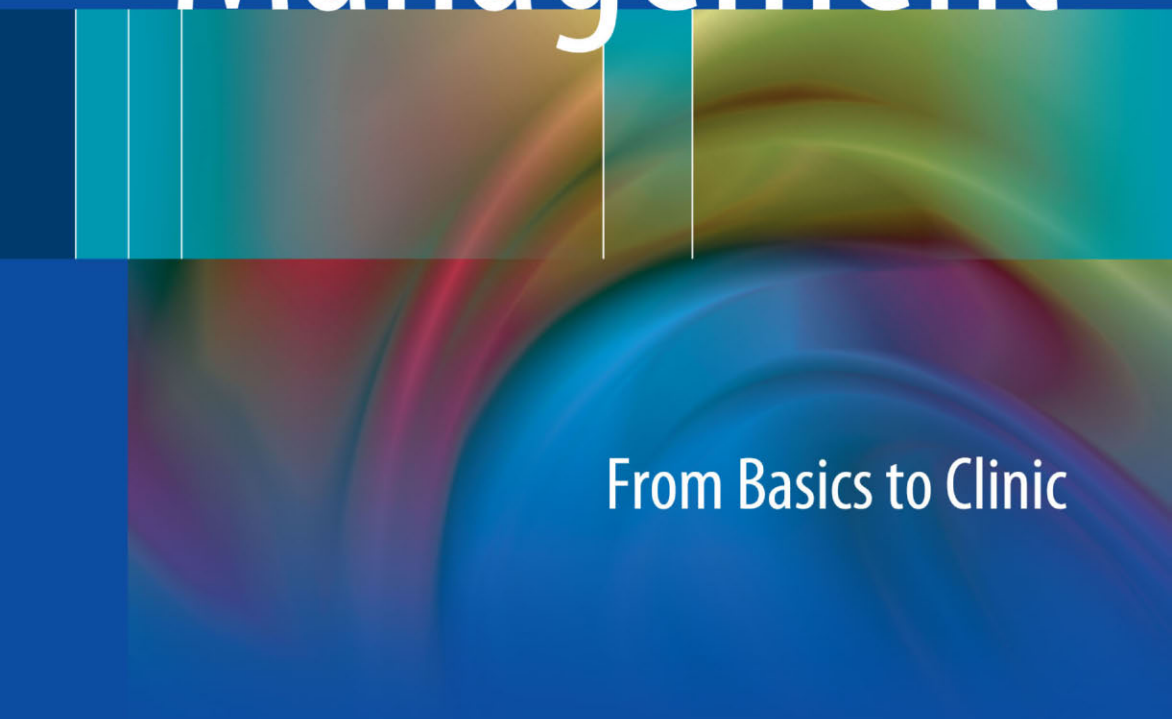
Hussein Yassine
Editor

Lipid Management



From Basics to Clinic

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This book is dedicated to my father, Naji, my mother Amne, and my wife Diala for their love and unwavering support.

To my daughters Leen and Lara, may the love of science bring you joy!

Hussein Yassine, February 2015

Preface

Over the last two centuries, the role of lipids in the etiology of cardiovascular has garnered significant attention, and in particular, the role of cholesterol on the development of atherosclerosis. The first studies involving cholesterol and cardiovascular disease date back to the early 1900s when Anichkov (an army pathologist) provided rabbits with high cholesterol diets and observed stiffening of their artery walls. In the 1950s, Ancel Keys reported in the Seven Countries study findings relating low fat intake and an association of low mortality form cardiovascular disease. His observations were misinterpreted into high carbohydrate diets that were particularly enriched with simple sugars. Together with sedentary life styles, large portion sizes, these changes contributed in part to the obesity epidemic that peaked toward the end of the twentieth century. The first lipid lowering medication, niacin, was discovered serendipitously after it was tested on a rabbit schizophrenia model. One of the side effects observed was lowering of cholesterol. In the 1960s, the coronary drug project ushered the first clinical trial for lipid lowering therapies featuring niacin use for the prevention of heart disease. Although immediately after the study the two groups did not statistically differ in heart disease rates, 20 years later, the participants assigned to niacin demonstrated a survival advantage. In the late 70s, a Japanese microbiologist Akira Endo first discovered natural products with a powerful inhibitory effect on cholesterol synthesis in a fermentation broth of *Penicillium citrinum*, during his search for antimicrobial agents. Concomitantly, Brown and Goldstein (later earning the Nobel Prize for their work in 1985) showed that HMG-co reductase inhibition represented the rate limiting step in cholesterol synthesis. These exciting basic and translational studies led to the production of statins. The first of many statin trials to come was the 4S study conducted in Scandinavian countries in the late 1980s and showed a significant reduction in cardiovascular events in participants assigned to statin therapy. Importantly, many later trials confirmed the benefits of statins in lowering heart disease risk. On another front, the publication of the Lyon Heart study (Mediterranean diet) in the mid-90s transformed our understandings of the dietary components that protect against heart disease. In that study, participants randomized to good fat (olive oil, nuts) survived longer following coronary bypass surgery than individuals kept on their regular diet. Several Mediterranean dietary studies have been conducted since confirming

a distinct role for fats in the pathogenesis of heart disease, and shedding light into healthy and less healthy fats.

In this book, we attempt to provide both basic concepts and clinical approaches to understanding and managing lipid related disorders that confer increased cardiovascular risk. The first 5 chapters cover basic aspects of lipoprotein metabolism. The remaining chapters focus on management of the patient with lipid disorders. In the first chapter, we go over the basics of lipoprotein metabolism inside the cell and how lipids are packaged in the circulation into lipoproteins. The second chapter focuses on genetic disorders of lipoprotein metabolism with an emphasis on Familial Hyperlipidemia, a common lipid disorder associated with increased heart disease risk. In the third chapter, we discuss the pathophysiology of atherosclerosis, focusing on the roles of lipids and inflammation. Dr. Abela and his colleagues discuss their land mark studies into the role of cholesterol crystals in inducing inflammation in the artery wall. In the third Chapter, Drs. Toledo-Corral, Alderete and Goran report to us important findings from their recent studies on the mechanisms linking obesity to atherosclerosis, particularly in the youth. In the fourth chapter, I discuss recent findings from studies aimed at raising HDL cholesterol but failing to improve outcomes, reviewing basic concepts of HDL metabolism. In the fifth chapter, we provide our approach to managing patients at risk for heart disease incorporating the recent AHA/ACC 2013 guidelines. We then present ten chapters that discuss the management of patients with lipid disorders and at risk for cardiovascular disease. In Chap. 6, Dr. Allevato discusses the latest evidence on dietary intervention trials that confer cardiovascular benefits with a focus on the Mediterranean diet. In Chap. 7, Drs. Abou Assi and Jordanov discuss statins, from trials to side effects and intolerance. Given the importance of statins as cornerstone therapies in the management of hyperlipidemia, Chaps. 9, 10, and 11 review the use of statins in three conditions: chronic kidney disease, non-alcoholic fatty liver and heart failure. In Chap. 12, Dr. Goldberg summarizes the evidence and use of non-statin therapies. In Chap. 13, Dr. Klapper reviews the use LDL apheresis as a modality to treat refractory dyslipidemias or severe familial hypercholesterolemias. Dr. Dube, a leading expert in the treatment of dyslipidemia in HIV presents the latest guidelines and approaches to treatment of dyslipidemia in HIV. Finally, Dr. Wong in Chap. 15 provides a concise summary on the new and emerging therapies for the treatment of hyperlipidemias. This book is intended for the public, scholars and physicians with interest in lipids. We hope that by coupling of basic concepts and management approaches to lipid disorders, we will assist the provider in making the best decisions in diagnosing and treating their patients.

Contents

About the Editor

Hussein Yassine, MD is an Assistant Professor of Medicine-Endocrinology at the University of Southern California, where he directs the lipid clinic. His research focuses on cholesterol transport in cardiovascular disease. He was the winner of several research and teaching awards including the Department of Veterans Affairs VISN 18 Outstanding Research Award, and the Endocrine Society Presidential Award.

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Chapter 1
Basics in Lipoprotein Metabolism

Hussein Yassine, Keenia Tappin and Muhammad Jawad Sethi

Abbreviations

APOA1	Apolipoprotein A-I
ApoB	Apolipoprotein B
APOE	Apolipoprotein E
CHD	Coronary heart disease
CMs	Chylomicrons
CVD	Cardiovascular disease
FH	Familial hyperlipidemia
FXR	Farsenoid X Receptor
HA	Hypoalphalipoproteinemia
HDL	High-density lipoprotein
HMG-CoA reductase	hydroxymethylglutaryl-coenzyme A reductase
IDL	Intermediate-density lipoprotein
LCAT	Lecithin-cholesterol acetyltransferase
CETP	Cholesterol ester transfer protein
LDL	Low-density lipoprotein
LDL-C	Low density lipoprotein- cholesterol
LDLR	Low density lipoprotein receptor
LRP	Lipoprotein receptor protein
LXR	Liver X ReceptorLp(a)—Lipoprotein(a)
LPL	Lipoprotein lipase
PPAR-alpha	Peroxisome proliferator-activated receptor alpha

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PPARs	Peroxisome proliferator-activated receptors
RBC	Red blood cells
RXR	Retinoic Acid Receptor
TG	Triglycerides
VLDL	Very-low-density lipoprotein

Overview of Lipoproteins

Structure of Human Plasma Lipoproteins Plasma lipoproteins contain a hydrophobic nonpolar lipid core of cholesteryl esters and triaclyglycerols and are illustrated in Fig. 1.1. They are surrounded on the surface by a more polar, hydrophilic coat of apolipoproteins, phospholipids, and unesterified cholesterol. The relative amount of core lipid to protein determines the size and density of the lipoprotein particles. Larger lipoproteins contain more core lipid and are less dense than the smaller lipoproteins. Lipoprotein transport in the plasma is made possible by apolipoproteins. These are amphipathic molecules that solubilize the nonpolar lipids. Apolipoproteins also have active roles in the metabolism of the lipoproteins and act as ligands for lipoprotein receptors and cofactors for lipolytic enzymes and lipid transferases. The apolipoproteins are named based on an alphabetical nomenclature, starting with A, B, C, and so forth. More than 12 apolipoproteins have been described. Among these, apo B and apo A-I have a paramount role. Elevated levels of the apo B–containing lipoproteins and low levels of the apo A-I–containing lipoproteins are associated with CHD.

Apo B–Containing Lipoproteins

These include chylomicrons, chylomicron remnants, VLDL, VLDL remnants (also known as IDL), LDL and Lipoprotein(a) (Lp[a]). Lp(a) consists of a molecule of

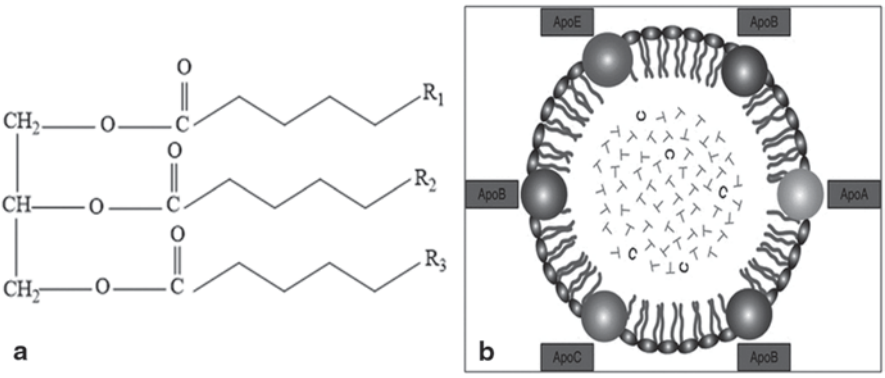

Fig. 1.1 Plasma lipoproteins. **a** Triacylglycerol. **b** Chylomicron with triacylglycerol and cholesterol ester core surrounded by a phospholipid and apolipoprotein membrane

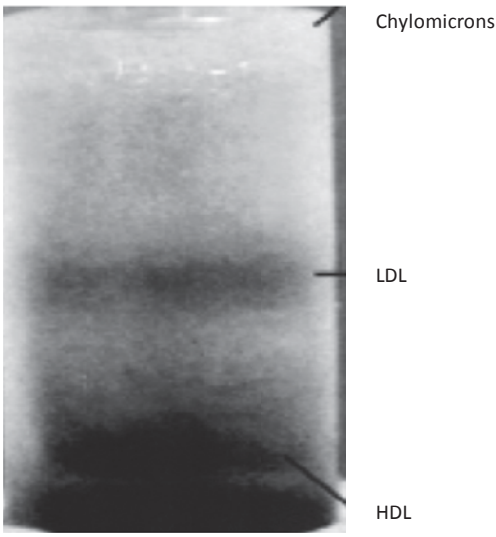

Fig. 1.2 Separation of plasma lipoproteins by density: This is an illustration of a plasma sample obtained after centrifugation demonstrating the separation of the plasma into distinct bands based on density. This sample reveals increased chylomicrons. Note that Chylomicrons are less dense, float and have a whitish appearance giving them the chyle description

LDL connected through a disulfide bridge on apo B to apo (a), a protein homologous to plasminogen [1].

ApoB containing lipoproteins are further classified based upon differences in the size and/or density. Generally VLDL and LDL are divided into large, intermediate, and small lipoprotein subclasses. An example of separation of lipoprotein by density is illustrated in Fig. 1.2 with centrifuged serum obtained from a hyperchylomicronemic patient postprandially. ApoB containing lipoproteins are lipid rich, and have an important role in carrying cholesterol and triglycerides in the circulation.

