeed, the concern should be with the measurement of LDL-C and, although less so, with non-HDL-C. Ultracentrifugation is the gold standard to measure LDL-C, but ultracentrifugation is not a practical method for routine clinical care. Indeed, LDL-C could only be introduced into clinical care because of the formula devised by Friedewald and his colleagues to calculate LDL-C based on TC, HDL-C, and TG [27]. This approach assumes the TG/VLDL-C ratio is constant – which it is not – and that HDL-C and TG can be measured precisely using standardized methods, which they cannot. At the time LDL-C was introduced into clinical care, these deficiencies were known, but did not matter since effective therapy did not exist and apoB lipoproteins had not been proven to cause CVD.

These deficiencies do matter now because effective therapies do exist now and apoB lipoproteins are proven to be a primary cause of CVD. Therefore, the adequacy of therapy needs to be accurately assessed. One approach has been the introduction of assays that directly measure LDL-C. Unfortunately, these have not been standardized, nor have they been validated in dyslipidemic samples, nor is there any evidence that directly measured LDL-C outperforms calculated LDL-C [72, 73]. There is, therefore, no evidence their use justifies the additional cost, a concern which has not been noted in any guidelines. It is curious, as regards the benefits versus the cost of a laboratory test, how high the bar has been set for apoB versus how low the bar has been set for directly measured LDL-C.

New methods to calculate LDL-C have been developed, and these seem particularly valuable at low levels of LDL-C, which has become an important issue in this era of PCSK9 inhibitors [5]. Interestingly, the apparent advantage of non-HDL-C over LDL-C is diminished or obliterated by such an approach. However, the physician must know which method was used to calculate LDL-C in order to compare the present result with previous results. Non-HDL-C is the arithmetic difference between TC and HDL-C. Measurement of TC is standardized, accurate, and inexpensive. Measurement of HDL-C is not standardized and not free of error and comes at a small but not insignificant cost. At low levels of TC, the error in HDL-C introduces significant error in the calculation of non-HDL-C [72, 73]. The bottom line is that measuring apoB involves only one measurement which is standardized, and one measurement which is standardized is preferable to multiple measurements which are not. The technology to measure apoB is, or should be, available in every clinical chemistry laboratory, and the costs of the test are not high.

Is ApoB All That Needs to Be Measured to Monitor Therapy?

At the present time, with three exceptions, type III hyperlipoproteinemia, type I hyperlipoproteinemia, and type V hyperlipoproteinemia, apoB is all that needs to be measured to monitor lipid-lowering therapy [13]. Type III hyperlipoproteinemia is a highly atherogenic dyslipoproteinemia, which generally appears in midlife, is more frequent in those with diabetes and abdominal obesity, and is characterized by the accumulation of massive numbers of cholesterol-enriched remnant chylomicron and VLDL particles [74, 75]. The pathophysiological defect is markedly impaired clearance of chylomicron and VLDL remnant particles, which persist in plasma for much longer than normal and therefore accumulate much more cholesterol than normal due to CETP-mediated CE-TG exchanges. However, the conversion of VLDL to LDL particles is severely reduced. Accordingly, there are many fewer LDL particles than normal. The result as shown in Fig. 29.7 is that total apoB is normal.

Type III is a treat-on diagnosis disorder, just like familial hypercholesterolemia. Diagnosis requires treatment. Calculation of risk is irrelevant. The problem is that with current lipid panels the diagnosis cannot be made, not even in specialized lipid clinics [76]. However, with just TC, TG, and apoB, the diagnosis could be made by any clinical laboratory. To the best of my knowledge, there are no studies that define how type III should be followed. Accordingly, I would recommend a conventional lipid panel plus apoB and use the apoB algorithm to ensure no significant numbers of abnormal remnants remain.

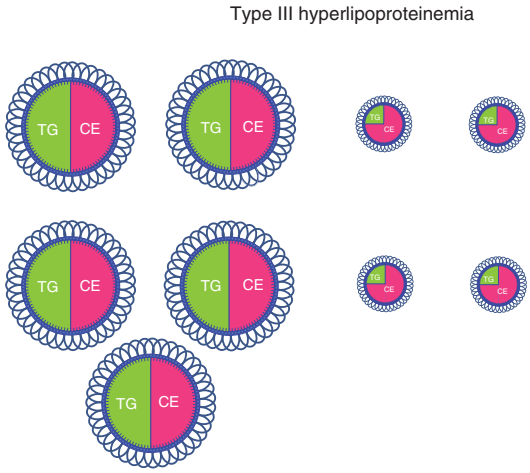

Fig. 29.7 This figure illustrates that at the same level of LDL-C (70 mg/dl), apoB particle number may vary substantially depending on whether the apoB particles are cholesterol-enriched (patient A), contain an average mass of cholesterol (patient B), or are depleted in cholesterol (patient C)

Type I and type V hyperlipoproteinemia are both characterized by marked hypertriglyceridemia, which can become severe hypertriglyceridemia, which can cause pancreatitis. Both disorders are quite uncommon and therefore do not warrant routine screening [13]. They can be diagnosed and differentiated with TC, TG, apoB, and application of the apoB algorithm. I do not recommend routinely measuring HDL-C other than to make an initial assessment of risk as I make no clinical decisions based on the result. TG are obviously the key criterion to follow these disorders. In my view, the evidence is now strong enough that I measure Lp(a) in every patient whom I evaluate for preventive statin therapy.

For the convenience of the reader, Table 29.1 summarizes the diagnostic criteria of the apoB algorithm for type III, type I, and type V, and Table 29.2 lists the levels of apoB that correspond to the equivalent population levels of LDL-C and non-HDL-C.

Table 29.1 Type III hyperlipoproteinemia, type I hyperlipoproteinemia, type V hyperlipoproteinemia, FH familial hypercholesterolemia; CVD cardiovascular disease; TG/apoB triglyceride/apoB ratio; TC/apoB total cholesterol/apoB ratio

Disorder	Disease	TG/apoB	TC/apoB	apoB mg/dl
Type III	CVD	<10	>6.2	<120
Type I	Pancreatitis	≥10		<75
Type V	Pancreatitis	≥10		≥75
FH	CVD			150

Summary

The three principal criticisms of apoB have been (1) that apoB does not increase the accuracy of the 10-year prediction of cardiovascular risk; (2) that apoB adds cost to a conventional lipid panel; and (3) that apoB is too complex for clinicians to understand. This chapter has outlined the limitations of the 10-year prediction of cardiovascular risk, including the fact that no lipid marker sig-

Table 29.2 Population percentiles and concentrations of LDL-C, non-HDL-C, and apoB (mg/dl)

Percentiles	10	40	75	90	98
LDL-C	70 mg/dl	100 mg/dl	130 mg/dl	160 mg/dl	190 mg/dl
Non-HDL-C	90 mg/dl	120 mg/dl	160 mg/dl	195 mg/dl	230 mg/dl
ApoB	65 mg/dl	80 mg/dl	105 mg/dl	125 mg/dl	150 mg/dl

All are rounded to the nearest 5 mg/dl and the nearest percentile
NHANES percentiles LDL-C levels and corresponding non-HDL and apoB values

nificantly improves the prediction of risk. Therefore, if this is the reason not to measure apoB, it is equally a reason not to measure any lipid, which is patently absurd. Second, for routine care, apoB adds to the conventional lipid panel? Therefore, why pay for a conventional lipid panel. If cost is the issue, apoB does not have to be a cost on top of a cost but rather a cost instead of a cost. Third, apoB equals the number of atherogenic particles. What could be simpler and clearer? It is the conventional lipid panel that is complex, confusing, and contradictory. LDL-C/non-HDL-C can be high but risk due to the atherogenic lipoproteins low. In which case, why measure LDL-C/non-HDL-C? Similarly, TG can be high but risk due to the atherogenic apoB lipoprotein particles low. Trapping of apoB particles within the arterial wall is fundamental to the initiation and maturation of atherosclerotic lesions within the arterial wall. ApoB is the most accurate measure of the number of apoB particles in plasma. Why rely on markers such as LDL-C and non-HDL-C, which are only indirect measures of apoB? Why order a lipid panel with five measures that need to be integrated and interpreted when a more accurate picture is given by one?

ApoB brings clarity, not complexity. ApoB integrates, summarizes, and amplifies the information from a conventional lipid panel. A simpler, more advanced process of care will result in better care. Nevertheless, apoB is not the only test that needs to be done. Lp(a) adds significantly to apoB, and TC and TG are necessary for full diagnosis of the atherogenic apoB dyslipoproteinemias. Introducing apoB into clinical care will require a major educational effort. But the simplification of the process of care and the improvement in the outcomes of care that results will be recognized and appreciated by primary care physicians as well as cardiologists and internists.

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30

C-Reactive Protein and Other Inflammatory Markers in Cardiovascular Disease: Inflammatory Disorders and Atherosclerosis

Eric J. Brandt

CRP Biology and Biochemistry

C-reactive protein (CRP) is a 206-amino acid plasma protein with high evolutionary conservation that is mostly synthesized in the liver [1, 2]. There are two forms of CRP, a native/pentameric form [5 identical subunits arranged symmetrically] and a modified/monomeric form, each of which have separate physiology [3]. Notably, the monomeric form may be synthesized outside the liver, in peripheral tissues. In fact, CRP messenger RNA has been found in various tissues, including adipose, lung, lymphocyte, renal cortical tubules, and atherosclerotic lesions (specifically within macrophages and cardiac smooth muscle cells). It is thought that the pentameric form can locally dissociate into the monomeric form, which may happen at the membranes of apoptotic cells and within activated platelets of atherosclerotic plaques [1].

Synthesis CRP varies in response to other inflammatory cytokines. Specifically, interleukin-1β (IL-1β), tumor necrosis factor (TNF), and interferon-γ are key inflammatory cascade factors that are released from macrophages and other inflammatory cells that then induce production of IL-6, which in turn promotes de novo CRP synthesis [1, 4–6]. Overall, CRP can be considered a more terminal component of an inflammatory cascade, which is initiated by IL-1 or TNF that then activates IL-6 which acts to signal CRP production. In fact, loss-of-function variants in IL-6 signaling have been found in Mendelian randomization studies to predict lower levels of hsCRP as well as cardiovascular events [7]. This suggests that IL-6 signaling plays a causal rather than bystander role in inflammation and ASCVD. Notably, IL-6 signaling increases the production of multiple acute-phase reactants beyond CRP, including plasminogen activator inhibitor-1 (PAI-1), serum amyloid A, IL-17, and TGF-β. Each of these factors may be connected to endovascular damage via their roles in inflammation, calcification, and fibrosis.

CRP itself has multiple potential downstream consequences for the development of atherosclerosis and atherothrombosis once it enters the serum. These include activation of the classic complement system, apoptosis, vascular cell activation, monocyte recruitment, lipid accumulation, and thrombosis [1]. Additional mechanisms include augmented expression of matrix metalloproteinases, disruption of nitric oxide synthesis, increased uptake of oxidized LDL (OxLDL), and promotion of fibrin formation [1, 8]. Interestingly, it is the monomeric form more so than the pentameric form that is thought to participate in the local detrimental mechanisms of CRP. However, the monomeric form is scarcely

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_30

detected in peripheral samples. As techniques to better measure the monomeric form evolve, this will increase understanding of differences in physiologic consequences of the polymeric versus monomeric forms and perhaps the ability of CRP to predict risk [1]. Lastly, the relationship between CRP and atherosclerosis is not unidirectional. As cholesterol is deposited in the subendothelium, the resultant endothelial dysfunction and activation can result in the release of inflammatory molecules (increased IL-6 and IL-1), including increased CRP production locally and hepatically. The inflammatory state results in elevated triglyceride-containing lipoproteins, small dense LDL particles and HDL particles, lipoprotein(a), and OxLDL [9]. CRP and OxLDL can both interact with lectin-like OxLDL receptor-1 (LOX-1), which increases endothelial dysfunction and monocyte deposition, thus completing a process that can feed back onto itself [10].

CRP as a Clinical Marker

The atherosclerotic and atherothrombotic processes are a result of the interaction of many factors, including adhesion molecules, cytokines, circulating monocytes, lipoproteins, and vascular endothelium [11]. CRP was initially described in the 1930s from its role in the acute-phase response [12]. Despite discovery in the 1930s, it wasn't until the mid-1990s that the association between acute coronary syndromes (ACS) was identified [11]. Initially, it was unclear if elevations in CRP were resultant from the ischemia or vice versa. However, it became apparent that chronic low-grade inflammation, as estimated by hsCRP, does precede clinical events [11, 13]. The contribution to clinical events more likely relates to risk of plaque rupture and thrombosis than to a representation of underlying atherosclerotic burden [11]. This knowledge has contributed to an understanding for various mechanisms in ACS. Notably, not all plaque ruptures occur in the presence of inflammation, as marked by CRP. In fact, half of ACS in one study occurred with normal levels of CRP [14]. Another study demonstrated with optical coherence tomography that in patients with ACS and plaque rupture, one third had no evidence of local inflammation in the region of the plaque [15]. Furthermore, many ACS are not due to plaque rupture at all and are resultant from plaque erosion. These data suggest multiple mechanisms for plaque rupture. Crea and Libby describe four potential mechanisms for ACS (see Fig. 30.1), which include the interplay of CRP: plaque rupture with inflammation, plaque rupture without inflammation, plaque erosion, and nonthrombotic etiologies (i.e., vasospasm) [16]. Thus, not all ACS might be attributable to inflammatory plaque eruption, although it is thought to interplay in a large proportion of such events. Further light has been shed to help us understand that plaques occur in a variety of stages that vary from stable and calcified to unstable and noncalcified, or a mix thereof. In a 2011 study of the MESA cohort, which only included patients that would have met criteria for the JUPITER trial, it was found that coronary artery calcium, but not hsCRP, was a predictor of coronary heart disease (CHD) or cardiovascular disease (CVD) [17]. However, another study found that among those without coronary artery calcium, hsCRP remained a predictor of risk for those with noncalcified plaques but not for calcified plaques or plaques with a mix of calcified and noncalcified plaque [18].

There remains a debate regarding the level of attribution that should be assigned to hsCRP. Is hsCRP a marker of a disease state or does it have a direct causal link to atherosclerosis or atherothrombosis? Investigations involving purified CRP, Mendelian randomization, and genome-wide association studies have helped to answer this question.

First, studies of infused pentameric recombinant or highly purified human CRP have failed to show any systematic physiologic changes. Next, Mendelian randomization methods use random and naturally assorted genetic variants to determine whether the relationship between a risk factor and an outcome is causative or merely an association. These methods have contributed to understanding the causality of several disease markers, including LDL-C, lipoprotein(a), blood

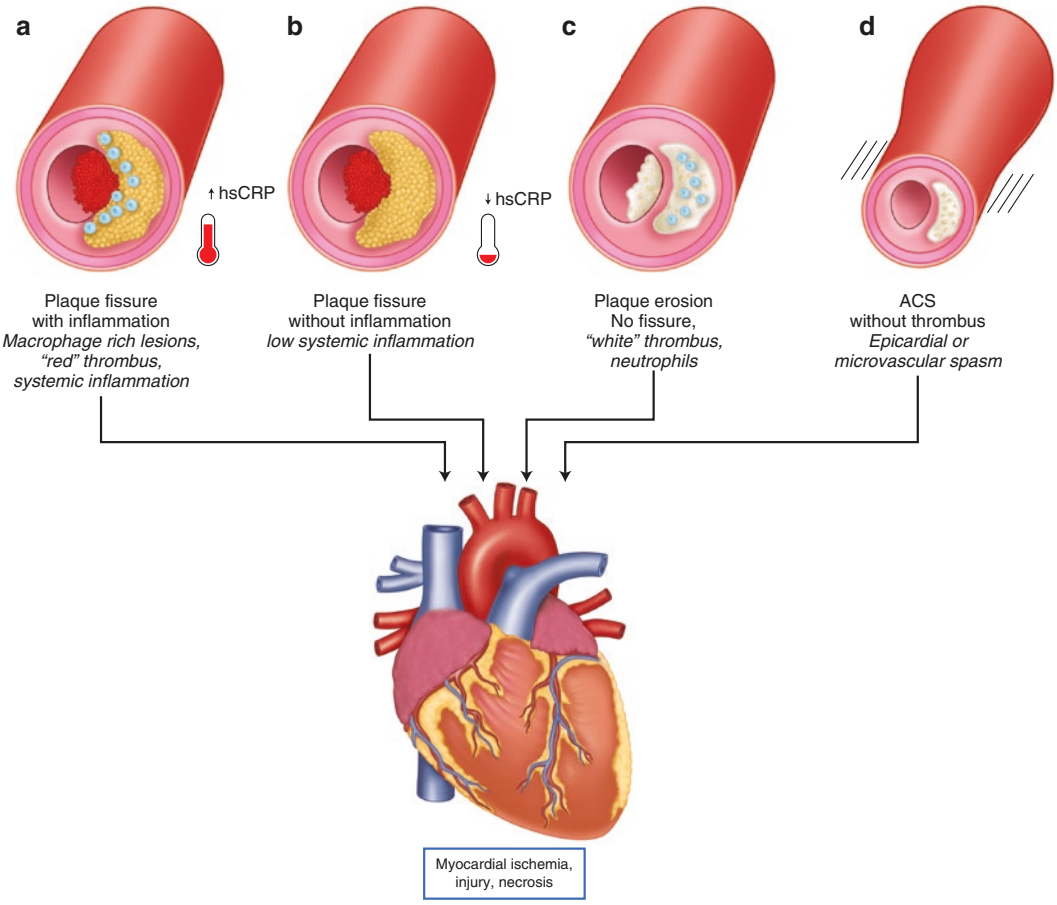

Fig. 30.1 Four diverse mechanisms cause acute coronary syndromes (ACS). (**a**) Plaque rupture, also referred to as fissure, traditionally considered the dominant substrate for ACS, usually associates with both local inflammation, as depicted by the blue monocytes, and systemic inflammation, as indicated by the gauge showing an increase in blood C-reactive protein (CRP; measured with a high-sensitivity [hsCRP] assay). (**b**) In some cases, plaque rupture complicates atheromata that do not harbor large collections of intimal macrophages, as identified by optical coherence tomography criteria, and do not associate with elevations in circulating CRP. Plaque rupture usually provokes the formation of fibrin-rich red thrombi. (**c**) Plaque erosion appears to account for a growing portion of ACS, often provoking non-ST-segment-elevation myocardial infarction. The thrombi overlying patches of intimal erosion generally exhibit characteristics of white platelet-rich structures. (**d**) Vasospasm can also cause ACS, long recognized as a phenomenon in the epicardial arteries but also affecting coronary microcirculation. (Adapted from Crea and Libby [16]. https://www.ahajournals.org/doi/full/10.1161/CIRCULATIONAHA.117.029870)

pressure, and BMI, among others [19]. For hsCRP, Mendelian randomization studies have failed to confirm it as directly causal of disease, but rather best serves as a disease marker [11, 20–22]. However, Zimmerman et al. do note that Mendelian randomization studies are not without their pitfalls, including the possibilities of linkage disequilibrium, pleiotropy of influential CRP single nucleotide polymorphisms that may positively or negatively affect risk attribution, gene-gene interactions, and canalization (changing genetic influence from various factors over

time) [23]. Hepatic CRP synthesis on the transcription level is exceptionally complex [24]. Lastly, genome-wide association studies have also failed to make the connection; one study among over 66,000 patients identified 18 CRP-associated loci, but no association with CHD [21, 25]. Regardless, this lack of direct causality does not dissolve its utility as a marker. Rather, the focus should be on upstream pathway factors for treatment targets and potential future biomarkers. In this regard, CRP might serve as a marker for other pathways, including the IL-6 receptor pathway or IL-1β [11]. In fact, Mendelian randomization studies suggest that IL-6 signaling pathway is causal for vascular events and influential to hsCRP levels [26]. On the whole, hsCRP can be considered a marker of plaque vulnerability and risk for thrombosis, but not as causal for ASCVD [8].

Interpretation of CRP

CRP and hsCRP are not interchangeable. Clinically the hsCRP is best suited for ASCVD risk assessment, whereas CRP is most adequate for monitoring major infections, inflammatory disorders, and endocarditis. One can often differentiate which test was ran based on the value alone. CRP are typically reported as mg/dL, whereas hsCRP are typically reported as mg/l [11].

Values of hsCRP can be separated in four categories [6, 11]:

- <1 mg/l – desirable/optimal, indicates low systematic inflammatory status and vascular risk
- 1–3 mg/l – moderate vascular risk
- >3 mg/l – higher vascular risk (must be interpreted within context of the individual patient)
- >10 mg/l – likely reflective of acute-phase response (i.e., acute infection), should be repeated in 2–3 weeks or upon resolution of the acute process

hsCRP values for an individual are 35–40% heritable, with the rest of the determination based on an individual's clinical milieu. It can be generally expected to slightly increase as one ages and vary significantly by race. Compared to White or Hispanic patients, blacks have higher levels [11]. Compared to Caucasian, Asian populations were found in a meta-analysis to have as follows: 22% higher levels for South Asians, 48% lower for Chinese, and 64% lower for Japanese, although there were few studies [27]. One should be conscious that these differences are likely reflective of varying levels of risk and not predetermined set points.

Some concern has been raised regarding hsCRP, including its specificity as a marker, repeatability, causality, and cost-effectiveness. Regarding specificity, hsCRP also predicts risk for other diseases, particularly for metabolic disorders (insulin resistance, adiposity, and type 2 diabetes) and all-cause mortality [11]. One should be sure to avoid measuring hsCRP in situations with known acute inflammatory responses to ensure they are getting an accurate value. Notably, hsCRP can shift up to 10,000-fold within 6 h and peaks 48 h after an acute event; the half-life is 19 h [23].

Other clinical factors are known to influence levels of hsCRP. These include the common scenarios in Western societies of metabolic syndrome, obesity, and insulin resistance. In fact, adipose tissue is an additional source of IL-6, the primary upstream factor for CRP production. In both the Dallas Heart Study and MESA studies, obesity was found to account for all or most, respectively, of the association between hsCRP and atherosclerosis [25, 28, 29].

To be sure, hsCRP has been found to have good repeatability with correlations ranging from 0.46 to 0.66. Although these values are moderate, in most studies hsCRP performed similar in repeatability to total cholesterol and blood pressure [11, 26, 30]. Interestingly, in the MESA and NHANES cohorts, repeat values have reclassified individuals from values of high risk to lower levels [30, 31]. Clinicians should use their best judgment to decide on appropriate scenarios to repeat CRP. Certainly, when the values are >10 mg/l, the clinician should repeat the study once the acute illness phase has resolved.

Disease Associations and hsCRP in the Context of Specific Diseases

hsCRP has been associated with a variety of clinical conditions. The list continues to lengthen, but this includes association with mortality (all-cause, cardiovascular (CV), and cancer-related) and the development of various CV conditions.

A recent meta-analysis by Li et al. found that among 83,995 participants in 14 studies, the highest to the lowest hsCRP category conferred an independent RR of 1.25 (95% CI 1.13–1.38) for cancer-related mortality, 2.03 (95% CI 1.65–2.50) for CV mortality, and 1.75 (95% CI 1.55–1.98) for all-cause mortality [32].

For CV disease, among patients with peripheral artery disease, it was found in a meta-analysis of eight studies that those in the highest vs lowest quantile had a higher risk of major CV events (HR 2.26, 95% CI 1.65–3.09, $p < 0.001$), with a HR of 1.38 (95% CI 1.16–1.63) per unit increase in $\log_e$ CRP [33]. For stroke, the RR of ischemic stroke was 1.46 (95% CI 1.27–1.67), but 0.82 (0.59–1.13) in hemorrhagic and 1.23 (95% CI 0.997–1.51) in all strokes when comparing highest to lowest groups [34].

Additional vascular diseases have also been associated. These include common carotid artery intima-media thickness, for which each standard deviation increase in hsCRP was associated with a significant 0.0082 mm increase ($p < 0.001$) [35]. For abdominal aortic aneurysm, it was found in meta-analysis that CRP was associated with presence of aneurysm and aortic diameter, but not with aneurysm growth rates. The area under the curve for CRP was 0.61. In this study the authors concluded it may not be a useful clinical marker in this context [36].

Autoimmune diseases represent a set of diseases that confer an increase in risk for ASCVD, which is not captured by current risk algorithms or calculators. Among patients with autoimmune disease, it is not yet clear how hsCRP can be integrated into risk estimation. Although experts in this area note that, along with other nontraditional risk estimation tools, i.e., coronary artery calcium scoring, hsCRP could be part of risk estimation and decision to initiate statin therapy [37].

In HIV, hsCRP has been evaluated in multiple studies as a potential additional risk estimator to predict incident ASCVD. Results have been mixed and in meta-analysis have not confirmed an additive benefit [38]. However, data are sparse; inclusion of hsCRP in future studies aimed to address the risk for ASCVD among those with HIV is encouraged [39].

Other diseases with associations include new-onset atrial fibrillation after acute myocardial infarction (MI) [40], pericarditis [41], new-onset hypertension, [42], venous thromboembolism [43, 44], and diabetes [45, 46]. Interestingly dental disease has also been found to influence systemic inflammation, treatment of which has been found in meta-analysis of randomized clinical trials to significantly reduce CRP levels ($p < 0.001$) [47].

Risk Estimation

Despite the availability of many risk prediction models, a considerable amount of those at risk for CV events remain unidentified by using traditional risk factors alone [23]. There have been many studies to estimate the risk association with hsCRP and CV disease. One meta-analysis that includes more than 160,000 subjects and 1.3 million person-years of follow-up found that each standard deviation increase in log-normalized hsCRP significantly increases risk 1.37 times (95% confidence interval (CI) 1.27–1.48) for coronary heart disease and 1.55 times (95% CI 1.37–1.76) for CV mortality. When compared to systolic blood pressure, total cholesterol, and non-high-density lipoprotein cholesterol, hsCRP in this study had at least the same magnitude of effect [48]. See Fig. 30.2.

Current guidelines provide recommendations on how to integrate hsCRP into clinical practice. The US guidelines offer a Class IIb recommendation to utilize hsCRP in risk estimation when there is uncertainty surrounding the decision to initiate statin therapy in intermediate-risk patients

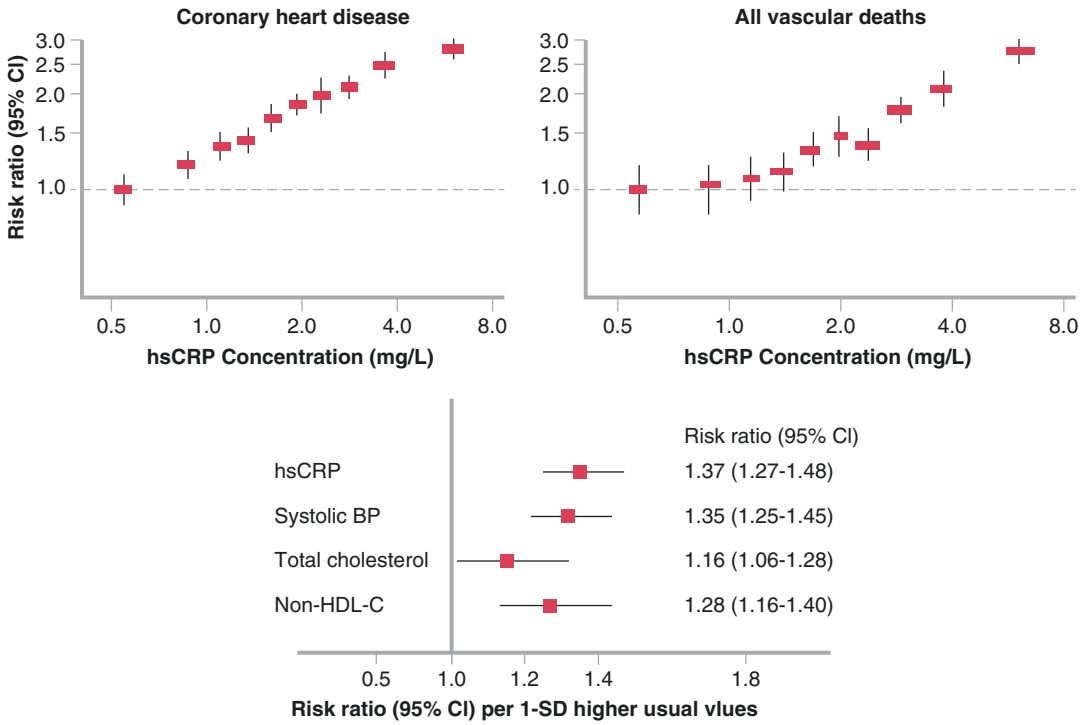

Fig. 30.2 Predictive usefulness of hsCRP in primary prevention. The relationship of hsCRP levels in healthy subjects to future risks of coronary heart disease and vascular deaths (*top*). The magnitude of cardiovascular risk associated with a 1-standard deviation (SD) change in hsCRP is at least as great as that associated with a similar change in systolic blood pressure, total cholesterol, or non-HDL-C (*bottom*). (Data from Kaptoge et al. [6]. *BP* blood pressure, *CI* confidence interval, *hsCRP* high-sensitivity C-reactive protein, *HDL-C* high-density lipoprotein cholesterol. Adapted from Ridker [11])

[11, 49]. The 2016 Canadian Cardiovascular Society guidelines recommend measuring in men (50 years and older) or women (60 years and older) with ≥1 additional risk factors who are at intermediate (10–19%) risk according to the Framingham Risk Score who would not otherwise qualify for lipid therapy [50]. The 2016 European Society of Cardiology guidelines give Class IIIB recommendation for assessment of circulating and urinary biomarkers, which include hsCRP [51].

To be sure, there are clinical risk estimation tools that integrate hsCRP into risk calculation. Perhaps the most widely utilized risk estimator with this feature is the Reynolds Risk Score [52]. Additional data needed for an estimation includes gender, age, smoking status, systolic blood pressure, total cholesterol, HDL cholesterol, and whether a parent had a MI before age 60. The Reynolds Risk Score is free to use and available at www.reynoldsriskscore.org.

Although one would expect incremental clinical utility in the addition of hsCRP to risk estimation, current studies have not confirmed this utility. Studies in this area typically utilize the net reclassification index, which is a calculation of the percent of patients having an event correctly reclassified divided by the percent of patients that did not have an event that were reclassified. Many studies have utilized this to assess hsCRP's additive utility to risk estimation, which suggest a net reclassification index lying between 1.5% and 12% [53]. The modest improvements in risk estimation have contributed to the aforementioned recommendations in the US guidelines. Notably, when compared to the Framingham Risk Model, the net reclassification index for the Reynolds Risk Score was

12.9% and with the Framingham model was 5.9% [54]. Other studies have had similar results; when comparing the Reynolds Risk Score, Framingham, and the Adult Treatment Panel III models, it was found that the Reynolds Risk Score outperformed both other measures, particularly in the group with 10-year risk between 5% and 10% [11]. Even when comparing the Reynolds Risk Score to the ACC/AHA Pooled Cohort risk score, it was found that the ACC/AHA risk score overestimated events by 78%, whereas the Reynolds Risk Score underestimated risk by 3% [11]. Thus, in each of these studies, it was the Reynolds Risk Score, which incorporates hsCRP, that was the superiorly calibrated risk estimator.

A recent meta-analysis was critical of the benefit of the addition of hsCRP to risk stratification or initiating preventive therapy. Lin et al. found that despite the large body of evidence for hsCRP, there lacks strong evidence that the addition of hsCRP greatly increases risk prediction [55]. They call to attention that there is only one study that evaluates the addition of hsCRP to the Pooled Cohort Equations, which found no change in discrimination with the addition of hsCRP to their model [56]. Furthermore, in the largest cohort, the Emerging Risk Factors Collaboration, the addition of hsCRP to the Framingham Risk Score among 166,596 patients added minimally to the model's discrimination ability (0.0039, 95% CI 0.0028–0.0050); the number needed to screen to prevent one event over 10 years among intermediate-risk (10–20%) individuals was between 400 and 500 people [57]. They concluded that evidence was insufficient in evaluating the incremental effect of hsCRP for risk assessment or preventive therapy [55, 58].

In situations of borderline risk estimation that might change decision to initiate therapy, one should consider multiple risk models. Although it might not be a valuable addition for all individual risk estimations, surely there remains a cohort of individuals of moderate risk for which hsCRP may alter their individual risk estimation and be an important key in shared decision-making regarding therapy initiation.