ApoA containing lipoproteins

These proteins form HDL particles and are also found on chylomicrons. They are critical components of reverse cholesterol and are excellent initial acceptors of cholesterol from peripheral tissues. The majority of HDL lipoproteins contain both AI and A-II. HDL lipoproteins are far more complex than LDL and VLDL, very heterogeneous and have a greater density due to enrichment with proteins. Recent studies suggest that these lipoproteins contain combinations of over 100 proteins [2–4] that are unified by having ApoA-1 as their major backbone.

ApoC and ApoE containing lipoproteins

These are "conductor" lipoproteins that can orchestrate the efficiency of lipoprotein metabolism. These two lipoprotein classes are being constantly exchanged between HDL and VLDL/LDL particles after meals [5]. Their capacity to move between particles regulates the rate of fatty acid, cholesterol and phospholipid turnover [6].

Table 1.1 Classification of plasma lipoproteins by density

Fraction	Density (g/mL)	Composition
Very low density lipoproteins/ chylomicrons	1.006	Apo B48 (Chylomicrons), ApoB100(VLDL), Apo E, Apo A-I and Apo CIII makes most of these proteins
Low density lipoproteins	1.006–1.06	Apo B-100 and Apo E defines the majority of these proteins
High density lipoproteins	1.06–1.21	Proteins with multiple amphipathic helical domains: Apo A-I and Apo A-II make 90% of these proteins HDL is characterized by an increase in surface phospholipid to cholesterol surface ratio, making it an excellent cholesterol acceptor
Non-lipidated plasma proteins	1.21	Albumin is a major component of this fraction

Table 1.2 Types and functions of apolipoproteins

Apo A-I	HDL structural protein, it activates LCAT and participates in reverse cholesterol transport
Apo A-II	Forms HDL and activates hepatic lipase
Apo A-IV	Activates LCAT. Also involved in triglyceride metabolism
Apo B-48	Structural component of chylomicrons. Binds to LDL receptor
Apo B-100	Structural component of all lipoproteins except HDL and chylomicrons. Binds to LDL receptor
Apo C-I	Inhibits lipoprotein binding to LDL receptor. Activates LCAT
Apo C-II	Activates lipoprotein lipase
Apo C-III	Inhibits lipoprotein lipase. Antagonizes Apo-E, inhibiting liver VLDL uptake
Apo-E	LDL and LRP receptor ligand, and is essential component of reverse cholesterol transport and triacylglycerol clearance

ApoC lipoproteins regulate lipoprotein lipase activity. ApoE lipoproteins determine the ability to efflux cholesterol in the periphery and the fate of cholesterol rich particles secondary to its affinity to the LDL receptor [7] (Table 1.1, Table 1.2).

Lipoprotein Metabolism

Chylomicron Metabolism

The average daily intake of lipids in the US is 81 grams, of which more than 90% is triacylglycerol (also known as triglycerides) [8]. The remaining dietary lipids consist of cholesterol, cholesterol esters, phospholipids and fatty acids. Triacylglycerols

are not soluble in the blood and therefore do not circulate free in the serum but are transported as chylomicrons and VLDL particles [9]. Following ingestion, TG, cholesterol esters and phospholipids are digested by lingual lipase and gastric lipase in the stomach. TG is further hydrolyzed by pancreatic lipase to a mixture of 2-monoacylglycerol and free fatty acids, while cholesterol esters are processed by cholesterol esterase to cholesterol and free fatty acids. These products are packed with bile salts and fat soluble vitamins into mixed micelles which are then taken up by the mucosal cells (enterocytes) of the intestinal villi. Within the enterocytes TG are reformed through re-acylation of the 2-monoacylglycerols by monoacylglycerol acyltransferase and diacylglycerol acyltransferase, while cholesterol is esterified with fatty acids by cholesterol acyltransferase [10]. The reformed TG and cholesterol esters are packaged as chylomicrons (lipid droplets surrounded by a phospholipid layer, unesterfied cholesterol and additional apolipoprotein B-48 and apolipoprotein A1), released into the lymphatic vessels where they are transported from the thoracic duct to the bloodstream. Each chylomicron particle contains a single molecule of apoB48 and has a hydrophobic core consisting mainly of triglyceride with a small amount of cholesteryl esters. The ratio of triglycerides to cholesterol in chylomicrons is 8:1 or greater. This differentiates them from VLDL, IDL and chylomicron remnants which have much lower triglyceride to cholesterol ratio. Approximately 80–90 % of chylomicrons are triglycerides, and 55 % of VLDL are triglycerides [9, 10]. It takes approximately 10–12 h to clear the blood of chylomicrons after a meal. Peak lipidemia is reached in approximately 3–5 h and persists for another 6–8 h [9].

Once in plasma, the apo B48 on the chylomicron surface activates lipoprotein lipase (LPL) that is present at the endothelial surface of capillaries in most tissues of the body. Activity of LPL results in hydrolysis of the triacylglycerol in chylomicrons. As a result of LPL activity, free fatty acids are released to peripheral tissues, either as a source of energy or, in the case of adipose tissue, for storage after being re-esterified into triacylglycerol. As chylomicrons lose triacylglycerols, their particle size decreases and become relatively cholesterol enriched. A second fate of circulating TG is their transfer to HDL particles. Cholesterol ester transfer protein (CETP) mediates transfer of TG to HDL in exchange for cholesteryl esters from HDLs, as shown in Fig. 1.3. In the case of chylomicrons, these metabolic processes result in the formation of smaller, cholesterol-enriched particles (known as chylomicron remnants) that are rapidly cleared by the liver and only rarely accumulate in significant amounts in plasma. Chylomicron remnants are responsible for transporting dietary cholesterol and very efficient at taking cholesterol from HDL and RBCs to the liver [11]. Thus, they are essential components of the reverse cholesterol transport pathway to the liver. Defective clearance with an increase in circulating chylomicron remnants, as seen in individuals with abnormal apo E genotypes is considered atherogenic.

VLDL Metabolism

The initial step in VLDL synthesis involves synthesis of Apo B-100 on ribosomes attached to the endoplasmic reticulum. An enzyme called “microsomal triacylglycerol

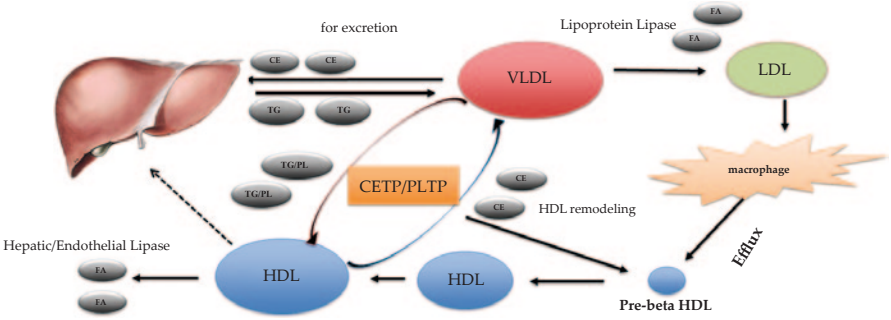

Fig. 1.3 Reverse cholesterol transport. This figure illustrates that the main mechanism for cholesterol transport back to the liver is through VLDL particles as a function of CETP in exchange for triglycerides. HDL can return cholesterol (*dashed line*), but this pathway contributes to less than 30% of the cholesterol ester pool returned to the liver [14]

transfer protein (MTTP or MTP)" assembles triacylglycerols and cholesterol with apolipoprotein B, E and a phospholipid. The next step involves transportation of fully lipidated VLDLs to the Golgi vesicles, where glycosylation proceeds, before they are transported to the plasma membrane and released into the space of Disse. Nascent VLDLs isolated from the Golgi apparatus contain newly synthesized apo E and apo C lipoproteins. They contain more phospholipids and much less unesterified cholesterol than plasma VLDLs. VLDL is secreted from the liver into the plasma. Triacylglycerols make up 50–60% of VLDL's weight. Triacylglycerols are the major fat to be transported from the liver into the bloodstream. VLDL also carries a lesser amount of cholesteryl esters in its core. It contains a number of apolipoproteins, but apo B-100 is necessary for its secretion from the liver. The circulatory half-life of VLDL particles is 30–60 min in humans. The contribution of VLDL cholesterol to the total cholesterol level is estimated by dividing the total triacylglycerol level by 5, because the average ratio of triacylglycerol to cholesterol on VLDL is 5–1. VLDL is metabolized in adipose tissue capillaries where Apo C-II on VLDL activates lipoprotein lipase (LPL) on adipose tissue capillaries. LPL is secreted into the interstitium by adipocytes and myocytes. It requires transport to the capillary lumen by Glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1 (GPIHBP1) [12]. LPL breaks down VLDL into fatty acids and glycerol. Fatty acids are taken up by the adipocytes for storage or for β oxidation in muscle.

During lipoprotein mediated lipolysis, VLDL is remodeled by the activities of cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP). CETP exchanges cholesterol esters between VLDL and HDL for triacylglycerols. PLTP facilitates the transfer of phospholipids from VLDL to HDL [13]. By this process, HDL unloads its cholesterol content to VLDL and LDL particles to be returned to the liver, primarily on VLDL particles as demonstrated in Fig. 1.3 [14] and acquires phospholipids that are essential for its capacity to accept cholesterol from cells and bind to steroidogenic cells. Recent studies confirm that HDL particles

(together with albumin) act as a "shuttle" to move cholesterol to other lipoproteins that have a greater capacity to carry and transport cholesterol (such as LDL) [15]. The remnant VLDL particle is called Intermediate density lipoprotein (IDL). IDL has Apo E which can bind to the LDL or LRP receptor and is taken up by the liver. IDL can also be acted upon by hepatic lipase which removes the remaining triacylglycerol leaving behind an Apo B containing triacylglycerol depleted particle known as LDL. IDL contains equal amounts of triacylglycerol and cholesterol.

The main mechanism that regulates VLDL secretion and uptake in the liver is under control of the Farsenoid X receptor (FXR) and SREBP1c transcription factors [16]. Very low density lipoprotein (VLDL) is synthesized in the liver in response to the ingestion of excess calories in the form of carbohydrates. Kinetic studies suggest that de novo lipogenesis contributes to only 3–5 % of VLDL production. However, high carbohydrate diet can increase VLDL synthesis up to 30 % and is an important mechanism for obesity induced dyslipidemia [17].Increased insulin and glucose following high carbohydrate diets stimulate SREBP1c transcription factor inducing the activity of several lipogenic enzymes (acetyl-CoA carboxylase, ATP citrate lyase, fatty acid synthase, and stearoyl-CoA desaturase). This process results in the synthesis of fatty acids that get conjugated to a glycerol backbone forming triacylglycerol. To export these fatty acids out of the liver, they are packaged with lipoproteins in the form of VLDL as described above. This is one of the mechanisms that explain the increased triglycerides (increased VLDL production) in metabolic syndrome. Clearance of hepatic triglycerides is regulated by the FXR system. Activation of hepatic FXR lowers plasma free fatty acid (FFA) and TG, likely resulting from (i) repression of hepatic TG and fatty acid (FA) synthesis as a result of SHP-dependent inhibition of SREBP-1c; (ii) induction of apoC-II and repression of apoC-III and ANGTPL3 in the liver, resulting in enhanced lipoprotein lipase (LPL) activity; (iii) induction of VLDL receptor (VLDLR) and human syndecan-1 (hSyndecan-1), thus promoting clearance of TG-rich lipoproteins; and (iv) induction of human PPARα and FA β-oxidation [18]. The use of FXR agonists in non-alcoholic fatty liver disease is discussed in Chap. 9.

LDL Metabolism

LDL is triacylglycerol depleted with only one apo B-100 particle. LDL particles have a great capacity to carry cholesterol that can facilitate cholesterol delivery to tissues for example: (1) cholesterol can be taken up by the liver via the LDL receptor for storage or repackaging (2) cholesterol can supplied to the gonads or adrenal glands with cholesterol for steroidogenesis or (3) donated for cell membrane biosynthesis. By weight LDL is approximately 50 % phospholipid and 50 % cholesteryl esters, unesterified cholesterol, and triacylglycerol. Thus, LDL carries more cholesterol per particle than other plasma lipoproteins. VLDL, IDL, and LDL particles each contain 1 molecule of apo B-100 per particle. As VLDL is converted into IDL and then LDL, the apo B-100 molecule remains with the lipoprotein particle until

it is removed from the blood. Total apo B in plasma therefore reflects the apo B on VLDL, IDL, and LDL. The LDL receptor is an essential mechanism for clearing both triglycerides and cholesterols from circulation. The liver is the main organ responsible for clearing LDL and remnant lipoproteins. Two apolipoproteins have important roles in regulating this process: Apo E and CIII. Underproduction of Apo E, or production of Apo E variants with decreased activity or levels (such as E4 genotype) delays clearance of both triglyceride and cholesterol enriched lipoproteins (chylomicron or VLDL remnants). Defects in cholesterol rich particle clearance are considered atherogenic. On one hand, overproduction of ApoE (as with the use of LXR agonists [19]) stimulates VLDL liver production and inhibits LPL mediated lipolysis. In this situation, clearance of cholesterol in TG rich lipoproteins is not impaired, and the ensuing hypertriglyceridemia is not considered atherogenic given that cholesterol from these particles is efficiently cleared by the liver LDL receptor. This mechanism might explain why isolated hypertriglyceridemia observed in familial hypertriglyceridemia syndromes is not associated with increased risk for atherosclerosis [20]. On the other hand, overproduction of both Apo E and Apo CIII (which is common after saturated fat or carbohydrate ingestion [5], and in diabetes/metabolic syndrome [21]) delays cholesterol clearance through competitive inhibition of CIII on Apo E mediated -VLDL receptor particle uptake [21]. Increased Apo CIII expression has been associated with both hypertriglyceridemia, small dense LDL formation [22] and atherosclerosis [23]. More recently, loss-of-function gene mutations of Apo CIII were associated with lower CHD risk [24, 25]. Small dense LDL particles interact more avidly with proteoglycans in the vascular wall and are more susceptible to oxidation [26], and thus considered an important contributor to atherosclerosis development.

HDL Metabolism

The HDL pathway is initiated by secretion of a nascent, disc-shaped apo A-I containing particle by hepatocytes and enterocytes, known as nascent HDL. The half-life of Apo A1 is about 4–5 days. Apo A-I in this nascent HDL particle activates the adenosine triphosphate (ATP)–binding cassette (ABC) protein, ABCA1, on the surface of peripheral cells such as macrophages. Once activated, the ABCA 1 protein transports unesterified cholesterol from the cell onto the nascent HDL particle. On the surface of HDL particle, the cholesterol is esterified by lecithin-cholesterol acyl transferase (LCAT) and its cofactor, apo A-I. As it circulates, nascent HDL particles are transformed into a mature, spherical HDL particle that contains cholesteryl ester in its core. The resulting cholesterol ester (CE) changes the shape of the HDL particle and is transferred via CETP to very low density (VLDL) and low density (LDL) lipoproteins after which it is finally taken up by the hepatic apo B, E receptors. The esterification of UC may be associated with remodeling of HDL sub-populations and with the formation of larger HDL particles that associate with less coronary artery disease (CAD). In physiological states, HDL particles are constantly shifting

between larger and smaller particles that facilitate the transport of cholesterol, triacylglycerol and phospholipids between the different lipoproteins and are important to the first step of reverse cholesterol transport. Obesity can accelerate HDL catabolism. TG enriched HDL in obesogenic states are susceptible to digestion by hepatic lipase resulting in smaller HDL particles that can get cleared by the kidney faster than larger HDL particles. An illustration of reverse cholesterol transport is provided in Fig. 1.3. Controversies on whether increasing HDL cholesterol represents improvements in reverse cholesterol transport are discussed in Chap. 5.

Phospholipid Metabolism

There are two major groups of phospholipids: glycerophospholipids with the glycerol backbone (such as phosphatidyl choline, ethanolamine or serine-PC, PE, PS), and sphingophospholipids with a sphingosine backbone (such as sphingomylin-SM and ceramide). An illustration of phospholipids is presented in Fig. 1.4. Phospholipids are the major structural components of the phospholipid bilayer of cell membranes. They are also very essential components of the lipoprotein surfaces. The majority of circulating phospholipids are on lipoproteins where they participate in cholesterol transport from and to tissues. Phospholipids have distinct roles in the process that leads to cardiovascular disease depending whether they circulate on HDL or LDL particles, and if they are oxidized or not. The major lipid constituents of HDL are phospholipids: HDL-PC, and HDL sphingomyelin (HDL-SM), followed by cholesterol and cholesteryl ester. There are three major mechanisms for phospholipid incorporation or assembly into lipoproteins. The first mechanism involves lipidation of ApoB containing particles in the liver by the activity of PLTP, where PC and SM are incorporated into the nascent VLDL particle. The second mechanism

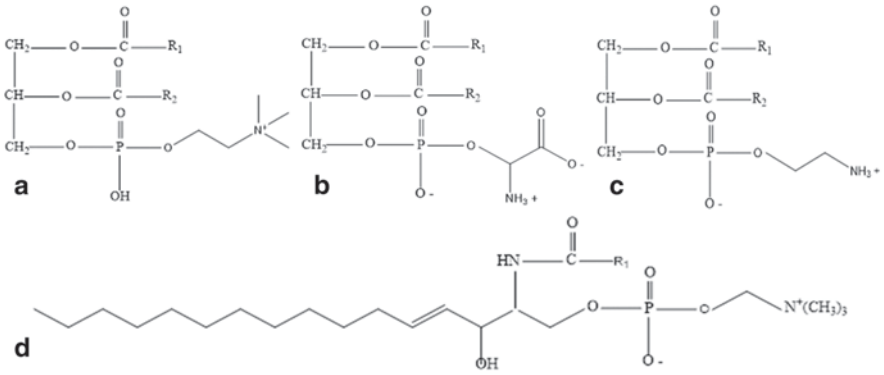

Fig.1.4 Phosphoplipids. **a** Phosphatidylcholine. **b** Phosphatidylserine. **c** Phosphatidylethanolamine. **d** Sphingomyelin. Phospholipids are amphiphilic molecules that have a polar head and a non-polar tail. These properties allow phospholipids to form the lipid bilayer portion of cell membranes and spontaneously form small vesicles in water

involves extracellular lipidation of nascent HDL as a function of ABCA-1 efflux of phospholipids. Recently, Sorci-thomas showed that the composition of nascent HDL is very similar to that of lipid rafts of plasma membranes and are formed by the activity of ABCA-1 [27]. The third mechanism involves the activity of phospholipid transfer protein (PLTP) in the plasma. PLTP transfers surface phospholipids from apoB containing particles to HDL during VLDL and chylomicron hydrolysis by lipoprotein lipase (LPL).

HDL-SM is considered atheroprotective and positively correlates with cholesterol efflux [28]. In contrast, LDL phospholipids are susceptible to oxidation and can be involved in promoting atherosclerosis. For example, LDL extracted from human atherosclerotic lesions is highly enriched with SM compared with plasma LDL, and SM carried into the arterial wall (associated with LDL) is acted upon by sphingomyelinase, increasing lesion ceramide levels, promoting LDL aggregation and foam cell formation [29]. More recent lipidomic studies suggest that certain species of shingolipids better define high risk CVD patients. Specifically, decreases in the long chain SM sphingolipids with an increase in the long chain ceramide sphingolipids characterize the plasma lipidomic profiles of high risk CVD.

Fatty Acids

Fatty acids have a hydrophilic carboxylic acid head and hydrophobic hydrocarbon tail. Fatty acids can be saturated or unsaturated (Fig. 1.5). Omega-3 fatty acids (also called ω-3 fatty acids or *n*-3 fatty acids) are polyunsaturated fatty acids (PUFAs) with a double bond (C=C) at the third carbon atom from the methyl (omega) end of the carbon chain. Fatty acids can be part of the cell membrane (part of phospholipids), or an energy source for the cell or a building block for more complex lipids. Fatty acid transportation and storage involve packaging fatty acids intro triglycerides (o triacylglycerols). Fatty acids can circulate unesterified, or bound to albumin. The regulation of fatty acid metabolism is coordinated in the liver, and their fate

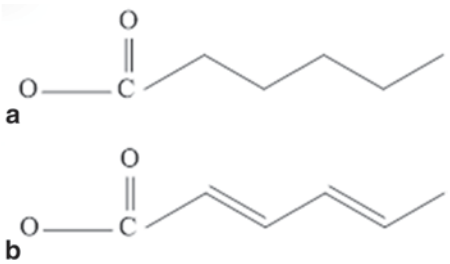

Fig. 1.5 Fatty Acids. **a** Saturated fatty acid (*Hydrocarbon chain only contains single bonds*). **b** Unsaturated fatty acid (*Hydrocarbon chain with single and double bonds*). Fatty acids have a hydrophilic carboxylic acid head and hydrophobic hydrocarbon tail. Fatty acids can be part of the cell membrane, an energy source for the cell or a building block for more complex lipids

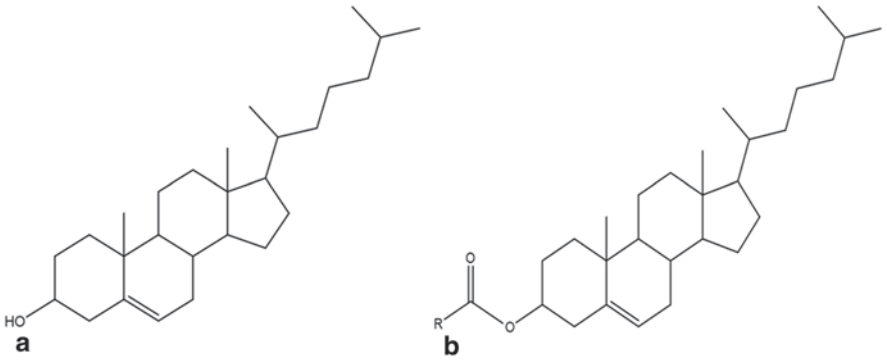

Fig.1.6 Cholesterol. **a** Cholesterol. **b** Cholesterol ester. Cholesterol is composed of a rigid ring structure, short hydrocarbon chain and polar hydroxyl group. Due to cholesterols stable structure cells are unable to degrade it. Therefore, it is either stored in the cell as a cholesterol ester or effluxed as free cholesterol

is dependent on lipase activities. In the muscle, lipoprotein lipase favors uptake of fatty acids for beta oxidation to generate energy. Each fatty acid molecule can liberate 9 Kcals of energy. Beta oxidation takes place in the mitochondria. In the adipose tissue, lipoprotein lipase favors storage of fatty acids in adipose cells where after uptake they are packaged into triacylglycerol droplets. In the liver, hepatic lipase regulates fatty acid liver uptake. Saturated fatty acid ingestion increases both LDL and HDL cholesterol. In contrast, ingestion of polyunsaturated fatty acids inhibits lipogenesis reducing triacylglycerol levels. Fatty acids regulate liver cholesterol and triacylglycerol by mechanisms that involve the liver sortlin 1 receptors and ERK signaling [30].

Cholesterol Metabolism

Cholesterol is composed of a rigid ring structure, short hydrocarbon chain and polar hydroxyl group and illustrated in Fig. 1.6. Cholesterol can circulate in plasma or can be packaged with lipoproteins in the form of free cholesterol or cholesterol esters. There is strong evidence from human and animals studies that link abnormal cholesterol metabolism to the development of atherosclerosis. Nikolai N. Anichkov first demonstrated the role of cholesterol in the development of atherosclerosis [31]. His classic experiments in 1913 involved feeding rabbits cholesterol-rich diets where he documented atherosclerotic lesions in the aorta. These experiments paved the way to our current understanding of the role of cholesterol in cardiovascular disease. Both human epidemiologic and genetic studies, together with the more recent statin intervention studies demonstrate a consistent strong association between non-HDL cholesterol levels and cardiovascular risk.

a) Sources of Cholesterol for the Humans There are two sources of cholesterol for the human body, de novo synthesis and diet. Biosynthesis of cholesterol accounts

for the majority of serum cholesterol (60–80 %) even when subjects are on a high cholesterol diet [32]. Intestinal absorption of cholesterol is the principal mechanism that regulates the contribution of dietary cholesterol to total cholesterol levels, with reduced cholesterol absorption at times of increased cholesterol consumption [32]. However, animal cholesterol intake efficiently shuts down liver synthesis of cholesterol and ultimately, cholesterol levels increase after prolonged cholesterol feeding. In contrast, ingestion of plant sterols can significantly decrease the amount of cholesterol absorbed and increase liver synthesis of cholesterol [33]. Niemann–Pick C1-Like 1 (NPC1L1) mediates intestinal cholesterol absorption and biliary cholesterol re-absorption [34]. This transporter is also the target of ezetimibe, an inhibitor of dietary cholesterol uptake which has been approved for the treatment of hypercholesterolemia. In contrast to NPC1L1, the heterodimer of ATP-binding cassette (ABC) transporters G5 (ABCG5) and G8 (ABCG8) has been shown to inhibit the absorption of cholesterol and plant sterols from the diet by mediating the efflux of these sterols from enterocytes back into gut lumen, and by promoting efficient secretion of cholesterol and plant sterols from hepatocytes into bile [35]. Sitosterolemia, a rare autosomal recessive disorder, is characterized by markedly elevated plasma levels of plant sterols and modest increases in plasma cholesterol, which is attributable to the hyper absorption of these sterols from the small intestine and reduced excretion into the bile [35].Our body synthesizes around 700 mg of cholesterol per day. The endoplasmic reticulum and cytoplasm are involved in the cholesterol biosynthesis. Although, any nucleated cell can synthesize cholesterol, the majority of blood cholesterol comes from the liver. Thus regulating the capacity of the liver to produce or catabolize cholesterol is critical to determining cholesterol levels in the blood. One example is the effect of statins on cholesterol metabolism. Statins can inhibit the production of cholesterol in the liver. This leads to the clearing of cholesterol from the body by up-regulating the liver LDL receptor.

b) Cellular Cholesterol Homeostasis There are three major mechanisms to control cellular cholesterol content. These are (1) de novo biosynthesis, (2) cholesterol uptake and esterification and (3) cholesterol efflux.