Lastly, the role for hsCRP goes beyond primary prevention. To be sure, hsCRP is able to predict recurrent coronary-related events, including acute ischemia in the post-angioplasty and bypass grafting settings, and renal failure [26]. In patients with stable CHD, those that had a recent acute event but hsCRP that remained elevated were found to be associated with increased risk of future events [59, 60]. The CANTOS trial, discussed further below, has helped to distinguish two different phenotypes (or a combination thereof) for residual risk: residual cholesterol-related risk and residual inflammatory-related risk [26]. In this mindset, one can use a patient's lipid profile and hsCRP to best devise a personalized regimen for an individual patient. As a clinical example, one might discover a post-MI patient that has sustained LDL-C >100 mg/dl but with low hsCRP (<1 mg/l) despite statin therapy and decide to utilize a PCSK9-inhibitor for further risk reduction. Alternatively, one might identify a patient with spectacular control of their lipid profile but with sustained hsCRP (>2 mg/l) that might be a candidate for direct anti-inflammatory therapy, i.e., canakinumab.

Figure 30.3 provides an algorithm for how one might utilize hsCRP in clinical risk assessment based on the current literature.

Lifestyle Interventions That Influence hsCRP or hsCRP-Associated Risk

Interventions that aim to alter high-risk health behaviors, such as lack of exercise, poor diet, and smoking cessation, are among the most powerful tools for risk reduction. For individuals with elevated hsCRP, these should be the earliest recommendations.

Smoking

Many studies have evaluated the association between smoking and hsCRP levels. More smoking is worse; CRP levels significantly

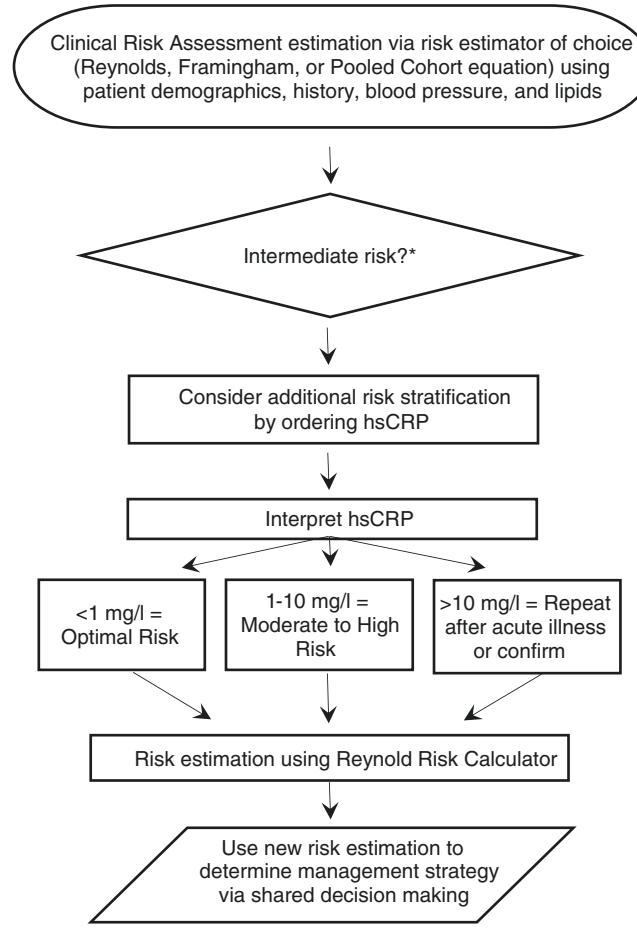

Fig. 30.3 Algorithm for utilization of hsCRP in clinical practice. *Intermediate risk for this algorithm is defined by 10-year risk for CV events between of >5% and <20%. hsCRP has highest likelihood to reclassify when risk is 5–10% [10]

increase ($p < 0.001$) per cigarette smoked per day. Also, smokers had higher levels than former smokers, who had higher values than non-smokers. When looking at current smokers only, those with the highest hsCRP levels also had higher event rates [61].

Diet

Several studies have investigated specific dietary patterns and components that influence hsCRP levels and risk for cardiac events. Notably, diets high in Western dietary components (refined starches, sugar, and saturated or trans-fatty acids) and low in fruits, vegetables, whole grains, and omega-3 fatty acids are associated with production of proinflammatory cytokines and reduced anti-inflammatory cytokines [62].

Two well-studied dietary patterns, the prudent and Mediterranean dietary patterns, are both known to reduce inflammation and also have support for improving the inflammatory milieu [63]. These dietary patterns are both known to reduce risk for CHD [64–66]. The prudent dietary pattern emphasizes a high intake of fruits, vegetables, legumes, whole grains, poultry, and fish while minimizing red and processed meat, sweets, desserts, potatoes and French fries, and refined grains [63]. In the Nurses' Health Study I (women) and Health Professionals Follow-up Study (men), the prudent pattern was associated with lower levels of CRP ($p = 0.02$ and 0.04, respectively) [67, 68]. An interventional study of 46 patients with hyperlipidemia found that the prudent diet had a 28% ($p = 0.02$) decline in CRP from baseline, which was similar to the effect from statin therapy (33% reduction in CRP,

$p = 0.002$); changes were observable at 2 weeks after intervention [69].

The Mediterranean dietary pattern is like the prudent diet with an emphasis on whole plant-based foods and minimization of processed meats and grains, although a bigger focus on olive oil and fish consumption. A recent meta-analysis found that in randomized control trials of adults, the Mediterranean diet was associated with a decrease in hsCRP by 0.98 mg/l (95% CI 0.49–1.48, $p < 0.0001$) [70]. Notably there were also lower levels of IL-6.

Exercise

hsCRP has infrequently been study in the context of exercise or sedentary behavior. One recent review found only five studies that reviewed CRP in the context of sedentary lifestyle, which the authors did not find adequate to conclude the presence or absence of a relationship [71]. However, exercise training has been associated with reductions in CRP, regardless of, but greater when accompanied by, weight loss [72]. Some investigators went further and found that both healthy adults and those with heart disease can significantly reduce hsCRP (standardized mean difference −0.53 mg/L; 95% CI, −0.74 to −0.33) [73]. Among these studies, the majority were with aerobic exercise although some included resistance exercise. Most regimens had 45–60 min sessions at three or more times per week with participants working between 50% and 90% VO_2 maximum.

Pharmacologic Interventions That Influence hsCRP or hsCRP-Associated Risk

Methotrexate: A Direct-Acting Anti-inflammatory Agent

There were recently two inflammatory cascade disrupting agents investigated in clinical trials. First was methotrexate, an agent that acts upstream in the inflammatory cascade and can have potent anti-inflammatory effects at small doses. Prior data found low-dose methotrexate to reduce risk for vascular events among those with psoriatic and rheumatoid arthritis [11, 74]. The Cardiovascular Inflammation Reduction Trial (CIRT) was a double-blind placebo-controlled randomized clinical trial among patients with coronary artery disease (CAD) plus either diabetes mellitus, metabolic syndrome, or both. This trial enrolled 4786 patients that were followed for 4 years with a mean age of 66 years to placebo or 15–20 mg weekly methotrexate. The trial failed to achieve reduction in the primary end point of major adverse cardiac events or biomarkers (IL-1β, IL-6, or hsCRP). Notably, the trial did not enroll patients based on pre-trial inflammatory biomarker elevation [75].

Canakinumab: A Direct-Acting Anti-inflammatory Agent

The second recently investigated agent with a more narrow biochemical focus is canakinumab, a human monoclonal antibody directed against IL-1β. As previously discussed, IL-1β is an important driver in the IL-6 pathway, a known causal pathway for atherosclerosis [11, 19]. In early studies that included high-risk patients with diabetes, canakinumab was found to decrease levels of fibrinogen, IL-6, and hsCRP by 15%, 45%, and 50%, respectively, without an effect on LDL-C [11, 76]. This was followed by the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS), a double-blind placebo-controlled randomized clinical trial among post-MI patients that, despite maximized therapy, had persistently elevated hsCRP > 2 mg/l [26]. Compared to placebo, canakinumab was found to have a median reduction in hsCRP of 26%, 37%, and 41% in those that received 50 mg, 150 mg, and 300 mg of the drug, respectively, with no significant reduction in LDL-C [77]. At 3.7 years of follow-up, the hazard ratios for the primary end point of nonfatal MI, nonfatal stroke, or CV death were found to be 0.93 (95% CI 0.80–1.07, P 0.30), 0.85 (95% CI 0.74–0.98, $p = 0.021$), and 0.86 (95% CI, 0.75–0.99, $p = 0.005$) for 50 mg, 150 mg, and 300 mg of canakinumab

injection every 3 months, respectively. Notably, canakinumab was found to be an effective treatment among those with chronic kidney disease. In secondary analysis, canakinumab reduced MACE in those with eGFR <60 ml/min/1.73m^2 (HR 0.82, 95% CI 0.68–1.00, $p = 0.05$) similarly to those with eGFR ≥60 ml/min/1.73m^2 (HR 0.86, 95% CI 0.77–0.97, $p = 0.012$) [78].

Notably, effect sizes differed based on achieved hsCRP. In the overall cohort of the CANTOS trial, reductions in MACE were greatest among those that achieved hsCRP < 2 mg/l (HR 0.75, 95% CI 0.66–0.85, $p < 0.001$) or >50% reduction in hsCRP (HR 0.81 (0.72–0.92), $p = 0.001$) after the initial dose [79]. This reduced the NNT to 16. Among those with CKD, the effect size was also larger in those that achieved hsCRP < 2 mg/L (HR 0.68, 95% CI 0.53–0.86) than those that did not (HR 0.84, 95% CI 0.66–1.06) ($P_{trend} = 0.0015$) [78]. This suggests that a clinical approach wherein patients have hsCRP tracked and then a treatment decision is made based on their response would identify the patients for whom continuing therapy would have the most likely benefit.

Secondary benefits of canakinumab included dose-dependent reduction in incident lung cancer and cancer mortality [80]. Notably, although there was an association with higher rate of fatal infection, there was no significant difference in all-cause mortality (HR for all doses vs placebo, 0.94, 95% CI 0.83–1.06, $p = 0.31$) [77].

Cost is currently a major limitation for use of canakinumab; the addition of canakinumab to treatment over 1 years was $832,000 with an estimated increase of 0.13 quality-adjusted life years (QALY); this equates to $6.4 million per QALY. The price would have to be reduced 98% to $1150 per year (or less) to meet the usual threshold of $100,000 per QALY. Even if restricted to those in whom treatment was only continued if there was a response to the first dose, the cost would be $819,000 per QALY and cost would have to be lowered to $6575 per year to reach the $100,000 per QALY threshold [81].

Overall, CIRT and CANTOS can be considered evidence that modulation of the inflammatory cascade may have a benefit in reduction of CV events as a pathway that is separate from lipoproteins and that modulation seeking to provide protection from ASCVD ought to be focused on cytokine inhibition, as in CANTOS, rather than broad-spectrum anti-inflammatory therapy [82]. Further investigations will help clarify when and who will benefit from these and other directed therapies. However, even in CANTOS, those that remained with elevated IL-18 and IL-1β had significant residual risk [83]. Thus, additional inhibition of components of the inflammatory cascade may offer additional risk reduction. Ongoing evaluations seek to evaluate new treatment targets and pharmacologic agents. This includes understanding the effects of inhibiting IL-6, IL-1, and the nucleotide-binding leucine-rich repeat-containing pyrin receptor 3 (NLRP3) inflammasome, which is responsible for activating IL-1β [82]. Further details on specific ongoing work are available later in this chapter.

Statins

There are several mechanisms by which statins might reduce hsCRP. The lipid-lowering effect of statins decreases OxLDL, which in turn disrupts plaque level induction of IL-1 and IL-6. Furthermore, by reduction of matrix metalloproteinase activity and presence of macrophages and lymphocytes, this further interrupts the inflammatory cascade. Additionally, through inhibition of protein isoprenylation, IL-6-induced production of CRP via gene expression is reduced [84]. In fact, trial level data has shown that among those with higher reduction in CRP, they also have a measurable decrease in plaque volumes [84–86].

Statins were first found to independently influence hsCRP in the Cholesterol and Recurrent Events (CARE) trial [87]. Following this, in 2001, the Air Force Coronary Arteriosclerosis Prevention Study (AFCAPS)/Texas Coronary Atherosclerosis Prevention Study (TexCAPS) trials suggested that in those with elevated hsCRP, statin therapy may reduce events, even while having well-controlled LDL-C. Those with LDL-C < 149 mg/dL but CRP > 1.6 mg/L had a 42% relative risk reduction

with lovastatin compared to placebo, whereas those with LDL < 149 mg/dL and hsCRP < 1.6 mg/L had a low event rate without significant decrease from placebo ($p = 0.74$) [84, 88]. From there, the PROVE-IT-TIMI 22, REVERSAL, and A-to-Z trials showed that outcomes (cardiac events, mortality, progression of atherosclerosis) were superior when hsCRP was <2 mg/l and LDL-C was <70 mg/dl [88–91]. The 2008 JUPITER trial followed these and was a pinnacle study in understanding the utility of hsCRP in relation to statin therapy [92]. JUPITER included healthy men and women with an LDL-C < 130 mg/dl who were at increased risk with hsCRP >2 mg/l that were randomized to rosuvastatin 20 mg/day or placebo. This resulted in significant reduction in MI (54%), need for revascularization (48%), stroke (47%), and all-cause mortality (20%). In the subgroup that had only age and hsCRP > 2 mg/dl as risk factors, there was a 37% reduction in all major vascular events. Furthermore, absolute risk reduction increased as hsCRP levels increased. Thus, the suggestion that hsCRP may be a valuable independent risk factor in assessing whether one may benefit from statin initiation for primary prevention.

Critics of JUPITER will point out that there was no control group of individuals with hsCRP < 2 mg/l; thus, it may be unfair to assign a benefit to the addition of testing hsCRP [25]. Furthermore, in additional post hoc testing, it was observed that there was a lower response in those with higher compared to lower levels of CRP, but if cut-offs of 3 and 4 mg/L were used, there was uniform relative risk reduction across all three groups. In fact, treatment response was only observed in those with at least one risk factor, but not among those with elevated CRP alone [84]. For another example, a post hoc analysis of the Heart Protection Study (HPS) found that simvastatin reduced events, irrespective of hsCRP levels [93]. Notably, in a meta-analysis of 22 studies, which included both JUPITER and AFCPAS/TexCAPS, statin-induced reductions in CRP were significantly associated with MI, but not for stroke, CV mortality, or all-cause mortality [94].

For secondary prevention there is no equivalent JUPITER trial that enrolled patients based on CRP. In the PROVE IT-TIMI 22 trial, it was found that those achieving both a reduced LDL-C and hsCRP had the lowest event rates ($p < 0.005$) [95]. In the A-to-Z trial, there was benefit that corresponded to the reduction in hsCRP with time of an increase in simvastatin from 40 mg to 80 mg/day [84, 96]. In Treating to New Targets (TNT), reduction in hsCRP correlated with reduction in major CV events [84, 97].

Meta-analyses of statin efficacy in relation to hsCRP have found that rosuvastatin is superior to atorvastatin in association reduction in hsCRP at dose ratios of 1:1 and 1:2 (rosuvastatin/atorvastatin) [98].

Ezetimibe

In a meta-analysis of trials that included ezetimibe, it was found that when added to statin therapy, more patients achieved a hsCRP of <1 mg/l and <3 mg/l than those not receiving ezetimibe (odds ratio (OR) = 1.2 for both) [99]. This study did not include the then ongoing IMPROVE-IT trial. In this trial of 18,144 patients, the ezetimibe plus simvastatin group had greater reduction in hsCRP than the placebo plus simvastatin group. When looking at the dual target of LDL-C < 70 mg/dL and hsCRP < 2 mg/L at 1 month, 50% achieved this in the ezetimibe plus simvastatin group compared to 29% in the simvastatin alone group ($p < 0.001$). This was associated with improved outcomes after multivariate adjustment. Notably, in patients that achieved both targets, regardless of assigned group, hazard ratio was 0.73 (0.66–0.81, $p < 0.001$) compared to those that met neither target [100].

Aspirin

Like other therapies, aspirin may be most beneficial in those with the highest levels of hsCRP. In the Physicians' Health Study, aspirin was significantly associated with reduction in MI among those in the highest quartile (55.7%, $P = 0.02$), but not those in the lowest quartile (13.9%, $p = 0.77$) [13]. However, subsequent studies

failed to show a specific anti-inflammatory effect in low-risk subjects [101]. This again emphasizes hsCRP as a valuable marker of the overall inflammatory state of a patient, which might improve risk estimation for some individuals.

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibitors

A meta-analysis of 16 studies ($n = 2546$ individuals) did not find any significant effect of PCSK9 inhibitors on hsCRP levels. This effect was the same for all PCSK9 inhibitors [102].

Post-Menopausal Hormone Therapy

The data regarding post-menopausal hormone therapy inducing changes in hsCRP levels has been mixed. However, in a meta-analysis of seven studies, it was not confirmed that there was a significant effect of therapy on hsCRP, regardless of dose or delivery method (topical or oral) [103].

Other Inflammatory Markers and Associated Therapeutics

The field of biomarker research is ever-expanding; thus, there are a plethora of potential novel biomarkers to discuss. To be sure, this is not a comprehensive list, although they are perhaps the most promising. There are additional therapeutics targeted at various inflammatory cascades under investigation.

Interleukin-6 (IL-6)

The IL-6 cascade has been intensely investigated. Within lipid metabolism, IL-6 increases VLDL production and secretion while decreasing clearance of triglyceride-rich lipoproteins, resulting in increased generation of small dense LDL and HDL [9]. Mendelian randomization studies found that the IL-6 cascade causally mediates CAD [11, 19]. IL-6 is known to be predictive of events for both primary and secondary prevention [104–106]. In fact, for patients on canakinumab in the CANTOS trial, on-treatment level of IL-6 was predictive of outcome [26]. However, IL-6 is more difficult to measure than hsCRP because of variation with circadian rhythm, short half-life, post-prandial variation, and assay instability. Il-6 is not currently available as a clinical inflammatory marker; thus, we rely on hsCRP as a downstream marker of the IL-6 pathway [107].

Tocilizumab, a humanized anti-IL-6 receptor antibody, was evaluated in a preliminary study and found to reduce hsCRP among NSTEMI patients. The ENTRANCTE study compared tocilizumab to etanercept with the primary outcome of vascular events among patients with moderate to severe rheumatoid arthritis and did not find a significant difference in events between the two groups [108], although LDL-C was found to be increased by 12% in the tocilizumab group compared to 1% in the etanercept group [109]. The mechanism of this increase is thought to be related to upregulation of apolipoprotein B [107].

Interleukin-1 (IL-1)

IL-1 is an upstream signaling pathway to both IL-6 and CRP and has emerged as a potential therapeutic target. There are two genetically coded proteins, IL-1α and IL-1β, which both bind to IL-1 receptor type 1. The α-form is primarily membrane bound and acts locally, whereas the β-form is circulating and has autocrine, paracrine, and endocrine effects [107]. IL-1β is converted to its active form by the NLRP3 inflammasome [23]. It was recently observed that those with proinflammatory IL-1 genotypes were found to modulate the risk of lipoprotein(a) for long-term CV events and CAD [110]. Neither IL-1α nor IL-1β is a measurable serum marker.

Differential predicted downstream effects of these two forms make it difficult to predict what

inhibition of this cascade will confer for CV risk. The aforementioned CANTOS trial has proven that direct inhibition of IL-1β can have beneficial effects [26]. It is yet unknown if nonspecific inhibition of both IL-1α and IL-1β, via anakinra, will have a similar result.

A third agent that is known to modify this pathway, colchicine, is also under evaluation. Colchicine is thought to have effects on the NLRP3 inflammasome, which can result in decreased IL-1β expression. Pilot observational studies with colchicine have shown in post-STEMI patients to have reduced CK-MB levels and infarct size [111]. In patients with stable CAD in the LoDoCo trial, it was found to reduce CV events (HR 0.33, 95% CI 0.18–0.59, $p < 0.001$) [112]. Also, 12 months of low-dose colchicine in patient with recent ACS were found to have reduced low attenuation plaque volumes, a marker of plaque instability [113]. Two double-blind placebo-controlled trials (the Colchicine Cardiovascular Outcomes Trial and the Colchicine and Spironolactone in Patients with STEMI/SYNERGY Stent Registry trial) will help to clarify the role for colchicine [114].

Fibrinogen

Fibrinogen is a plasma protein that, like CRP, is an acute-phase reactant. It binds activated platelets via glycoprotein IIb/IIIa which contributes to platelet activation and, when cleaved by thrombin, forms fibrin and increases plasma viscosity [115]. Fibrinogen has been linked to CV events. In meta-analysis the highest vs lower tertile had an OR of 2.3 (95% CI 1.9–2.8) for CV events [116]. In a more recent meta-analysis, the addition of fibrinogen to risk prediction resulted in a minimal increase in outcome detection (increase c-index by 0.0027, $p < 0.001$, and net reclassification improvement of 0.83%), with a number needed to test to prevent 1 event of 400–500 [57]. The National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines concluded that there are sufficient evidence for fibrinogen as an independent marker of CVD risk, although they raise concerns about analysis methods, insufficiency of assay standardization, and uncertainty in treatment strategies. Thus, they fail to recommend (grade IIIA) measuring levels for clinical application [117]. Ongoing studies will help to clarify a role for fibrinogen in risk assessment.

Phospholipase A_2(PLA2)

Secretory PLA2 (sPLA2) is an enzyme that promotes lipoprotein retention, induces platelet activation, and facilitates LDL oxidation. It is produced in response to other inflammatory mediators, and elevated levels were found to have adverse prognostic implications [118]. Varespladib, a nonspecific inhibitor of secretory phospholipase A2 (PLA2), was found in the VISTA-16 trial to significantly lower LDL-C and hsCRP, but resulted in higher rates of MI [119].

Lipoprotein-associated PLA2 (Lp-PLA2) is small molecule carried on LDL, HDL, and VLDL particles. It is thought to generate mediators, which activate macrophages and smooth muscle cells, resulting in endothelial dysfunction. Elevated levels are associated with CV risk independent of other factors with OR ranging from 1.15 to 2.0 [118]. Darapladib, a direct selective inhibitor of lipoprotein-PLA2, failed to reduce events [11, 114].

Oxidized LDL (OxLDL)

OxLDL is highly proinflammatory and atherogenic and thought to be a more damaging form of LDL. In the aforementioned processes of inflammation leading to increased small dense LDL and HDL particles, there is also an increase in OxLDL. Increased circulating levels predict CHD events independent of other factors, wherein the highest tertile compared to the lowest tertile had a HR of 4.25 (95% CI 2.09–8.63, $p < 0.001$) [120].

Trimethylamine N-Oxide (TMAO)

First, gut flora generate trimethylamine (TMA) from dietary sources of choline or phosphatidylcholine (primarily from animal products [eggs, milk, liver, red meat, poultry, shellfish, and fish]) [118]. TMA is then converted to TMAO in the liver, which has proinflammatory effects and induces lipid uptake by macrophages [121]. In this study by Wang et al., higher levels of TMAO were associated with higher atherosclerotic burden and CVD risk. TMAO is a clinically available test, although no current guidelines currently integrate its utilization in risk assessment.

Adipose Tissue and Various Markers

As previously mentioned, adipose tissue is a highly active organ in relation to inflammatory regulation [122]. Visceral fat is known to release several proinflammatory compounds, including IL-6, TNF-α, and plasminogen activator inhibitor-1. In addition, adiponectin and leptin are both known to vary in response to differing metabolic states, particularly within obesity and metabolic syndrome. Specifically, adiponectin is a potent anti-inflammatory agent that decreases as visceral fat increases [123]. CAD was found to be twice as prevalent in those with adiponectin levels <4 μg/mL [124]. Leptin has been found to increase IL-6 and TNF-α and hepatic synthesis of CRP [37, 125].

MicroRNAs (miRNA)

miRNA are small, 20–25 bases, single-stranded, non-coding RNAs that can be measured in plasma. More recently, it has been observed that various miRNA are found in higher or lower concentrations during acute coronary syndromes and in the setting of vulnerable coronary plaques [123]. There is no clinical test yet that integrates miRNA into risk estimation. However, research is ongoing to understand which miRNA are best for risk prediction. This may be a tool for risk stratification in the future.

Other Biomarkers

To be sure there are other biomarkers that have relation to the inflammatory system that we did not delve into here. These include matrix metalloproteinases, myeloperoxidase, oxidized apo A1, placental growth factor, and monocyte chemo-attractant protein-1, among others [118]. Future research is needed to inform us on whether there is clinical significance to measuring these markers.

Conclusion

It has been almost a century since the discovery of CRP. The most recent data suggest that CRP is not directly causative of atherosclerosis or atherothrombosis. However, hsCRP does act as a marker of the inflammatory state and a prognostic factor for risk of atherosclerotic events. This ability to prognosticate can be utilized to improve risk prediction for cardiac events, particularly for those with intermediate risk. Recent studies have helped to better understand the role of inflammation as a pathway for risk reduction for those with cardiac events. Future trials will help to better understand the optimal points in the inflammatory cascade to target, with the ultimate goal of reduced risk for cardiac events.

Statement This publication was made possible by CTSA Grant Number TL1 TR001864 from the National Center for Advancing Translational Science (NCATS), a component of the National Institutes of Health (NIH). Its contents are solely the responsibility of the authors and do not necessarily represent the official view of NIH.

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Coronary Artery Calcium and CT Angiography

31

Shone Almeida, Kashif Shaikh, and Matthew Budoff

Abbreviations

CAC	Coronary artery calcification
CAD	Coronary artery disease
CT	Computed tomography
CVD	Cardiovascular disease
EBCT	Electron beam computed tomography
FRS	Framingham risk score

Introduction

Calcification of the coronary arteries is widely recognized as a marker of subclinical atherosclerosis. Dubbed as the "mammogram of the heart", calcium scoring allows for the early detection of coronary disease and prognostication of cardiovascular risk. Over the last 30 years, the field has made significant inroads with wide acceptance and implementation in preventive cardiology and guidelines [1]. Over the last decade, coronary computed tomography angiography (CCTA) has emerged as a cost-effective and powerful strategy for non-invasive evaluation of coronary arteries. Unlike functional testing, CCTA today is utilized to not only rule severe stenosis but also quantify atherosclerotic plaque burden and characterize morphology of non-obstructive and obstructive atherosclerotic plaque. In the current era, most of the patients who undergo some form of diagnostic test for chest pain are low to intermediate risk without ischemic obstructive lesions. Several studies have established association of non-obstructive CAD and future risk of cardiovascular events. Robust evidence suggests that CCTA is impactful in encouraging preventive care and leads to significant relative risk reduction of future incident MI [2–4]. Furthermore, CCTA has been utilized to monitor plaque progression to evaluate the impact of lifestyle changes and pharmacotherapy, suggesting CTA may hold future to personalize individual therapies.

The first part of this chapter will discuss the role of coronary calcification in the existing risk prediction framework, the interpretation of the calcium score, and the power of zero. It will also address the technical aspects from image acquisition to calcium quantification as well as CCTA. The second part will discuss the prognostic value of CCTA beyond CAC and compared to functional testing and role of CCTA in monitoring the efficacy of lifestyle changes and pharmacotherapy.

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_31

Coronary Artery Calcification

Coronary Artery Disease: Risk Prediction Framework

Coronary artery disease is the leading cause of death in the developed world, accounting for an estimated 17.3 million deaths globally [5]. The 2017 American Heart Association (AHA) statistics on heart disease and stroke estimates that over 92 million adults in the United States (US) carry a diagnosis of cardiovascular disease (CVD) with nearly 44% of the US population projected to have some form of CVD by 2030 [5] (1). With advances in medical therapy, the death rates from CVD have declined by 25.3% from 2010 to 2014 [5]. However, the economic impact associated with diagnosis and management of coronary disease is substantial, approximating $165 billion in 2009 [6].

The diagnosis of CAD is complex, incorporating an understanding of disease prevalence, an assessment of individual risk factors, and recognizing pre-test probability [7]. Traditional risk factors for CAD include hypertension, hyperlipidemia, diabetes mellitus, family history, smoking history, and increasing age [8]. Clinical cardiology guidelines as recently as 2010 relied on population-based studies to predict the likelihood of cardiovascular events [9]. The Framingham Heart Study demonstrated that age, gender, smoking, diabetes, blood pressure, and cholesterol levels can be used to estimate the risk of cardiovascular events. Nearly 8500 participants were followed for a 12-year period and monitoring for outcomes of coronary heart disease and cerebrovascular disease [10]. This data led to a population-based multivariable algorithm, the Framingham risk score (FRS), to better stratify coronary disease risk in asymptomatic patients [10].

Many population-based risk assessments exist (SCORE, QRISK1, PROCAM), the most widely used being the FRS [11]. The major limitation of these risk scores is the selection of a narrow population from which the algorithm is derived and limited scope of outcome data focusing primarily on coronary heart disease. The Framingham Heart Study, for example, enrolled an exclusively white population. Because of limited applicability to diverse, real-world populations, the American College of Cardiology (ACC) and American Heart Association moved away from FRS in the 2013 revised Guideline on Assessment of Cardiovascular Risk, focusing instead on Pooled Cohort Equations based on representative cohorts of US whites and African Americans to estimate lifetime risk of atherosclerotic cardiovascular disease (ASCVD) [12]. The guideline's working group notes however that these risk assessment tools have not been formally evaluated in randomized trials and that risk estimation is based on population averages. This data has to be interpreted by the clinician in consideration of the history and focused physical exam to determine individual cardiovascular risk.

Thusly, clinicians are confronted with two key questions in assessing cardiovascular risk: (1) Is the patient at increased risk for a cardiovascular event? (2) Does my patient warrant initiation of lipid-lowering therapy? In comparison to three primary prevention cohorts (the Women's Health Study, the Physicians' Health Study, the Women's Health Initiative Observational Study), Ridker et al. found that the ACC/AHA risk prediction algorithm overestimates observed risk as much as 75–150% (Fig. 31.1). Accordingly, the addition of an additional risk marker with strong negative predictive value to the traditional risk prediction framework will enable clinicians to better adjudicate patients whom are more likely to benefit from lipid-lowering therapies and those in whom foregoing statin therapy may be considered owing to very little net clinical benefit [13, 14].

Coronary Artery Calcium (CAC)

History

Early work in coronary artery calcification (CAC) relied on cardiac cinefluoroscopy for visualization. In a report of 360 patients undergoing coronary angiography, coronary calcification was seen in 154 cases, and over 97% of these had severe coronary artery disease, defined as luminal stenosis >70% [15]. Follow-up work solidified

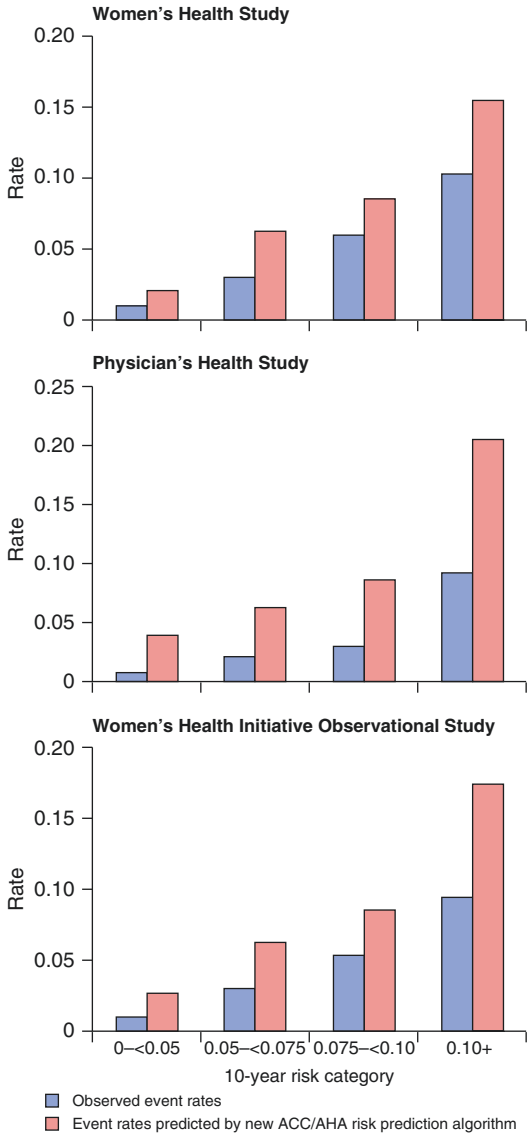

Fig. 31.1 Observed CVD event rates versus predicted rates by the 2013 ACC/AHA Pooled Cohorts ASCVD algorithm. (Adapted from Ridker and Cook [14]. With permission from Elsevier)

the association between coronary calcification and atherosclerosis in a review of clinical, post-mortem, and angiographic studies [16]. The development of electron beam computed tomography (EBCT) in 1979 enabled rapid, high-resolution image acquisition of the coronary arteries. This *ultrafast CT* was shown to be twice as sensitive as fluoroscopy in detecting coronary calcium, making it ideal for screening [17].