b.1.Cholesterol Biosynthesis The cholesterol biosynthesis is initiated when two molecules of acetyl-CoA condense to form acetoacetyl-CoA. This reaction is catalyzed by cytosolic thiolase. Acetoacetyl-CoA then condenses with another molecule of acetyl-CoA to form HMG-CoA; this reaction is catalyzed by HMG-CoA synthase. HMG-CoA is reduced to mevalonate by HMG-CoA reductase. This is the principal regulatory step in the pathway of cholesterol biosynthesis and the target of statins. The next stage is the formation of isoprenoid units from mevalonate by decarboxylation. Six isoprenoid units gather to form squalene. This in turn folds into lanosterol which is then converted into cholesterol in the membrane of the endoplasmic reticulum. Brown and Goldstein demonstrated an important mechanism by which the cell regulates its cholesterol content [36] through a cholesterol sensor system known as the sterol-regulatory-element-binding protein (SREBP-2) cleavage-activating protein (SCAP). When cholesterol levels are high, SREBP-2/SCAP is retained in the ER by binding to ISIG, a resident ER protein. When cholesterol

is low, the SREBP-SCAP complex exits from the ER, and SREBP undergoes two proteolytic cleavages. This releases the cytosolic domain of SREBP, which is then translocated into the nucleus regulating the transcription of many genes, including the LDL receptor and HMG-CoA reductase, the rate-limiting enzyme in cholesterol synthesis. Thus, this system regulates both the synthesis of cholesterol and its uptake by lipoproteins

b.2 Cellular Cholesterol Uptake and Esterification The major mechanism of cellular cholesterol uptake is through endocytic uptake of lipoproteins such as low-density lipoprotein (LDL) and hydrolysis of their cholesterol by cholesterol ester hydrolases [37]. Accumulation of free cholesterols in cells can lead to cell death [38], since cells cannot digest the cholesterol nucleus. To maintain a critical level of free cholesterol in the cell, it is stored in cells as cholesterol ester through the function of Acyl-Co A cholesterol acyl transferase (ACAT), forming lipid droplets. When there is a need for free cholesterol, cholesterol esters are hydrolyzed by neutral cholesterol ester hydrolases. The cholesterol released from the droplets can be used for cell membranes and, in steroidogenic cells, for steroid hormone synthesis. The cycle of cholesterol esterification and hydrolysis may provide an important buffering mechanism for maintaining cholesterol levels in cells. The activity of ACAT is regulated by cholesterol levels [38]. In the setting that favors the development of atherosclerosis, macrophages ingest modified LDL through scavenger receptors, a process that is independent of the LDL receptor and thus not subject to the cholesterol feedback mechanisms. Cholesterol esters build up forming foam cells. To prevent cell death, the macrophage lysosomes forms autophagosomes [39]. Cholesterol ester is hydrolyzed by the activities of lysosomal acid lipase to generate free cholesterol. These cellular cholesterol pools are dependent on the ABCA-1 transporter for cholesterol efflux as discussed below. Studies of Niemann-Pick disease type C (NPC), an inherited lysosomal storage disorder that leads to accumulation of cholesterol, have shown that a luminal protein (NPC2) and a transmembrane protein (NPC1) in late endosomes are required for efflux of cholesterol from these organelles [40].

b.3 Cholesterol Efflux and Excretion At the peripheral level (macrophages, that are of relevance to atherosclerosis), there are two major mechanisms for cholesterol efflux out of the cell to be incorporate into lipoproteins for liver excretion: First, there is an active mechanism that relies on the ABCA-1 transporter that usually gets activated after cholesterol loading of cells via Liver X Receptor (LXR) signaling. Second, there is a passive mechanism that relies on the cholesterol/phospholipid gradient between the cholesterol donor and acceptor. In the active process, the most avid cholesterol acceptor is lipid poor, Apo A-I. In the passive pathways, larger HDL particles with a large surface phospholipid to cholesterol ratio are the primary cholesterol acceptors. In addition to HDL, albumin, RBCs and other plasma proteins can accept cellular cholesterol and participate in reverse cholesterol transport [15, 41]. After cholesterol returns to the liver (chylomicrons in the fed state, VLDL and HDL in the fasting state), SREBP-1c is activated to assist with cholesterol storage, efflux or liver elimination in bile. This pathway links cholesterol and fatty

acid metabolism, perhaps as a means for the cell to achieve the appropriate ratio of cholesterol to other lipids and thereby maintain cellular membrane integrity. When insulin levels are high, SREBP-1c is transcribed at extremely high levels, and the resultant nuclear SREBP-1c activates genes necessary to produce fatty acids, which are incorporated into triglycerides. LXR-regulated genes include cholesterol 7α-hydroxylase (CYP7A1; [42]) which facilitates bile acid formation for elimination, the ATP-binding cassette transporter-1 (ABCA1; [43]) which is important for cholesterol efflux, and sterol regulatory element binding protein 1c (SREBP-1c, [44]), which governs the expression of stearoyl CoA desaturase (SCD-1) leading to the production of oleic acid needed for cholesterol esterification. Bile acids, the end products of hepatic cholesterol catabolism, are important for lipid digestion and absorption from the intestinal lumen, serve as signaling molecules, and also represent the principal means of eliminating cholesterol from the body. Importantly, in order to maintain whole body cholesterol homeostasis, approximately 5% of the bile acids secreted from the gall bladder into the duodenum are not reabsorbed and thus are excreted in the feces. FXR is a key sensor for bile acids and has a central role in maintaining bile acid homeostasis, as it regulates all aspects of bile acid metabolism, including bile acid synthesis, conjugation, secretion, absorption and refilling of the gall bladder [18]. More recently, a trans-intestinal mechanism has been described where cholesterol can be excreted directly through the intestine bypassing the liver [45].

Summary

Lipid metabolism is an integrated process through which peripheral tissues exchange cholesterol, fatty acids and phospholipids, and is mainly orchestrated in the liver. At times of high energy needs, fatty acids are oxidized in the muscle to produce energy. At times of excess calories, lipids are packaged and stored in the adipose tissue. Genetic defects in the LDL receptor or defects in particle clearance in obesity confer an increased risk for atherosclerosis, by favoring increases in circulating lipid species. The increased exposure to cholesterol or oxidized phospholipids favors inflammation in the artery wall, ultimately leading to wall thickening, plaque formation and rupture. Understanding the molecular mechanisms that facilitate lipid metabolism is critical to designing appropriate therapies to address the CVD risk.

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Chapter 2
Genetics of Lipid Disorders

Marija Stojanova Jordanov

Abbreviations

ABL	Abetalipoproteinemia
APOA1	apolipoprotein A-I
ApoB	Apolipoprotein B
APOE	apolipoprotein E
CHD	coronary heart disease
CHD	coronary heart disease
CMs	chylomicrons
CVD	cardiovascular disease
EGF	Epidermal growth factor-like domain
EGF-CA	Calcium-binding EGF-like domain
FCH	Familial combined hyperlipidemia
FDA	US Food and Drug Administration
FH	Familial hyperlipidemia
FHBL	familial hypobetalipoproteinemia
FLD	fatty liver disease
HA	hypoalphalipoproteinemia
HDL	High-density lipoprotein
HeFH	Heterozygous familial hypercholesterolemia
HMG-CoA reductase	hydroxymethylglutaryl-coenzyme A reductase
HoFH	Homozygous Familial Hypercholesterolemia
IDL	Intermediate-density lipoprotein
LCAT	lecithin-cholesterol acetyltransferase
LDL	low-density lipoprotein
LDLa	LDL receptor domain class A
LDLb	LDL receptor repeat class B

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LDL-C	low density lipoprotein- cholesterol
LDL-C	Low-density lipoprotein cholesterol
LDLR	low density lipoprotein receptor
Lp(a)	Lipoprotein(a)
LPL	lipoprotein lipase
LPL	lipoprotein lipase
mg	milligrams
mg/dl	milligrams/deciliter
ml	milliliter
mmol/l	milimol per litre
PAI-1	Plasminogen activator inhibitor-1
PCSK9	proprotein convertase subtilisin/kexin type 9
PLG	plasminogen
PPAR-alpha	Peroxisome proliferator-activated receptor alpha
PPARs	peroxisome proliferator-activated receptors
VLDL	Very-low-density lipoprotein

Genetics of Lipid Disorders

Hyperlipidemias is a group of disorders that can be classified as familial or primary caused by specific genetic abnormalities, or secondary to alterations in plasma lipid and lipoprotein metabolism [1]. Hyperlipidemia can be idiopathic, if the cause is not known. Based on which types of lipids are elevated, hyperlipidemias are classified as: hypercholesterolemia, hypertriglyceridemia or if both then combined hyperlipidemia. Increased levels of Lipoprotein (a) may also be classified as a form of hyperlipidemia. Fredrickson classification is the most common approach to classifying the types of the Familial hyperlipidemias and is based on the results of either the electrophoresis or ultracentrifugation and summarized in Table 2.1 [2]. In the first section of this chapter, we discuss the genetic basis for hyperlipidemias classified by Fredrickson. The second section of the chapter discusses the genetic basis of lipid disorders that have been characterized more recently.

Mendelian Randomization Studies as a Tool to Differentiate Lipid Disorders that Confer Increased Cardiovascular Disease Risk

Recently, genetic epidemiology has increased our understanding of lipid disorders that directly contribute to heart disease. Since genes are randomly assigned during meiosis (which gives rise to the name “Mendelian randomization”), carriers of certain genes that affect a marker of interest will not be systematically different from carriers of other alleles in any other respect, and in consequence there should be no

Table 2.1 Fredrickson classification of hyperlipidemias

Hyper-lipoproteinemia		Synonyms	Defect type	Increased lipoprotein	Main symptoms	Serum appearance	Estimated prevalence
Type I	a	Buerger-Gruetz syndrome or familial hyperchylomicronemia	Decreased lipoprotein lipase (LPL)	Chylomicrons	Acute pancreatitis, lipemia retinalis, eruptive skin xanthomas, hepatosplenomegaly	Creamy top layer	1 in 1,000,000
	b	Familial apoprotein CII deficiency	Altered ApoC2				
	c		LPL inhibitor				
Type II	a	Familial hypercholesterolemia	LDL receptor deficiency	LDL	Xanthelasma, arcus senilis, tendon xanthomas	Clear	1 in 500 for heterozygotes
	b	Familial combined hyperlipidemia	Decreased LDL receptor and increased ApoB	LDL and VLDL		Clear	1 in 100
Type III		Familial dysbetalipoproteinemia	Defect in Apo E 2 metabolism	IDL	Tubo-Eruptive Xanthomas & Palmar Xanthomas	Turbid	1 in 10,000
Type IV		Familial hypertriglyceridemia	Increased VLDL production and Decreased elimination	VLDL	Can cause pancreatitis at high triglyceride levels	Turbid	1 in 100
Type V			Increased VLDL production and Decreased LPL	VLDL and Chylomicrons		Creamy top layer & turbid bottom	

confounding. For example, Low-density lipoprotein LDL and high–density lipoprotein HDL are cholesterol fractions among the most commonly measured biomarkers in clinical medicine. Studies have shown that LDL and HDL cholesterol have opposing association with heart disease. For LDL cholesterol, the results of randomized trials of LDL-cholesterol-lowering treatments and from human mendelian diseases are similar and suggest that plasma LDL cholesterol is causally related to risk of myocardial infarction. This is not the case for HDL cholesterol disorders. The results from several Mendelian randomization studies challenge several established views about plasma HDL cholesterol [3] One example was greater HDL cholesterol levels in carriers of an endothelial lipase gene variant (that does not change levels of LDL or triglycerides) was not associated with a decreased risk of myocardial infarction. Hence, solo abnormalities in plasma HDL cholesterol cannot be assumed to be causally related to cardiovascular disease [3]

Genetic Basis for Fredrickson Classes

Familial Chylomicronemia

Synonyms: **hyperlipoproteinemia type I**, Lipoprotein lipase deficiency, chylomicronemia syndrome

Chylomicrons (from the Greek chylo, meaning juice or milky fluid, and micron, meaning small particle) are lipoprotein particles that consist of triglycerides (85–92 %), phospholipids (6–12 %), cholesterol (1–3 %), and proteins (1–2 %) [4]. They transport dietary lipids from the intestines to other locations in the body.

Chylomicrons are one of the five major groups of lipoproteins (chylomicrons, VLDL, IDL, LDL, HDL) that enable fats and cholesterol to move within the water-based solution of the bloodstream [4].

The chylomicronemia is characterized by severe hypertriglyceridemia and fasting chylomicronemia. Genetic causes of the syndrome are rare and include deficiency of lipoprotein lipase (LPL), apolipoprotein C-II, and presence of apolipoprotein CIII which is an inhibitor of LPL. Patients with familial forms of hypertriglyceridemia in combination with secondary acquired disorders (nephrotic syndrome, chronic kidney disease, Cushing's syndrome, and hypothyroidism) account for most individuals presenting with chylomicronemia [5].

Type I hyperlipoproteinemia (chylomicronemia) [6] exists in several forms (Table 2.1):

1. **Type Ia Chylomicronemia** is due to a deficiency of lipoprotein lipase (LPL) or altered apolipoprotein CII, resulting in elevated chylomicrons, the particles that transfer fatty acids from the digestive tract to the liver.
2. **Type Ib Chylomicronemia** is a condition caused by a lack of apolipoprotein CII that is lipoprotein lipase activator.

3. **Type Ic Chylomicronemia** is due to the presence a circulating inhibitor of lipoprotein lipase and hepatic lipase.

Type I hyperlipoproteinemia usually presents in childhood with eruptive xanthomata and abdominal colic. Complications include retinal vein occlusion, acute pancreatitis, steatosis and organomegaly, and lipaemia retinalis

Treatment

The treatment of patients with genetically inherited LPL and apoC-I1 deficiency primarily involves restriction of dietary fat to approximately 15% of total calories [7]. The degree of fat restriction (10 to 15 g of fat daily) required to achieve an acceptable plasma triglyceride concentration may be variable. Both unsaturated and saturated fats should be limited. Patients can be given supplements with medium-chain triglycerides as their cooking oils. Medium-chain triglycerides are directly absorbed into the portal vein and do not contribute to the formation of chylomicron triglycerides. However, reports of liver fibrosis have been associated with medium chain triglycerides, and thus they should be used with caution [8]. Treatment of acquired hypertriglyceridemias is covered in Chapter 6.

Familial Hypercholesterolemia

Synonyms: **Type IIA Familial Hypercholesterolaemia,** Hypercholesterolemia, Autosomal Dominant Hyperlipoproteinemia [9]

Familial hypercholesterolemia is a genetic disorder characterized by high cholesterol levels, specifically very high levels of LDL cholesterol (LDL-C) that cause atherosclerotic plaque deposition in arteries and a markedly increased risk of coronary artery disease at an early age. Cholesterol deposits are found in the tendons (xanthomas, Fig. 2.1) and/or around the eyes (xanthelasmas, Fig. 2.2) [10]. The

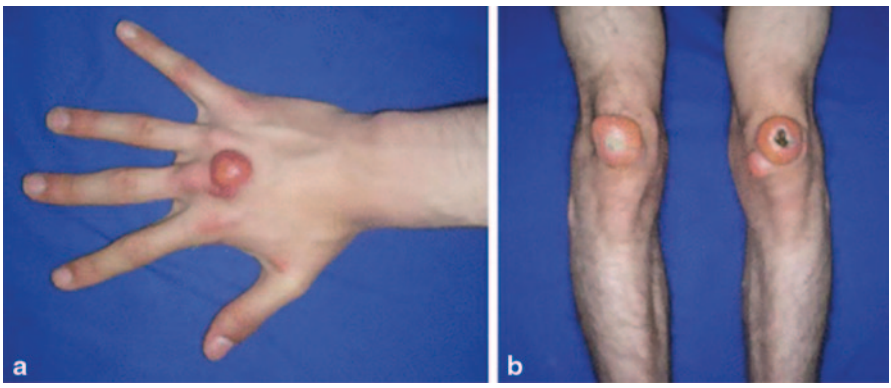

Fig. 2.1 Tendinous xanthoma, b. Bilateral ulcerated xanthomas on the extensor knee surface

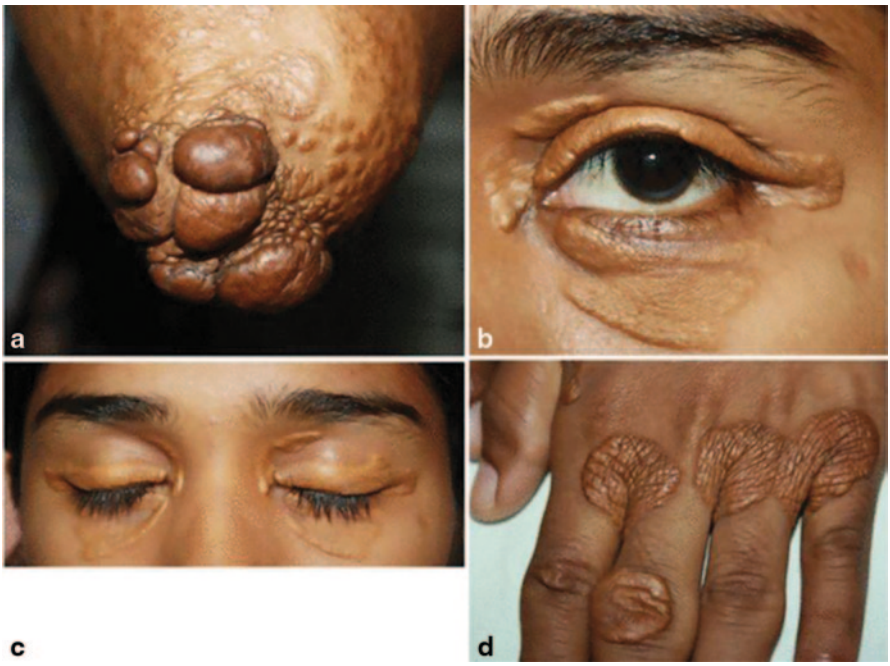

Fig. 2.2 a. Tuberous xanthoma of elbow b. Cutaneous xanthoma around the eye. c. Xanthelasma palpebrarum, arcus juvenalis. d. Intertrigenous xanthoma.

most common cardiovascular disease in FH is coronary heart disease (CHD), which may manifest as angina and myocardial infarction; stroke occurs more rarely.

Clinical Description

High cholesterol levels are not usually symptomatic [10]. Cholesterol deposits can be seen in different places on the body such as in the tendons of the hands, elbows, knees and feet, particularly the Achilles tendon (known as a tendon xanthoma), the eyelids (known as xanthelasma palpebrarum), and the outer margin of the iris (known as arcus senilis corneae).

The underlying cause of cardiovascular disease is the accelerated deposition of cholesterol in the walls of arteries which leads to atherosclerosis. FH causes development of coronary artery disease at a much younger age than would be expected in the general population [11]. This leads in many cases to angina pectoris or heart attacks. The arteries of the brain are less commonly affected, and this may lead to transient ischemic attacks or stroke. Peripheral artery occlusive disease occurs mainly in people with FH who smoke. Atherosclerosis risk is increased further with age and in those who smoke, have diabetes, high blood pressure and a family history of cardiovascular disease [12].

Mode of Inheritance

The two forms of FH, heterozygous familial hypercholesterolemia (HeFH) and homozygous familial hypercholesterolemia (HoFH) are inherited in an autosomal dominant manner. Patients who have one abnormal copy of the LDLR gene are heterozygous and patients who have two abnormal copy of the LDLR gene are homozygous. Heterozygous FH is a common genetic disorder, occurring in 1:500 people in most countries [13]. Homozygous FH is much rarer, occurring in 1 in a million births.

Total cholesterol levels of 350–550 mg/dL are typical of heterozygous FH while total cholesterol levels of 650–1000 mg/dL are typical of homozygous FH [14]. LDLR mutations are more common in certain populations. The Africans, French Canadians, Lebanese Christians, and Finns have high rates of specific LDLR mutations that make FH particularly common in these groups. ApoB mutations are more common in Central Europe (Fig. 2.3).

Approximately all affected individuals that are diagnosed with HeFH have an affected parent. If the pathogenic variant found in the affected person cannot be detected in leukocyte DNA of either parent, two possible explanations are germline mosaicism in a parent or de novo mutation in the affected person [9] Even though most individuals diagnosed with HeFH have an affected parent, the family history may appear to be negative because of failure to recognize the disorder in family members, early death of the parent before the onset of symptoms, or late onset of the disease in the affected parent.

The risk to the siblings of the affected person depends on the genetic status of the parents. If a parent is affected or has a pathogenic variant, the risk to the siblings is 50%. If both parents are affected with HeFH or have a pathogenic variant, the risk to siblings of having HeFH is 75% (50% chance of HeFH and a 25% chance of HoFH) [9].

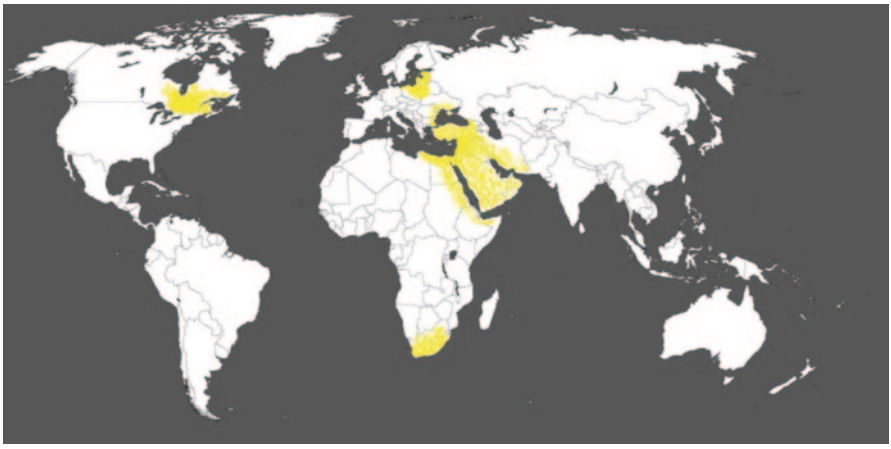

Fig. 2.3 Familial Hypercholesterolemia concentrations in the world's populations. Groups with increased prevalence of FH are highlighted

Molecular Genetics

The most common genetic defects in FH are LDLR mutations (prevalence 1 in 500, depending on the population), ApoB mutations (prevalence 1 in 1000), PCSK9 mutations (less than 1 in 2500) and LDLRAP1 [10].

a. LDLR

LDLR encodes a mature protein product of 839 amino acids. LDLR has four distinct functional domains that can function independently of each other [9]:

- LDL receptor domain class A (LDLa)
- Epidermal growth factor-like domain (EGF)
- Calcium-binding EGF-like domain (EGF-CA)
- LDL receptor repeat class B (LDLb)

LDLR is made of cell surface proteins involved in endocytosis of LDL cholesterol (LDL-C). Once LDL-C is bound at the cell membrane, it is taken into the cell and to lysosomes where the protein moiety is degraded and the cholesterol molecule suppresses cholesterol synthesis via negative feedback.

Pathogenic variants in LDLR usually reduce the number of LDL receptors produced within the cells or disrupt the ability of the receptor to bind LDL-C. Either way, people with a heterozygous pathogenic variant in LDLR generally have high levels of plasma LDL-C.

b. ApoB

ApoB is 42,216 base pairs in length, comprising 28 introns and 29 exons. The gene product is the main apolipoprotein of chylomicrons and low density lipoproteins. ApoB has four functional domains [9]:

- Synthesis, assembly, and secretion of hepatic triglyceride-rich lipoproteins
- Binding of lipids and serving as a structural component of very low density lipoproteins (VLDL) and LDL
- Binding of heparin and various proteoglycans found in the arterial wall
- Interaction with the LDL receptor, important for clearance of LDL from plasma

ApoB is generally involved in aiding the binding of LDL-C to its receptor on the cell surface. ApoB pathogenic variants alter the ability of protein to effectively bind LDL-C to LDLR, causing fewer LDL-C particles to be removed from the blood.

c. PCSK9 (proprotein convertase subtilisin/kexin type 9)

PCSK9 [15] is the gene located on the short (p) arm of chromosome 1 at position 32.3. This gene encodes a protein consisting of 692 amino acids and three main

domains. Mutated alternates in this gene have been linked both with hypercholesterolemia and hypocholesterolemia. “Gain-of-function” is description for mutations that are responsible for hypercholesterolemia because they appear to increase the activity of the PCSK9 protein or to give the protein a new, different function. The consequence of the overactive PCSK9 protein is the significant reduction in the number of LDL receptors on the surface of liver cells. The excess cholesterol is placed abnormally in tissues such as the skin, tendons, and coronary arteries, which greatly increases a person’s risk of having a heart attack.

Other genetic changes in the PCSK9 gene result in an opposite effect – reduced blood cholesterol levels (hypocholesterolemia). These mutations decrease the activity of the PCSK9 protein or decrease the amount of this protein that is produced in cells. This type of mutation is described as “loss-of-function.” The nonsense mutation (*PCSK9142X PCSK9679X*) is the most common “loss-of-function” mutation in the PCSK9 gene and leads to an increase in the number of low-density lipoprotein receptors on the surface of liver cells. These additional receptors can remove low-density lipoproteins from the blood more rapidly than usual, which reduces the amount of cholesterol circulating in the bloodstream. Different studies advocate that people with reduced cholesterol levels caused by PCSK9 mutations have a significantly lower-than-average risk of developing coronary heart disease [15].

Treatment Guidelines for the management of Familial Hyperlipidemia are discussed in Chapter 6. All individuals with FH should be classified as high risk for cardiovascular disease (CVD) and should be aggressively treated actively to lower their cholesterol levels [9].

Heterozygous FH is typically treated with statins [14]. Statins efficiently lower cholesterol and LDL levels, even though sometimes supplemental therapy with other drugs is necessary, such as bile acid sequestrants (cholestyramine or colestipol), nicotinic acid preparations or fibrates [10].

Homozygous FH is harder to treat. In individuals with Homozygous FH the LDL receptors are minimally functional, if at all. Only high doses of statins, often in combination with other medications, are modestly effective in improving lipid levels [11]. If medical therapy is not successful at reducing cholesterol levels, LDL apheresis may be used, this filters LDL from the bloodstream in a procedure similar to kidney dialysis [10].

Lomitapide, an inhibitor of the microsomal triglyceride transfer protein was approved by the FDA in December 2012 as an orphan drug for the treatment of homozygous familial hypercholesterolemia [16] In January 2013, The FDA also approved mipomersen, which inhibits the action of the gene apolipoprotein B [17].

Children should be considered for drug treatment with statin-based regimens when:

LDL-C levels are ≥190 mg/dL (≥4.9 mmol/L).

LDL-C levels are ≥160 mg/dL (≥4.1 mmol/L) and at least two other risk factors are present.

A multidisciplinary expert panel in 2006 advised on early combination therapy with LDL apheresis, statins and cholesterol absorption inhibitors in children with homozygous FH at the highest risk [13]

Familial Combined Dyslipidemia

Synonym: **hyperlipoproteinemia type IIb**

Combined hyperlipidemia also known as "Multiple-type hyperlipoproteinemia" is a subtype of hypercholesterolemia characterized by increased LDL and triglyceride concentrations, frequently accompanied by decreased HDL. It is the most common inherited lipid disorder, with occurrence of 1/200 persons. In fact, almost 20% from the people who develop coronary heart disease before the age of sixty will have this disorder [1]. The elevated triglyceride levels (>90 mg/dl) are generally due to an increase in VLDL (very low density lipoprotein), a class of lipoprotein that is prone to cause atherosclerosis.

There are two forms of this lipid disorder. This disease is common in patients with metabolic syndrome ("syndrome X", incorporating diabetes mellitus type II, hypertension, central obesity and CH).Excessive free fatty acid production by various tissues leads to increased VLDL synthesis by the liver. Initially, most VLDL is converted into LDL until this mechanism is saturated, after which VLDL levels elevate.Fibrate drugs are used for treatment of both forms and they act on the peroxisome proliferator-activated receptors (PPARs), specifically PPARα, to decrease free fatty acid production. Statin drugs, especially the synthetic statins (atorvastatin and rosuvastatin) can decrease LDL levels by increasing hepatic reuptake of LDL due to increased LDL-receptor expression. The management of this disease is discussed in more detail in Chap. 6.

Type III Dyslipidemia

Synonyms: **Familial dysbetalipoproteinemia**

Type III dyslipidemia, also called type III hyperlipoproteinemia, it is partially caused by mutation in the APOE gene. The effect of this mutation is decrease in the hepatic uptake of APOE-containing lipoproteins and reduction in the conversion of VLDL and IDL to LDL particles [18]. If other factors are not present, remnants do not accumulate to a degree enough to cause hyperlipidemia.