Biology of Arterial Calcification

Vascular calcification is now understood to be an active process rather than one of senility. In the coronary arterial bed, calcification is driven by a combination of metabolic and inflammatory factors. Studies have previously reported that the arterial wall has a subpopulation of cells that have the ability to undergo osteoblastic differentiation and mineralization [18]. Vascular smooth muscle cells normally express proteins that inhibit calcification [19], a process that is disrupted by inflammation and oxidized low-density lipoprotein (LDL). The presence of oxidized LDL particles upregulates osteogenic differentiation of the vascular smooth muscle cells and thus promotes vascular calcification [20].

Inflammation is a critical driving factor for atherosclerotic plaque formation and arterial calcification. The accumulation of oxidized LDL promotes endothelial dysfunction and release of pro-inflammatory cytokines. The secretion of these cytokines and adipokines from perivascular fat [21] creates a milieu that promotes the infiltration of inflammatory cells such as macrophages within the arterial wall [22]. This regional inflammation and oxidative stress further promote vascular calcification.

CAC vs. Risk Cohorts

The Framingham risk score offered an intuitive CV risk assessment based on readily available variables (age, gender, smoking, blood pressure, cholesterol). Since then, finer calibration of these risk prediction models has allowed wider applicability by including more diverse populations. The discriminative power of these models is continuously challenged by the addition of new risk factors such as C-reactive protein [23], carotid intima-media thickness test [24], and lipoprotein(a) [25]. Coronary artery calcium is an imaging biomarker that essentially provides direct visualization of coronary atherosclerosis. In a prospective, observational population-based study of 1461 asymptomatic adults with coronary risk factors, coronary calcium was shown to rank CVD risk independent of the FRS [26]. The addition of CAC score provided the greatest improvement in discrimination (Fig. 31.2). Similarly,

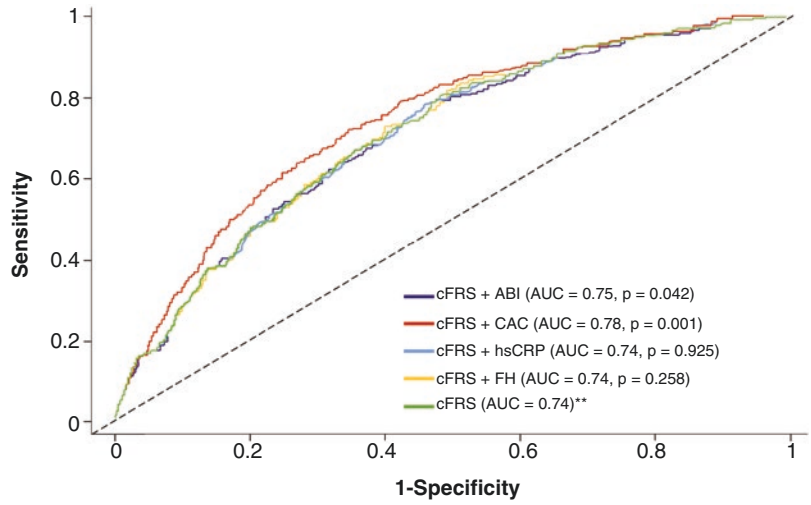

Fig. 31.2 Receiver operating characteristic curves showing the area under the curve (AUC) for calibrated FRS (cFRS) alone or plus coronary artery calcium score. ABI ankle brachial index, hsCRP high sensitivity C-reactive protein, FH family history. (Adapted from Yeboah et al. [95]. With permission from Elsevier)

Taylor et al. demonstrated that CAC independently predicts incident premature coronary heart disease over standard CV risk factors [27]. The relationship between CAC and future CV events was also studied in the Multi-Ethnic Study of Atherosclerosis (MESA) cohort. CAC scanning was performed on 6722 men and women in MESA, of which 27.6% were black, 21.9% Hispanic and 11.9% Chin*ese* [28]. Over a median follow-up period of 3.8 years, 162 coronary events were noted. Compared with participants without any coronary calcification, the risk of coronary events increased by a factor of 7.73 with CAC scores 101–300 and a factor of 9.67 with scores >300 ($p < 0.001$). Importantly, there was no difference in the predictive value of CAC across different ethnic groups. In the MESA cohort, the traditional CAD risk factors of older age, male gender, Caucasian race, hypertension, and diabetes were all associated with the development and progression of coronary artery calcification [29].

The predictive value of CAC has also been compared to the newer pooled risk cohorts. In a large Korean population of 4194 individuals without known cardiovascular disease, the odds ratios for CAC progression in low- (pooled risk 5 to <7.5%), intermediate- (7.5 to <10%), and high-risk (≥10%) groups were 1.85 (95% confidence interval (CI) 1.52–2.25), 2.63 (95% CI 2.01–3.46), and 3.58 (95% CI 2.73–4.70), respectively [30]. The study demonstrated that the newer pooled risk cohorts were predictive of the incidence and progression of CAC. However, when the pooled risk algorithm was applied to MESA, it performed suboptimally with C-statistics of 0.6–0.7, whereas the C-statistic for CAC prediction of coronary events was 0.8 [31, 32]. Thus, CAC may indeed perform more robustly than the ASCVD pooled risk algorithm alone.

An analysis of the observed versus predicted risk of cardiovascular events by DeFilippis et al. revealed that the 2013 ACC/AHA prevention guidelines overestimated CV risk in the MESA cohort (9.16% predicted vs. 5.16% observed) [33]. This discordance was noted throughout the continuum of cardiovascular risk. Risk overestimation may translate into preventive therapy such as statin drugs applied to patients who are unlikely to benefit and of course increased costs. Nasir et al. applied the pooled risk equations to 4758 statin-naive patients of the MESA cohort. By the 2013 ACC/AHA guidelines, 50% were eligible for statin therapy [34] (3). When looking at the distribution of CAC by statin eligibility, 41% of the 2377 participants recommended for moderate- to high-intensity statin by ACC/AHA guidelines had CAC = 0. CAC of zero may indeed reclassify nearly 50% patients as much lower risk than predicted by pooled cohorts and thus not favorable for statin therapy.

In every day clinical practice, the clinician is faced with a vast amount of data with which to appropriately classify cardiovascular risk. The integration and interpretation of traditional risk factors with coronary calcification scores has to be personalized to the patient. Knowledge of the patient's pre-test probability based on traditional risk models is critically important to interpretation of the CAC score. Pletcher et al. elegantly demonstrated how the coronary artery calcium score can be integrated with conventional cardiovascular risk factors to estimate future risk [31]. The study modeled the National Cholesterol Education Panel's Adult Treatment Panel III guideline's version of the Framingham risk score in addition to race/ethnicity to estimate 10-year heart disease risk compared with CAC score. For example, a 60-year-old white male with systolic blood pressure 120 millimeters of mercury (mmHg), total cholesterol 150 mg/dL, and high-density lipoprotein (HDL) 65 mg/dL has a 10-year heart disease risk estimate based on the modeled FRS of 5% (low-intermediate risk). However, the finding of a CAC score of 101–300 increases that risk estimate to 10%, affecting clinical decision-making [31]. Similarly, a high-risk patient based on traditional FRS risk factors (≥10%) with a CAC score of zero reclassifies into a 10-year coronary heart disease risk of 2% (see section "Power of Zero"). Thus, in cases where a high CAC score might be expected based on risk factors alone, a score of zero or moderately elevated (CAC 1–100) may be reassuring to some degree. An online MESA risk calculator is available to clinicians to integrate traditional risk factors and the CAC in different ethnic groups (Caucasian, Hispanic, African American, and Chinese) – *https://www.mesa-nhlbi.org/CACReference.aspx*. The tool incorporates age; gender; ethnicity; presence of risk factors such as diabetes, tobacco use, and hypertension; as well as objective data points such as systolic blood pressure, total cholesterol, and calcium score [35].

Cost-Effectiveness of CAC

CAC scans typically range $100–200 in out-of-pocket costs. The cost-effectiveness of cardiac imaging is dependent on the prognostic capability, the finer discrimination of risk, and finally the ability to reclassify patients based on revised risk assessment. The EISNER (Early Identification of Subclinical Atherosclerosis by Noninvasive Imaging Research) study evaluated the clinical impact of the addition of CAC to conventional risk factors [36]. Of 2137 patients randomized to CAC scan or no scan, those who underwent calcium scanning showed improvements in blood pressure ($p = 0.02$) and LDL ($p = 0.04$) as well as a tendency toward weight loss, though statistical significance was not reached. Overall downstream testing and costs did not differ between the scan and no scan group; however, within the scan group, higher quartiles of CAC showed increased utilization of downstream testing (electrocardiogram [EKG], stress testing, coronary CTA, catheterization, revascularization, or carotid ultrasonography).

The cost-effectiveness of calcium scoring for CAD risk prediction and guiding statin allocation was evaluated in the MESA cohort [37] (4). The study simulated a model to assess the clinical and economic effects of a one-time CAC study in intermediate-risk patients. Two treatment strategies were evaluated: statin therapy for CAC ≥1 or CAC ≥100. Treating intermediate-risk patients with CAC ≥1 averted an average of 5.1 coronary events compared with 3.9 events in a treat-all strategy. Only treating patients with CAC ≥100 prevented fewer coronary events; however, it also reduced the number of patients experiencing statin-related adverse effects. Overall the study concluded that treatment on the basis of calcium score is more effective in preventing coronary events and also allows for identification of patients who would benefit from high-intensity statin therapy while also increasing medication adherence.

Power of Zero

Coronary artery calcification has been consistently shown to strongly predict cardiovascular events. CAC offers improved risk stratification where other prediction algorithms fall short – ethnic populations, women, and those at low-intermediate risk. Lakoski et al. studied over 3600 asymptomatic women in MESA who were deemed low-risk for 10-year coronary heart dis-

ease risk based on FRS [38] (5). The prevalence of CAC >0 in this cohort was 32% ($n = 870$), and compared with women with CAC = 0, this cohort had a much higher risk for coronary heart disease (hazard ratio 6.5; 95% CI 2.6–16.4) (5). The addition of CAC to traditional risk algorithms such as FRS improved the risk prediction of coronary heart disease and CVD events.

The event rate with CAC zero is substantially lower. Thus, the presence of atherosclerotic plaque or so-called vulnerable/unstable plaque is highly unlikely with cardiac event rates approaching 0.1% per year [39]. In a pooled analysis of 35,765 asymptomatic persons, Shareghi et al. demonstrated that in a subset of patients with CAC = 0, the annual event rate approached 0.027% and estimated 10-year event rate approximately 0.3% [40]. Budoff et al. provided further support for CAC as a predictor of future cardiac events, showing unadjusted Kaplan–Meier cumulative event curves for major coronary events in males and females (Fig. 31.3) [41]. Similarly, in a large registry of 25,253 persons, those with CAC = 0 scores showed survival of 99.7% over a 6.8-year period (Fig. 31.4) [42].

Role in Symptomatic Patients

The power of zero for coronary artery calcium scoring has the highest yield when applied to asymptomatic populations. When symptoms are introduced, the pre-test probability of disease increases substantially, and the negative predictive value falls. Nevertheless, the role of CAC in symptomatic patients has been previously evaluated. Higher CAC scores are associated with increased likelihood of detecting stenosis >50% [43]. In early work by Guerci et al., patients with CAC score >170 were far more likely to have obstructive coronary disease on invasive angiography regardless of number of risk factors [44]. A CAC score cutoff of 100 showed a high sensitivity and specificity for detecting high-grade stenosis (>75%) by invasive angiography, 95% and 79%, respectively [45] (6). In the multicenter PROMISE (Prospective Multicenter Imaging Study for Evaluation of Chest Pain) trial, Budoff et al. compared the prognostic value of CAC in symptomatic patients to functional testing. CAC strongly predicted future cardiovascular events, C-statistic similar to functional testing (0.67 vs. 0.64), although functional studies were more specific [46] (Table 31.1).

Caution must be exercised in applying the "power of zero" to clearly symptomatic patients. Applying the Bayes theorem, which invokes that the efficiency of a diagnostic test is reliant on the frequency of disease in the population tested, clinicians must be wary of using a CAC = 0 to rule

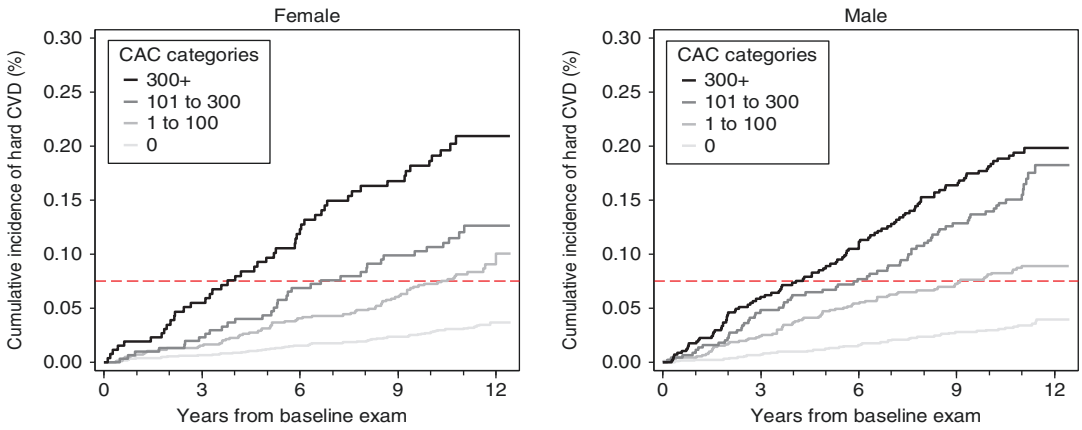

Fig. 31.3 Unadjusted Kaplan–Meier cumulative incidence of major coronary events stratified by CAC score and sex. (Adapted from Budoff et al. [41])

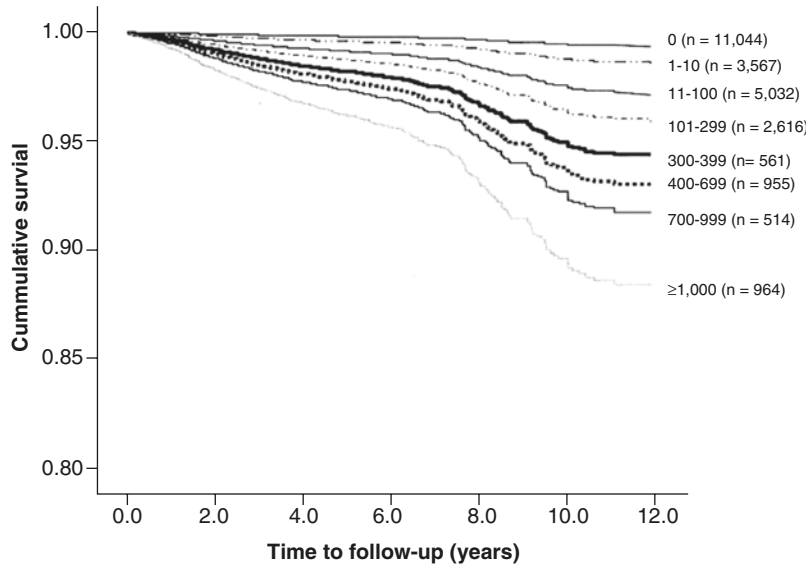

Fig. 31.4 Cumulative survival stratified by CAC subsets from 0 to >1000. Increasing calcium scores are associated with worsened survival. (Adapted from Budoff et al. [42]. With permission from Elsevier)

Table 31.1 Summary of existing guidelines and expert consensus statements on the addition of CAC scoring to traditional cardiovascular risk assessment tools in asymptomatic persons

Guideline/statement	Summary	COR	LOE
2010 ACC/AHA Guideline on the Assessment of Cardiovascular Risk [7]	Measurement of CAC is reasonable for cardiovascular risk assessment in asymptomatic adults at intermediate risk (10–20% 10-year ASCVD risk)	IIa	B
2016 European Guidelines on Cardiovascular Disease Prevention in Clinical Practice [8]	Coronary artery calcium scoring may be considered as a risk modifier in CV risk assessment	IIb	B
2018 United States Preventive Services Task Force [9]	In asymptomatic adults, the current evidence is insufficient to assess the balance of benefits and harms of adding CAC score to traditional risk assessment of CV disease prevention	I	
2018 Guideline on the Management of Blood Cholesterol [10]	In adults 40–75 years of age without diabetes mellitus and LDL levels ≥70–189 mg/dL, at a 10-year ASCVD risk of ≥7.5–19.9%, if a decision about statin therapy is uncertain or selected borderline risk (5% to <7.5% 10-year ASCVD risk), consider measuring CAC. A CAC score of 1–99 favors statin therapy, especially in those ≥55 years of age. For any patient, if the CAC score is ≥100 Agatston units or ≥75th percentile, statin therapy is indicated	IIa	B-NR
2017 Expert Consensus Statement from the Society of Cardiovascular Computed Tomography [11]	It is appropriate to perform CAC testing in the context of shared decision-making for asymptomatic individuals without clinical ASCVD who are 40–75 years of age in the 5–20% 10-year ASCVD risk group and selectively in the <5% ASCVD group, such as those with a family history of premature coronary artery disease		

CAC coronary artery calcium, *CV* cardiovascular, *AHA* American Heart Association, *ACC* American College of Cardiology, *COR* class of recommendation, *LOE* level of evidence, *ASCVD* atherosclerotic cardiovascular disease, *LDL* low-density lipoprotein, *NR* nonrandomized

out obstructive coronary disease in a symptomatic, higher-risk population [47]. Results from the Core64 substudy which consisted of primarily intermediate to high pre-test probability of obstructive CAD demonstrated that while CAC = 0 reduced the likelihood of obstructive disease on invasive angiography (15% for CAC = 0, 58% for CAC >10), it cannot be used to exclude CAD in a high-risk, symptomatic cohort [48].

However, there may be a role for assessing coronary calcium in the low-risk symptomatic patient presenting to the emergency department. Current expert consensus statements advocate for the use of CAC in triaging chest pain patients in the emergency department. The authors argue that CAC = 0 has sufficiently high sensitivity (98%) such that a low-risk symptomatic patient with a score of zero can be safely discharged without further testing [49]. Such a fast rule-out model applied to the right patient population may translate to significant cost savings on the healthcare system.

Guidelines

A summary of current guidelines and expert consensus statements on the use of coronary artery calcium scoring is provided in Table 31.1. The 2018 Guideline on the Management of Blood Cholesterol incorporated CAC assessment to determine need for statin therapy, moving to a class IIa recommendation for any adult 40–75 years of age with CAC >100 [10, 50]. A recent study from Walter Reed Army Medical Center evaluated the impact of statins on ASCVD outcomes stratified by CAC score. Over a median follow-up period of 9.4 years and enrollment of 13,644 patients, the investigators found that statin therapy reduced MACE events in patients with CAC (adjusted subhazard ratio 0.76; 95% CI 0.60–0.95; $p = 0.015$) but not in patients without coronary calcification (adjusted subhazard ratio: 1.00; 95% CI 0.79–1.27; $p = 0.99$) [12]. The number needed to treat (NNT) in patients with CAC >100 was 12 ($p < 0.0001$), whereas CAC 0 showed no significant effect and CAC 1–100 showed NNT 100 ($p = 0.095$) [51].

Technical Aspects

Image Acquisition

In current modern-day, multi-detector CT scanners, the acquisition of coronary artery calcium scans is standardized across vendors and imaging centers. Images are acquired prospectively with EKG gating at a slice thickness of 2.5–3 mm [52]. CAC scans are acquired without the use of intravenous contrast. Scanner settings can alter the density of calcified plaque through increased blooming artifact. Nonetheless, image acquisition time remained too slow for imaging rapidly moving heart to accurately assess the coronary arteries, until the early 2000s, when faster CT systems with capability to acquire thin slices were introduced. For example, 64-slice CT system was available around 2005 with rotation time of 330 milliseconds (ms) and slice thickness of 0.6 millimeter (mm) with the capability to cover the entire heart in three partial rotations. Some of the latest scanners have 256/320 rows of detectors. They provide a rotation speed of 280/300 ms. At a collimated slice thickness of 0.6/0.5 mm, scan volume of 16 cm can be covered, sufficient to cover the heart in one single partial resolution. The Society of Cardiovascular Computed Tomography (SCCT) has specified CAC and CCTA scan acquisition at a voltage of 120 kVp with tube current variable based on body habitus [53].

Radiation

The ALARA (as low as reasonably achievable) principle applies to coronary artery calcium scans just as with any other medical imaging that utilizes ionizing radiation. The lifetime risk of cancer relates to the cumulative radiation dose, making it all the more important to keep dose low in each study when possible. The SCCT requires that all CT laboratories record radiation dose in each patient as dose-length-product (DLP; units of milligray*cm) and effective radiation dose (millisievert [mSv]) [53]. The average DLP should not exceed 200 mGy*cm with effective radiation dose averaging 1.0–1.5 mSv [53]. Importantly, there has been dramatic reduction in

radiation doses since the last decade for CCTA as well. Median effective dose estimates were 12.4 mSv in 2007 decreasing to 2.7 mSv by 2017, resulting in 78% reduction in radiation doses according to large prospective multicenter trial. Notably, the number of non-diagnostics coronary CTAs did not increase [54]. Low radiation with capability to not only rule out obstructive disease but characterize atherosclerotic plaque severity and morphology makes CCTA a unique and attractive non-invasive imaging modality.

CAC Scoring

Several methods exist for quantifying coronary artery calcification (Fig. 31.5). Each has its own benefits and limitations; however, quantifying the degree of coronary calcification is essential to its predictive value for cardiovascular disease.

The Agatston score is the most widely used scoring system in clinical practice and remains the reference standard since introduction by Dr. Arthur Agatston in 1990 [17]. The per-lesion score is the product of area (mm^2) and lesion density weighting factor (DWF). The density weighing factor is obtained from the maximal CT attenuation of a given lesion where 130–199 Hounsfield Units (HU) =1, 200–299 HU = 2, 300–399 = 3, and >400 = 4. The total Agatston score is the summed score of all calcified lesions.

Alternate methods for describing coronary calcium burden include a volume-based score that relies upon similar scanning protocols as the Agatston score. The number of voxels exceeding a cutoff of 130 HU and area ≥1 mm^2 multiplied by the volume per voxel yields the per-lesion volume score [55]. This methodology does not account for density of a particular plaque. Another method for scoring calcium burden is to measure the total mass of coronary calcium. This method involves the use of phantoms for calibration and is not widely used. Finally, the density score is another scoring system that has gained increased attention. This method uses the Agatston score and the total volume score to back-calculate the average density factor. In MESA, Criqui et al. demonstrated that CAC density showed an inverse relationship with CVD events. Consideration of calcium density may be of most value in extremes of age – younger patients with low calcium density in whom intermediate Agatston scores may underestimate risk or older patients in whom highly dense lesions with borderline Agatston scores may lower risk estimates [13, 55].

Regardless of scoring methodology, high-quality image acquisition is paramount to high reproducibility and accuracy of calcium scoring. Motion can result in overestimation of calcium, particularly in the right coronary artery which is prone to such artifact. Similarly, poor spatial res-

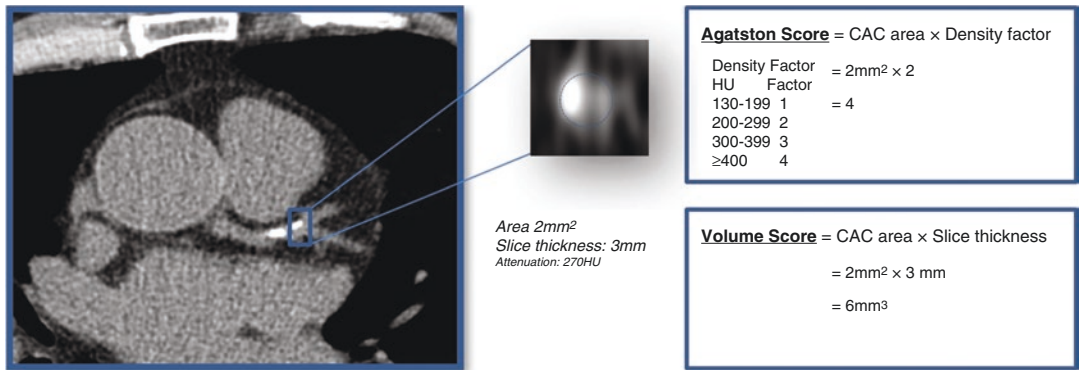

Fig. 31.5 Quantification of coronary calcification using the Agatston score and volume-based score. CAC coronary artery calcification, HU Hounsfield units

olution and noisy images may underestimate the total calcium score. Calcification outside the coronary arteries, such as valvular calcification, mitral annular calcification, and aortic root calcification, can all contribute to overestimation of the calcium score and must be excluded. Vessel segments with stents must also be excluded from analysis.

CAC from Nongated Chest CT

On average in the United States, 14,000,000 chest CT scans are obtained annually for non-coronary purposes [56]. While vascular calcification may be noted on formal reports, quantification of CAC is typically not undertaken. This presents a tremendous opportunity to screen and identify patients at risk for future cardiovascular events and, importantly, capture this data across a variety of clinical settings (i.e., primary care, emergency department) and for myriad indications (lung cancer screening, chronic obstructive pulmonary disease). Prompt implementation of secondary prevention strategies from cholesterol reduction to risk factor modification could have a significant impact on population-based cardiovascular risk. Recent work from our lab demonstrated a strong correlation in Agatston score between gated calcium scans and nongated chest CTs with a weighted Cohen's kappa = 0.86 (95% CI: 0.84–0.89). Measurement of coronary calcium from nongated chest CTs presents an opportunity for earlier identification of coronary disease and implementation of targeted primary prevention measures.

CT Angiography

Prognostic Value of Coronary CT Angiography

Semi-quantitative CT Measures

Atherosclerotic plaque is assessed on per segment basis on CCTA. Coronary arteries usually >2 mm are evaluated. Coronary plaques are defined as structures >1 mm^2 within and/or adjacent to the coronary lumen, which could clearly be distinguished from the surrounding pericardial fat tissue and contrast-enhanced vessel lumen. Normal coronary arteries are defined as absence of obstructive or non-obstructive atherosclerotic plaque [57]. The parameters that are used for semi-quantitative analysis on cardiac CT are as follows.

Segment involvement score (SIS)- is determined by adding the number of segments with any coronary lesion, providing a number of segments of the coronary tree with stenosis present. The Total Plaque score (TPS) is derived by the amount of plaque in each segment. Plaque is quantified as mild (score-1), moderate (score of 2), or severe (score of 3). Total plaque score is determined by summation of the severity of plaque in each coronary segment. Segment stenosis score (SSS): Severity of stenosis for each segment is determined as score of 0 for normal, 1 for 1–49% stenosis, 2 for 50–69%, and 3 for >70% stenosis. SSS is calculated as the sum of the maximal stenosis score in each segment [57, 58].

Furthermore, morphology of coronary artery plaques is determined visually. Non-calcified plaques are defined as those with no calcifications, while partially calcified or mixed plaques have <50% calcification and calcified plaques as presence of >50% calcifications [58] (Fig. 31.6).

The earlier studies evaluated the prognostic value of CCTA mostly utilizing the worst lumen stenosis [59, 60]. A meta-analysis of 9592 patients showed that the presence of >50% stenosis on CCTA had incidence of death or MI 3.2% as compared to 0.15% in those without CAD [61]. Moving beyond stenosis, subsequent studies evaluated the prognostic value of CTA utilizing several other markers such as SIS, TPS, and SSS as described above. CONFIRM (COronary CT Angiography EvaluatioN For Clinical Outcomes: An InteRnational Multicenter) Registry which comprises 27,125 consecutive patients from 12 cluster sites in 6 different countries has played a pivotal role in establishing prognostic value of CCTA. It comprises patients with known coronary artery disease (CAD), patients with suspected but without known CAD, or asymptomatic persons undergoing CTA [58].

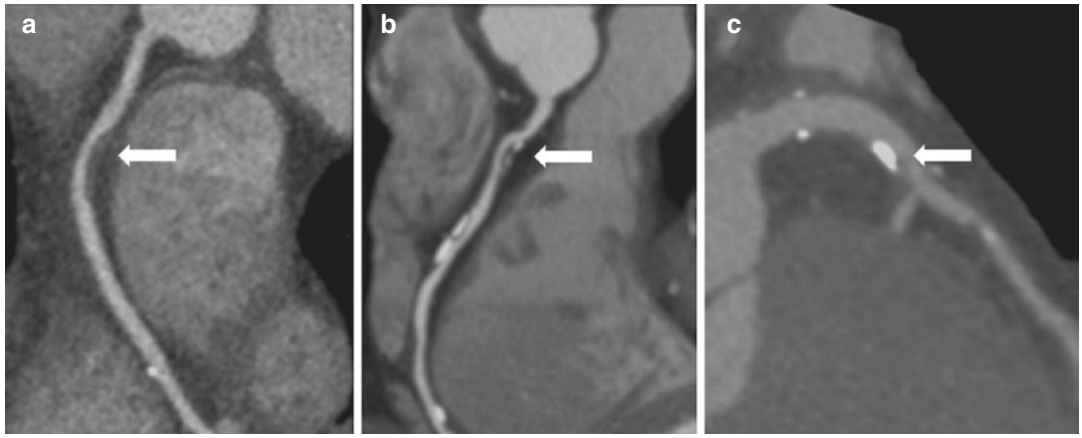

Fig. 31.6 Examples of atherosclerotic plaque types, (**a**) non-calcified plaque, (**b**) mixed plaque, and (**c**) calcified plaque

In CONFIRM Registry, individuals without prior CAD and with no known medically modifiable CAD risk factors including hypertension, dyslipidemia, diabetes mellitus, and family history were evaluated. Non-obstructive disease defined as >1 coronary segment involved was associated with increased mortality as compared to those with no atherosclerosis (9.48% vs. 3.95%, $p < 0.001$) over a mean long-term follow-up of 5.6 years. In this cohort of patients with no-modifiable risk factors, 92% were classed as either low or intermediate pre-test likelihood of obstructive CAD, according to the Diamond and Forrester model. However, 24% patients had obstructive CAD and 26.3% non-obstructive CAD, highlighting the inconsistency in clinical assessment of CAD and extent of atherosclerosis on coronary CT [62].

CONFIRM investigators created a CONFIRM score based on test sample of 17,792 patients and validation sample of 2506 patients. It integrated the National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) III score, with assessment of most predictive CCTA parameters including plaque and stenosis in proximal segments. Proximal segments include proximal and mid left anterior descending, proximal and mid right coronary artery, proximal left circumflex, and first obtuse marginal. Deseive and colleagues showed that among all clinical risk scores, NCEP ATP III performed better (c-index 0.675), followed by the Framingham score (c-index 0.661) and Morise score (c-index 0.606) for all-cause mortality. However, CONFIRM score provided best prediction for all-cause mortality (c-index 0.69) with reclassification of 34% of patients when compared with the NCEP ATP III score. Furthermore, the authors conducted subgroup analyses in women and asymptomatic individuals. Predictive value of CONFIRM scores remained robust in these subgroups. This study underscores the importance of utilizing CCTA parameters, which could potentially reclassify around one third of patients. The CONFIRM score also provided significantly better prediction for all-cause mortality in comparison to other CCTA-based parameters, and c-indices for SIS, SSS, and Leaman score were 0.648, 0.653, and 0.646 ($P < 0.001$) for all-cause mortality [63].

Recent guidelines recommend deferring statins in patients with CAC-0 in general population except in individuals with specific conditions such as diabetes. There are reports that CCTA provides an added prognostic value over CAC in asymptomatic individuals with diabetes. Min et al. reported age, gender, and CACS in asymptomatic diabetics provided c-index of 0.64, which improved by the addition of CCTA parameters such as SSS (c-index 0.78) [64]. However, two meta-analyses showed a conflicting result about predictive value of coronary CTA as a screening test in asymptomatic diabetics [65, 66].

Currently, CCTA is not recommended as screening test, but CTA may hold a place in screening high-risk patients with diabetes and those with chronic inflammatory conditions such as HIV and rheumatoid arthritis. Nonetheless, more work would need to be done before making screening CTA a routine in these groups.

Quantitative Volumetric Analysis

Invasive imaging tools such as intravascular ultrasound (IVUS) and optical coherence tomography (OCT) offer the closest information to match histopathology of atherosclerotic plaque information [67–70].