Dysbetalipoproteinemia happens when an ApoE defect (almost always the E2/E2 genotype) occurs in combination with a second genetic or acquired defect that causes either overproduction of VLDL (such as FCHL) or a reduction in LDL receptor activity (such as occurs in heterozygous FH or hypothyroidism). The frequency of the Dysbetalipoproteinemia is estimated to be about 1/10,000.There other less frequent causes for dysbetalipoproteinemia are ApoE variants such as ApoE3-leiden and ApoE2 (lys146→Gln) can also be causes. Typical for patients with dysbetalipoproteinemia is to have elevated levels of both cholesterol and triglycerides. They are likely to develop premature CVD and are at increased risk for peripheral vascular disease. Clinical signs of dyslipidemia show differently in both genders, and usually do not develop before adulthood in men or before menopause

in women. Pathognomonic physical signs are palmar xanthomas, and orange lipid deposits in the palmar creases may or may not appear. Tubero eruptive xanthomas are occasionally found at pressure sites on the elbows, buttocks, and knees.

Candidates for dysbetalipoproteinemia are patients with elevated total cholesterol and triglyceride levels that range from 300 to 1000 mg/dl and are roughly equal. Ultracentrifugation can be used to determine the presence of cholesterol-enriched VLDL particles. The useful way to determine the dysbetalipoproteinemia is by demonstrating the presence of E2/E2 genotype

Familial Hypertriglyceridemia

Synonyms: **Hyperlipoproteinemia type IV**

Familial hypertriglyceridemia type IV is subtype of hyperlipidemia, inherited in an autosomal dominant manner. It is a frequent condition with 1 % occurrence in the whole population. This disorder is characterized by elevated triglycerides levels as a result of excess hepatic production of VLDL or heterozygous LPL deficiency. The level of the cholesterol is not affected. Premature coronary disease is not associated with familial hypertriglyceridemia. The triglyceride level ranges from about 250 to 1000 mg/dl in approximately one half of first-degree relatives [19].

It is not typical for persons with familial hypertriglyceridemia to experience any symptoms. A strong indicator of this disorder is the familiar history. However, there is an increased risk of developing pancreatitis.

Hypertriglyceridemia Type V

Synonyms: **Hyperlipoproteinemia type V**

Both type I and type V hyperlipoproteinemia are characterized by severe hypertriglyceridemia due to an increase in chylomicrons. Type I hyperlipoproteinemia is caused by genetic abnormalities of the lipoprotein lipase (LPL)- apolipoprotein C-II system, whereas the cause of type V hyperlipoproteinemia is more complicated and more closely related to acquired environmental factors (heavy drinking, type 2 diabetes,

hormonal therapy using steroids and estrogen, and drugs such as diuretics and β –blockers) resulting in elevations of both VLDL and chylomicrons. Since the relationship of hypertriglyceridemia with atherosclerosis is not as clear as that of hypercholesterolemia, and since type I and V hyperlipoproteinemia are relatively rare, few guidelines for their diagnosis and treatment have been established. Type I and V hyperlipoproteinemia are clinically important as underlying disorders of acute pancreatitis, and appropriate management is necessary to prevent or treat such complications [6].

The Genetic Basis for Other Lipid Disorders

Lipoprotein(a)

Lipoprotein(a) [Lp(a)] is a part of lipoprotein subclass made up of an LDL-like particle and the specific apolipoprotein(a) [apo(a)], which is covalently bound to the apoB of the LDL like particle. Plasma concentrations of Lp(a) are genetically determined and are mostly controlled by the apolipoprotein(a) gene [LPA] located on chromosome 6q26–27 [20]. Lipoprotein(a)'s structure is very similar to plasminogen and tPA (tissue plasminogen activator) and it competes with plasminogen for its binding site, leading to reduced fibrinolysis. Also, since Lp(a) stimulates secretion of Plasminogen activator inhibitor-1 (PAI-1), it leads to thrombogenesis. Lp(a) also transports cholesterol and thus contributes to atherosclerosis [16]. In addition, Lp(a) transports the more atherogenic proinflammatory oxidized phospholipids, which attract inflammatory cells to vessel walls and leads to smooth muscle cell proliferation. The concentrations of Lp(a) vary widely between individuals, from <0.2 to >200 mg/dL. These concentrations differences are observed in all populations studied. Different world populations show wide variations in mean and median concentrations, sometimes two to three folds higher from one to another. For example, Lp(a) plasma concentration of populations of African descent compared to Asian, Oceanic, or European populations is two to three folds higher. The physiological function of Lp(a)/apo(a) is still unclear. High concentrations of Lp(a) in blood is related to increased risk of coronary heart disease (CHD), cerebrovascular disease (CVD), atherosclerosis, thrombosis, and stroke [21]. The connection between Lp(a) levels and stroke is not as strong as that between Lp(a) and cardiovascular disease [22]. Lp(a) concentrations may be linked to certain disease states, (for example kidney failure), but are only slightly affected by diet, exercise, and other environmental factors.

High Lp(a) increases the risk of early atherosclerosis in patients with no other known cardiac risk factors, including high concentrations of LDL cholesterol. In patients who already have cardiovascular disease, Lp(a) contributes to an additional coagulant risk of plaque thrombosis. Lp(a) has domains that are very similar to plasminogen (PLG). Accumulation of Lp(a) in the vessel wall it inhibits binding of PLG to the cell surface that results in reducing plasmin generation, which increases clotting. Lp(a) also promotes proliferation of smooth muscle cells. All these features of Lp(a) cause generation of clots, atherosclerosis which consequently leads to coronary artery disease [21] It is found that in isolated homogeneous tribal population of Tanzania, vegetarians have higher levels of Lp(a) than fish eaters. This is raising the possibility that pharmacologic amounts of fish oil supplements may be helpful to lower the levels of Lp(a) [21].

Treatment

The results of many trials and meta analyses using statin medications suggests that lipid reducing drugs, with exception of atorvastatin [23]have little or no effect on

Lp(a) concentration. Aspirin and Nicotinic acid (Niacin) are drugs known to significantly reduce the levels of Lp(a) in some individuals with high Lp(a). They are safe, easily available, and inexpensive and should be used under the supervision of a qualified physician.

Sitosterolemia

Synonym: Phytosterolemia

Sitosterolemia is a lipid metabolic disorder that is inherited autosomal recessively. It is found in patients who have increased absorption and decreased biliary excretion of dietary sterols leading to hypercholesterolemia [24]. Several plant sterols accumulate in the body under this disorder, Sitosterol being one of them. Because plant sterols are not produced in the body but part of food intake, the signs and symptoms of this disorder appear early in life once the food containing plant sterols are consumed. On a worldwide level, only 45 cases have been reported in the medical literature, making this disease relatively rare. It is likely that Sitosterolemia is misdiagnosed in many patients with hyperlipidemia [24].

Clinical Description

Sitosterolemia is clinically very similar to familial hypercholesterolemia (FH). It is characterized by the appearance of tendon xanthomas in the first 10 years of life and the development of premature atherosclerosis. However, unlike FH patients, sitosterolemia patients usually have normal to moderately elevated total sterol levels and very high levels of plant sterols (sitosterol, campesterol, stigmasterol, avenosterol) and 5α-saturated stanols in their plasma. Plasma sitosterol levels in sitosterolemia patients are 10–25 times higher than in normal individuals (8–60 mg/dl). Not all patients with sitosterolemia have tendon xanthomas, therefore its absence should not be used to exclude this diagnosis [24]. Lipid plaques (xanthomas) may appear at any age, even in childhood. These may be present as subcutaneous xanthomas on the buttocks in children or in usual locations (e.g., Achilles tendon, extensor tendons of the hand) in children and adults. Corneal arcus and xanthelasma are less common. Decreased range of motion with possible redness, swelling, and warmth of joints due to arthritis may be present. In addition, sitosterolemia patients may develop hemolytic episodes and splenomegaly. Untreated, the condition causes a significant increase in morbidity and mortality. Coronary heart disease and its inherent health consequences are the primary causes of illness and premature death in untreated patients [24].

Genetics

Sitosterolemia is autosomal recessively inherited. The genetic causes are related to mutations of two opposite genes (ABCG5 and ABCG8) located in chromosome 2

in band 2p21. These genes are responsible for encoding for ABC transporter proteins named sterolin-1 and sterolin-2, respectively. As a result of this mutation, the mechanism for active pumping back into intestine of passively absorbed plant sterols is disrupted and hepatic secretion is decreased due to the accumulation of these sterols. Over time, the liver functions deteriorate. Although bile acid synthesis remains unchanged, the total amount of sterols in the bile is decreased by 50%. The mechanism for decreased hepatic secretion is unclear. The whole body cholesterol biosynthesis associated with suppressed hepatic, ileal, and mononuclear leukocyte hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA reductase), the rate-controlling enzyme in the cholesterol biosynthetic pathway is noticeably reduced in these patients. The down-regulation due to accumulated sitosterol is controversial but most recent data indicate that secondary effects of unknown regulators other than sitosterol can lead to reduced HMG-CoA reductase activity in the disease. This is coupled with significantly increased low-density lipoprotein (LDL) receptor expression.

Treatment

By adjusting the diet and significantly reducing the consumption of the food that contains plant sterols such as: vegetable oils, olives, avocados, etc. this disorder can be managed effectively. Since plant sterols are present in all foods, diet adjustment may not be sufficient to control this disease. Statins are usually prescribed medication to lower cholesterol levels and to protect from atherosclerotic disease. Also, bile acid-binding resins such as cholestyramine or colestipol could be considered. In October 2002, ezetimibe, received US Food and Drug Administration (FDA) approval for use in sitosterolemia. This drug is now the standard of care, as it blocks sterol entry and can be used in combination with bile-acid resins. Finally, ileal bypass has been performed in select cases to decrease the levels of plant sterols in the body, though this therapy was undertaken prior to the advent of ezetimibe.

Low HDL Syndromes

Synonyms: **Hypoalphalipoproteinemia**

The deficiency of high-density lipoprotein (HDL) causes hypoalphalipoproteinemia that are summarized in Table 2.2. The mechanism of how HDL accelerates the development of atherosclerosis is not established, but may involve impairments in reverse cholesterol transport, or alterations in oxidations or inflammation. As discussed in the introduction, low HDL cholesterol as an isolated finding is not causally related to cardiovascular disease. There are no characteristic physical findings in the mild forms of hypoalphalipoproteinemia (HA), except some patients may have premature coronary heart or peripheral vascular disease, as well as a family history of low HDL cholesterol levels and premature CHD. Severe HDL deficiencies caused by rare autosomal recessive disorders, including familial hypoalphalipoproteinemia

Table 2.2 Hypoalphalipoproteinemia

Variant	Molecular Defect	Inheritance	Metabolic Defect	Lipoprotein abnormality	Clinical features	Premature atherosclerosis
Familial apo A-I	Apo deficiency	Autosomal codominant	Absent apo A-1 biosynthesis [31]	HDL < 5 mg/dL; TGs normal	Planar xanthomas, corneal opacities [32–34]	Yes
Familial apo A-I structural mutations	Abnormal apo A-I	Autosomal dominant	Rapid apo A-1 catabolism [35, 36]	HDL 15–30 mg/dL; TGs increased	Often none; sometimes corneal opacities [37, 38]	No
Familial LCAT	LCAT deficiency (complete)	Autosomal recessive	Rapid HDL catabolism [39]	HDL < 10 mg/dL; TGs increased	Corneal opacities, anemia, proteinuria, renal insufficiency [40-42]	No
Fish-eye disease	LCAT deficiency (partial)	Autosomal recessive	Rapid HDL catabolism [43]	HDL < 10 mg/dL; TGs increased	Corneal opacities [44–46]	No
Tangier disease	Unknown	Autosomal codominant	Very rapid HDL catabolism [47, 48]	HDL < 5 mg/dL; TGs usually increased	Corneal opacities, enlarged orange tonsils, hepatosplenomegaly, peripheral neuropathy [49, 50]	No to yes
Familial HA	Unknown	Autosomal dominant	Usually rapid HDL catabolism [51]	HDL 15–35 mg/dL; TGs normal	Often none; sometimes corneal opacities [52, 53]	No to yes

(HA), familial lecithin-cholesterol acetyltransferase (LCAT) deficiency, and Tangier disease [25]

Familial Hypoalphalipoproteinemia or Familial ApoA-I Deficiency

Criteria for the definition of familial HAs are:

(1) A low HDL cholesterol level in the presence of normal VLDL cholesterol and LDL cholesterol levels
(2) (An absence of diseases or factors to which HA may be secondary, and
(3) The presence of a similar lipoprotein pattern in a first-degree relative

Familial HA is a relatively common disorder and is frequently associated with decreased Apo A-I production or increased Apo A-I catabolism [25]

Apo A-I Milano was first identified by Dr. Cesare Sirtori in Milan, who also demonstrated that its presence significantly reduced cardiovascular disease, even though it was associated with a reduction in HDL levels and an increase in triglyceride levels. ApoA-I Milano (also ETC-216, now MDCO-216) is a naturally occurring mutated variant of the apolipoprotein AI protein found in human HDL, the lipoprotein particle that carries cholesterol from tissues to the liver and is associated with protection against cardiovascular disease [26]

Tangier Disease

Tangier disease is caused by mutations in the ABCA1 gene. Patients with this disease have nerve function disorders; swollen orange-colored tonsils; and corneal clouding. In contrast, it is not typical for the patients with familial HDL deficiency to have these additional features [27].

Familial Lecithin Cholesterol Acyltransferase Deficiency (LCAT deficiency)

This is a very rare autosomal recessive disorder. The disease has two forms [28]:

a. a. Familial LCAT deficiency in which there is complete LCAT deficiency.
b. b. Fish eye disease in which there is a partial deficiency.

Both variants are autosomal recessive disorders whose origin is in mutations of the LCAT gene located on chromosome 16q22. A deficiency of LCAT causes buildup of unesterified cholesterol in body tissues. Cholesterol flows out from cells as free cholesterol and it is transported in HDL as esterified cholesterol. LCAT is the enzyme that esterifies the free cholesterol on HDL to cholesterol ester and allows the maturation of HDL.

When LCAT deficiency happens, it does not allow for HDL maturation, resulting in its rapid catabolism of circulating apoA-1 and apoA-2. The remaining form of

HDL resembles a beginning form of HDL. Symptoms of the familial form include scatter corneal opacities, target cell hemolytic anemia and proteinuria with renal failure. Fish eye disease only causes progressive corneal opacification.

Low LDL Syndromes

Synonym: **Hypobetalipoproteinemia**, **Abetalipoproteinemia**

Abetalipoproteinemia (ABL) and **familial hypobetalipoproteinemia (FHBL)** are rare hereditary disorders of lipoprotein metabolism that cause low cholesterol levels. Persons with these two conditions exhibit an enhanced tendency to develop fatty liver disease (FLD) [29]. The hallmark of these disorders is a profound reduction of LDL cholesterol that may confer a decreased risk for heart disease. ABL is a rare disease where LDL and very low-density lipoprotein (VLDL) are essentially absent. The clinical signs of this disorder are: fat malabsorption, spinocerebellar degeneration, acanthocytic red blood cells, and pigmented retinopathy. The genetic cause for it a homozygous autosomal recessive mutation in the gene for microsomal triglyceride transfer protein MTP. The function of the MTP is to mediate the intracellular lipid transport in the intestine and liver and consequently ensures the normal function of chylomicrons (CMs) in enterocytes and of VLDL in hepatocytes [30].Affected infants may appear normal at birth, but by the first month of life, they develop steatorrhea, abdominal distention, and growth failure. Children develop retinitis pigmentosa and progressive ataxia, with death usually occurring by the third decade. Early diagnosis, high-dose vitamin E (tocopherol) therapy, and medium-chain fatty acid dietary supplementation may slow the progression of the neurologic abnormalities. Obligate heterozygotes (i.e., parents of patients with ABL) have no symptoms and no evidence of reduced plasma lipid levels [12].

FHBL is also a rare disorder of apolipoprotein B (apoB) metabolism characterized by levels of plasma cholesterol and LDL cholesterol that are less than one-half normal in heterozygotes and are very low (< 50 mg/dL) in homozygotes. FHBL is caused by several mutations that include an autosomal, codominant mutation in the gene for apoB (APOB) [12], which is carried on chromosome 2. This mutation results in a shortened form of apoB. Mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9) can also produce a similar phenotype.

Homozygotes present with fat malabsorption and low plasma cholesterol levels at young age. They develop progressive neurologic degenerative disease, retinitis pigmentosa, and acanthocytosis, similar to patients with ABL. Although heterozygotes are usually asymptomatic, they exhibit decreased LDL cholesterol and apoB levels and possibly have a decreased risk of atherosclerosis [31].

There are non-familial forms of hypobetalipoproteinemia that are typically secondary to a number of clinical states, such as occult malignancy, malnutrition, and chronic liver disease.

Treatment The cornerstone of treatment include adherence to a low-fat diet, and supplementation with essential fatty acids and high oral doses of fat soluble vita-

mins. Prognosis is variable, but early diagnosis and strict adherence to treatment can recover normal neurological function and halt disease progression [32].

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Chapter 3
Lipid and Inflammation in Atherosclerosis

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Introduction

Inflammation is an adaptive response that is triggered by many agents and conditions, and its regulation is dependent on a complex network of cytokine and chemokine signaling between key cells including endothelial cells, monocytes, and lymphocytes.

The role of inflammation in atherosclerosis was recognized many decades ago by Rudolph Virchow (1821–1902) who coined the term 'endarteritis deformans' in describing atherosclerosis [1]. Subsequent work by Russell Ross in the 1970s reintroduced the concept of vascular injury as a basis for the development of atherosclerosis [2]. More recent investigations by Peter Libby and Paul Ridker have built a strong case for the role of inflammation as an important player in atherosclerosis

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while using serum markers as a risk factor to predict cardiovascular events [3–4]. However, the agents that incite inflammation in the arterial wall have remained largely unrecognized while the primary focus of investigations has been on biomarkers of inflammation.

Although infection is the best understood trigger of inflammation, its role in atherosclerosis has not been well established. One study demonstrated that endogenous substances were found to initiate the inflammation leading to atherosclerosis in germ-free animals, suggesting that infectious agents may not be major initiators of atherosclerosis [5]. Other studies were conducted to connect non-infectious agents that trigger inflammation to the formation of atherosclerosis. In the 1970s, Donald Small evaluated cholesterol crystals and their potential role in atherosclerosis but concluded that cholesterol crystals were an 'inert' element [6]. More recently, Daniel Steinberg et al. demonstrated that oxidized low density lipoprotein (OxLDL) is a primary trigger that activates macrophages in the plaque [7]. OxLDL was demonstrated to disrupt the endothelial cell surface, promote inflammation and stimulate the immune system via cytokine release from macrophages and antibody production.

Various types of serum biomarkers have been implicated in the inflammation associated with atherosclerosis, in particular with cardiovascular disease, and the list continues to grow steadily. The most prominent biomarker has been high-sensitivity C-reactive protein (hs-CRP) and others including Interleukin 6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), myeloperoxidase (MPO), and granulocyte-macrophage colony stimulating factor (GM-CSF). Evidence from several clinical trials has demonstrated that inflammatory biomarkers could be predictive of future acute cardiovascular events [4, 8–13]. High-sensitivity C-reactive protein has been the most studied and promising marker, and was recently used in the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) study to select patients for treatment with rosuvastatin [14]. The results of the study demonstrated a dramatic reduction in cardiovascular events favoring the statin treated cohort based on elevated hs-CRP levels.

Many reports have demonstrated that hyperlipidemia could trigger an inflammatory response and activate various types of cells [15–18]. Hyperlipidemia was found to provoke vascular wall cells including endothelial cells and smooth muscle cells to produce cytokines, which is an early stimulus for the recruitment of circulating inflammatory cells [15]. Hypercholesterolemic mice were found to have high levels of monocytes that exhibit particularly pro-inflammatory functions in the peripheral blood and spleen [16, 17]. These cells exit the spleen and can accumulate in atheromas [18]. Another report demonstrated an early predominance of special CD4+ cells in mouse atheromas that could accelerate atherogenesis [19, 20]. Moreover, Ox-LDL was identified as potential endogenous antigen, which may stimulate adaptive immunity in atherosclerotic plaques [21]. Recently, the role of crystalline cholesterol has become more prominent based on studies by George Abela et al. [22]. The role of cholesterol crystals in atherosclerosis development was not appreciated until modification in tissue preparation revealed the early presence of cholesterol crystals in atherosclerotic plaque and their capacity to trigger inflammation [22, 23]. Moreover, cholesterol crystals are not only critical in the triggering of

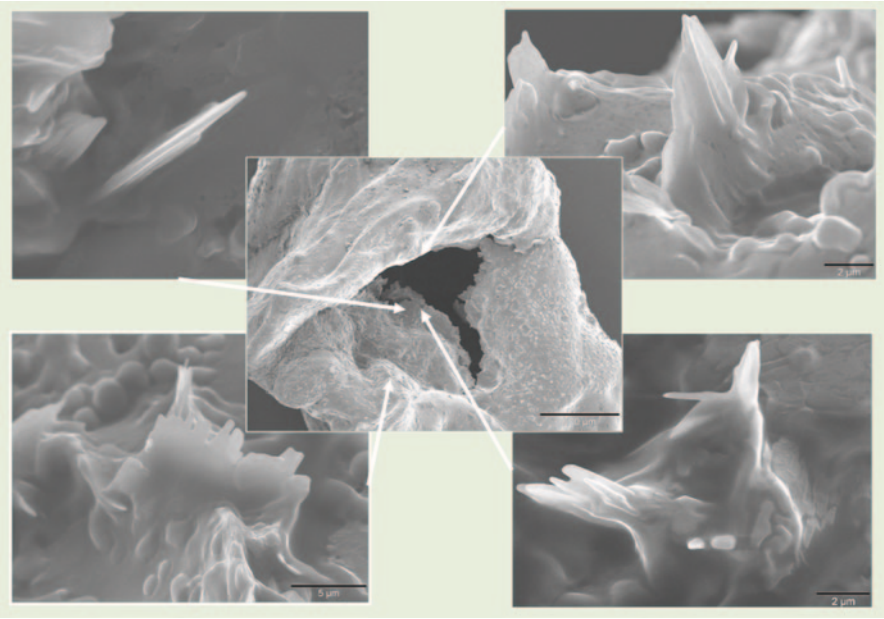

Fig. 3.1 Scanning electron micrograph at low power of *left* anterior descending coronary artery from a patient who died with an acute myocardial infarction (*center* image). This was just *below* the plaque rupture site. Extensive cholesterol crystals are noted perforating the intimal surface. (modified from [116])

inflammation but also in inducing plaque rupture (Fig. 3.1). These aspects will be discussed later in the chapter.

Inflammatory and Other Biomarkers of Cardiovascular Risk Biomarkers

Sterile inflammation is a characteristic of atherosclerosis and a large number of inflammatory biomarkers have been investigated as potential indicators of severity of atherosclerosis and risk for cardiovascular events. Inflammation in atherosclerosis involves both the innate and adaptive immune systems that are activated in response to physical and biochemical insults associated with atherogenesis [24]. Initial injury to the arterial endothelium leading to endothelial dysfunction can be induced by mechanical and oxidative stress and is an early step in the development of atherosclerosis [25, 26]. The activated endothelial cells produce the adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). These promote the adhesion of leukocytes (primarily monocytes and to a lesser extent T cells) that eventually migrate into the intima where monocytes differentiate into macrophages. The increased permeability of a dysfunctional endothelium allows LDL particles to more easily penetrate into the

arterial wall and accumulate in the intima where they are phagocytosed by macrophages that transform into foam cells. Macrophages and T helper cells produce several inflammatory cytokines, many of which further promote the progression of atherosclerotic lesions. A discussion of some of the most researched cytokines and other inflammatory biomarkers in atherosclerosis follows.

Monocyte Chemotactic Protein-1

Monocyte chemotactic protein-1 (MCP-1) is a chemokine cytokine that is produced by several types of cells, including monocyte, endothelial and smooth muscle cells [27]. MCP-1 plays an important role in the development of atherosclerotic lesions by increasing recruitment of monocytes to sites of inflammation [28], promoting neointimal hyperplasia [29] and mediating angiogenesis [30, 31]. Studies on human and animal atherosclerotic vascular tissue have demonstrated that MCP-1 is highly expressed in these tissues [32, 33]. Several studies have found an association between plasma MCP-1 levels and risk for atherosclerotic disease [34–36], while other studies found that MCP-1 plasma levels were not correlated with atherosclerosis [37, 38].

Interleukin-1ß

Interleukin-1β (IL-1β) is a proinflammatory cytokine that is produced by macrophages in response to pathogens or physical injury. Studies have demonstrated that cholesterol crystals commonly present in atherosclerotic plaque induce inflammation by acting as damage-associated molecular pattern molecules (DAMPs) via the NLRP3 inflammasome and Caspase-1, leading to secretion of IL-1β [23, 39]. Upon release, IL-1β induces the formation of other inflammatory cytokines, such as IL-6, and activates other cells to produce MCP-1 as well as reactive oxygen and nitrogen species that cause membrane damage and lead to the formation of pro-atherogenic oxidized LDL [40]. Duewell demonstrated that mice deficient in NLRP3 or IL-1β had smaller atherosclerotic lesions compared to wild type mice [23]. Based on these observations, a monoclonal antibody against IL-1β is being tested in human subjects for reducing rates of cardiovascular events associated with atherosclerosis [41]. However, it should be noted that genetic inactivation of the IL-1 pathway in atherosclerotic mice resulted in an increase in atherosclerotic plaque instability and reduced lumen size in the brachiocephalic artery [42], reflecting the complex nature of cytokine action in inflammatory processes.

Tumor Necrosis Factor-α

Tumor necrosis factor-α (TNF-α) is a cytokine that is produced by macrophages and other leukocytes and is expressed in atherosclerotic lesions where it exerts multiple pro-atherogenic effects. TNF-α promotes foam cell formation and apoptosis in macrophages, impairs endothelial function and permeability, induces the production of reactive oxygen species and enhances smooth muscle cell migration and proliferation [43]. In humans, the level of TNF receptor levels in plasma is correlated with carotid artery plaque thickness [44] and TNF-α levels is also positively correlated with intimal medial thickness [45]. TNF-α antagonists used as therapy for inflammatory diseases such as rheumatoid arthritis are reported to reduce intima media thickness [46] and provide transient improvement in endothelial function [47].