However, their invasive nature precludes their utilization for cardiovascular risk assessment. Volumetric nature of CCTA provides an opportunity to assess the atherosclerotic plaque burden in the entire coronary artery tree, thus making it unique among various imaging modalities (Fig. 31.2). CCTA identifies twice as many atherosclerotic plaques compared to invasive coronary angiography [71, 72]. Submillimeter isotropic resolution of CCTA allows the assessment of morphology of coronary atherosclerosis. Several studies have shown that plaque detection and characterization evaluated on CCTA correlate well with IVUS [67, 68, 73]. Motoyama et al. [74] showed that total atheromatous plaque volume progression over time on a volumetric basis was an independent predictor of future acute coronary syndrome (ACS) as compared to non-progressors (14.3% vs. 0.27%) over a median follow-up of 4 years. In a case-control study, M.M Hell et al. [75] showed that total plaque volume >179 mm^3, non-calcified plaque volume >146 mm^3, and low-attenuation plaque >10.6 mm^3 were significant predictors of cardiac death over a mean 5-year follow-up period [75]. Similarly, several other studies have shown that software-based objective assessment of plaque burden, specifically non-calcified plaque, is associated with future major adverse cardiovascular events [76]. Verteylen et al. [76] showed that volumetric plaque quantification and characteristics provided additional prognostic value over clinical risk factors and conventional CT reading (including CAC, segment stenosis, lesion severity, and number of segments with non-calcified plaques (AUC 0.64–0.79, $p = 0.047$)). Currently plaque quantification and characterization using semi-automated software takes on average 20–30 minutes making it hard to incorporate in routine clinical practice. Nonetheless, with machine learning algorithm getting better might make plaque quantification part of routine clinical algorithm [75].

Adverse Plaque Features

Three coronary atherosclerotic plaque characteristics – positive remodeling, low-attenuation plaque, and spotty calcification – have been identified as high risk of coronary CTA (Fig. 31.7). Motoyama et al. [77] studied 38 patients with ACS and compared them with 33 patients with stable chest pain. The presence of positive remodeling, spotty calcification, and low-attenuation plaque was significantly more in ACS lesions. In a nested case-control ICONIC (Incident COronary EveNts Identified by Computed Tomography) study, patients with high-risk plaque features, defined as ≥2 of the above-described features, had 60% increased risk of future acute coronary syndrome [78]. Interestingly, 75% of acute coronary syndrome culprit lesion precursors at baseline showed <50% stenosis [79]. In patients who experienced ACS versus those who did not, adverse plaque features were present in 52% and 33%, respectively, implying the dynamic evolving nature of plaque and that even stable asymptomatic patients may have these underlying high-risk plaque features that makes them vulnerable. Furthermore, recent analysis from Scottish COmputed Tomography of the HEART Trial (SCOT-HEART) [80] showed that adverse plaque features were predictive of MACE over a 2-year but not at 5 years' follow-up, suggesting that these plaque features might identify patients at near-term risk.

CTA Versus Standard of Care in Patients with Stable Chest Pain

Two large prospective multicenter randomized trials compared initial strategy of CCTA versus traditional strategy of functional testing or usual

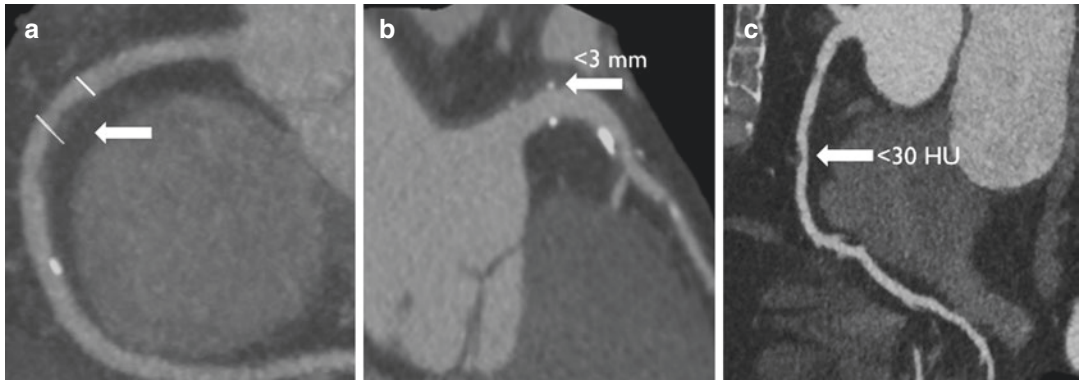

Fig. 31.7 CCTA-based examples of adverse plaque features. (**a**) Positive remodeling characterized by ratio of vessel diameter at lesion (white arrow) site to reference vessel >1.05. (**b**) Spotty calcification (white arrow) characterized by <3 mm calcification. (**c**) Low-attenuation plaque (arrow) characterized by <30H

care in patients presenting with stable chest pain. The PROMISE (Prospective Multicenter Imaging Study for Evaluation of Chest Pain) study showed there was no significant decrease in MACE in CCTA arm as compared to functional testing. However, there was a significant reduction of the number of patients receiving invasive catheterization without obstructive disease in CTA versus functional strategy (28% vs. 52%) [81]. However a priori planned subgroup analysis showed that patients with diabetes who underwent CCTA had a lower risk of death/MI compared with functional testing (CCTA: 1.1% vs. stress testing: 2.6%; a; $p = 0.01$ [82]). A recent landmark 5-year clinical outcome result for SCOT-HEART showed a 40% reduction of coronary heart disease death or non-fatal MI in CCTA arm compared to standard of care [83]. There is an evidence that these results are likely due to initiation or intensification of preventive therapies in patients undergoing CTA [4]. The capability of CCTA to see and quantify atherosclerosis leads to post-care pattern that is quite dissimilar from that of functional testing [3].

CTA Versus Standard of Care in ER

Four large randomized trials (CT-COMPARE, ROMICAT II, ACRIN-PA, and CT-STAT) compared current standard including stress testing with CCTA strategy [84–87]. These trials demonstrated that patients who underwent CCTA had shorter length of stay and shorter time to discharge. Importantly these trials demonstrated the safety of a negative CCTA with very low subsequent events (<1%). It is estimated that more than 6 million people in the United States alone go to emergency departments due to acute chest pain. Very few percentages of these patients have obstructive coronary artery disease. In majority of these patients, CP is unrelated to heart. Along with faster discharge, CCTA provides an opportunity to initiation and intensification of preventive therapies in patients with non-obstructive coronary artery disease on CTA (Fig. 31.8).

Monitoring Therapy with Serial Coronary CT Angiography

Serial studies utilizing IVUS and coronary angiography provided an insight into natural history of atherosclerotic coronary artery disease. Besides, serial measurements of coronary plaque volume using IVUS have served as remarkable tool to gauge drug efficacy in atherosclerosis progression [69, 88]. Nonetheless, invasive nature of IVUS limits the routine use of this modality. Given the capability of CCTA to assess the plaque morphology. Several studies have utilized serial CCTAs to evaluate changes in morphology and progression of plaque after a specific therapy [69] (Fig. 31.9). Shin et al. [89] performed semi-automated quantitative coronary CT plaque assessment in 467 patients with median scan

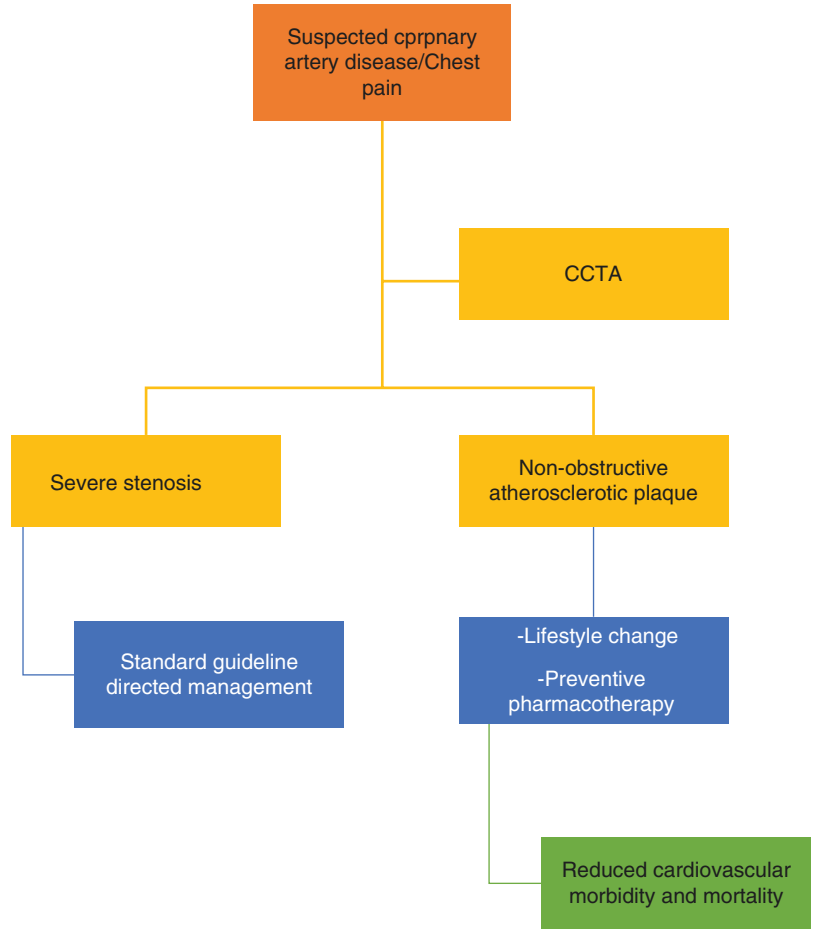

Fig. 31.8 Pathway to improve outcomes in patients who underwent CT for acute chest pain or stable chest pain of suspected coronary origin

period of 3.2 years. Patients who achieved LDL-C of <70 were compared to those with >70. Patients with LDL-C levels below 70 had significantly less progression of plaque as compared to those with >70 mg/dl (12.7 + 38.2 vs. 44.2 + 73.6 mm, respectively = 0.014).

Kaivan et al. [90] performed serial coronary CT study to assess the impact of colchicine on plaque over a mean follow-up of 12.6 months. They showed that colchicine therapy significantly reduced LAPV as compared to control group (mean 15.9 mm [−40.9%] vs. 6.6 mm [−17.0%]; p = 0.008). In a serial prospective study of 32 patients, 24 on statins and 8 not on statins, Kaori et al. [91] assessed the efficacy of fluvastatin. Serial CTAs were performed after a median follow-up of 12 months. In the fluvastatin-treated patients, total plaque volume and low-attenuation plaque volume were significantly reduced over time (92.3 ± 37.7 vs. 76.4 ± 26.5 mm, $p < 0.01$) and (4.9 ± 7.8 vs. 1.3 ± 2.3 mm, $p = 0.01$), respectively. Control subjects had no change in total atheroma plaque volume and LAP. Other studies utilizing serial coronary CTA showed the less coronary plaque progression in patients treated with statins, in concordance with previous IVUS literature. Budoff et al. [92] recently evaluated impact of testosterone on coronary atherosclerosis. Testosterone treatment compared to placebo was associated with a significant increase in non-calcified plaque volume from baseline to 12 months as compared to placebo (estimated difference 47 mm 3; 95% CI, 13–80 mm; $P = 0.006$).

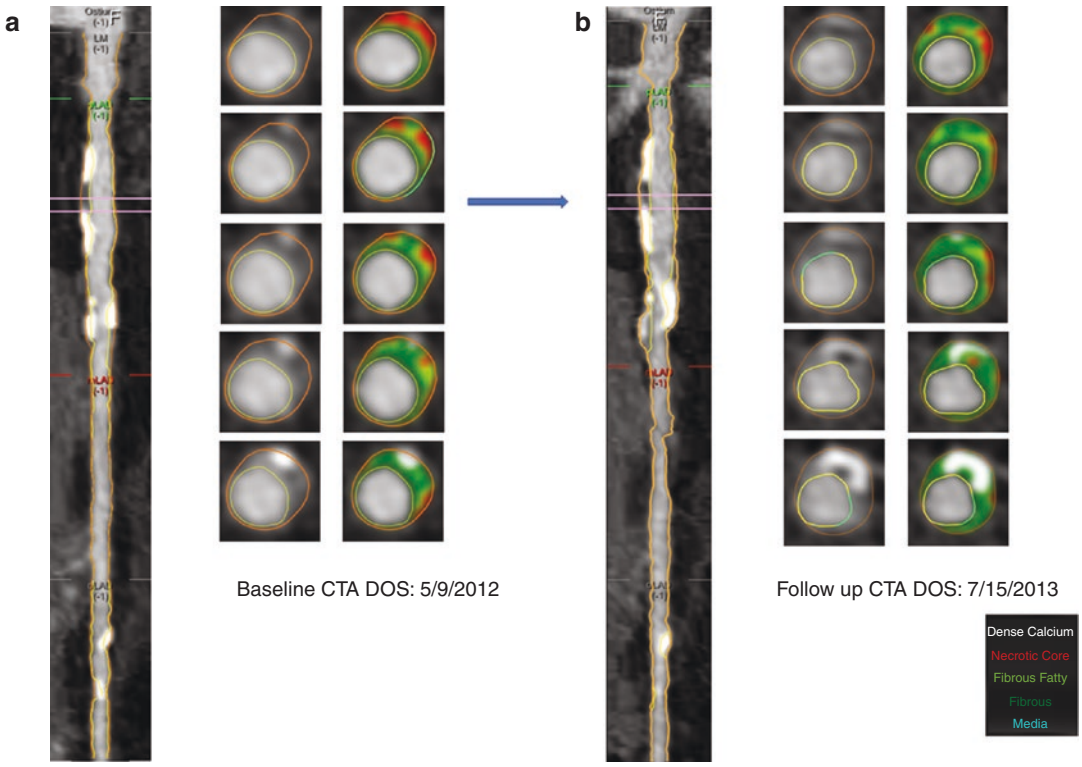

Fig. 31.9 Volumetric assessment of coronary plaque, (**a**) plaque burden at mid left anterior descending artery and (**b**) plaque progression at follow-up scan over a year later

Our lab and others have evaluated the efficacy of alternative therapies in halting coronary plaque progression over time. For example, aged garlic extract compared to placebo was shown to cause regression in low-attenuation plaque volume on serial coronary CT over a period of 1 year in patients with metabolic syndrome and diabetes [93, 94]. There was 20% reduction in LAP in participants taking aged garlic extract as compared to those on placebo [93].

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The Clinical Use of Ultrasound for Atherosclerosis Imaging

32

Steven Feinstein and Anupama K. Rao

Introduction

Atherosclerosis is the leading cause of vascular disease worldwide. There is increasing recognition of atherosclerosis as a complex, multisystem process that starts at a young age and progresses as an indolent process for decades until clinical symptoms occur [1]. The eventual clinical sequelae of peripheral arterial disease, ischemic stroke, and coronary artery disease account for the morbidity and mortality resulting from atherosclerosis. While there have been dramatic declines in the incidence of ischemic heart disease and stroke in high-income countries, developing countries have seen less pronounced and more varied trends [2, 3]. Much of this decline can be attributed to advances in knowledge about the pathophysiology of atherosclerosis, with improved methods of detection and availability of novel therapeutic targets. These recent advances have heightened the need for noninvasive imaging techniques to allow for earlier diagnosis and accurately monitor treatment response. Modern technology has made available a myriad of noninvasive options for atherosclerosis imaging. It is imperative that these imaging methods offer incremental information over traditional risk factors and allow for earlier detection in a cost-effective manner.

Ultrasound is a particularly attractive imaging tool, given the wide availability, avoidance of exposure to ionizing radiation, ease of use, and relatively low cost. A number of ultrasound techniques have emerged, with potential utility in both clinical and research settings. These techniques have made it possible to accurately assess the extent and functional significance of atherosclerosis in order to prevent devastating clinical consequences before they occur. This chapter will explore the use of ultrasound imaging of atherosclerosis in the clinical setting. Specifically, we will delve beyond the simple anatomic assessment of stenosis and understand how ultrasound technology can help assess physiologic consequences such as alterations in arterial wall biomechanics. We will explore the various methods of assessing vulnerable plaque and discuss advances in ultrasound technology, including contrast-enhanced ultrasound and molecular imaging.

Brief History of Ultrasound

The Austrian neurologist Karl Dussik was the first to use ultrasound for diagnostic purposes in 1942, when he attempted to detect brain tumors

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_32

by passing a beam emitted by an elaborate ultrasound generator through the human skull [4]. A-mode or "amplitude-mode" ultrasonography and shortly after B-mode or "brightness-mode" technology followed in the late 1940s and early 1950s. The 1960s and 1970s gave rise to various techniques of assessing blood flow including continuous wave, pulse wave, color, and spectral Doppler imaging. Early applications of vascular imaging included using Doppler shift to detect disturbances in flow and velocity. This evolved further into 3D vascular imaging and measurement of plaque area with increasingly sophisticated ultrasound techniques, accompanied by improvements in image quality and ease of use. Today, ultrasound probes have become even more compact and efficient, linking to personal handheld devices with ever-increasing ease of use, accuracy, and convenience.

Ultrasound Imaging

Ultrasound imaging involves balancing spatial and temporal resolution with adequate penetration. Resolution improves with increasing frequency, but at the expense of reduced depth of penetration. Modern clinical ultrasound systems use linear array transducers operating at a frequency between 5 and 18 MHz. While this frequency range is adequate for superficial vessels such as the carotid arteries, deeper structures such as coronary arteries cannot be optimally imaged. A 5 MHz system cannot sufficiently visualize plaque within a coronary artery due to extremely poor resolution. Invasive intracoronary vascular ultrasound systems such as intravascular ultrasound (IVUS), which operate at high frequencies of 20–40 MHz, can be utilized to characterize plaque within the coronary arteries. Piezoelectric material is mounted on the tip of an invasive catheter, which can be guided in a retrograde manner through the aorta into the coronary arteries. This technology allows for tomographic views of the coronary wall and can assess the extent of atherosclerosis, which is not visible by traditional invasive coronary angiography. High-resolution images in the axial and lateral directions can be obtained using linear array transducers, containing up to 256 elements in a single row. When such transducers are utilized, high frame rates of up to 50 frames/second can be achieved, allowing for both geometric assessment and analyzing arterial wall dynamics.

Anatomic Imaging

B-Mode Ultrasound

B-mode ultrasound is a fundamental method to visualize atherosclerotic plaque with excellent delineation of vessel wall and plaque geometry. The common carotid artery as well as the internal and external carotid arteries can be readily imaged given the proximity to the skin surface (Fig. 32.1). B-mode ultrasound provides a gray-scale image that provides detailed information about the extent and type of plaque. The ultrasound probe is generally positioned parallel to the course of the artery and circumferential scans are obtained from both anterior and posterior angles. Due to differences in acoustic impedance between blood and the intima, the transition appears as a bright reflection on B-mode imaging. The medial layer is less echogenic than the intima and the adventitial layer appears more echogenic than the media, enabling discrimination between the layers of the vessel wall. Atherosclerotic plaque appears as protrusions of the intima-media layer. The total plaque area and total plaque volume are measurements that are well established as independent predictors of cardiovascular death and coronary events [5, 6]. Carotid plaque area, when defined as the sum of cross-sectional area of all carotid plaques, has been well correlated with increased future cardiovascular risk [7]. In one study of elderly patients, the number of carotid plaques conferred a higher overall risk of mortality, with a 2.9-fold risk of death when 1–2 plaques were present and 4.9-fold risk when more than four plaques were present [8].

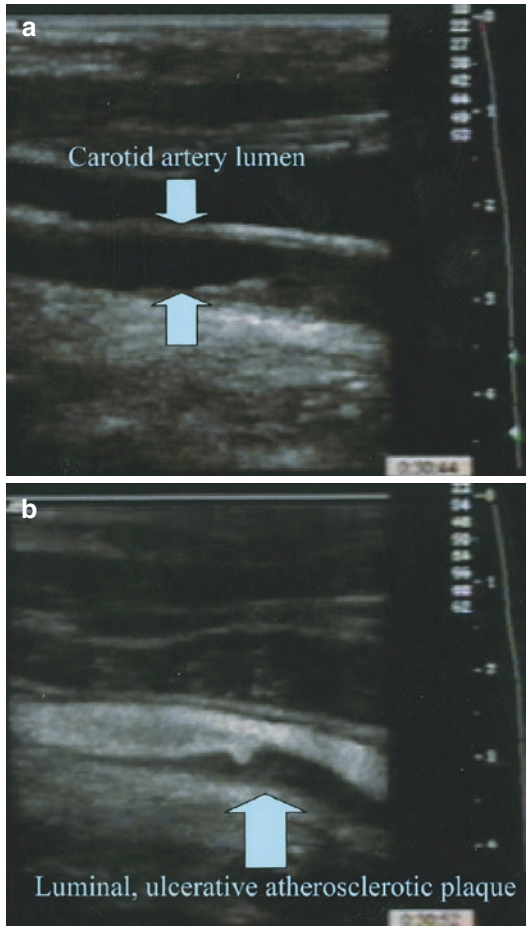

Fig. 32.1 B-mode ultrasound showing the lumen of the carotid artery without contrast (**a**) and with ultrasound contrast (**b**). The lumen appears as an echo-dense (white) structure along with ulcerative plaque. Feinstein [67]

Doppler Ultrasound

Doppler ultrasound can augment the information obtained from grayscale imaging with the ability to measure flow across a vascular region utilizing both pulse Doppler and color Doppler techniques. Grayscale images can be superimposed on color Doppler to provide information about direction of flow and flow velocity. For example, the ratio of flow between the internal carotid and common carotid arteries can help distinguish a compensatory increase in flow from a contralateral artery occlusion over true stenosis. However, there are a number of caveats to using Doppler flow to estimate the degree of vessel stenosis including higher velocity in females and reduced accuracy in the setting of vessel tortuosity, contralateral vessel stenosis, calcification, or when stents are present [9–12]. Additionally, Doppler ultrasound may not be able to detect complete versus partial vessel occlusion and requires trained personnel to yield clinically useful and reproducible measurements. Despite some of these pitfalls, Doppler information is a readily available technique that can enhance anatomic information.

Plaque Characterization

There are a number of sonographic characteristics of plaque that have been found to be predictive of adverse clinical outcomes including plaque echolucency, neovascularization, and presence of ulceration and mobility within the plaque. These high-risk features are readily assessed with ultrasound techniques and can identify vulnerable plaque.

Plaque Echogenicity

The echogenicity of plaque can provide further information regarding propensity for clinical events. Plaques that are composed of a lipid core appear echolucent, while fibrosis and calcification render an echogenic appearance. Histologically, plaque echolucency correlates with the presence of a lipid-rich necrotic core or intraplaque hemorrhage [13]. In patients with carotid plaque, echolucent plaques appear to have a much higher risk of stroke, with increasing echolucency over time predicting cerebrovascular events [14, 15]. Furthermore, echolucent carotid plaque appears to be a significant and independent predictor of future coronary events in patients with stable angina [16].

Vasa Vasorum Neovascularization

Healthy arteries show vascularity of the adventitia with the intima and media being supplied by nutrients from the luminal blood flow. However,

with progression of atherosclerosis, there is neovascularization of the vasa vasorum into the media and intima layers [17]. These neovessels are thought to promote plaque progression and development of a necrotic core [18]. Plaque neovascularization has been correlated with plaque echolucency, a marker of high-risk plaque [19]. Ultrasound techniques such as contrast-enhanced imaging, which will be discussed further, can detect these pathologic vessels. Qualitative and quantitative data have shown correlation with plaque vulnerability and with clinical coronary and cerebrovascular events [20]. Additional studies are needed in using this technique to predict future clinical events.

Plaque Ulceration and Mobility

Plaque ulceration is readily detectable via ultrasound and is well recognized as a cause of clinical events such as ischemic stroke due to local thrombosis and embolism. Ultrasound findings validated by pathologic specimens from patients undergoing carotid endarterectomy include a concave appearance of plaque with the basal border echo weaker than that of adjacent plaque surface [21].

CIMT

Plaques that are prominent enough to be visualized by ultrasound are a late clinical finding in the time course of atherosclerosis. Thickening of the carotid intima-media layer occurs early on in atherosclerosis before the intravascular lumen is compromised [22]. Thus, carotid intima-media thickness (CIMT) is considered to be a preclinical marker of atherosclerosis. It is well established that CIMT is associated with all forms of atherosclerotic disease, including coronary heart disease, stroke, and peripheral vascular disease [23–25]. Moreover, CIMT has been shown to regress after pharmacologic therapy such as lipid-lowering treatments [26]. Therefore, CIMT is considered to be a surrogate marker for cardiovascular disease as well as an independent risk factor and a valuable tool for the early detection of atherosclerosis. CIMT is defined as the distance between the lumen-intima interface and the media-adventitia interface (Fig. 32.2). Typical measurements are taken at the near and far walls of the right and left common carotid arteries, the carotid bifurcation, and the proximal portion of the internal carotid arteries. B-mode ultrasound with frequency ranges between 5 and 15 MHz is commonly used to measure CIMT. The composite CIMT in an individual is an average of various arterial segments [22, 27]. Normal CIMT values at age 10 are approximately 0.4–0.5 mm, progressing to 0.7–0.8 mm after the fifth decade [28]. In general, values about 1 mm are considered abnormal although standard values differ depending on age, gender, and ethnicity.

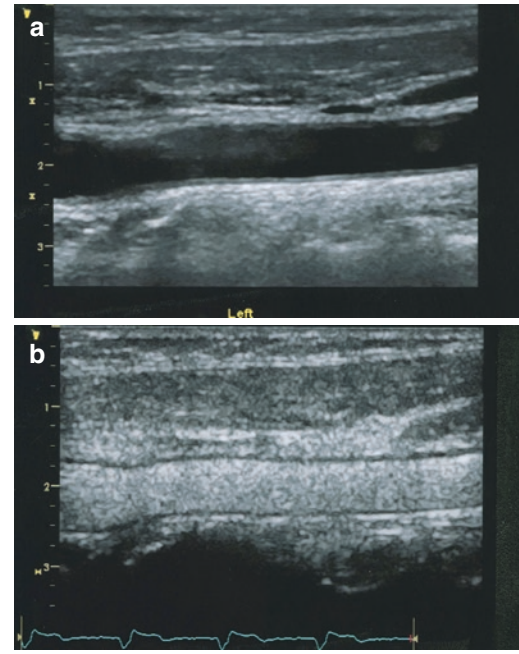

Fig. 32.2 Unenhanced (**a**) and contrast-enhanced (**b**) ultrasound of the near wall of the carotid artery clearly delineating the carotid intima-media thickness. Feinstein [67]

A number of early studies showed an association between CIMT and risk of cardiovascular events. For example, the Kuopio Ischaemic Heart Disease study revealed an 11% increase in the risk of myocardial infarction with each 0.1 mm increase in CIMT [29]. In the Atherosclerosis

Risk in Communities (ARIC) study, hazard ratios for coronary heart disease events in women and men with mean CIMT >1 mm compared to those with <1 mm CIMT were 5.07 and 1.85, respectively [30]. However, despite this early data, CIMT does not appear to improve risk stratification when added to traditional risk scores. When mean common CIMT was added to the Framingham 10-year risk score for myocardial infarction and stroke, a net reclassification only occurred in 0.8% of subjects and in 3.6% of those at intermediate risk [31]. Two meta-analyses have yielded conflicting results regarding the value of CIMT in risk assessment. Lorenz et al. reported a relative CVD risk of CVD events of 1.15 for every 0.1 mm increase in CIMT, while Den Ruijter et al. found no value to addition of CIMT to usual risk prediction models [31, 32]. Given these conflicting results and modest ability to reclassify risk profiles beyond traditional risk prediction models, CIMT is not considered to be useful for prediction of cardiovascular risk. As a result, the 2013 American College of Cardiology/American Heart Association guidelines for CVD risk assessment do not recommend the routine use of CIMT for risk assessment purposes [33].

CIMT has been frequently used as a surrogate endpoint in multiple pharmaceutical trials investigating the efficacy of a number of drugs including statins and antihypertensives on the cardiovascular system [26, 34, 35]. For example, in the REGRESS study of patients treated with pravastatin, a 0.05 mm reduction in CIMT was shown to decrease absolute 2-year cardiovascular event rate by 10% [36]. However, modifying therapy based on CIMT results does not translate into improvements in hard clinical endpoints such as death, myocardial infarction, and stroke. Hence, until there is additional data, there has been a shift away from using CIMT as a surrogate endpoint in clinical trials.

Intravascular Ultrasound

Intravascular ultrasound (IVUS) is a valuable invasive technique to elucidate information about plaque within the coronary arteries. Unlike conventional angiography which only assesses for luminal stenosis, IVUS allows for the detection of early plaques with eccentric remodeling that do not encroach on the vessel lumen. In fact, IVUS studies have revealed that many acute coronary syndromes are due to non-flow limiting eccentric plaques [37]. Grayscale IVUS allows for assessment of atheroma burden and eccentricity which closely correlates with histology [38, 39]. Calcified plaques can appear bright on grayscale IVUS with less dense, or "soft" plaques appearing less echo-dense. There are emerging studies investigating the use of IVUS to get more detailed information regarding plaque composition. For example, integrated backscatter data from the radiofrequency signal can be color coded to provide information regarding plaque composition [40].

Biomechanical Imaging

While much of the early clinical use of ultrasound was directed towards diagnosing the extent and type of plaque, the focus has expanded to detecting pathologic alterations in the biomechanical properties of the arterial wall. Progression of atherosclerosis is characterized by fibrosis, calcification, and smooth muscle proliferation within the wall of the vessel, leading to increased stiffness and decreased deformability [41]. This loss of elasticity can be measured by pulse wave (PW) Doppler, which correlates with atheroma burden and cardiovascular events [42]. Additionally, advancing age and hypertension show a strong association with increased pulse wave velocity [43]. It is possible to invasively evaluate the elastic properties of coronary arteries with intravascular ultrasound (IVUS) using elastography [44, 45]. This method uses radiofrequency data to assess local tissue displacement and derive local strain maps of the vessel wall, with fatty plaques that are at potentially higher risk of rupture demonstrating higher strain values than fibrous plaques. Another similar technique is intravascular palpography, which provides information about the luminal surface of plaques [46]. Higher strain values have been found for

plaques with necrotic cores and inflammation, with more deformable plaques also correlating with inflammatory markers such as C-reactive protein. These emerging techniques have revealed the intricate interactions between plaque morphology and composition and changes within the vessel wall.

Contrast-Enhanced Ultrasound

Contrast-enhanced ultrasound (CEUS) utilizes microbubble contrast agents to provide information about plaque morphology and perfusion. These agents make it possible to accurately visualize the vessel lumen, delineate the surface of plaque, and detect plaque neovascularization. Commercially available contrast agents are gas-filled microbubbles measuring between 2 and 8 μm, the size of circulating red blood cells, ensuring that they stay within the vasculature when injected intravenously. These gas-filled microbubbles serve as excellent intravascular contrast agents due to their marked acoustic mismatch relative to surrounding tissue. CEUS is performed after standard B-mode, color Doppler, and spectral Doppler ultrasonography utilizing the same transducers. Typically, the acquisition is set to a contrast-specific low mechanical index (MI) (0.06–0.5) setting to prevent microbubble destruction by the ultrasound beam. The transmitted frequency is aligned with the microbubble resonance frequency, thus generating harmonic signals from the contrast agent. Advantages of CEUS include an excellent safety profile without the risks of nephrotoxicity or thyrotoxicity as iodinated contrast agents. Additionally, no specific laboratory testing is needed prior to administration and the risk of allergic or anaphylactic reactions is exceedingly low [47].

CEUS has been shown to be superior to color Doppler imaging for grading of luminal stenosis and delineation of the plaque surface [48, 49]. CEUS has also been shown to improve diagnostic accuracy of ultrasound for carotid disease and strongly correlates with angiography for degree of stenosis [50, 51]. A recent study by Ventura and colleagues showed that CEUS was more effective than conventional Doppler ultrasound and equivalent to CT angiography to distinguish between truly occlusive carotid disease and pseudo-occlusive disease in patients referred for vascular surgery [52]. Plaque ulceration, often not well visualized with unenhanced ultrasound, is a high-risk feature that is optimally detected by CEUS. In a study of 29 carotid artery specimens from 20 patients with carotid stenosis, the sensitivity and specificity of CEUS for detection of plaque ulceration were 89% and 59% using CT angiography as the gold standard [53]. Additionally, CEUS can be utilized in surveillance for restenosis following carotid endarterectomy or stenting and allows visualization of the entire length of the stent without stent-related artifacts encountered with conventional ultrasound [54–56].

Intraplaque Neovascularization

A unique feature of CEUS is the ability to detect pathologic microvessels within atherosclerotic plaque, a phenomenon called intraplaque neovascularization (IPN). While the vasa vasorum (VV) is confined to the adventitial layer in healthy blood vessels, progression in atherosclerosis is accompanied by extension of the VV into the developing plaque to provide oxygen and nutrients [57]. Due to the lack of structural support by pericytes, these pathologic VV are especially prone to rupture leading to intraplaque hemorrhage, contributing to overall plaque instability [58, 59]. Feinstein was the first to report the use of CEUS for direct visualization of the carotid artery IPN and adventitial VV in 2004 [60] (Fig. 32.3). Since then, there have been numerous studies highlighting the adverse implications of IPN visualized by CEUS on plaque severity and vulnerability. Saito and colleagues, in a study of 50 patients who underwent CEA, showed that increased intensity of echo-contrast of the plaque shoulder correlated with a higher density of neovessels and a higher incidence of ruptured plaques [61]. The presence of IPN within carotid plaques has been shown to be an independent predictor of future coronary and cardiovascular

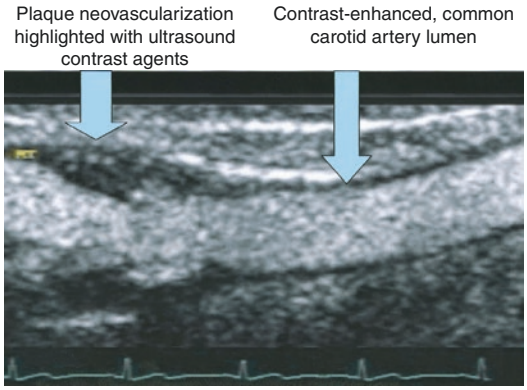

Fig. 32.3 Ultrasound contrast agents highlight the vessel lumen and reveal intraplaque angiogenesis arising from the vasa vasorum. Feinstein [67]

events even after adjusting for traditional cardiovascular risk factors [62].

Molecular Imaging

Microbubble contrast agents can be conjugated with ligands, allowing for targeted molecular imaging. Conventional imaging data regarding flow, structure, and function can be synthesized with molecular level data to provide comprehensive pathophysiologic information. As microbubbles remain exclusively within the vascular compartment, this technology is well suited for the detection of intravascular molecular processes. For example, CEUS has been used to detect and quantify vascular cell adhesion molecule (VCAM-1) expression within the aortic wall, signifying vascular inflammation [63]. CEUS probes against VCAM-1 and P-selectin have been used to detect early endothelial activation preceding any changes in intima-media thickness in primates fed a Western diet [64]. Microbubbles targeted to ICAM-1 have been shown to detect transplant rejection in a murine model, and probes targeted to CD3 antibody have directly assessed the role of T-lymphocytes in a transplant model [65, 66]. These preclinical advances have primed the stage for clinically applicable molecular CEUS imaging. Translation of these advances into routine clinical care will require contrast agents approved for human use and will need to demonstrate incremental value to existing clinical pathways.

In summary, CEUS is a rapidly growing tool that overcomes some of the limitations of conventional unenhanced ultrasound to provide incremental information regarding plaque composition, morphology, and vascularity. CEUS is especially valuable in detecting features of high-risk plaque including plaque neovascularization. There is immense potential to use CEUS for targeted molecular imaging of atherosclerosis.

Conclusion

Ultrasound is a versatile clinical tool for the imaging of atherosclerosis. New ultrasound technologies have expanded the assessment of atherosclerosis beyond basic anatomy to include the physiologic consequences of plaque. Available technology has allowed for the early detection of atherosclerosis as well as identification of high-risk plaque. Novel technologies such as contrast-enhanced ultrasound and molecular imaging have further improved the ability to quantify the physiologic consequences of atherosclerosis. Continued advances will enhance our ability to detect and potentially prevent the devastating consequences of atherosclerosis.

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33 Emerging Therapies for Regulating Dyslipidaemias and Atherosclerosis

Natalie C. Ward and Gerald F. Watts

Introduction

Population and family genetic studies have paved the way for recent advances in the development of new drug therapies for dyslipidaemias. This has been supported by more traditional approaches to drug discovery based on drug screening protocols and extant knowledge of lipid biochemistry and metabolism [1]. From a clinical perspective, the need for new agents arises from the demands in managing patients with severe dyslipidaemia and those that are intolerant to standard pharmacotherapies, such as statins. There is also the need to treat the gap related to the concept of residual cardiovascular risk, referred to below. All new therapies aim to prevent atherosclerotic cardiovascular disease (ASCVD), as with atherogenic dyslipidaemia, or acute pancreatitis and hepatic steatosis, as with severe hypertriglyceridaemias [1, 2].

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Atherosclerosis is a multifaceted disease process that is driven by dyslipidaemia [3–5] and inflammation [6, 7]. Therapies to reduce the risk of atherosclerosis include lifestyle interventions, blood pressure-lowering medications, statin therapy, anti-platelet agents and, in select groups of patients, revascularisation procedures. Despite this, significant residual risk remains, particularly in patients with existing atherosclerosis [8]. Residual risk can largely be attributed to two factors: firstly, the need for further and more aggressive reduction of low-density lipoprotein cholesterol (LDL-c) and, secondly, the presence of vascular inflammation [9]. Understanding the contributions of both these factors is crucial for optimal treatment of patients, particularly in the era of precision medicine [8, 10].

This chapter will principally review new and emerging therapies for regulating atherogenic lipoproteins and as a consequence the pathogenesis of atherosclerosis. It will also cover agents that can effectively lower severe hypertriglyceridaemia (e.g. chylomicronaemia) and mitigate the risk of acute pancreatitis and steatohepatitis.

Dyslipidaemia and Atherosclerosis

Increased plasma concentrations of cholesterol-rich apolipoprotein B (apoB)-containing lipoproteins, including low-density lipoprotein cholesterol (LDL-c), lipoprotein (a) [Lp(a)] and

M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_33

triglyceride-rich lipoproteins (TRLs), have been causatively linked to the development of atherosclerotic cardiovascular disease (ASCVD) [1, 11]. Calculation of most risk estimations and the majority of drug trials have focused on the benefits of LDL-c reduction, which is proportional to the degree and duration of lowering [1, 11]. In addition to widespread consensus on the value of lowering LDL-c to reduce risk [4] are multiple trials supporting clinically significant reductions in cardiovascular mortality [5]. Large prospective epidemiological studies have consistently demonstrated that high levels of LDL-c are predictive of future ASCVD events [12]. Meta-analysis highlights the benefits of LDL-c reduction, with every 1 mmol/L (38.7 mg/dL) reduction associated with a significant 22% relative risk reduction in major vascular and coronary events [4]. The Cholesterol Treatment Trialists Collaboration (CTTC) demonstrated that in men and women with a wide spectrum of clinical characteristics, there was a consistent relative risk reduction in major vascular events per change in LDL-c level [13].

Lp(a) is thought to contribute to increased cardiovascular risk through three main mechanisms: the atherogenic nature of its LDL-like moiety, the anti-fibrinolytic effects of its apolipoprotein (a) [apo(a)] moiety and the pro-inflammatory effects of its oxidised phospholipid content [14]. Its contribution to increased ASCVD risk may be further exacerbated by the pro-atherogenic properties of both LDL-c and apo(a) [14]. Multiple epidemiological studies confirm a positive association between circulating Lp(a) levels and risk of ASCVD [15–22], a finding that is consolidated by Mendelian randomisation and genome-wide association studies [23–28]. More recent analysis has revealed that the risk of coronary heart disease (CHD) is proportionally associated with the absolute change in Lp(a) mass concentration, where Lp(a) must be lowered by ~100 mg/dL to achieve the same CHD risk reduction as lowering LDL-c by 38.67 mg/dL [29]. Meta-analysis of long-term prospective studies reveals a continuous, independent and modest association of Lp(a) concentration and risk of CHD and stroke. This association appears to be exclusive to vascular outcomes and presents under a wide range of clinical circumstances [15].

Elevated triglycerides are also a common risk factor for ASCVD and a biomarker for the accumulation of circulating TRLs and their metabolic remnants in plasma [2, 30]. It often presents with obesity, insulin resistance, hepatic steatosis, ectopic fat deposition and diabetes mellitus [2]. In addition to elevated ASCVD risk, severe hypertriglyceridaemia is also associated with an increased risk of pancreatitis [30]. The AMORIS study recently revealed that elevated lipids, including cholesterol, triglycerides, LDL-c and apoB, as well as glucose levels measured two decades before a cardiovascular event in patients <50 years may account for half of all events in this population [31]. Furthermore, hypertriglyceridaemia may contribute to residual CVD risk, in the presence of statin therapy, through uncorrected atherogenic dyslipidaemia, predominantly due to hepatic oversecretion and/or hypocatabolism of TRLs [32].

Standard Lipid-Lowering Therapies

Statins are a widely prescribed class of drugs to lower cholesterol, primarily via inhibition of hydroxymethylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol biosynthesis pathway [33]. The effect of statins on CVD outcomes is driven by the extent of LDL-c lowering, which is further enhanced by the addition of ezetimibe [12]. The utility and drawback of statins were recently reviewed [34, 35]. While their clinical value is well established [36], key questions remain concerning their role in primary prevention (with the exception of FH), in the elderly (>75 years), in chronic kidney disease and younger patients with diabetes [35].

Fibrates, which predominantly lower triglyceride levels, have not been demonstrated to improve CVD outcome measures [37]. In patients with type 2 diabetes, fenofibrate reduced the number of total CVD events, despite no difference in the risk of the primary outcome of coronary events. This was mainly due to fewer nonfatal myocardial infarctions and revascularisations [38]. In addition, fenofibrate reduced the

need for laser treatment for diabetic retinopathy [39] and the need for amputations, particularly minor amputations without known large-vessel disease [40], with both of these beneficial effects apparently unrelated to changes in plasma lipid levels. In contrast, the ACCORD study revealed that when given in combination with a statin, fenofibrate did not have any effect on the rate of fatal cardiovascular events, nonfatal myocardial infarction or nonfatal stroke compared to statin alone. However, it should be noted that ACCORD did show favourable effects on microangiopathy and cardiovascular events in a subgroup of patients with high triglycerides and low HDL-c [41, 42].

Bile acid sequestrants, including the newer colesevelam, lower LDL-c and have been demonstrated to be safe and efficacious, either alone or in combination with statins [43]. Additional benefit may come from colesevelam's ability to also lower high-sensitivity C-reactive protein (hsCRP) levels when given in combination with statin therapy [44]. When given in combination with ezetimibe, colesevelam was shown to significantly lower LDL-c, total cholesterol, non-HDL-c and apoB levels as well as increase apoA-I levels, with no effect on triglycerides [45]. Colesevelam has also been shown to improve glycaemic control and lower LDL-c in type 2 diabetes, although there was also a significant increase in triglyceride levels [46]. However, the use of bile acid sequestrants is limited by gastrointestinal side effects related to bloating and constipation.

The newer proprotein convertase subtilisin/kexin type 9 (PCSK9) monoclonal antibodies have demonstrated significant reductions in LDL-c (and some other lipids) and cardiovascular outcomes in high-risk patients already on statin therapy [47–49]. In contrast, plasma levels of Lp(a), which are not routinely tested, have very few established or clinically approved drug treatments with the exception of apheresis [50], which is only approved in Germany and the USA. Statin treatment does not appear to have any effect and there are reports that their use may increase circulating levels of Lp(a) by 10–20% [14], with a recent study suggesting an Lp(a) level >50 mg/dL is associated with an increased risk of recurrent events in patients on statin therapy [51]. Other pharmacological therapies including niacin, microsomal triglyceride transfer protein (MTP) inhibitors and cholesteryl ester transfer protein (CETP) inhibitors all affect lipid levels to varying degrees; however, their clinical use remains limited [52, 53].

Guidelines for Use of Existing Lipid-Lowering Therapy

The 2018 American Heart Association and American College of Cardiology (AHA/ACC) Guideline on the Management of Blood Cholesterol recommends the use of statin therapy to reduce risk in a range of patient populations (clinical ASCVD, diabetes mellitus and hyperlipidaemia), where the greater the LDL-c reduction, the greater the subsequent risk reduction, with recommendations to reduce levels by ≥50%. Addition of ezetimibe is recommended for those not achieving lipid targets on maximally tolerated statins, while a PCSK9 inhibitor is only recommended for third-line therapy in specific patient populations [54, 55]. The 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease also recommends statin therapy as first-line treatment for primary prevention of ASCVD in patients with elevated LDL-c (≥190 mg/dL or ≥4.9 mmol/L), patients with diabetes aged between 40 and 75 years and patients considered to be at sufficiently high ASCVD risk, based on their 10-year absolute ASCVD risk calculation, following consultation with their clinician [55].

The AHA/ACC guidelines currently only recognise elevated Lp(a) as a "risk-enhancing" factor in the development of ASCVD. Indications for its measurement are a family history of premature ASCVD or a personal history of ASCVD not explained by major risk factors. Enhanced risk is associated with Lp(a) levels ≥50 mg/dL or ≥125 mmol/L, although in women it is recommended that this also be accompanied by hypercholesterolaemia [54]. Triglyceride-lowering drugs, fibrates and niacin, have mild LDL-c-lowering properties, although their use as add-on therapy to statins is not supported by ran-

domised controlled trials [54]. Many patients with severe hypertriglyceridaemia have multiple ASCVD risk factors (e.g. diabetes, hypertension) and are considered to be at enhanced risk. In such patients, initiation of statin therapy is recommended to lower atherogenic LDL and non-HDL cholesterol, but hypertriglyceridaemia needs to be independently addressed by correcting secondary causes (e.g. obesity and diabetes) using lifestyle and non-statin approaches. To prevent acute pancreatitis associated with severe hypertriglyceridaemia, a low-fat diet should be implemented, in addition to adding a fibrate or omega-3 fatty acid [54]. More severe forms, likely due to genetic factors, such as familial chylomicronaemia syndrome (FCS) require more specialised diagnosis and therapies, as discussed below [56–58].

Emerging Lipid-Lowering Therapies

Despite existing treatments, most notably the widely used statins, poor lipid management resulting in significant residual risk of ASCVD remains an ongoing problem. This can be due to various factors including an inability to achieve lipid targets despite maximally tolerated therapy, statin intolerance, an existing genetic condition that results in significantly elevated lipid levels or an aversion/inability to take standard pharmacotherapy (children, pregnancy, cost, cultural reasons). As a result, there is an ongoing need for new therapies to reduce lipids and subsequent ASCVD risk. The purpose of this chapter is to provide an update on emerging lipid therapies and their effect on various lipid parameters and atherosclerotic cardiovascular outcomes.

LDL-C-Lowering Therapies

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibitors

PCSK9 inhibitors function by reducing the concentration or activity of circulating and possibly intracellular PCSK9, preventing the degradation of LDL receptors and thus increasing clearance of LDL-c from the plasma [59, 60]. Multiple strategies for reducing circulating PCSK9 are being investigated, including preventing PCSK9 from binding to the LDL receptor (monoclonal antibodies) and targeting PCSK9 synthesis and processing (RNA-targeted therapies) [33, 61–64]. To date, the monoclonal antibodies, which bind to the catalytic domain and prodomain of PCSK9, blocking its binding to and degradation of the LDL receptor and neutralising the effect of PCSK9 in the plasma, have been the most effective approach for lipid lowering [59, 61, 64, 65]. Two PCSK9 monoclonal antibodies, alirocumab (formerly SAR236553/REGN727) and evolocumab (formerly AMG145), have been approved for use by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) in patients with familial hypercholesterolaemia (FH) and those with clinical ASCVD who require additional lipid lowering despite maximal statin dose and diet control [61, 66]. Their significant LDL-c-lowering effectiveness has been demonstrated in a number of clinical trials and a range of patient populations through the FOURIER and ODYSSEY clinical trial programs [67–80].

inclisiran is a GalNAc-conjugated siRNA that binds to PCSK9 mRNA to inhibit hepatocyte production of the protein and thus reduce LDL-c levels. Its mode of action is distinctly different to the monoclonal antibodies in that it decreases hepatocyte PCSK9 production rather than binding to PCSK9 in the circulation [64, 81, 82]. A phase I dose escalation study in healthy volunteers with elevated cholesterol revealed significant reductions in both circulating plasma PCSK9 protein and LDL-c levels [83]. Subsequent studies in patients at high risk for CVD and with elevated cholesterol levels observed dose-dependent reductions in both PCSK9 and LDL-c levels [84]. Patients with elevated LDL-c despite maximally tolerated statin therapy had significant reductions in apoB, non-HDL cholesterol and VLDL-c following a single dose of inclisiran, with a second dose providing additional lowering, although there was large inter-individual variation in VLDL-c, triglyceride and Lp(a) reductions [85].

Additional approaches to targeting PCSK9 include development of anti-PCSK9 vaccines, new monoclonal antibodies and targeted gene editing, although these are all currently still in the developmental pre-clinical phase [1, 64, 86].

Apolipoprotein B Inhibitors

Apolipoprotein B (apoB) is the main structural and receptor-binding component of atherogenic lipoproteins, including LDL-c [12]. Mipomersen is a second-generation antisense oligonucleotide (ASO) that targets apoB-100, inhibiting protein translation at the mRNA level and reducing levels of apoB-containing lipoproteins [12, 81]. It is approved for use in the USA for treatment of homozygous FH patients [87] and has been demonstrated to lower LDL-c, apoB and Lp(a) levels [12, 88–91]. In heterozygous FH patients, mipomersen showed similar efficiency when added to conventional lipid-lowering therapy, with significant reductions in LDL-c [92]. In heterozygous FH patients with CAD and on maximally tolerated statin therapy, mipomersen resulted in significant reductions in both LDL-c and Lp(a) [90]. More recently, four phase III trials, which included patients with homozygous FH, heterozygous FH with CAD, severe hypercholesterolaemia and hypercholesterolaemia at high risk of CAD, found significant reductions in Lp(a) with mipomersen. This was most frequent in the homozygous FH and severe hypercholesterolaemic patients. Modest correlations were observed between apoB-100 and Lp(a) and LDL-c and Lp(a) [93]. Prospective post hoc analysis of three randomised controlled trials demonstrated that in addition to lowering atherogenic lipoproteins, mipomersen also reduced the number of cardiovascular events in FH patients. This reduction coincided with the mean absolute reduction in LDL-c, non-HDL cholesterol and Lp(a) [94]. In statin-intolerant patients at high risk of CVD, mipomersen significantly reduced LDL-c, apoB and Lp(a) [95].

Mipomersen has several important limitations, including its restricted use in very specified patient populations and its side effect profile. This includes injection site reactions, flu-like symptoms and elevated liver transaminase levels. Also noted is an increase in hepatic fat accumulation, which appears to diminish with continuous exposure beyond 1 year and is not accompanied by fibrosis [12, 81, 90]. These concerns have resulted in it not being licenced in Europe, which when combined with its limited clinical use makes its future uncertain.

Bempedoic Acid (Adenosine Triphosphate (ATP)-Citrate Lyase Inhibitor)

Bempedoic acid (ETC-1002) is an oral drug that is able to simultaneously inhibit adenosine triphosphate-citrate lyase (ACL) and activate adenosine monophosphate-activated protein kinase (AMPK), although in humans, the former is thought to be the dominant mechanism [1]. This dual activity results in a reduction of hepatic cholesterol synthesis and increased LDL receptor expression when the drug is converted in the liver to its active metabolite. The additional activation of AMPK may also confer benefit through its anti-inflammatory properties [1, 12, 96]. Early phase I and II studies demonstrated its LDL-c-lowering ability when used as monotherapy (27% reduction), when added to statin (additional 24% decrease) and/or ezetimibe (total 48% reduction) treatment and in patients with statin intolerance. It was also shown to improve cardiometabolic risk factors, including in patients with type 2 diabetes, without worsening glycaemic control [96–98]. In statin-intolerant patients, bempedoic acid was shown to significantly lower LDL-c, non-HDL-c, total cholesterol and apoB. Accompanying this were reductions in hsCRP. Triglycerides and HDL-c did not change, and there was no difference in muscle-related adverse effects between the bempedoic and placebo groups [99]. In a phase IIb study, bempedoic acid was found to significantly lower LDL-c, non-HDL-c, total cholesterol, apoB and hsCRP, with or without concomitant ezetimibe treatment, more so than ezetimibe treatment alone [100]. Similar results were seen in patients receiving stable statin ther-

apy [101] and in statin-intolerant patients on ezetimibe [102].

The CLEAR-OUTCOMES trial (NCT02993406), a randomised, double-blind, placebo-controlled trial investigating the effect of bempedoic acid on cardiovascular events in high-risk patients who are also statin intolerant, is due for completion in 2021.

Gemcabene (Carboxylase Acetyl-Coenzyme A Inhibitor)

The carboxylase acetyl-coenzyme A (Ac-CoA) inhibitor, gemcabene, is a dicarboxylic acid for which the lipid-lowering mechanism has not yet been fully elucidated, although it appears to be independent of effects on peroxisome proliferator-activated receptors (PPARs). Studies have shown it reduces the production of hepatic triglycerides and LDL-c as well as enhances the clearance of VLDL. Gemcabene also has a potentially anti-inflammatory effect, which may enhance the impact of its lipid-regulating properties on ASCVD [1, 12]. A phase II safety and efficacy trial demonstrated significant increases in HDL and both apoA-I and A-II levels. This was accompanied by significant reductions in both triglycerides and LDL-c, with proportionate decreases in apoB, although these appeared to be dependent on the dose of gemcabene administered and baseline triglyceride levels [103]. When used as add-on to stable statin therapy, gemcabene provided dose-dependent and significant reductions in both LDL-c (>20%) and CRP (>40%) compared to placebo [104].

COBALT-1 was a phase II open-label dose-finding study to assess safety, efficacy and tolerability of gemcabene in patients with homozygous FH on stable lipid therapy (NCT02722408). Although the study was completed in 2017, findings have yet to be published, with preliminary results presented at the 2017 FH Global Summit (http://ir.gemphire.com/phoenix.zhtml?c=254241&p=irol-newsArticle&ID=2302595). These reveal a significant LDL-c-lowering effect for FH patients, including reductions of 39% in heterozygous FH patients and significant reductions on top of PCSK9 inhibitors. The higher dose was also found to reduce hsCRP. ROYAL-1 investigated the safety, efficacy and tolerability of multiple doses of gemcabene 600 mg in patients with hypercholesterolaemia not adequately controlled on moderate- or high-intensity statins (NCT02634151). As with COBALT-1, preliminary results demonstrated gemcabene significantly reduced LDL-c and hsCRP.

The Study of Gemcabene in Adults with FPLD (NCT03508687) is investigating the safety and efficacy of two dosing regimens (300 mg/day for 24 weeks or 300 mg/day for 12 weeks followed by 600 mg/day for 12 weeks) in patients with familial partial lipodystrophy, with elevated triglycerides and non-alcoholic fatty liver disease. This study is due for completion in 2020. Both short- and long-term studies on the safety and potential toxicity of gemcabene are required before this drug is FDA approved for clinical use.

Apolipoprotein E Mimetics

Apolipoprotein E (apoE) is an alternative ligand for the LDL receptor and mediates the clearance of triglyceride-rich lipoproteins. In addition, it binds to other hepatic receptors, including LDL receptor-related protein, and to heparan sulphate proteoglycans, thereby effectuating the hepatic clearance of atherogenic lipoproteins. Ac-hE18A-NH_2 is a dual-domain apoE mimetic peptide that associates with LDL and other atherogenic lipoproteins and has been shown to enhance LDL uptake in cell culture [105, 106]. The mimetic has undergone safety and efficacy trials (NCT02100839) and been demonstrated to have significant lipid-lowering activity and beneficial effects on arterial wall cells that relate to its anti-inflammatory and antioxidant properties that may be independent of changes in plasma lipoproteins [107]. New analogues have also been designed and demonstrated to reduce plasma cholesterol in animal models, with enhanced potency at lower levels and thus warranting further investigation [106]. Phase I clinical trials are planned for early 2020.

Nutraceuticals

Nutraceuticals are natural plant and food-derived compounds that represent a non-pharmacological approach to lipid lowering. Over 40 nutraceuticals have been identified as having promising effects, either as individual or combination treatments [108]. Several small studies have shown the beneficial effects of a nutraceutical combination containing berberine, red yeast rice (RYR) and policosanol [109], a combination of RYR, polyunsaturated fatty acids (PUFAs) and phytosterols [110] as well as the combination of berberine, RYR and policosanols with ezetimibe in reducing LDL-c and triglycerides in hypercholesterolaemic patients [111]. Studies investigating the lipid-lowering effects of the patented Armolipid Plus have observed beneficial effects when combined with pravastatin [112] or in combination with dietary recommendations [113]. In patients with documented statin intolerance, Armolipid Plus was found to reduce LDL-c and total cholesterol when given alone or in combination with ezetimibe [114]. More recently, several reviews and meta-analyses have described the benefits of Armolipid Plus on lipid profiles in a range of patient populations [115–117].

Lipoprotein (A) Lowering

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Inhibitors

Secondary analysis of the FOURIER study demonstrated that the PCSK9 monoclonal antibody evolocumab significantly reduced Lp(a) levels in statin-treated patients with stable ASCVD, with the greatest reduction seen in those with the highest baseline levels. There was also a modest positive correlation in the percent change in Lp(a) and LDL-c in those treated with evolocumab. The highest clinical benefit of evolocumab was observed in patients who had the highest baseline levels of Lp(a). Although the relationship between Lp(a) and coronary risk remained similar throughout the range of LDL-c levels, the relative risk reduction was greatest in those who achieved reductions in both LDL-c and Lp(a) [53]. This was underscored somewhat by recent data, which reveals that patients with very high Lp(a) levels treated with evolocumab had only a 14% reduction in Lp(a) levels, which resulted in persistent Lp(a) elevation and no significant impact on arterial inflammation [118]. Inclisiran has also been demonstrated to lower Lp(a) levels although the inter-individual variation following treatment appeared large [85].

Apolipoprotein (a) Inhibitors

IONIS-APO(a)$_{Rx}$ binds to the exon 24–25 splice site of the mature apo(a) transcript, with additional potential to bind to 11 alternative sites within the transcript. A phase I study in healthy adults with Lp(a) ≥25 nmol/L investigated single dose (50 mg, 100 mg, 200 mg or 400 mg) or multi-dose (100 mg, 200 mg or 400 mg for total dose of 600 mg, 1200 mg or 1800 mg) of IONIS-APO(a)$_{Rx}$. While the single-dose injections did not alter Lp(a) levels, multi-dose injection resulted in significant dose-dependent decreases (39.6%, 59.0%, 77.8%) in plasma Lp(a) levels over 4 weeks. Similar reductions in the amount of oxidised phospholipids associated with apoB-100 and apo(a) were also observed. Mild injection site reactions were the most common adverse event [119].

A phase II trial to assess efficacy and safety was conducted in patients with elevated Lp(a) with escalating subcutaneous doses (100 mg, 200 mg, 300 mg) for 4 weeks followed by 300 mg a week up to 12 weeks. Significant reductions in Lp(a) were observed (66.8–71.6%), with additional reductions in LDL-c, apoB-100 and oxidised phospholipids also observed. Two serious adverse events (myocardial infarction) were reported, one in the IONIS-APO(a)$_{Rx}$ and one in the placebo group, while 12% of IONIS-APO(a)$_{Rx}$ injections reported injection site reactions [120].

In a phase I/IIa first-in-man trial, a newly developed ligand-conjugated apo(a) ASO, IONIS-APO(a)-L$_{Rx}$, was investigated in healthy volunteers with baseline Lp(a) levels ≥75 nmol/L, either as a single dose (10–120 mg) or multiple

doses (10 mg, 20 mg or 40 mg) in ascending dose design. Unlike its predecessor, IONIS-APO(a)-L_{Rx} is triantennary *N*-acetylgalactosamine ($GalNAc_3$) conjugated, which results in enhanced hepatic specificity and allows for greater potency with 20–30-fold lower dosing [81]. The study found IONIS-APO(a)-L_{Rx} was considerably more potent than its predecessor, with significant dose-dependent reductions observed for all single-dose groups. The multi-dose groups had significant mean reductions in Lp(a) of 66% with 10 mg, 80% with 20 mg and 92% with 40 mg. Improved tolerability was also observed with no injection site reactions or adverse effects reported [120]. On the basis of these findings, Akcea Therapeutics Inc. conducted a phase IIb trial in patients with established CVD and elevated Lp(a). In a late-breaking presentation at the 2018 American Heart Association Meeting, results presented demonstrated that 98% of patients in the 20 mg/week cohort and 81% in the 60 mg every 4 weeks cohort achieved clinically significant reductions in Lp(a), bringing them below the recommended risk threshold of <50 mg/dL. These reductions were also associated with decreases in LDL-c, apoB and oxidised phospholipids associated with apoB and apo(a). Injection site reactions occurred in 26% of patients, with 1 discontinuing treatment (http://ir.ionispharma.com/node/24326/pdf).

Thyroid Mimetics

Eprotirome is a thyroid hormone analogue that contains two bromides and has minimal uptake in non-hepatic tissues compared to triiodothyronine. A randomised controlled trial to assess the safety and efficacy of this drug in statin-treated patients with hypercholesterolaemia found that in addition to lowering LDL-c, significant reductions were also seen for Lp(a), as well as apoB and triglycerides [121]. In patients with FH, eprotirome was found to reduce LDL-c when added to conventional statin therapy, with or without ezetimibe; however, this was accompanied by significant elevations in liver function tests, raising doubts about its future use [122].

Nutraceuticals

Several natural compounds have been proposed to exert significant Lp(a)-lowering effects, including L-carnitine, coenzyme Q10, Xuezhikang, pectin, fibernat, *G. biloba*, flaxseed, resveratrol, curcumoids and chenodeoxycholic acid. Although small human intervention trials have demonstrated reductions ranging from 9% to 28.6%, further investigation in robust, long-term randomised controlled trials is needed [123].

Triglyceride-Rich Lipoprotein (TRL)-Lowering Therapies

Many of the therapies reviewed in the previous sections also have triglyceride-lowering effects, but their use is mainly targeted at reducing LDL-c, apoB-100 and Lp(a). The following section describes agents that specifically lower triglycerides not only for reduction in risk of pancreatitis and steatohepatitis but also for the reduction in residual risk of ASCVD related to lowering TRLs [124].

Apolipoprotein C-III Inhibitors

Apolipoprotein C-III is present on all lipoproteins, including LDL, HDL and Lp(a), where it is found in varying amounts. It functions as an inhibitor of lipoprotein lipase and is also able to inhibit clearance of all triglyceride-rich lipoproteins via the LDL and LRP1 receptors, which leads to elevated VLDL-c and triglycerides, as well as chylomicronaemia [81]. Volanesorsen is an ASO targeted to apoC-III that has been demonstrated to reduce both plasma apoC-III and triglyceride levels in pre-clinical models and a phase I clinical model in healthy volunteers [125]. A small study in patients with familial chylomicronaemia revealed significant reductions in plasma apoC-III (71–90%) and triglycerides (56–86%) following volanesorsen treatment [126]. A phase II study in patients with hypertriglyceridaemia revealed dose-dependent reductions in plasma triglyceride levels when

administered as a single therapeutic or when given in conjunction with fibrates [127]. More recently, APPROACH, a phase III trial, revealed significant reductions in both triglycerides and the incidence of abdominal pain, with no episodes of pancreatitis reported in the treatment group compared to placebo. The most common adverse events were injection site reactions, although five patients terminated the study early due to platelet count reductions, which warrants further investigation [128].

Angiopoietin-like 3 Inhibitors

Angiopoietin-like (ANGPTL) proteins are involved in the regulation of plasma lipid metabolism via their inhibition of lipoprotein lipase. Three members of this family, ANGPTL3, ANGPTL4 and ANGPTL8, are considered important for this process, with ANGPTL3 exclusively produced in the liver. Loss-of-function mutations in *ANGPTL3* lead to familial hypobetalipoproteinaemia-2, a disorder characterised by low plasma LDL-c, HDL-c and triglyceride concentrations. As such, ANGPTL3 shows promise as a target for lipid-lowering therapy [129]. This was confirmed in the DiscovEHR human genetic study where participants with heterozygous loss-of-function variants in *ANGPTL3* had significantly lower levels of serum triglycerides, HDL cholesterol and LDL-c. There were also fewer cases of patients with CAD who had these variants compared to controls [130].

ASOs targeting ANGPTL3 (IONIS-ANGPTL3-L_{Rx}) have demonstrated dose-dependent reductions in mouse hepatic Angptl3 mRNA and protein, as well as triglyceride and LDL-c levels. This was accompanied by reductions in hepatic triglyceride content and atherosclerosis progression and increased insulin sensitivity. Hypertriglyceridaemic humans were also investigated and reductions were also seen for ANGPTL3 protein, triglycerides, LDL-c, VLDL-c, non-HDL cholesterol, apoB and apoC-III [131]. A phase II study (NCT03371355) investigating the efficacy of different doses and dosing regimens of IONIS-ANGPTL3-L_{Rx} in type 2 diabetic patients with fatty liver disease and elevated triglycerides is due for completion in mid-2019.

Evinacumab is a monoclonal antibody targeting ANGPTL3, which acts mainly in the circulation to bind to ANGPTL3 and potentiate plasma lipase activity, in contrast to the ASOs, which reduce ANGPTL3 production in the liver [1]. A phase I safety and efficacy study using single ascending doses in healthy volunteers revealed significant reductions in triglycerides (~75%) and LDL-c (23%) [130]. A follow-up phase II open-label study in homozygous FH patients revealed significant reductions in triglycerides (47%), LDL-c (49%) and HDL-c (36%) [132].

Polyunsaturated Fatty Acids (PUFAs)

AMR101, an ω-3 fatty acid that contains ≥96% pure icosapent ethyl, the ethyl ester of eicosapentaenoic acid (EPA), was investigated in a phase III randomised controlled trial (ANCHOR) in high-risk statin-treated patients with residually elevated triglycerides but controlled LDL-c levels. After 12 weeks of treatment, 4 g/day significantly decreased triglycerides, non-HDL-c, LDL-c, apoB, total cholesterol, VLDL-c, lipoprotein-associated phospholipase A(2) and hsCRP. Decreases in triglycerides and non-HDL-c were greatest in patients with higher efficacy statin treatments and those with highest baseline triglyceride levels [133]. An earlier study demonstrated similar results in diet-stable hypertriglyceridaemic patients with or without background statin therapy [134].

Pemafibrate

Pemafibrate is a selective PPARα modulator which has been shown to enhance reverse cholesterol transport and lower serum triglycerides in addition to other atherogenic lipids [135, 136]. A phase II trial investigating its safety and efficacy revealed significant improvements in triglycerides, HDL-c and other lipid parameters compared to placebo or fenofibrate. There were no increases

in adverse events [137]. More recent investigation into patients with type 2 diabetes and hypertriglyceridaemia demonstrated significant reductions in triglycerides (~45%) compared to placebo, with accompanying decreases in non-HDL-c, remnant lipoprotein cholesterol, apoB-100, apoB48 and apoC-III levels. There were significant increases in HDL-c and apoA-I levels and no changes in fasting glucose, insulin or HbA1c, despite reduced HOMA-insulin resistance scores [138]. The PROMINENT study (NCT03071692) investigating whether pemafibrate will delay the time to first occurrence of composite CVD endpoints in patients with type 2 diabetes and dyslipidaemia is due for completion in 2022.

High-Density Lipoprotein Cholesterol (HDL-C) Therapies

Cholesteryl ester Transfer Protein (CETP) Inhibitors

The cholesteryl ester transfer protein (CETP) role is to exchange cholesteryl ester for triglycerides between HDL and triglyceride-rich lipoproteins, resulting in a reduction in HDL-c. Inhibition of this protein therefore results in an increase in HDL-c and a reduction in both triglycerides and LDL-c [12]. Early genetic studies of CETP deficiency resulting from rare *CETP* loss-of-function mutations showed patients had elevated HDL-c levels and seeming protection from ASCVD [139]. This has been contradicted somewhat by Mendelian randomisation studies, which have demonstrated significantly decreased risk, marginal increased risk or no change in risk of ASCVD, despite increased HDL-c levels [1].

Several studies using small molecule inhibitors of CETP have looked at the effect of increasing HDL-c and subsequent CVD risk with mixed results. The ILLUMINATE study investigated torcetrapib in combination with atorvastatin versus atorvastatin alone in patients at high cardiovascular risk. Despite significant increases in HDL-c (72.1%) and decreases in LDL-c (24.9%), there were significant increases in systolic blood pressure, risk of cardiovascular events and death from any cause, which the authors suggest may be in part due to off-target effects [140]. In patients with a recent acute coronary syndrome, dalcetrapib on top of standard care was compared with standard care. Despite significant increases in HDL-c (31–40%), there was no significant reduction in risk of recurrent cardiovascular events [141]. The ACCELERATE trial, which investigated evacetrapib in patients with high-risk vascular disease in addition to standard medical therapy, saw that despite increases in LDL-c (31.1%) and increases in HDL-c (133.2%), there was no effect on the rate of cardiovascular events [142]. In contrast, a study in patients with ASCVD who were receiving intensive atorvastatin therapy, anacetrapib significantly increased HDL-c (104%) and resulted in a significantly lower incidence of major coronary events [143].

Recombinant HDL-c

Apolipoprotein Al (apoA-I) is a major protein component of HDL particles in plasma, promoting cholesterol efflux through interaction with ABCA1 [12]. ETC-216 contains apoA-I Milano, derived from 40 inhabitants of a village in Italy who carry a genetic variant of apoA-I that presents as very low HDL-c, yet appear to be at low risk of ASCVD. In a phase II study, ETC-216 was infused into patients with acute coronary syndrome and demonstrated that repeated infusion induced plaque regression measured by intravascular ultrasonography [144]. An improved formulation, MDCO-216, was investigated to determine its effect on plaque burden in statin-treated patients with an acute coronary syndrome. At day 36, there were no effects on LDL-c levels, significant reductions in HDL-c levels (−6.3 mg/dL) and no effect on incremental plaque regression [145].

CER-001 is a lipoprotein complex that consists of phospholipid and recombinant human apoA-I that mimics the structure and function of HDL but is not an exact copy due to its negative charge [12]. The CHI-SQUARE study in patients with a recent acute coronary syndrome found no

reductions in coronary atherosclerosis, as measured by intravascular ultrasonography or quantitative coronary angiography following serial CER-001 infusions [146]. The MODE study in patients with homozygous FH demonstrated that 12 biweekly infusions of CER-001 resulted in significant reductions in carotid mean vessel wall area, suggestive of a reversal of atherogenic changes to the vessel wall [147]. However, the TANGO study (NCT02697136) investigating CER-001 infusions in patients with familial primary hypoalphalipoproteinaemia and proven CVD on appropriate lipid-lowering therapy was terminated early due to a lack of efficacy. The CARAT study investigating the effect of weekly infusions of CER-001 in patients with acute coronary syndrome on statin treatment demonstrated no changes in LDL-c, HDL-c or percent atheroma volume at day 78 [148].

The safety and tolerability of CSL112, an infusible, plasma-derived apoA-I [149], were investigated in patients with a recent acute myocardial infarction in the phase IIb AEGIS-I trial. Four weekly infusions were feasible, well-tolerated and not associated with any abnormal biochemistry or safety concerns. In addition, CSL112 was confirmed to acutely enhance cholesterol efflux [150]. The phase III AEGIS-II study (NCT03473223) will investigate the safety and efficacy of CSL112 on reducing the risk of major adverse cardiovascular events in patients with acute coronary syndrome and is due for completion in 2022.

Probucol

Probucol is an antioxidant with additional pleiotropic effects that include anti-inflammatory properties as well as promotion of cholesterol efflux and enhancing reverse cholesterol transport by activation of CETP, with subsequent beneficial effects on HDL-c levels [151]. Studies have shown that it is able to decrease plasma ANGPTL3 and HDL phospholipids, while increasing prebeta-1 HDL, suggesting that it induces HDL remodelling via an endothelial lipase-mediated pathway [152]. Despite this, in 1995 it was withdrawn from use in the USA after studies showed reductions in HDL-c and possible detrimental effects on the heart leading to ventricular arrhythmias. Use of probucol, however, remains debatable, with some studies suggesting the arterial benefits seen from CETP activation may be important, despite the negative effects on HDL-c and the disappointing cardiovascular protection [153]. The PROSPECTIVE study will investigate the use of probucol for secondary prevention in patients with prior CHD [154].

Effects on Atherosclerosis

Despite the widespread development of new lipid-lowering therapies, many of these treatments are used in specific patient populations, with cost limiting their widespread use. Ultimately, the clinical use of any new lipid-lowering therapy should be supported by clinical outcome trials, as reviewed in the following section, with the exception of rare conditions and when it is unethical to do so (e.g. homozygous FH patients).

PCSK9 Inhibitors

To date, three major outcome studies have been conducted to investigate the effect of PCSK9 monoclonal antibodies on ASCVD outcomes [47–49]. SPIRE-2 investigated the effect of bococizumab in high-risk CVD patients. Although terminated early, the study observed a significant reduction in the primary endpoint (cardiovascular death, myocardial infarction, stroke, urgent revascularisation) at 1 year in high-risk patients treated with bococizumab, despite no beneficial effect on major adverse cardiovascular events in lower-risk patients [49].

The FOURIER trial investigated the effect of evolocumab in patients with stable ASCVD. At 2.2 years of follow-up, there was a significant reduction in the primary endpoint (composite of cardiovascular death, myocardial infarction, stroke, hospitalisation for unstable angina or coronary revascularisation) and the secondary

endpoint (composite of cardiovascular death, myocardial infarction or stroke). Although there were no significant differences in relative risk reduction when patients were stratified for baseline LDL-c levels [47], pre-specified secondary analysis revealed that patients who achieved lower LDL-c levels had progressively fewer cardiovascular events with no evidence for a plateau and no increase in adverse events [155]. Further analysis revealed a significant reduction in the risk of cardiovascular events in patients with peripheral arterial disease and a reduced risk of major adverse limb events that was consistently associated with lower LDL-c levels [156]. Reductions in risk were also seen in patients with a recent MI, with multiple prior MIs or with residual multivessel coronary artery disease [157].

ODYSSEY OUTCOMES investigated the effect of alirocumab in patients with a recent acute coronary syndrome. At 2.8 years of follow-up, there was a reduction in the primary composite endpoint (death from CHD, nonfatal myocardial infarction, fatal and nonfatal ischaemic stroke and unstable angina requiring hospitalisation) [48]. The risk reduction was of a similar magnitude to that seen in FOURIER. Interestingly, in ODYSSEY, the risk reduction was greatest in patients with higher baseline LDL-c levels (>2.6 mmol/L or 100 mg/dL), which is consistent with the findings in SPIRE-2. Further analysis revealed significant reductions in several secondary endpoints, including a CHD event, a major CHD event, a cardiovascular event and a composite of death from any cause, nonfatal myocardial infarction or nonfatal ischaemic stroke [48]. Additional pre-specified analysis revealed a reduction in total nonfatal cardiovascular events and death, with alirocumab preventing twice the total number of nonfatal cardiovascular events and deaths than the number of first events [158].

Additional ASCVD outcomes have been investigated in smaller studies, including the GLAGOV study [159], which demonstrated a significant decrease in nominal change in percent atheroma and total atheroma volume and more plaque regression with evolocumab treatment. Although underpowered for this endpoint, there were also fewer adverse cardiovascular outcomes, nonfatal myocardial infarctions and coronary revascularisation procedures in the evolocumab-treated patients [159, 160]. The OSLER trials included patients who had completed evolocumab phase II and III trials and were designed to gather long-term safety data and pre-specified exploratory analysis on cardiovascular outcomes. In addition to reductions in LDL-c, the rate of cardiovascular events at 1 year was reduced in the evolocumab group [161].

Limiting the widespread use of PCSK9 monoclonal antibodies is their high cost. Economic models have generally been based on hypothetical patient populations utilising inclusion criteria and baseline characteristics of randomised controlled trials. Carried out by both industry and academia, they have produced varied and confusing results [162]. To date, only the FOURIER trial has presented economic analysis, which found that a statin plus PCSK9 monoclonal antibody had a low probability (<1%) of being cost-effective at the generally accepted $100,000 per quality-adjusted life-year societal threshold. In addition, PCSK9 monoclonal antibodies produced a negative return on investment for 86% of private payers, with threshold analysis suggesting that the price of the drug would need to drop by 62% to meet conventional cost-effectiveness standards [163].

Polyunsaturated Fatty Acids (PUFAs)

The recently completed REDUCE-IT study has demonstrated a beneficial effect of 4 g/day AMR101 in patients with established CVD or diabetes plus other risk factors, who were on statin therapy and had residually elevated triglycerides. In patients receiving icosapent ethyl, there was a significant reduction in the primary endpoint composite of CVD death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularisation or unstable angina. Similar reductions were observed for the secondary endpoint composite of CVD death, nonfatal myocardial infarction and nonfatal stroke, as well as

additional ischaemic endpoints. A higher proportion of icosapent ethyl patients had serious bleeding events or were hospitalised for atrial fibrillation [164]. Pre-specified analysis also revealed that icosapent ethyl substantially reduces the burden of first, subsequent and total ischaemic events, reducing the total primary endpoint events, each component of the primary composite endpoint and total secondary endpoint events [165]. Cardiovascular benefit is clearly related to a panoply of effects, including triglyceride lowering, anti-inflammatory, antithrombotic and improved endothelial function that requires further studies to unbundle [124].

The STRENGTH trial (NCT02104817) is investigating the combination of Epanova (high-dose omega-3 carboxylic acids) and statin versus statin and corn oil on a composite of CVD endpoints in patients with hypertriglyceridaemia, low HDL-c and high CVD risk. The study is due to be completed in late 2019.

Nutraceuticals

Clinical studies in various populations, ranging from healthy people to high-risk patients, have reported reductions in total cholesterol (16–31%), LDL-c (22–32%) and triglycerides (0–36%) at doses of 0.2–3.6 g/day of RYR with additional benefits on all-cause and cardiovascular mortality [166]. In perhaps the only long-term study looking at the effect of RYR on cardiovascular endpoints, it was observed that Xuezhikang (a partially purified extract of RYR) significantly decreased the recurrence of coronary events and the occurrence of new events in a Chinese population who had previously had a myocardial infarction. In addition, the treatment was well-tolerated, safe and improved lipid profile (total cholesterol reduced by 10.9% and LDL-c reduced by 17.6%) [167].

Additional Therapies

Reduction in inflammation is also a key component to reducing ASCVD. Although this is discussed in detail in Chap. 32, it is worth noting several important therapies that have demonstrated significant clinical impact on cardiovascular events and mortality. The CANTOS trial investigated the use of canakinumab, a therapeutic monoclonal antibody targeting interleukin-1β (IL-1β) in patients with a previous myocardial infarction and elevated hsCRP levels. Treatment with canakinumab at 150 mg every 3 months resulted in significantly lower rates of recurrent cardiovascular events compared to placebo. Interestingly, despite statin treatment, the incidence rate of both primary and secondary cardiovascular endpoints was high, confirming the importance of residual inflammatory risk. Furthermore, the benefits of canakinumab, which also reduced hsCRP and were greatest in the patients who had the largest reductions in both hsCRP and IL-6, were independent of lipid lowering [168]. The CIRT trial investigated the effect of low-dose methotrexate in patients with a previous myocardial infarction or multivessel CAD, who additionally had either type 2 diabetes mellitus or the metabolic syndrome. Despite its wide use as a treatment for a variety of inflammatory conditions, low-dose methotrexate did not reduce levels of IL-1β, IL-6 or hsCRP and did not result in fewer cardiovascular events compared to placebo [169]. When considering the CANTOS and CIRT findings, it is important to note the level of inflammation already present prior to treatment and the inflammatory pathway that is targeted by the treatment.

Additional anti-inflammatory treatments, including colchicine and more specific NLRP3 inhibitors, are currently undergoing investigation and may prove more beneficial [170, 171]. The LoDoCo2 (ACTRN12614000093684), COLCOT (NCT02551094) and CLEAR-Synergy (NCT03048825) trials are all exploring the beneficial effects of colchicine in patients with CAD. Lastly, the role of apabetalone, an inhibitor of bromodomain and extra-terminal proteins, which modulate lipoprotein and inflammatory factors, was evaluated in a pooled analysis of patients with CAD. In addition to increasing apoA-I, and HDL-c, apabetalone also reduced hsCRP. Although there was no effect on atherogenic lipoproteins, patients treated with apabet-

alone had fewer major adverse cardiovascular events, which was even more significant in patients with diabetes, low baseline HDL-c or high baseline hsCRP levels [172].

Conclusions and Perspectives

The discovery of the new and evolving lipid drugs reviewed in this chapter has primarily resulted from genetic studies in human populations, the success of this approach being particularly exemplified by the development and use of PCSK9 inhibitors. Many of the biologics reviewed are expensive and will have a narrow spectrum of clinical indications. Those with sustained pharmacodynamic effects (siRNA-based therapies for defined targets) are likely to be less expensive and could have wider applications. Of the oral agents, bempedoic acid and pemafibrate have the greatest promise and if the clinical outcome trials are favourable will have broad market indications. Gemcabene has a theoretically very diverse and unique mechanism of action, but more efficacy and toxicity studies are required in humans to establish its value.

Developing clear indications for the use of new pharmacotherapies is paramount for registration and reimbursement. Clear demonstration of metabolic and clinical efficacy underpinned by good long-term safety data and favourable health economic evaluations are essential for the success of drug entry into the market. There are multiple agents available to target residual risk of ASCVD. The use and application of biomarkers that address not only exotic dyslipidaemias but also inflammation, metabolic syndrome, platelet aggregation and coagulation will make therapeutic choices more justified and potentially more cost-effective in the era of precision medicine. The use of HDL as a therapeutic target will require a re-evaluation of its functional role in atherothrombosis and verification that such HDL properties (e.g. apoA-I transport) are causally related to the development of ASCVD. While clinical outcome trials will strictly still be required to verify the value treating a particular pathway of residual ASCVD risk, the same will not apply to agents with orphan or restricted indications, for which trials based on surrogate endpoints of lipid metabolism will suffice.

The broad indications of the drugs reviewed are for patients with severe dyslipidaemias, such as FH, elevated Lp(a) and chylomicronaemia, that increase their risk of ASCVD and acute pancreatitis and remain refractory or intolerant to standard therapies. Statin intolerance is a particular indication for new agents that selectively lower LDL cholesterol. Irrespective of intolerance, non-adherence to statins is a continuing problem in clinical practice that will limit the efficacy of new LDL lowering, such as PCSK9 inhibitors. This means that the success of such agents relies on a multidisciplinary team approach that pragmatically addresses adherence to standard lifestyle and drug therapies. This involves the collaboration and integrated efforts of cardiologists, lipidologists, family doctors, nurse practitioners, specialist nurses, dietitians and pharmacists, with the patient at the centre of all shared decisions. Ensuring global equity and wide availability of all forms of new lipid drugs with proven benefit on the clinical outcomes discussed above remains a central aim of the international community of scientists, clinicians, industry and politicians that champion the prevention and reversal of ASCVD.

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34 The Allied Health Professional's Role in the Management of Dyslipidemia and Accreditation Council for Clinical Lipidology Certification Program

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Introduction

Allied health professionals play an integral role in healthcare today. This chapter focuses on several critical aspects of this concept within the framework of diagnosis and management of dyslipidemia. In a team-based collaborative approach, it is important to identify who should be represented in the team with specific individuals' roles, how collaboration between the team improves patient outcomes and satisfaction, and strategies to develop an effective collaborative team.

Statement of the Problem

The role of dyslipidemia in development and progression of cardiovascular disease has been well documented in clinical literature derived from both large epidemiologic and observational trials as well as peer-reviewed clinical trials. Unfortunately, coronary artery disease (CAD) remains the number one killer of patients in the United States (US) and around the world. Per the 2018 American Heart Association Statistics, approximately 2300 Americans die of cardiovascular disease (CVD) each day, for an average of 1 death every 38 seconds [1]. CVD further imposed a costly burden for America. In 2016, CVD cost was $555 billion, and in 2035, CVD is predicted to cost $1.1 trillion [2].

Despite this, much that can be done to prevent heart disease has not been fully utilized. American Heart Association data from the National Cardiovascular Data Registry Proactive Innovation and Clinical Excellence [3] observed that 43.9% of cholesterol treatment-eligible primary prevention patients were receiving a statin medication and up to 35.9% were not receiving any lipid-lowering therapy [3]. Risk factors for CVD are common among adults per the American Heart Association Statistics Committee and Stroke Statistics Subcommittee, which reports that 47% of US adults have at least one of the three key independent risk factors for CVD [4]. Smoking appears to have a multiplicative effect in the presence of other major risk factors for CHD such as diabetes and hyperlipidemia. Long-term exposure to elevated cholesterol levels can lead to CHD later in life, and one study of >1 million adults with hypertension determined the lifetime risk of CVD at age 30 years was 63.3% compared with 46.1% for those with normal blood pressure [4]. Many individuals with high blood pressure and dyslipidemia also have type 2 diabetes mellitus, which is known to be a major risk factor. Several of these patients will further present with >3 components of metabolic syndrome and contribute to approximately 80%

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_34

death from CVD from diabetes [5]. While not modifiable, increased age, male sex, and a positive history of premature CAD further increase the patient's risk for CVD [4]. Patients with these multiple comorbidities will require comprehensive care that is best managed utilizing a team-based concept.

Evolution of Guidelines for the Management of Dyslipidemia

A long history of guidelines exists for cholesterol management; however, to date these have not been successful in considerably moving the needle for cardiovascular disease or dyslipidemic management. Early guidelines were focused on the science associated with CVD and dyslipidemia, while the most recent ACC/AHA guideline allows for a more personalized patient-centered concept with specific recommendations for clinicians to discuss options with patients and use of a comprehensive healthcare team fostering utilization of the strength of multiple specialists to accomplish goals.

Guidelines were first presented by the NIH-sponsored National Cholesterol Education Program (NCEP) in 1988. The First Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel (ATP)) was followed by NCEP II and III. Each edition of the guidelines was focused toward specific goals. ATP I outlined a strategy for primary prevention of coronary heart disease (CHD) in persons with high levels of low-density lipoprotein (LDL) cholesterol (≥160 mg/dL) or those with borderline-high LDL cholesterol (130–159 mg/dL) and multiple (2+) risk factors. ATP II affirmed the importance of this approach and added a new feature: the intensive management of LDL cholesterol in persons with established CHD. For CHD patients, ATP II set a new, lower LDL cholesterol goal of ≤100 mg/dL. ATP III moved forward with a call for more intensive LDL-lowering therapy in certain groups of people, in accord with recent clinical trial evidence, but the core recommendations were based on ATP I and ATP II. ATP III did highlight intensive treatment of patients with CHD; however, its major new feature was a focus on primary prevention in persons with multiple risk factors. Many of these patients have a relatively high risk for CHD and will benefit from more intensive LDL-lowering treatment than recommended in ATP II [6].

In 2013, the American College of Cardiology (ACC) and American Heart Association (AHA) Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Disease in Adults was presented. This document was notably different than existing guidelines in that lipid targets and goals were eliminated and adults aged 40–75 who fit into four "major statin benefit groups" were recommended to be started on either high- or moderate-dose statin depending on their risk. A new risk calculator was introduced, "The Pooled Cohort Equations." which is used for enhanced assessment in primary prevention. Only randomized clinical trials were used to formulate these guidelines and only statins were considered for treatment. These guidelines were met with both debate and criticism. Guidelines in Europe, Canada, and by the National Lipid Association (based on both randomized clinical trials as well as epidemiologic and other trials) continued to have lipid targets and goals and utilize both statins and non-statins.

The 2013 [7, 8] ACC/AHA guidelines recommended a multifaceted lifestyle approach; however, no specific reference regarding the notion of a comprehensive multidisciplinary-based team was indicated. Mention is eluded to within the lifestyle section of the guideline referring to nurse case management and nutrition counseling, along with advanced practice nurses, exercise specialist, and clinical psychologist, but does not go as far as to comment on or suggest the necessity or value of development a total comprehensive team [9]. At the time of implementation of these guidelines, most existing teams, while not necessarily as comprehensive as the totality of what a team would be today, were found in specialty practices such as a lipid clinic. While previous NCNP guidelines were under the auspices

of the National Institutes of Health (NIH), the work of the 2013 writing group was transferred over to the ACC/AHA to be completed. Under the ACC/AHA restrictions were imposed dictating the literature that could be reviewed, cost of the project, and how many questions would be considered, thereby driving outcomes [6].

In the interim since the 2013 ACC/AHA guidelines, several other organizations published statement and recommendations for diagnosis and lipid management. In 2015, the NLA issued their Recommendations for Patient-Centered Management of Dyslipidemia: Part 1 [10]. Of note, the NLA continued with lipid targets and goals as well as use of non-statin medications. In 2015, Part 2 of the recommendations by the NLA was published utilizing a multidisciplinary panel of writers and was focused on five specific areas related to prevention and/or treatment of ASCVD [11] (Table 34.1).

In 2016 and 2017, the ACC responded to the changing environment related to new research and approval of a new class of medication with updates to augment their 2013 ACC/AHA guidelines, "Focused updates of the ACC expert consensus decision pathways on the role of non-statin therapies for LDL-cholesterol lowering in the management of atherosclerotic cardiovascular disease risk" introduced and serve to provide an interim pathway for use pending the completion and release of the 2018 Formal Guidelines [12, 13].

Table 34.1 Specific areas related to prevention and/or treatment of ASCVD

Sections	Related specialties and author discipline
Lifestyle therapies	Nutrition Exercise
The lifespan – children to seniors	Children and adolescents Women's health Older patients
Ethnic and racial groups	Hispanics/Latinos African Americans South Asians American Indians/Alaska Natives
High-risk conditions and residual risk	HIV-infected patients Patients with rheumatoid arthritis Patients with residual risk despite statin and lifestyle management
Improving patient outcomes	Patient adherence Team-based collaborative care

Adapted from Jacobson et al. [11]. Used with permission from NLA National Lipid Association

In 2017, the American Association of Clinical Endocrinologists and the American College of Endocrinology issued their Guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease [14]. In contrast to other cardiovascular or lipid associations, they issued their guidelines and advanced goals for LDL with <55 mg/dL recommended for those at highest risk.

By 2018, there was a call and need for the ACC/AHA to publish updated guidelines related to changes in technology, research, and the need for a more inclusive patient-centered philosophy utilizing a multidiscipline team of clinicians. In 2018, the ACC, AHA, NLA, and many other organizations came together to issue the "Guideline on the Management of Blood Cholesterol" that synthesized recommendations of all groups. This systematic review provided evidence for non-statin lipid-modifying therapies to traditional statin therapy to reduce ASCVD. Evidence was found benefiting use of ezetimibe and PCSK9 inhibitors but did not include niacin or cholesterol-ester transfer protein inhibitors [7, 15].

Additionally, differences in the development of the 2018 guidelines are noted with regard to the writing committee member composition. This diverse panel, charged with development of the document, included members of various medical professional backgrounds and within the implementations section strongly reinforced, for the first time, the role of a comprehensive multidisciplinary team-based concept. Newest concepts illustrate that primary and secondary prevention is different among age groups and populations which require expertise of specialist from the fields of primary care, pediatrics, geriatrics, and cardiology as well as lipid specialists [7]. Tools for identification and assessment of risk are illustrated, using the newly introduced risk-enhancing factors, which provide additional factors to determine individualized risk

and treatment options and enhanced assessment. Use of risk calculators was reinforced, and when appropriate coronary artery calcium assessment (CAC) recommended as a tiebreaker when decisions are unclear for optimal assessment in primary care. Familial hypercholesterolemia is called out, reinforcing the underdiagnosis and undertreatment of this common genetic lipid disorder, with specific guidelines for children and adolescents [7, 16]. More specifically, application of newest guidelines into practice includes the need for a paradigm change to include education of all clinicians, staff, and patients, to consider health literacy, and consideration for the implementation of a comprehensive healthcare team, highlighting the use of allied healthcare professionals. Successful implementation of these incentives will require time from already busy clinicians; however, use of a team-based concept with specific roles for the allied health professional team will facilitate the process.

Defining the Comprehensive Team

Allied healthcare providers represent a distinct and diverse group of professionals who in tandem with physicians are able to provide a comprehensive and seamless partnership of care with the patient. This group of professionals applies their rich diversity of talent, capabilities, and expertise to prevent disease transmission, diagnose, treat, and rehabilitate people of all ages and conditions [17]. Working together as a team, all members within specific areas of expertise, direct patient care, rehabilitation, treatment, diagnostics, and health improvement interventions to restore and maintain optimal physical, sensory, psychological, cognitive, and social functions.

In 2012, the Institute of Medicine proposed a definition of team-based healthcare adapted from Naylor et al. [18] as the provision of health services to individuals, families, and/or their communities by at least two health providers who work collaboratively with patients and their caregivers, to the extent preferred by each patient, to accomplish shared goals within and across settings to achieve coordinated, high-quality care.

The National Lipid Association (NLA) fully endorses the concept of need for an allied professional team and is described in their Part 2 Recommendations for Patient-Centered Management of Dyslipidemia [11]. The NLA interpretation indicates that team-based collaborative care has emerged related to the complexity of today's modern healthcare and the need to comply with current national guidelines. They indicate that providers are not only responsible for a greater volume of patients but also have accountability for quality indicators based on multiple evidence-based clinical practice guidelines [11]. Providers today must be equipped to integrate new techniques to assist patients to not only prevent and manage health issues but must communicate with various other practitioners involved in patient care. An effective allied healthcare team is widely recognized as necessary for this type of coordinated health delivery system. Central to the success of a team-based care team is their ability to work together to make best use of the expertise within the group.

In 2011, the Community Guide Branch of the CDC published a systematic review of the effectiveness of team-based care in improving blood pressure outcomes [19]. Data and results from this review and model suggest a reasonable application to team-based care in the setting of lipid management.

For many years, the medical field was dominated by a treatment approach that emphasized providers working in *silos* [20]. This approach has frequently resulted in fragmented outcomes, medical errors, and less than ideal patient care. A movement away from the *silos* approach is occurring with the identification and adaption of team-based care. Taking a team or a holistic view of a patient means that medical issues are addressed as they intersect with social, emotional, and legal needs. Team-based care is particularly helpful in providing care to an aging population and within an increasingly complicated medical system. A single provider may struggle to fully help a patient given the complexity of the healthcare system. The interdisciplinary team approach while improving quality of care also enhances the perceptions of care among patients.

Approaches to care in lipid management are a prime example where the implementation of a team concept is not only useful but pivotal in assisting patients to achieve appropriate thresholds of care. The 2015 ACC Health Policy Statement in the Role of Advanced Practice Providers [17] took a first step in the journey toward articulating the ACC's vision of optimal use of team care, outlining the training, qualifications, and role of some core team members that comprise current health teams [17]. Each discipline within the healthcare team requires specific educational requirements, testing for licensure, residency, fellowships, maintenance of licensure, as well as maintenance of certification (MOC) [17] (Table 34.2).

A retrospective study conducted by a of group of advanced practice providers (APPs) in a preventive cardiology clinic (PCC) sought to analyze the effectiveness of risk stratification, initiation of recommended medical therapies, and resultant changes in global ASCVD risk by APPs with indirect oversight by a cardiologist utilizing locally developed treatment algorithms based on published guidelines. They initially hypothesized that APP interventions would reasonably fill in gaps created by physician shortages and improve adherence/compliance with preventive ASCVD interventions of patients enrolled in the APP PCC [21]. Patients were stratified using the Framingham risk score (FRS) and coronary artery calcium scoring (CACS). Baseline demographics were balanced between study groups of 595 patients in each group. Study results indicated that in primary care patients, use of CACS resulted in reclassification of 30.6% of patients to a higher risk category, including statin therapy in 26.6% of low-FRS PCC patients with CACS ≥75th MESA percentile. Aspirin initiation was higher for high- and intermediate-FRS patients in the PCC ($P < 0.001$). Post-intervention means LDL-C, non-HDL-C, and triglycerides (all $P < 0.05$) were lower in the PCC group. Compliance with appropriate lipid treatment was higher in intermediate- to high-FRS patients ($P = 0.004$) in the PCC group. Aggressive LDL-C and non-HDL-C treatment goals (<70 mg/dL, $P = 0.005$, and <130 mg/dL, $P < 0.001$, respectively) were more commonly achieved in high-FRS PCC patients. Median post-intervention SBP was lower among intermediate- and low-FRS patients ($P = 0.001$ and $P < 0.001$, respectively). Cumulatively, this resulted in a reduction in median post-intervention PCC FRS across all initial FRS risk categories ($P < 0.001$ for all). Researchers concluded based on study findings that APPs within a PCC effectively risk-stratify and aggressively manage ASCVD risk factors, resulting in a reduction in post-intervention FRS [21].

Disciplines Represented in the Allied Healthcare Team

Attributes of a team-based unit are centered around five personal values that are frequently present among members of a well-functioning team [17] and include honesty, discipline, creativity, humility, and curiosity. These values are particularly relevant in lipid management related to care which sometimes can be perplexing and stressful for the team as well as the patient and often requiring care throughout the patient's lifespan. Team members need to be disciplined with regard to their roles and responsibilities and understand when other team members with more knowledge or expertise in a given situation [9, 17] need to be consulted. Several disciplines may be included in healthcare teams for optimal lipid management. Members of the comprehensive team will include physicians and/or other allied health professionals, but the patient is at the core of the team. The patient and patient's family should be included in all discussions about treatment and have the final say on the overarching goals of care. These goals should be clearly articulated and understood by all members of the care team. Brush suggests a useful motto for team-based care is "shared goals and clear roles" [17]. This motto when implemented will create greater stability in all team members. Shared goals should be monitored by measurement with feedback of the team's processes and outcomes, providing a clear mechanism for correcting any deficiencies [22]. Each team member should have a clear

Table 34.2 Education, licensing, credentialing, and advanced training requirements of cardiovascular team members [17]

Requirement	Cardiologist	APRN	PA	PharmD	RN
Education	4 years for an MD or DO degree (after obtaining undergraduate degree) in a school accredited by the LCME of the AAMC and AMA or the AOA	2 years for a MSN (after obtaining an RN/BS or BSN degree) and 2–3 additional years for those who choose to obtain a DNP or 2–4 additional years for a PhD (completed within 7 years) in a school accredited by the AACN	2–3 years for a PA master's degree in a school accredited by the ARC-PA (after obtaining undergraduate degree) and 1–3 additional years for those who choose to obtain a DHSc or DScPA degree	4 years for a PharmD (after 2–4 years of undergraduate study) in a school accredited by the ACPE	To 4 years for a diploma in nursing, associate's degree, or baccalaureate degree in nursing in a school accredited by the CCNE or ACEN
Testing for licensure	USMLE examination for physicians with an MD degree, or the COMLEX-USA examination for physicians with a DO degree, parts 1–3	Examination administered by the ANCC, AANP, and AACN	PANCE administered by the NCCPA	NAPLEX and state-specific examinations	NCLEX
Licensure	State board of medicine	State board of nursing (in some states, both the board of nursing and board of medicine)	State board of medicine (a few states have a separate physician assistant board)	State board of pharmacy	State board of nursing
Added certifications	Not applicable to physicians, who have official board certifications NLA – ABCL	E.g., heart failure, lipid management, anticoagulation (ANCC, AACN, or other certifying organizations) NLA – ACCL	E.g., cardiovascular and thoracic surgery, emergency medicine, hospital medicine (NCCPA), lipid management NLA – ACCL	E.g., lipid management, anticoagulation, BCPS AQ-Cardiology NLA – ACCL	E.g., heart failure, lipid management, anticoagulation ANCC NLA ACCL
Residency	3 years in a program accredited by the ACGME or AOA; certified by ABIM, AOBIM, or ABP	Not available	Limited availability	1–2 years optional (ASHP, ACCP)	Not available
Maintenance of licensure	Every 2 years	Every 2 years	Every 1–2 years	Every 1–2 years	Every 2 years
Maintenance of certification	ABIM secure examination every 10 years with record of MOC activity every 2 years and demonstration of 100 points of MOC activity every 5 years, or OCC for DO physicians	Every 5 years; requires documentation of practice hours, of CE (100 hours), and of scholarly activities; retesting if certification has lapsed	PANRE examination every 10 years and documentation of at least 100 hours of CME every 2 years (administered by the NCCPA)	BCPS (AQ- Cardiology) portfolio review every 7 years	Every 5 years

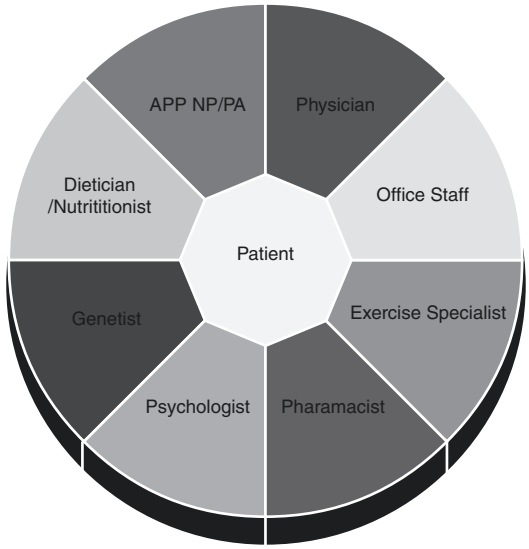

Fig. 34.1 Members of the allied healthcare team

understanding of his or her functions, responsibilities, and what to expect from him or her. The team may be inclusive of office clerical staff, the patient's primary healthcare provider, nurses, nurse practitioners (NPs), pharmacists, physician assistants (PAs), Registered Dietitian Nutritionists (RDNs), exercise specialists, social workers, community health workers, as well as licensed professional counselors, psychologists, and health educators. The patient should be recognized as an active partner in the team. It is important to note that the primary care provider may be a physician, nurse practitioner, or physician assistant, while other team members complement the activities of the primary care provider(s) (Fig. 34.1).

Team members often share responsibilities for medication management, active patient follow-up, lifestyle adherence, and self-care support. Lifestyle counseling and instructions are best provided by healthcare team members with expertise in these areas, such as registered dietitian nutritionists, exercise specialists, and licensed professional counselors or psychologists for purposes of dealing with life stressors, anger management, and lifestyle coaching. Each team's membership will vary related to the type of practice, location of practice, and availability of specific team members.

Roles of the Members of the Healthcare Team

Role of the Patient

The patient is at the center of the team with his/her involvement pivotal to implementing and sustaining a quality risk reduction plan for either primary or secondary care. The patient needs to be made aware of his/her rights with regard to inclusion into their care. This concept is reinforced within the 2018 guidelines [7] for the patient to be wholly involved with decision-making throughout the entire medical encounter. The Institute of Medicine (IOM) defines patient-centered care as "Providing care that is respectful of, and responsive to, individual patient preferences, needs and values, and ensuring that patient values guide all clinical decisions" [23]. While ultimately the patient is responsible for his or her own health, a good patient-provider partnership is essential for effective health outcomes. A collaborative care approach not only enhances the care plan and improves treatment outcomes but also can increase patient adherence, safety, and satisfaction [9, 24, 25]. This type of patient engagement can often lead to more tailored healthcare interventions and allow patients to be actively involved in the decision-making process around what works best for their health. Patients need to learn as much as possible about their medical issues, appropriate treatment options, strategies to maintain health, diet and rest requirements needed to maintain energy and participate in valued community leisure and work activities. Working together with health professionals, the engaged patient becomes empowered to meet the challenges of their illness with knowledge, confidence, and energy [9].

Role of the Physician

The physician is not an allied health professional but remains an integral part of the healthcare team. The primary care physician (PCP) can be seen as the director of care, orchestrating possible solutions for different conditions, which may

require treatment in the setting of multiple comorbidities [20]. The PCP, often in concert with advanced practice providers who are members of the allied healthcare team, will determine differential diagnoses based on both knowledge and judgement as well as develop management and treatment plans for those conditions/diseases. Once developed, the PCP will include members of the allied healthcare team appropriate to meet the individual needs of the patient.

Role of the Lipid Specialist

Lipid specialists are credentialed clinicians either by the American Board of Clinical Lipidology (ABCL) (physicians) or the Accreditation Council for Clinical Lipidology (ACCL) Healthcare Professional Certification Program (physicians, nurses, NPs, PAs, pharmacists, dietician, exercise specialists, and psychologists). ACCL and ABCL members will come from a variety of disciplines who have received specialized training in employing dietary, lifestyle, and pharmacy modalities which are effective strategies for the specific treatment of CVD, with related risk factors [26]. Specialist roles include patient education, involvement with clinical research, as well as providing education to the community to improve quality of patient care. Specialists provide excellent resources for patients with severe lipid disorders, patients with multiple comorbidities requiring medications that may lead to drug to drug interactions, and those who are unable to reach appropriate thresholds for LDL-C by either the inability to tolerate medications or related to the severity of their disease such as FH. Specialists may be found in large academic centers as well as small practices throughout the United States. In children with FH, parents are advised to see a pediatric lipid specialist. In some areas, there may only be one certified lipid specialist for large populations; however, specialists may be consulted for advice with developing a plan of care if a patient is unable to be seen in person. The National Lipid Association (NLA), the Foundation of the NLA, and the FH Foundation provide information on their websites to assist providers to locate a specialist in any particular demographic area [26].

Role of Nurses

Nurses have multiple and various roles within the lipid clinic. Nurses provide process support and are often the first line of communication between the patient and the provider for health issues when the patient is at home; consequently, they may start to form a relationship with the patient prior to the visit. The practice nurse may be charged to gather pre-appointment data to ensure a timely and optimal visit to the practice for the patient. The nurse through telephone interaction or other forms of communication such as email will collect clinical and demographic background, reason for the visit, allergies, medication regimen, and pertinent information related to medications, especially any medication failures with lipid-lowering medications. Family history can also be gathered at that time, along with lifestyle behaviors such as exercise, alcohol consumption, smoking, and dietary habits. The nurse may collect insurance information for the chart as well. The patient will be advised of any documents or studies that may be useful for the clinician and will be advised to present to the clinic in a fasting state. Once the patient has arrived for the visit, the nurse or medical assistant will bring the patient to the treatment room and record additional data in the EMR from records brought to the practice. In some clinics, the nurse may draw blood and provide information with regard to lifestyle management such as smoke cessation programs. A combination of strategies is most effective for facilitating adherence after the visit, such as educational approaches, behavioral counseling, and supportive techniques which are elaborated in the current guidelines [7, 9]. After the initial visit, the office staff nurse will employ a variety of strategies to improve adherence to lifestyle changes and medication. These Interactions may include contacting with patient to reinforce the treatment plan by mail or telephone contact, providing of prompts and reminders, as well as a plan for follow-up will be

reinforced along with instructions for follow-up labs will be confirmed. The nurse with appropriate qualifications is encouraged to receive certification as a clinical lipid specialist through the Accreditation Council for Clinical Lipidology Certification (ACCL).

Role of the Advanced Practice Professional (APP)

Nurse practitioners may be focused on chronic disease management, patient education, and transitions of care. The clinical nurse specialist may be focused on developing and improving specific cardiovascular programs as well as research within a practice [9, 17]. In most states, APPs have prescriptive authority and the practice may bill directly for their services. In many lipid specialty clinics, the patient is scheduled with the APP for follow-up appointments. Patients with metabolic syndrome and other complex healthcare issues benefit from close follow-up for weight management, dietary reinforcement, exercise, blood pressure, and laboratory studies to ensure that consistent lipid management and other risk reduction goals established are met [9]. The APP is qualified by training to perform tasks including history taking, physical examination, diagnosis, and patient management. APPs often collaborate with physicians in the development of clinic policy and procedures. They may take the lead on developing assessment forms and patient education materials within the EMR. The APP is encouraged to receive certification as a clinical lipid specialist through the Accreditation Council for Clinical Lipidology Certification (ACCL).

Role of the Physician Assistant (PA)

Tasks of the PA will vary depending on the clinical setting and tend to be modeled on the technical and clinical tasks of the physician, thus enhancing the overall capabilities of the physician [17]. The PA is qualified by their training to perform tasks traditionally performed by doctors including history taking, physical examination, diagnosis, and patient management. PA functions include performing diagnostic, therapeutic, preventive, and health maintenance services. The PA is encouraged to receive certification as a clinical lipid specialist through the Accreditation Council for Clinical Lipidology Certification (ACCL).

Role of the Pharmacist

Pharmacists serve as important resources to healthcare providers including assessments related to medication or drug-drug interactions that could lead to poor outcomes and assessing the patient's ability to understand and adhere to complex medication regimens. General pharmacist is responsible for medication reconciliation, managing potential for drug-drug interactions, care and managing complex drug therapies, medication adherence, and care during transitions from hospital to home [9]. Specialty pharmacist is often utilized for assistance with new and costly medications such as PCSK9 inhibitors, thus relieving the clinician of the burden of prior authorization [7]. In the community setting, pharmacists are directly accessible to patients as a resource for health and medical information and can conduct preventive health testing, including services for cholesterol, blood glucose, and glycated hemoglobin levels. The pharmacist is encouraged to receive certification as a clinical lipid specialist through the Accreditation Council for Clinical Lipidology Certification (ACCL).

Role of the Registered Dietician/ Nutritionist (RDN)

The practice of dietetics/nutrition has been defined by the American Diabetic Association (ADA 1991) as the integration and application of the principles derived from the sciences of nutrition, biochemistry, food, and physiology to achieve and maintain the health of individuals through the provision of nutrition care services. The nutritionist will further define nutrition care services to include assessment of the nutritional

needs of individuals and groups and determining resources and constraints in the practice setting. The nutritionist will establish priorities, goals, and objectives that meet nutrient needs and are consistent with available resources and constraints. While the nutritionist will provide nutritional counseling to the general population, they play a pivotal role in the management of diet in specific disease states. The NLA has published strong evidence-based recommendations supporting patient referral to the RDN registered in their Recommendations for Patient-Centered Management of Dyslipidemia: Part 1 and Part 2 [10, 11]. The referral for medical nutrition therapy (MNT) is in concert with several other national guidelines, including the AHA, the ACC, the Obesity Society, and the ADA. These recommendations and guidelines strongly reinforce the role of the RDN for issues such cardiometabolic risk factors, including dyslipidemia, HTN, overweight/obesity, metabolic syndrome, prediabetes, and type 2 DM. The four components of the MNT process by an RDN include (1) nutritional assessment, (2) nutritional diagnosis, (3) nutritional intervention, and (4) nutritional monitoring and evaluation [27]. Within the lipid clinic, the RDN must be prepared to appropriately counsel those with various environmental and genetic disorders. For example lipid disorders, such as familial chylomicron syndrome (FCS), in which the current treatment today is totally dependent to a diet very low to no fats, the RDN is pivotal in assisting the patient and family with diet to avoid episodes of pancreatitis. In FH the dietician will advise the type of diet within current guidelines for this genetic disorder as part of lifestyle management. The nutritionist will be responsible for developing, implementing, and managing nutritional care systems as well as to evaluate, make changes, and maintain appropriate standards of quality in food and nutrition care services while individually taking into mind the patient's lipid disorder, lifestyle, culture, and the presence of other cardiovascular risk factors [23, 28]. A team-based approach for nutritional management is optimal; however, in private offices and specific practices, unfortunately there often is not availability for patients to see the nutritionist. This opportunity may not be possible and the education will need to be provided by other team members. The patient may need to make a separate appointment which may or may, which may limit access to this service by the patient, not be covered by insurance. Ideally within the team-based concept such as a lipid center, the patient receives this consultation as part of the total assessment and is not charged independently for this service. The nutrition specialist is encouraged to receive certification as a clinical lipid specialist through the Accreditation Council for Clinical Lipidology Certification (ACCL).

Role of the Psychologist

The psychologist assesses, diagnoses, and treats the psychological problems and the behavioral dysfunctions resulting from or related to physical and mental health [29]. Additionally, they play a major role in promoting healthy behavior, preventing disease, and improving patient's quality of life [29]. Psychologists as behavioral health providers play a major role in understanding how biological, behavioral, and social factors influence health and illness, which are integral in treating the whole patient, physically and mentally. Although a psychologist is usually not a staff member in a lipid clinic, he/she is often consulted and provides a valuable resource. While gathering a patient's medical history, providers often identify extreme sources of stress in a patient's life or ineffective ways of managing stress which interfere with necessary lifestyle changes. Psychologists are skilled in assisting patients to effectively manage their stress and learn specific coping mechanisms and stress reduction techniques. Psychologists can provide in-depth counseling when necessary on behavioral change strategies that facilitate the therapeutic plan for patients [9]. Within the field of dyslipidemia and especially with issues of genetic origin, parents may suffer with guilt and even depression related to children who may have inherited their disorder. Families as well as patients with FCS, for example, will need intensive assistance for family members related to the

very strict and isolating diet required to prevent recurrent episodes of pancreatitis. Isolation related to the diet is common since eating out at restaurants is difficult as well as dining with family and friends [30]. Related to specific training in their field, psychologists have the flexibility to serve multiple roles within primary care [31, 32]. It is likely that by focusing and highlighting the roles of *clinicians*, *team-builders*, and *system specialists*, psychologists can do their part in promoting a cultural shift [33]. Psychologists can utilize common clinical factors in easing tensions, communicating, and bringing providers together for a better interdisciplinary team. Psychologists are trained to assist other team members to *reframe*, or a view a situation in a more positive light, which is important with busy teams in a bureaucratic setting. The psychologist is encouraged to receive certification as a clinical lipid specialist through the Accreditation Council for Clinical Lipidology Certification (ACCL).

Role of the Genetic Counselor

Genetic counseling is the process of helping people understand and adapt to the medical, psychological, and familial implications of the genetic contributions to disease [34–36]. The process includes interpretation, risk assessment, education, and counseling. Genetic counselors may be employed in specialist genetic centers or within other specialist units. Frequently, they contribute to patient care as one member of a multidisciplinary team in areas within oncology, ophthalmology, cardiology, metabolic clinics, or obstetrics. The roles of the genetic counselor include both information giving and exploration of the client's circumstances and needs [36, 37]. As members of a healthcare team, genetic counselors provide information and support to families affected by or at risk for a genetic disorder. They serve as a central resource of information about genetic disorders for other healthcare professionals, patients, and the general public. The role of genetic counseling includes helping to identify families at possible risk of a genetic condition by gathering and analyzing family history and inheritance patterns and calculating chances of recurrence. They provide information about genetic testing and related procedures. They are trained to present complex and difficult-to-comprehend information about genetic risks, testing, and diagnosis to families and patients. Genetic counselors help families understand the significance of genetic conditions in relation to cultural, personal, and familial contexts. They also discuss available options and can provide referrals to educational services, advocacy and support groups, other health professionals, and community or state services. Genetic counselors can serve as a central resource of information about genetic conditions for other healthcare professionals, patients, and the general public [38]. If genetic testing is performed, the genetic counselor often acts as the point person to communicate results. However, the posttest session involves more than the provision of medical information and often focuses on helping families cope with the emotional, psychological, medical, social, and economic consequences of the test results. In particular, psychological issues such as denial, anxiety, anger, grief, guilt, or blame are addressed, and, when necessary, referrals for in-depth psychosocial counseling are offered. Information about community resources and support groups can be provided to the patient/family. This is particularly relevant to the patient with lipid disorders [34]. The genetic counselor is encouraged to receive certification as a clinical lipid specialist through the Accreditation Council for Clinical Lipidology Certification (ACCL).

Role of the Exercise Specialist

Patients seen in the lipid clinic may be followed for primary or secondary risk management and therefore are likely to have diverse and individual exercise prescriptions. Exercise training improves all modifiable risk factors and specifically benefits the lipid panel by reducing triglycerides (TG), raising high-density lipoprotein cholesterol (HDL-C), and reducing non-HDL-C. The majority of patients with stable CHD should be engaged in age- and health status-appropriate regular exer-

cise. Patients who have had a recent cardiac event with percutaneous intervention or coronary artery bypass grafting should be referred to an established cardiac rehabilitation program staffed by an exercise specialist. Exercise therapists typically hold a master's degree or doctorate in exercise physiology, while ancillary staff may include a nurse, with specialized training in exercise science, or members of related disciplines [9]. Prior to the inception of an exercise program the CHD will perform a symptom-limited exercise test to assess maximal heart rate as well as to determine the presence of ischemia or arrhythmias that may impact the safety and development of an individualized exercise program. Exercise specialists are trained to assess the results of exercise testing and formulate an exercise prescription tailored to the individual's functional capacity. Typically, exercise patients are seen in the program three times per week with an intensity of 70–85% of the measured peak heart rate while supervised and monitored [9]. In addition to aerobic exercise, calisthenics, flexibility, and strength training are often part of the program. The exercise specialist is encouraged to receive certification as a clinical lipid specialist through the Accreditation Council for Clinical Lipidology Certification (ACCL).

Role of the Office Staff

Office staff play a pivotal role in the success of the healthcare team. They are responsible in many cases to obtain prior authorizations, which can be particularly time consuming in the era of PCSK9 inhibitors as well as other medications. They are often responsible for coordination of the patient through the maze that is often healthcare today. They are frequently the first persons that the patient speaks with when setting up an appointment, helping to obtain critical data to provide for a comprehensive office visit when the patient is first seen in the practice. Office staff answer the phone when patients call for questions and often are able to provide reassurance with new diagnosis and medical regimens, which frequently creates a bond and trust which is essential for optimal patient outcomes. The staff member may also be involved with billing as well as other routine office tasks.

Principles of Patient-Centered Care

The Picker Institute and Harvard Medical School established a concept which illustrates eight principles of patient-centered care [39] as a guide for what clinicians and all members of the healthcare team need to understand and include in their interactions with patients and family. These principles examine what it means to be truly patient-centered and were developed based on findings from multiple focus groups. Within the model, they define patient-centered care for patients as well as families in ways that are meaningful as well as valuable to the individual patient. The concept includes listening to, informing, and involving patients in their care. Principles are designed with regard to concepts related to issues of patient respect, coordination and integration of care, information and education, physical comfort, emotional support and alleviation of fear and anxiety, involvement of family and friends, continuity and transition, and access to care. This model is useful for the development of the healthcare team and is articulated with regard to specific actions related to each principle.

The Lipid Clinic

Patients with dyslipidemia can be seen in practices from primary care to specialist in cardiac care, internal medicine, women's health, or endocrinology. Involvement of an integrated multidisciplinary team has been shown to be of value for improving care for this often high-risk population [27]. The provider roles will vary depending on the setting in which care is provided. A lipid center may be located within a large academic center or be located in a small community with minimal ancillary staff. Resources will often dictate the level of care able to be accomplished within any lipid center. Small centers with minimal staff and resources will often not have the time to deal with rare genetic disorders or complicated complex patients and will need to

refer the patient to another setting for the initial workup and development of the plan of care. In cases with rare disorders or complex care, patients may be offered the opportunity to participate in clinical research protocols if amenable and qualified. Once a thorough diagnosis has been made and the patient inclusion into the plan through the clinician-patient discussion is complete, the patient may be returned to the original provider for implementation, evaluation, ongoing care (Box 34.1).

Box 34.1 Settings and Practitioners

Settings		
Academic/ hospital	Independent practices	Small practices
Access to many professionals	Group offices	Individual provider(s)
	Cardiology	Primary care
	Endocrinology	Internal medicine
Who may be part of the practice?		
Physician NP/PA Office nurse Dietician With access to Pharmacist Geneticist Psychologist	Physician may have NP/PA office nurse and staff	Physician Office staff

Case Study: HeFH Patient with DM, Metabolic Syndrome, and SAMS

SD presents to the PCP with concerns regarding very strong family history of premature CAD, father's fatal myocardial infarction (MI) at age 43, and mother's fatal MI at age 46; now his first cousin suffered MI at age 37. In spite of parents' premature disease, SD was never instructed to have cholesterol tested at that time. Neither parent had ever been treated for dyslipidemia; therefore, no lipid diagnosis was possible, although a genetic correlation was suspected. The PCP had laboratory work in chart dating back to his commissioning in the Marine Corps revealing LDL-C at 195 mg/dL at age 22. No recommendations for treatment were made at that time. Sporadic laboratory work was obtained over the years which revealed LDL-C ranging from 178 to 202 mg/dL. He was counseled to limit animal fats in his diet and continue with his normal exercise program consisting of playing basketball at a community center at least 3 days per week. The patient did not have routine physical examinations; in spite of suggestion, he only sought care when ill with minor issues. He is now 41 and with the premature MI of his cousin he now is greatly concerned about his risk. New had laboratory work was completed prior to the appointment as ordered by the PCP. A comprehensive metabolic and lipid panel revealed TC 264, mg/dL TGs 203 mg/dL, HDL 38 mg/dL, LDL-C 185 mg/dL, and non-HDL 226 mg/dL. Glucose was found to be at 110 mg/dL and TSH within normal limits. He was diagnosed with familial combined hypercholesterolemia. Treatment plan consisted of continuation of exercise program, again to limit saturated and total fat in the diet, to limit alcoholic beverages to <2 per day, and to lose weight since his body mass index was 26. Simvastatin was started at 20 mg and he was advised to report increase in muscle ache and pain. Two weeks into therapy he complained of severe bilateral pain to lower extremities which were limiting his ability to play basketball. The PCP changed statin to 40 mg of atorvastatin with similar symptoms. The patient told he was allergic to statins and should seek out further treatment with the lipid specialist which was part of the healthcare system.

On presentation to the academic lipid center, housed within the healthcare system, SD was off of all lipid-lowering or other medications. Per the protocol of the center, new laboratory studies were ordered with the addition of Lp(a), ApoB, HS-CRP, and an ultracentrifugation for lipid analysis and direct LDL-C. Further studies including a comprehensive metabolic panel were ordered along with a TSH and HbA1c. Per the protocol, a pre-visit telephone interview was scheduled with the patient, in which data was obtained including family history, social history, past medical history, medication history, and lifestyle behaviors. The patientt reports that he had a remote history of smoking one pack of cigarettes per day for about 5 years, stopping approximately 6 years ago, and

he drinks alcoholic beverages <4 times per week and does ongoing exercise. He indicates that he has not made changes with diet since he really did not understand recommendations that were made. He has one sister who is alive and well who is also not aware of her cholesterol levels.

At presentation he reports being well with no cardiovascular signs or symptoms and was reluctant to start any statin therapy since it was determined that he was "allergic" and was concerned over his future now that he had two children at ages of 10 and 12.

Laboratory studies revealed TC 283 mg/dL, TG 226 mg/dL, HDL 34 mg/dL, non-HDL 249 mg/dL and direct LDL 237 mg/dL, glucose 126 mg/dL, and HbA1c 7.9%. Evaluation of risk-enhancing factors revealed Lp(a) elevated at 108 mg/dL with normal range ≥50 mg/dL, ApoB elevated at 189 mg/dL, and HSy -CRP elevated at 2.8 mg/L. Additional risk-enhancing factors included family history, persistently elevated LDL-C, metabolic syndrome (glucose, TG, and HDL) and persistently elevated TGs ≥175 mg/dL.

Physical examination revealed a blood pressure of 146/84 mm Hg, a weight of 204 lbs, a waist circumference of 39 inches, and stigmata of hypercholesterolemia identified with bilateral Achilles tendon xanthomas.

Based on family history, current laboratory studies, and physical examination, SD was diagnosed with heterozygous familial hypercholesterolemia with superimposed elevated TGs, type 2 DM, metabolic syndrome, statin-associated muscle symptoms (SAMS), hypertension, and family history of premature CHD. Findings placed him in a very high-risk category with the recommendation of high-intensity statin therapy with the aim to reduce LDL-C levels by 50% or more.

Plan:

1. Meet with practice dietician on the same day of initial visit for recommendations to reduce weight, to limit total and saturated fats in the diet, as well as to limit salt intake.
2. Make appointment with genetic counselor for education and recommendations for genetic disorder with the need to have children and other pertinent family members tested.
3. Clinician-patient discussion consistence with 2018 guidelines initiated [7] to discuss risk assessment, endorse lifestyle management, continuation of exercise, implement changes with diet related to meeting with dietician, potential net clinical benefit of pharmacotherapy in spite of SAMS in the past with fear of statins, cost considerations all concluding with shared decision-making. The Pt was encouraged to verbalize feelings with statin therapy, available options, and risk/benefits; the patient was invited to ask questions, express values and preferences, and state willingness to adhere to lifestyle changes and medications, along with agreement to the follow-up plan [7].

The patient was shocked with the discussion of risk and diagnosis. Discussion with regard to medication regimen included the need to institute a statin regimen that was acceptable as well as tolerable and begin metformin for type 2 DM, ACE inhibitor for HTN, and ASA at 81 mg. He was concerned with the need to take multiple medications when he had not been taking anything other than the statin trials in the past. Given his reluctance, it was agreed that he would benefit from further evaluation and a coronary artery calcium (CAC) test was ordered. He was able to have that study performed on the day of the visit which revealed a score placing him at the 75th percentile for his age and placing him in a category of a cardiac equivalent. Given these results and continued discussion with the NP in the practice regarding all the data gathered at the comprehensive visit, it was agreed that he was willing to once again challenge statin therapy. It was agreed that he would start rosuvastatin at 5 mg given two times per week. He would communicate with the NP via email for toleration of mediation regimen, titration of statin, and positive reinforcement of lifestyle management. After gradual titration to 5 mg of rosuvastatin to daily, which he was able to tolerate, he did not achieve ≥50% LDL-C reduction, and statin was increased to 10 mg daily. After 4 weeks of being able to tolerate statin therapy at that level bloodwork was obtained to evaluate the reduction of LDL-C. Findings reveal LDL-C at 117 md/dL, with threshold established to be

≤70 mg/dL. An increase of statin to 20 mg daily was implemented with a return of SAMS; he was instructed to return to the 10 mg dose and scheduled to return to the clinic for further treatment recommendations.

Follow-up appointment: SD returned to the clinic looking and feeling well overall. He was tolerating his medication regimen of the lower dose of statin and continued with recommended lifestyle changes. He was greatly concerned that his 12-year-old son was diagnosed with heterozygous FH and was scheduled to see a pediatric lipid specialist at the attached children's hospital. His wife accompanied him to this appointment and had the option to meet with the dietician for recommendation of a reasonable family dietary plan, especially concerning the multiple diagnoses of her husband, and now son diagnosed with HeFH. He further reported that given the recommendation from the geneticist at his initial visit, who was able to provide a letter provided for family members to understand the need for cascade screening, it was found that patient's sister was diagnosed with elevated LDL-C as well as another cousin. The recommendation from the geneticist was responsible for the identification of three family members with FH who now could be treated to avoid a premature cardiac event.

Results of recommendations made at his initial visit were evaluated with positive changes noted with lifestyle management including a 10 lb weight reduction, continued exercise, toleration of metformin, ACE inhibitor with BP now 128/72 mm Hg, and glucose 89 mg/dL. He was given positive reinforcement and praise for all efforts made. Since LDL-C was still suboptimal, and after further discussion, ezetimibe 10 mg was added with instruction to continue communication via email with the NP at least on a weekly basis or more if SAMS occur once again. No further problems with toleration were reported with statin therapy and after 4 weeks new laboratory results revealed TC 140 mg/dL, direct LDL-C of 71 mg/dL, TGs were now at 101 mg/dL, HDL increased to 38 mg/dL and non-HDL 102 mg/dL. While very happy with his efforts and medication, he tells the staff nurse that he feels he has become depressed since his son was diagnosed with HeFH and felt responsible. He was not sleeping well and felt uncomfortable discussing his feelings with his wife, since he did not want to worry her. He was reassured that this was not an uncommon occurrence and an appointment was made with the team psychologist to discuss his feelings.

In the interim of stratifying SD's risk and implementation of a treatment plan, the PCP was kept up to date with information available to him from the EMR, since he was a provider within that hospital system. The identification by the PCP of the need for a more specific evaluation and individual treatment requiring increased resources and time in an already busy practice was beneficial for SD to receive a full spectrum of care.

Case Study: Improving Care Through Collaboration in Patient with CAD and HIV

Mr. P is a 63-year-old male with known CAD who developed chest pressure and tightness while on a motorcycle trip. He continued to ride and soon began to feel clammy and nauseated as well. He rode to a hospital in the next town where he underwent cardiac evaluation revealing a non-ST-segment elevation and acute coronary syndrome. Catheterization revealed a high-grade stenosis in the proximal left anterior descending (LAD) artery which was angioplastied and stented. He had residual disease with a known chronic total occlusion (CTO) in the right coronary artery (RCA) and his primary cardiologist agreed that medical management was appropriate.

Past cardiac history revealed CAD 8 years prior presented as a myocardial infarction (MI) with staged percutaneous coronary interventions (PCI) with stenting of the LAD followed by stenting of an obtuse marginal branch. Recurrent symptoms 5 months later lead to PCI to a de novo lesion in the distal LAD.

Other past pertinent medical history reveals asthma, deep-vein thrombosis and pulmonary embolus treated with warfarin, and human immunodeficiency virus (HIV).

Risk factor evaluation related to the progression of his arteriosclerotic cardiovascular disease (ASCVD) identified type 2 diabetes, hypertension, obesity, metabolic syndrome with dyslipidemia, and HIV.

Medications at presentation included valsartan 80 mg, clopidogrel, ipratropium inhaler, and warfarin as well as ritonavir (protease inhibitor), abacavir/lamivudine (nucleoside/nucleotide reverse transcriptase inhibitors (NNRTIs)), darunavir, and atorvastatin at 20 mg, even though he reported an allergy to statins with pravastatin and trialed rosuvastatin. Atorvastatin was increased to 40 mg since he appeared to tolerate it. Lifestyle management was strongly encouraged, which resulted in a 30 lb weight reduction.

Given his history and multiple comorbidities, he was referred to the lipid clinic. Laboratory findings on maximally tolerated statin revealed total cholesterol (TC) 219 mg/dL, low-density lipoprotein cholesterol (LDL-C) 112 mg/dL, non-high-density cholesterol (non-HDL-C) 174 mg/dL, high-density lipoprotein cholesterol (HDL) 45 mg/dL, glucose 109 mg/dL, and hemoglobin A1c (Hgb A1c) 5.9%.

Treatment plan: Ezetimibe was added to his regimen and lifestyle reinforced.

On follow-up clinic visit, glucose was increased at 137 mg/dL, TC 315 mg/dL, TG 467 mg/dL, non-HDL-c 273/dL, and HDL 42 mg/dL. He reported stopping his ezetimibe which he felt was worsening his asthma and was experiencing leg pain as well. He agreed to a modified dosing of ezetimibe. Repeat labs on that regimen demonstrated TC 292 mg/dL, TG 668 mg/dL, non-HDL-C 250 mg/dL, and glucose 127 mg/dL which prompted the addition of low-dose fenofibrate and metformin following the next lab draw revealing TC 203 mg/dL, direct LDL-C 153 mg/dL, non-HDL-C 159 mg/dL, TG 216 mg/dL, and HDL-C 44 mg/dL.

At this point, he was on maximally tolerated statin and ezetimibe and low-dose fenofibrate therapy. Based on the complexity of the condition and with the patient's consent, a discussion followed with colleagues from the National Lipid Association (NLA) with known expertise in managing patients with HIV. The protease inhibitors were identified as the problem and changes to the antiretroviral therapy were recommended. As a result of this collaboration, the immunologist agreed to change the ART which resulted in a much-improved lipid profile as illustrated in accompanying chart/table.

HIV-infected patients are at an increased risk for development and progression of ASCVD with increased incidence of both MI and CVD mortality. Per the NLA, HIV infection should be considered equivalent to one additional ASCVD factor. When HIV patients are treated with ART, they present with a worsening cardiometabolic risk profile as noted with this patient, noted by adverse effects on not only lipid parameters but also insulin resistance and hyperglycemia. These patients experience further potential complications related to drug-drug interactions related to metabolism of medications by the same CYP450 pathways. Given the severity of this patient's ASCVD, high-intensity statin is recommended but caution must be taken because of the potential interactions with his ART [40].

The case illustrated how a seemingly straightforward case can represent a complex management dilemma requiring collaboration of multiple specialists (Table 34.3).

Table 34.3 Case #2 flow chart of patient treatment [40]

Metabolic parameter in mg/dL	Atorvastatin 40 mg	−30 lbs plus ezetimibe	Stopped ezetimibe	Plus modified ezetimibe dose	Plus fenofibrate and metformin	New ART
Total cholesterol	219	183	315	292		124
Triglyceride	308	236	467	668		102
HDL-C	45	43	42		44	41
LDL-C	112	93			153 direct	63
Non-HDL-C	174	140	273	250	159	83
Fasting glucose	108	127	137	127		
AI%	5.9					

As noted with the above case studies, individual practices will have different staff members and varied time frames to be spent with patients for the implementation of a collaborative plan based on available resources. This development and implementation of a treatment plan may also vary depending on the size and setting of a practice, state regulations, and workforce availability [17]. The PCP remains the gatekeeper for patients and most often will be able to diagnose and treat the majority of patients with dyslipidemia within the practice, but today patients are older and sicker and have more complicated conditions than ever before. PCPs are encouraged to use available resources to assist with keeping up with the ever-changing guidelines and available treatment modalities. The National Lipid Association Organization [26] through its membership provides each type of practice with knowledge and ongoing educational opportunities to inform the best practices in the treatment of dyslipidemia. This multidisciplinary organization brings members of various disciplines together to enhance the practice of all providers. Through publications such as the *Journal of Clinical Lipidology* and the *LipidSpin*, along with the Annual National Meeting and Regional Chapters, the NLA affords providers from all types of practice to share in the expertise of each other's specific knowledge [26]. The NLA further encourages providers from all levels of practice to become members and complete certification either as a physician with the ABCL or allied health programs with the ACCL certification program to validate their competency in clinical lipidology [41].

Strategies for Effective Team-Based Collaborative Care

Central to successful primary care teams, researchers have indicated that a cultural shift toward improved interdisciplinary care will need to occur [42]. The move from a culture of fragmented care to a culture of interdisciplinary care is a challenge [43]. Mistrust of the skill level of other providers or misperceptions of skill level frequently result in less use of team-based care [43, 44].

Strategies for effective team-based collaborative care have been offered to improve the success of the comprehensive healthcare team. In 2014, Proia et al. [19] under the Community Guide Branch of the CDC conducted a systematic review related to blood pressure control and published a guide offering several strategies for effective team-based care. Results of this review provide a model that can be applied in the setting of lipid management. In their work, team-based care increased the proportion of people with controlled BP and reduced both systolic and diastolic BP, especially when pharmacists and nurses were part of the team. Findings were applicable to a range of US settings and population groups [45]. Implementation of this multidisciplinary approach requires health system level organizational changes and could be an important element of the medical home. Strategies offered suggest several approaches for effective team-based collaborative care. These strategies can be applied to facilitate communication and coordination of care and support among various team members and include enhanced use of evidence-based guidelines by providers, establishing regular structured follow-up mechanisms to monitor patients' progress and schedule additional visits as needed, and actively engaging patients in their own care by providing them with education about medication, adherence support, and tools and resources for self-management, including behavior change [11, 46].

Competencies and Responsibilities of the Comprehensive Healthcare Team

Traditionally, there has been a hierarchy of roles in the healthcare team which is counter to the combined goals of team. Recognizing the individual responsibilities and opinions of every member of the team is critical to ensuring the best outcomes for the patient. Vega [24, 25] introduces specific competencies for consideration to assistance with clarification of roles and responsibilities of interprofessional care team members. Within this model, it is the responsibility of each team mem-

ber to communicate their role to the patient as well as the other healthcare team. It is critical that each team member understands and recognizes their limitations related to skills, knowledge, and abilities. When limitations are noted within the team, the member identifying the problem is required to engage another affiliate who is prepared by education and experience to complement and develop strategies to meet the patient's needs. Members of the team must be able to articulate roles and responsibilities of each of the team members and how the team is designed to work together to provide optimal patient care. Each team member is charged to foster use of their full scope and responsibilities, knowledge, skills, and abilities to provide care that is safe, timely, efficient, effective, and equitable. Once a plan is in place, each member of the team must be able to clarify each member's specific responsibility in executing components of the plan. To ensure effectiveness of the team, each member must develop interdependent relationships with other members of the team to improve care and advance learning while continuing to engage in interprofessional development which will enhance team performance.

Recommendations for Team-Based Collaborative Care from the NLA: Part 2

The NLA has created recommendations for team-based collaborative care. NLA recommendations indicate that this team should be multidisciplinary and provides examples of those disciplines to be considered for representation on the healthcare team. Team composition is consistent with other recommendations but specifically allocates recommendations based on the strength of the recommendation from quality of the recommendations (Tables 34.4 and 34.5).

Table 34.4 Grading of the strength of recommendations and quality of evidence

Evidence grading: strength of recommendation – Grade Strength of recommendation

	Grade Strength of recommendation
A	Strong recommendation There is high certainty based on the evidence that the net benefit† is substantial
B	Moderate recommendation There is moderate certainty based on the evidence that the net benefit is moderate to substantial, or there is high certainty that the net benefit is moderate
C	Weak recommendation There is at least moderate certainty based on the evidence that there is a small net benefit
D	Recommend against There is at least moderate certainty based on the evidence that it has no net benefit or that the risks/harms outweigh benefits
E	Expert opinion There is insufficient evidence or evidence is unclear or conflicting

Evidence grading: quality of evidence	Quality rating
Well-designed, well-executed RCTs that adequately represent populations to which the results are applied and directly assess effects on health outcomes Well-conducted meta-analyses of such studies Highly certain about the estimate of effect; further research is unlikely to change our confidence in the estimate of effect	High
Moderate RCTs with major limitations Nonrandomized controlled studies and observational studies with major limitations affecting confidence in, or applicability of, the results Uncontrolled clinical observations without an appropriate comparison group (e.g., case series, case reports) Physiological studies in humans Meta-analyses of such studies	Moderate
Low certainty about the estimate of effect; further research is likely to have an impact on our confidence in the estimate of effect and is likely to change the estimate	Low

Adapted from Jacobson et al. [11]. Used with permission from NLA

Team-Based Collaborative Care and Improved Outcomes

The effectiveness of team-based care has been evaluated with respect to several outcomes: lipid levels and LDL-C goal attainment, medication and lifestyle adherence, behavioral change, management of statin adverse effects, and cardiovascular risk reduction.

Table 34.5 Strength and quality of treatment recommendations

Recommendation	Strength	Quality
Members of the healthcare team may include, where available: The patient The patient's primary healthcare provider Lipid specialist Nurses Nurse practitioners Pharmacists Physician assistants Registered dietitian nutritionists, including certified diabetes educators in some practices Exercise specialists Social workers Community health workers Licensed professional counselors Psychologists Health educators	A	High
Healthcare team members should Coordinate care support among various team members Use evidence-based guidelines/recommendations for dyslipidemia management Establish a structured plan for monitoring patient progress Provide patients with a variety of tools and resources to improve their own care	A	High
Team-based collaborative care may be incorporated into the patient-centered medical home as a strategy to address shortfalls in patient healthcare quality, access, continuity, and cost	E	Low

Adapted from Jacobson et al. [11]. Used with permission from NLA

In the Community Guide Systematic Review on team-based care and improved blood pressure control, lipid outcomes were evaluated in several studies [19]. Studies included within the review were required to have a comparison group or an interrupted time-series design with at least 2 measurements before and after the intervention. Proia noted that team-based care resulted in improvements in blood pressure but also had favorable outcomes with regard to total and LDL-C. Improvements on multiple risk factors related to interventions were found to be accomplished with the addition of nurse practitioners with prescriptive authority [19].

Another 2-year prospective trial was designed to evaluate a physician-pharmacist comprehensive intervention utilizing remote interaction with 6963 patients, received care from 68 physicians in 9 clinics [47]. Participants were at least 18 years of age and identified by a diagnosis of DM. Clinicians within the study had access to the health information tool CareManager, which was able to provide automated DM related point of care prompts, a Web-based registry, as well as performance feedback. Outcomes of the study included the difference in LDL-C goal attainment, mean LDL-C, prescribed lipid-lowing therapy, and patient satisfaction between arms. Results revealed that intervention arm participants were more likely to achieve LDL-C targets. The rate of lipid testing was also found to be higher in the intervention group and was more likely to have lipid-lowering medication prescribed. Even with a more positive outcome in the intervention group, there was no significant difference in patient satisfaction between study arms. Results indicate further that even with a remotely located physician-pharmacist team care revealed a significant improvement in LDL-C, achieving pre-specified goal attainment was achieved among patients with DM [9, 19, 47].

An additional study was designed to evaluate the impact of a structured screening and nurse practitioner intervention on improvement of the cardiovascular risk profile. All patients enrolled were diagnosed with established cardiovascular disease. Patients were assessed along with lifestyle intervention utilizing an automated questionnaire. A NP-led program was implemented utilizing a best practices model which included an individual plan of care based on these assessments, including lifestyle and medical

management. After a 1-year intervention, LDL-C and systolic blood pressure were significantly reduced. A reduction in the amount of smoking, alcohol consumption, and unhealthy eating habits was observed. However, the amount of physical activity was unaffected, and body mass was increased. Results indicated that a structural evaluation of cardiovascular risk factors and an integrated nurse-led approach can successfully reduce risk in cardiovascular patients [48].

Current guidelines focus on the need for interventions for improving adherence to prescribed therapy [7, 46, 49]. A Cochrane systematic review demonstrated that intensification of patient care interventions improves both short- and long-term adherence to medication as well as meaningful reduction in LDL-C levels [7]. Interventions to foster desired outcomes may include telephone reminders, calendar reminders, integrated multidisciplinary educational activities, simplification of the drug regimen to once-daily dosing, and pharmacist-led interventions are prime examples for optimal use of the comprehensive healthcare team [7]. In a large Veterans Affairs study, Rodrigues et al. [50] found that a cohort study of patients with ASCVD and overall high-statin adherence revealed a graded, inverse association between statin adherence and mortality. This association was observed across patient subgroups and by statin intensity. Using a national sample of Veterans Affairs patients with ASCVD, they found that a low adherence to statin therapy was associated with a greater risk of dying. Women, minorities, younger adults, and older adults were identified as less likely to adhere to statins. These findings underscore the importance of finding methods to improve adherence. They further identified an inverse, graded association between long-term statin adherence and all-cause mortality in this national sample. These findings suggest that there is a substantial opportunity for improvement in the secondary prevention of ASCVD through optimization of statin adherence [51]. This dilemma is not specific to the VA population, and recent data indicates that non-adherence occurs in many cases within months of being prescribed medication and is associated with increased risk for cardiovascular events in patients with known CAD.

It is therefore desirable to identify medication non-adherence and to facilitate strategies to improve adherence by helping patients overcome real, or perceived, barriers to adherence. In the very busy primary care or specialty practice, it often is difficult to find the time to work with the patient with regard to adherence to the treatment plan. Within the comprehensive team-based care model, there are multiple providers and staff that are available and can be trained to provide assistance to meet this need. Optimal strategies for practices will be variable related to available team members. Training staff in communication and consolidating roles and workflow is beneficial to facilitate more provider and staff time with patients. This type of communication can include face-to-face interactions, phone call, email, and text messaging and will be instrumental in increasing patient compliance not only with medication but also lifestyle management [11]. In order for any plan to be successful, the patient must be ready and willing to cooperate with implementation of the plan. More conversation with the patient and repetition in the discussion can lead to enhanced adherence. Utilization of the comprehensive healthcare team and multiple providers working within their specialty can increased the likelihood of success.

The NLA reinforces a multifaceted approach, consistent with other recommendations, which should be employed by clinicians to improve medication adherence (Box 34.2).

Team-Based Collaborative Care and Improved Patient Satisfaction

The impact of team-based care on patient satisfaction remains inconclusive as noted in a clinical trial by Pape et al. [47]. This study with a physician-pharmacist team-based approach to cholesterol management did not show a difference with patient satisfaction within the study arms. In a systemic review by Wen [52] of 26 randomized controlled trials (RCTs) with over 15,000 patients results indicated that team-based

Box 34.2 Multifaceted Approach Recommended by the NLA

(a) Simplify the regimen
(b) Provide clear education using visual aids and simple, low-literacy educational materials
(c) Engage patients in decision-making, addressing their specific needs, values, and concerns
(d) Address perceived barriers of taking medication
(e) Identify suboptimal health literacy and use "teach-back" techniques to increase patient understanding of those behaviors needed to be successful
(f) Screen and eliminate drug-drug and drug-disease interactions leading to low adherence or drug discontinuation
(g) Praise and reward successful behaviors

Adapted from Jacobson et al. [11]. Used with permission from NLA

care improved patient satisfaction compared to usual care; however, in 7 studies with combined continuous data, no difference was reported between team-based care and usual care [52]. Results of this work indicate that some evidence showed that team-based care is better than usual care in improving patient satisfaction. However, further large-scale and high-quality randomized controlled trials comparing team-based care and usual care are needed.

Incorporation of Team-Based Collaborative Care into the Patient-Centered Medical Home Model (PCMH)

Team-based collaborative care is central to the patient-centered medical home (PCMH) model and can provide a structure to improve the efficiency of care delivery, support patient access to resources for self-management activities, and achieve a coordinated and comprehensive approach to treating lipid disorders, other risk factors, and chronic cardiovascular conditions [9, 19, 41, 49, 53, 54].

The PCMH focuses on a patient-centered approach to care, including enhanced access to a personal clinician who coordinates care through an integrated team with continuous relationships based on trust, an emphasis on communications and care coordination, involvement of family and caregivers, patient advocacy by providers, and care of the whole person.

The Agency for Healthcare Research and Quality [55] has defined the PCMH around five attributes: (1) patient-centered orientation, (2) comprehensive team-based care, (3) coordinated care, (4) access to care, and (5) a systems-based approach to quality and safety. The PCMH is promoted as a component of the required changes needed to address healthcare quality, access, continuity, and cost shortfalls in the United States. The 2018 joint guidelines [7] reinforce this notion throughout, especially with discussion regarding the comprehensive care team utilizing all appropriate and necessary members of the allied health framework to ensure safe, up-to-date, and cost-effective care for patients. The in-depth emphasis on the clinician-patient discussion speaks to use of the enhancing risk factors [7] for primary prevention stratification, as well as implementation of a patient-centered treatment plan for reduction of ASCVD risk, benefits of treatment, adverse event potential, potential of drug-drug interactions related to other medication that the patient may be taking for other health issues, and patient preferences [7].

Accreditation Council for Clinical Lipidology Certification (ACCL)

The mission of the Accreditation Council for Clinical Lipidology is to establish, certify, and maintain the clinical competencies of qualified healthcare professionals who have a focus in clinical lipidology [26].

The clinical lipid specialist (CLS) certification program is an advanced certification pathway open to licensed physicians, nurses, nurse practi-

tioners, physician assistants, pharmacists, registered dietitian/nutritionists, clinical exercise physiologists, and other healthcare professionals who meet qualifying criteria. The concept of a certification program was spearheaded by the National Lipid Association to provide opportunities for professional development and formal recognition of the specialized expertise of healthcare professionals with a focus in clinical lipidology. The Accreditation Council for Clinical Lipidology was incorporated as an independent organization in 2006 to develop and administer the clinical lipid specialist exam. The CLS program certifies and validates one's professional credentials to provide specialized care to patients with dyslipidemia and related cardiometabolic conditions. This independent certifying organization has established standards along with an examination in the field of clinical lipidology for a growing number of healthcare professionals involved in lipid management. This certification is timely in the milieu of team-based collaborative practice and provides recognition for this group of healthcare professionals who have successfully met the credentialing criteria and have successfully completed the written examination. Certification of these allied health professionals benefits the practitioner but enhances the profession as well by adding qualified providers in diverse fields of practice. Benefits of certification have been associated with improvement in the quality of patient care, setting benchmarks of clinical competency for lipid specialists, encouraging ongoing learning, and improvement which is essential for the maintenance of excellence for a lifetime of practice. Certification reinforces the recognition of specialized training and qualifications of healthcare professionals, enhancing their credibility within the medical community and to their patients as well. The ACCL is a self-governing and separate entity from the NLA; however, the two organizations work collaboratively to ensure adequate educational and professional development opportunities to meet the prerequisites of the certifying examinations.

Qualifications To become credentialed, candidates must meet requirements of basic eligibility, patient care, educational and training. The requirements are rigorous and designed to provide an avenue for advanced healthcare providers with demonstrated knowledge and experience in lipidology to become certified as clinical lipid specialists (CLS). Maintenance of certification requires renewal every 10 years with meeting established criteria. Renewal of certification (ROC) process is designed to ensure those holding this credential have maintained professional competency and have met the standards of knowledge and skills required to maintain their certification (Table 34.6).

ACCL Designations/Use of Credentials

Candidates who successfully pass the clinical lipid specialist exam will earn the designation "clinical lipid specialist." This credential may be abbreviated and used in the signature of professionals as follows: John Smith, PharmD, CLS.

Or this credential may be stated as "Diplomate, Accreditation Council for Clinical Lipidology."

After 10 years, the CLS must apply for maintenance of certification. There are specific requirements for re-cetification that include holding a current active license to practice and current CLS certification through the ACCL and demonstration of completion of at least 1000 practice hours in the 5 years prior to expiration of certification as well as meeting criteria of either pathway 1 or 2.

Pathway 1: Lipid-Focused Continuing Education

Demonstrate 75 hours of lipid-focused continuing education, completed in the 5 years prior to expiration of certification. The ACCL endorses the NLA's Core Curriculum in Clinical Lipidology. Continuing education programs that cover anything outlined in this curriculum meet ACCL's definition of "lipid-focused." All NLA programs are accepted.

Table 34.6 Eligibility and criteria for NLA certification

Clinician	Basic eligibility requirement	Criteria
Physician	US or Canadian resident Licensed to practice	1. Certification in a primary board (50 points) 2. Subspecialty certification in endocrinology, diabetes, and metabolism (EDM), cardiology, nephrology (50 points), or other relevant advanced training and/or certification, including certified diabetes educator, clinical nutrition, gastroenterology, and pediatric cardiology/endocrinology 3. Specialist in clinical hypertension (10–50 points[a]) 4. Clinical research and/or scholarly publications in the management of lipid disorders (10 points each publication – up to 50 points) 5. Balance of points not obtained in 1–4, must be earned through lipid-focused continuing medical education credit obtained within the previous 5 years (2 points per credit hour earned)

Clinician	Basic eligibility requirements	Degree	Licensure status	Patient care requirement	Criteria
Nurse practitioner/ clinical nurse specialist/registered nurse	US or Canadian resident with license to practice	Master's or higher in related health science Bachelor's in related health science	Licensed/ registered	2000 hours of demonstrated clinical experience in the management of patients with lipid or other related disorders (~1 year)* 3000 hours of demonstrated clinical experience in the management of patients with lipid or other related disorders (~1.5 years)*	1. Certification in a primary board (e.g., ANCC, AANP) or other appropriate certificate programs (50 points) 2. Higher education level: doctoral degree or other relevant advanced training (50 points) 3. Relevant academic practice and faculty appointment at a recognized institution (i.e., relevant to lipid practice) (50 points) 4. Clinical research and/or scholarly publications in the management of lipid disorders (10 points per publication up to 50) 5. Balance of points not obtained in 1–4, must be earned through lipid-focused continuing education credit obtained within the previous five calendar years (2 points per credit hour earned)
Pharmacist	US or Canadian resident with license to practice	Master's or higher in related health science*	Licensed/ registered	2000 hours of demonstrated clinical experience in the management of patients with lipid or other related disorders (~1 year)*	1. Certification in a recognized board (e.g., Board of Pharmaceutical Specialties (50 points)) or advanced licensure recognized by the state or other appropriate certificate programs (50 points)
		Bachelor's in related health science*	Licensed/ registered	3000 hours of demonstrated clinical experience in the management of patients with lipid or other related disorders (~1.5 years)*	2. Relevant accredited postgraduate training of at least 1 year (if not accounted for in basic requirements) or higher education level (Postbaccalaureate, PharmD) (50 points)

Table 34.6 (continued)

					3. Relevant academic practice and faculty appointment at a recognized institution (i.e., relevant to lipid practice) (50 points)
					4. Clinical research and/or scholarly publications in the management of lipid disorders (10 points per publication)(up to 50 points)
					5. Balance of points not obtained in 1–4, must be earned through lipid-focused continuing education credit obtained within the previous five calendar years (2 points per credit hour earned)
Physician assistant	US or Canadian resident with license to practice	Master's or higher in related health science*	Licensed/ registered	2000 hours of demonstrated clinical experience in the management of patients with lipid or other related disorders (~1 year)*	1. Certification in a primary board (e.g., NCCPA) or other appropriate certificate programs (50 points) 2. Higher education level: doctoral degree or other relevant advanced training 3. Relevant academic practice and faculty appointment at a recognized institution (i.e., relevant to lipid practice) (50 points) 4. Clinical research and/or scholarly publications in the management of lipid disorders (10 points per publication up to 50) 5. Balance of points not obtained in 1–4, must be earned through lipid-focused continuing education credit obtained within the previous five calendar years (2 points per credit hour earned)
		Bachelor's in related health science*	Licensed/ registered	3000 hours of demonstrated clinical experience in the management of patients with lipid or other related disorders (~1.5 years)*	Same as 1–5 above
Dietitian/nutritionist and exercise physiologist/ specialist	US or Canadian resident with license to practice	Master's or higher in related health science*	Licensed/ registered	2000 hours of demonstrated clinical experience in the management of patients with lipid or other related disorders (~1 year)*	1. Certification in Adult Weight Management from the ADA, certification from other relevant advanced training, including certified diabetes educator or FADA, or other appropriate certificate programs (50 points)

Table 34.6 (continued)

		Bachelor's in related health science*	Licensed/ registered	3000 hours of demonstrated clinical experience in the management of patients with lipid or other related disorders (~1.5 years)*	1. Higher education level: doctoral degree or other relevant advanced training (50 points) 2. Relevant academic practice and faculty appointment at a recognized institution (50 points) 3. Clinical research and/or scholarly publications in the management of lipid disorders (10 points per publication up to 50) 4. Balance of points not obtained in 1–4, must be earned through lipid-focused continuing education credit obtained within the previous five calendar years (relevant to lipid practice) (2 points per credit hour earned)
Other applicants This pathway is for those who do not hold a license, registration, or degree as described in basic eligibility requirements and/or education requirements. Must show proof of 4000 hours (2 years) of demonstrated clinical experience in the management of patients with lipid and other related disorders	US or Canadian resident with license to practice			A minimum of 200 points is required to successfully credential for certification. Points are earned through primary and subspecialty board certifications, relevant academic practice, involvement in relevant clinical research, and participation in lipid-focused continuing medical education activities	1. Certification in a recognized board (50 points) 2. Higher education: doctoral degree or other relevant advanced training/certificates (50 points) 3. Academic practice and/or relevant faculty appointment (50 points) 4. Clinical research and/or scholarly publications in the management of lipid disorders (10 points per publication up to 50) 5. Balance of points not obtained in 1–4, must be earned through lipid-focused continuing education credit obtained within the previous five calendar years (2 points per credit hour earned)

Adapted from NLA website [26]. Used with permission from NLA

[a]The combined points earned from these categories may not exceed 10

Pathway 2: Examination

Pass the current CLS exam by December 31 of renewal year. Once ROC application is processed and approved, candidates receive an email with instructions on how to schedule and set up exam.

The CLS certification is now recognized by the American College of Cardiology as qualifying criteria to become an Associate of the American College of Cardiology (AACC), a distinguished designation honoring those with advanced training and education in the field of cardiology.

The national board certifications for AACC designation were selected by the CCA Designation Working Group and approved by the ACC's Credentialing and Membership Committee. Selection of these national board certifications was based on the amount of cardiology-specific content on the board examination.

Benefits of Certification

NLA participants value the distinction of achieving this certification as is evidenced by statements from members. One member states that "I have experienced a heightened level of respect and clout from my peers, colleagues, and collaborative physicians – both in the in-patient and out-patient settings. I have been referred patients by other doctors, patients have found me online and scheduled appointments to see me, I get texts or emails frequently with difficult cases from clinicians, I am asked to participate in research, etc. Additionally, I have been invited to speak at multiple conferences around the country with clinicians, researchers, and doctors that I would never have dreamed I would be standing next to as a colleague" [56]. Another clinical lipid specialist indicates that passing the certification exam 10 years ago boosted her self-esteem since passage reinforced the knowledge base to provide quality care to my patients. It further provided me with greater credibility among my peers and other medical providers. I felt it helped define my specialty [57].

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35 The American Board of Clinical Lipidology Physician Certification Program

Christopher W. DeVille

Introduction

The concept of a certification program for clinical lipidologists was first spearheaded by the National Lipid Association (NLA) in 2002. With the goal of providing formal recognition of the specialized expertise of physicians focused on lipid management, the American Board of Clinical Lipidology (ABCL) was created. Incorporated as an independent organization in 2003, the ABCL was established to develop and administer a certification examination which would demonstrate measurable competence in the field. The mission of the ABCL is to reduce morbidity and mortality *from dyslipidemia and related diseases by certifying and evaluating knowledge and training in clinical lipidology* [1]. *Clinical lipidology is defined as a* multidisciplinary branch of medicine focusing on lipid and lipoprotein metabolism and their associated disorders [2]. A *lipidologist* is defined as a physician who has demonstrated expertise and commitment in the field of clinical lipidology through certification by the American Board of Clinical Lipidology [3].

The ABCL created the first official program for certification in clinical lipidology with a 20-year goal of achieving recognition as a subspecialty of medicine through the American Board of Medical Specialties (ABMS). Several milestones would need to be achieved with the help of the NLA including the development of a curriculum, competencies, and pathway for independent course study. These milestones along with the establishment of an examination mirroring standards and requirements of the ABMS would be necessary before formal recognition could be achieved.

The ABCL is an independent certifying board, established as a nonprofit 501c3 organization in 2003. At this time, the board is not recognized by the ABMS or any other accrediting agency. The leadership of the NLA and the ABCL is committed to the formal recognition and designation of specialty status for the field of clinical lipidology by the ABMS; however, this is a long-term pursuit. The process of applying for ABMS recognition is rigorous, and many requirements must be met before ABMS will consider an application from a new board, including the approval of ACGME-recognized training programs in the field. Several states restrict a physician's use of the term board certification to those boards recognized by the American Board of Medical Specialties (ABMS). The ABCL adheres to the "AMA Guidelines for Truthful Advertising Physician Services," used to ascertain the legitimacy of certifying boards, although the ABCL has not been recognized by the ABMS. Because several states prohibit a physician from

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M. H. Davidson et al. (eds.), *Therapeutic Lipidology*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-030-56514-5_35

advertising board certification by a board not recognized by the ABMS, physicians should consult with state law before explicitly advertising board certification by the ABCL. Use of the term "Diplomate of the American Board of Clinical Lipidology" is acceptable in all states.

Rationale for Board Certification

Atherosclerotic events, including myocardial infarction and stroke, account for approximately one-third of all deaths in the United States. Lipid management employing dietary, lifestyle, and pharmacologic modalities has been demonstrated to be one of the most effective strategies for the prospective treatment of cardiovascular disease. Yet, the number of specialists with expertise in lipid management is inadequate to address this large population of patients [3].

The ABCL was established to assess the level of knowledge required to be certified as a clinical lipidologist, to encourage professional growth in the practice of lipidology, and to enhance physician practice behavior to improve the quality of patient care. Clinical lipidologists are physicians who come from a variety of backgrounds including internal medicine, cardiology, endocrinology, family practice, osteopathy, and obstetrics and gynecology.

The ABCL physician certification program establishes a consistent benchmark of expertise in the field of clinical lipidology. The commitment to achieve certification by the ABCL requires hard work, time, and effort. The recognition carries with it significant, long-term value. Certification by the ABCL signifies that you have documented your commitment to continued professional development in clinical lipidology. Certification provides assurance to the public, your colleagues, and the medical profession that you have successfully completed a course of education in lipid management and have passed an additional rigorous examination in clinical lipidology. Certification contributes to your professional stature and credibility in the field and provides stronger credentials for enhanced professional and advancement opportunities [4].

A Diplomate of the ABCL has successfully credentialed and passed the certification exam. A Diplomate is endorsed by the ABCL as displaying a high level of experience, knowledge, and competence in clinical lipidology [2].

Eligibility Criteria

To become credentialed, candidates must submit evidence that they have earned 200 points through primary care board certification, advanced training and education, experience, an academic appointment, or clinical research in the field of lipid disorders. Eligibility requirements are described in Table 35.1. Although rigorous, the requirements have been designed to provide any physician with demonstrated knowledge and experience in lipids an avenue to become certified as a clinical lipidologist. Candidates must be a currently licensed physician (MD or DO) in the United States or Canada and be certified by a primary care board (ABMS or equivalent) (i.e., internal medicine, family practice, pediatrics, obstetrics-gynecology, geriatrics, and neurology/vascular neurology), or have completed a 2-year minimum relevant accredited fellowship (e.g., cardiology, endocrinology), or have 2 years of demonstrated appropriate experience and practice activity in the management of patients with lipid disorders [5].

Table 35.1 Eligibility criteria

Criteria	Points awarded
Certification by a primary board	50
Subspecialty certification or other relevant training and/or certification	50
Relevant academic practice or appointment at ACGME-recognized institution	50
Clinical research and/or scholarly publications related to management of lipid disorders	Up to 50
Lipid-focused continuing medical education obtained within the previous 3 years	2 points per credit hour

Courtesy of the American Board of Clinical Lipidology (ABCL)

The Certification Exam

The subject content of the certifying examination reflects the knowledge, skills, and attitudes deemed essential for the competent practice of lipidology. The 4-hour examination is constructed at the upper level of difficulty and consists of approximately 200 multiple-choice questions. The exam blueprint is described in Table 35.2 [6].

How to Apply

Applications are accepted three times per year with a 6-week testing window immediately following the application deadlines. Applications must be submitted along with the applicable fees via mail. Applications, applicant handbook, and frequently asked questions are available at www.lipidboard.org [7].

Maintenance of Certification

The ABCL is committed to establishing and maintaining a rigorous and clinically valid certification process to assure clinical competency by physicians in the field of clinical lipidology. Of even greater importance, the certification process helps achieve good patient outcomes by promoting a standard of excellence in clinical care. The ABCL Board of Directors has carefully and intentionally designed an MOC program that offers flexibility and accommodation for diplomates, while staying true to the six core competencies of MOC set forth by the ABMS: professionalism, interpersonal skills, communication skills, procedural skills, system-based practice, and patient care.

As with the MOC process established by the ABMS constituent boards, the ABCL MOC program encourages, measures, and recognizes the essential task of maintenance of competency through lifelong learning. The ABCL MOC program is an ongoing educational process made up of four parts: Part I, Licensure and Professionalism; Part II, Lifelong Learning and Self-Assessment; Part III, Practice Performance Assessment; and Part IV, Cognitive Expertise [8].

Table 35.2 ABCL exam blueprint

Content area	Percentage of exam
Classification, measurement, and metabolism of lipids and lipoproteins	9%
Pathophysiology and vascular biology	4%
Pathophysiology and genetic dyslipidemias	13%
Evidence-based medicine and statistics	8%
CVD risk factors and risk indicators	11%
Therapeutic lifestyle change	13%
Treatment of dyslipidemia (includes 2018 guidelines)	30%
Special populations and consultative issues	12%

Courtesy of the American Board of Clinical Lipidology (ABCL)

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