C-reactive Protein

C-reactive protein (CRP) is a protein that is synthesized in the liver and increases in plasma in response to inflammatory conditions, including atherosclerosis, but is not a specific indicator of the presence of atherosclerosis. There are conflicting reports on the association between CRP and risk for cardiovascular disease (CVD). Several animal studies demonstrate a lack of a pro-atherogenic role for CRP in atherosclerosis. In a study using genetically modified mice deficient in CRP no reduction in atherosclerosis was detected [48], indicating a lack of pro-atherogenic role for CRP. Similarly, overexpression of CRP in transgenic mice and rabbits had no effect on the level of atherosclerosis [49–51]. Moreover, meta-analysis of data from the C Reactive Protein Coronary Heart Disease Genetics Collaboration (CCGC) studies [52] involving 194,418 participants, including 46,557 with coronary heart disease (CHD) indicates that CRP is not a causal factor in CHD [53]. However, results from the Multiple Risk Factor Intervention Trial (MRFIT) in men found a strong association between plasma CRP levels and risk for mortality caused by CHD [54] and similar findings were reported by Ridker et al. [55] where CRP was noted to be a good predictor of risk for cardiovascular events. Results from the Women's Health Study (WHS) indicate that plasma levels of high sensitivity CRP (hsCRP) were also reported to be a better indicator of risk for future cardiovascular events than plasma LDL cholesterol [12]. The conflicting evidence regarding the utility of hsCRP as a robust indicator of risk for cardiovascular events reflect the variability in its plasma levels with gender, ethnicity and age [56], thus limiting its usefulness as a specific biomarker for atherosclerosis.

Myeloperoxidase

Myeloperoxidase (MPO) is an enzyme that is produced by monocytes and neutrophils and catalyzes the production of reactive oxidant molecules that cause lipid peroxidation and protein modification. While these actions are beneficial within the context of the innate immune system fighting pathogens, MPO-mediated protein and lipid modifications during sterile inflammation can have detrimental effects on cell metabolism. High levels of active MPO are present in atherosclerotic lesions [57] where it catalyzes the oxidation of phospholipids and apolipoproteins in low density and high density lipoproteins. Oxidation of LDL results in the formation of atherogenic oxLDL which is phagocytosed by macrophages leading to the formation of foam cells in atherosclerotic plaque [58]. Lipid and protein modification of HDL catalyzed by MPO impair the protective function of HDL in reverse cholesterol transport out of cells [59], thus exacerbating the accumulation of cholesterol in arterial walls and the formation of fatty streaks. Epidemiological studies indicate that an elevated serum level of MPO is a useful biomarker for risk for mortality and recurrence of cardiovascular events in patients with established atherosclerosis [60].

Matrix Metalloproteinases

Matrix Metalloproteinases (MMP) are a family of gelatinase enzymes that are involved in tissue remodeling by catalyzing the degradation of extracellular matrix (ECM) components, including collagen and elastin. In atherosclerotic tissue, metalloproteinases are dysregulated in foam cells resulting in excessive synthesis and release of various MMPs while the levels of tissue inhibitors of MMP (TIMP) are decreased [61]. The degradation of ECM components of the intima by MMP can cause thinning of the fibrous cap of plaques and increases the risk of rupture and thrombosis [62]. Studies in genetically modified mice indicate that MMP-2 and MMP-9 are the primary gelatinases involved in atherogenesis [63]. MMP-2 and MMP-9 have also been reported to be elevated in human patients with acute coronary syndrome [64] and other studies indicate that patients with coronary artery disease who have high levels of MMP-9 are at a higher risk for mortality resulting from a cardiovascular event [65].

Plasminogen Activator Inhibitor-1 (PAI-1)

Plasminogen Activator Inhibitor-1 (PAI-1) is a protein that is produced by endothelial cells and acts as an inhibitor of fibrinolysis by inhibiting tissue plasminogen activator and urokinase, thus promoting thrombosis. Elevated levels of PAI-1 are present in atherosclerotic arteries [69, 70]. The involvement of PAI-1 in atherogenesis was demonstrated in a mouse model of atherosclerosis where PAI-1 deficiency

limited the progression of atherosclerosis [71]. Plasma levels of PAI-1 have been reported to be correlated with carotid artery intimal medial thickness and plaque score in hemodialysis patients [72]. These and other studies indicate that high plasma levels of PAI-1 are associated with endothelial dysfunction and atherosclerosis; however, the value of PAI-1 as an additional biomarker for atherosclerosis has been questioned due to its strong correlation with traditional CVD risk factors which provide a similar assessment of risk [73].

Novel Inflammatory Biomarkers

Advances in the fields of genomics and proteomics allow for a more robust approach for the identification of novel biomarkers for atherosclerosis and other diseases. Comparisons between the proteomic profiles of atherosclerotic and healthy tissue, plasma and cell secretions (secretomes) have revealed the existence of a large number of differences, with some proteins being more highly expressed in atherosclerosis while the expression of others is diminished [74]. Investigations in this field are still at an early stage and none of the newly identified potential biomarkers have been thoroughly validated, however studies have already identified potential biomarkers that have a strong correlation with risk for cardiovascular events in humans such as osteopontin [75] and adipocyte fatty acid binding protein (FABP4) [76].

Lipoprotein(a)

Lipoprotein(a) Lp(a) is an LDL –like lipoprotein that is produced in the liver and is composed of apolipoprotein(a) and apolipoprotein B100 [66]. Elevated levels of plasma Lp(a) have been associated with an increased risk for CVD [67]. Lp(a) accumulates in the walls of arterial vessels and exerts multiple atherogenic effects by promoting monocyte chemotaxis, inducing production of adhesion molecules, promoting proliferation of smooth muscle cells and foam cell formation, and acting as a pro-thrombotic agent [68].

Prognostic Value of Inflammatory Biomarkers

The prognostic effect of several biomarkers, including hsCRP, as well as novel biomarkers (e.g., Lp(a), PLAC2, leucocyte count) were recently evaluated by Tzoulaki et al. [77] who analyzed 31 meta-analyses of randomized controlled trials and observational studies. They found that observational studies gave a stronger prognostic effect than controlled trials and in the case of CRP and Lp(a) the prognostic effect was very small. It is important to note that levels of many inflammation

biomarkers can increase in response to inflammation that may be caused by diseases other than atherosclerosis. Therefore, these inflammation biomarkers can only be realistically used as indicators of the presence of atherosclerosis in conjunction with other indicators of cardiovascular disease. However, a combination of various biomarkers with Framingham risk score and radiological imaging (e.g. calcium score by CT scan, PET scanning) may help in enhancing the predictive outcomes. [78–80] Helfand et al. proposed several criteria for evaluating the clinical value of new biomarkers, including. [81]:

1. Ease and reliability of measurement,
2. Ability to act as an independent predictor of risk for cardiovascular events in individuals with no history of cardiovascular disease,
3. Ability to result in re-classification of intermediate risk persons into a higher risk category,
4. Reclassification based on the biomarker should result in a change in disease management and a reduction in risk for cardiovascular events for reclassified patients.
5. If two risk factors are equivalent, the convenience and cost and safety should determine choice.

A number of other novel biomarkers are being investigated including: Lipoprotein associated phospholipase A2 mass and activity, serum amyloid, leucocyte count, and tissue plasminogen activator antigen [74, 82]. All these have yet to demonstrate their validity based on the criteria for being a clinically useful biomarker.

Oxidized LDL and Inflammation

The concept that oxidized LDL and inflammation play important roles in atherosclerosis is not new [7]. Both past and recent research suggest that the development and progression of atherosclerosis is predicated on a positive feedback cycle. Oxidized LDL plays an early role but it may not always be the inciting factor, thus the triggers that induce the initial injury may vary greatly. Oxidative stress and cardiovascular disease have been investigated by epidemiologic studies, animal models of atherosclerosis, and cell lines. The results of these studies have suggested that higher oxidative stress and elevated levels of oxLDL may be correlated with increased cardiovascular risk [83]. LDL becomes oxidized in two stages, the initial stage occurs without alterations in the apolipoprotein B100. This results in the formation of minimally oxidized LDL. However, recruitment of macrophages drives the second stage of LDL oxidation into the ‘Oxidized LDL’ [84]. Studies focused on the proatherogenic mechanisms of oxLDL have identified many pathways. These include chemotactic actions with macrophages, smooth muscle activation, and inactivation of certain protective enzymes like PON1 and up-regulation of proatherogenic mechanisms like LOX-1 receptors or metalloproteinase enzymes (MMPs) [85]. Studies in humans have found a strong correlation between cardiovascular

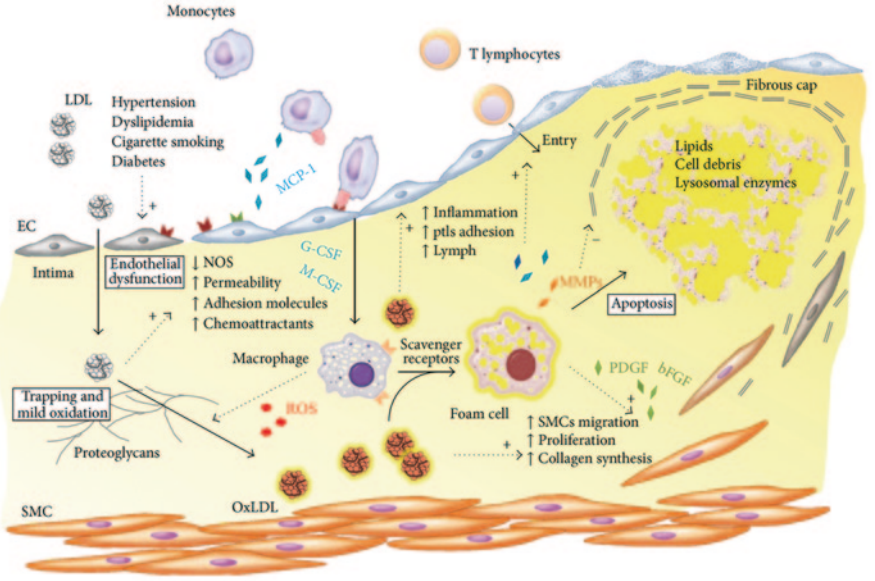

Fig. 3.2 Putative pathway of oxidized low-density lipoprotein (oxLDL) in the atherogenic process according to the oxidative hypothesis of atherosclerosis. Reproduced under the creative commons attribution license [86]

disease and oxidative stress (Fig. 3.2) [86]. However, outcomes of treatment with antioxidants to reduce oxidative stress, oxLDL, and cardiovascular disease have had mixed results. It has been suggested this may have been in part due to the inclusion of patients at mild to moderate risk as opposed to high risk patients who are diabetic, smokers, or on hemodialysis and with CVD.

Oxidized LDL exerts a positive feedback in the development of atherosclerosis at multiple levels. In addition to contributing to the formation of lipid-laden macrophages, oxLDL has other atherogenic effects (Table 3.1).

OxLDL interacts with platelets via the platelet CD36 receptor [87]. This may be significant since lipid laden platelets have been found in macrophages, and inhibition of cyclooxygenase by aspirin (ASA) was found to suppress platelet monocyte interaction in response to oxLDL. Valente et al. demonstrated that oxLDL, via LOX-1 receptors on endothelial cells, induce the expression of various proinflammatory cytokines, chemokines, and adhesion molecules in part via TRA- F3IP2 protein which is an upstream regulator of the NF-κB and AP-1 pathways [93]. This also creates an environment of oxidative stress that promotes cardiomyocyte injury. They found that activation of this pathway and the induction of cell death by oxLDL were inhibited by the knockout of the TRAF3IP2 gene. An important finding of that study was that HDL3 blocked oxLDL-induced endothelial cell death adding to the well-known primary anti-atherogenic function of HDL in promoting cholesterol efflux. However, this positive effect can be overcome by modifications to HDL during chronic oxidative and inflammatory conditions leading to the oxidation of

Table 3.1 Inflammatory and other atherogenic effects of oxidized LDL

Site of action	Physiological effect	Reference
Monocytes/ macrophages	Enhances cholesterol uptake	Badrnya [87]
	Reduces reverse cholesterol transport	
	Induces production of MCP-1 chemotactic activity of monocytes via CXCR2 (IL-8 receptor)	Hashizume [88]; Lei [89]
	Stimulates monocyte binding to endothelial cells	
	Increases expression of intercellular adhesion molecule-1 and vascular-cell adhesion molecule-1	Holvoet [90]
	Reduces the motility of macrophages, leading to accumulation of macrophages in vascular walls and development of plaque	
Arterial wall, endothelium and smooth muscle cells	Induces production of IL-8 in endothelial cells	Terkletaub [91]; Claise [92]; Valente [93]
	Induces endothelial dysfunction	
	Induces platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF) production by endothelial cells and macrophages, which in turn stimulate migration of smooth muscle cells (SMCs) and SMCs proliferation, respectively	Jimi [94], Rajavashisth [95], Xu [96], Loidl [97]
	Induces collagen production	
	Induces production of metalloptroteinases (mmps), and reduces MMP inhibitors from SMCs	
	Cytotoxic to vascular cells, induces apoptosis and release of intracellular lipids and lysosomal enzymes from vascular cells	Schwartz [98], Cathcart [99], Sata [100], Hardwick [101], Thorin [102], Li [103]
	Reduces nitric oxide production and increases prostacyclin which impacts vasoconstriction and increases platelet adhesion and aggregation	
	Decreases the secretion of the tissue-type plasminogen activator, increases plasminogen activator inhibitor-1, and reduces the fibrinolytic activity of endothelium	Kugiyama [104], Grafe [105], Allison [106]

HDL and the consequent loss of its protective effects [93, 107]. The diminished protective effects of oxHDL in chronic inflammation may explain why patients with inflammatory disorders like rheumatoid arthritis are known to have high risk of CVD despite high HDL levels.

Yang et al. demonstrated that oxLDL enhances the release of CD147, known as the extracellular MMP inducer, from coronary smooth muscle cells [108]. MMP in turn is thought to make plaques more vulnerable by destabilizing the fibrous cap. In their study platelets treated with ox-LDL exhibited a significant increase in the expression of CD147 whereas HDL or anti-LOX-1 monoclonal antibody decreased these effects. The expression of soluble CD147 increased in a dose dependent manner with the concentration of ox-LDL. Holvoet et al. identified PPAR-γ as a regulator of oxidative stress and inflammation in a murine model [83]. Also, the induction of PPAR-γ results in an increase in SOD1 that is associated with a reduction of LDL oxidation by decreasing reactive oxygen species. Rosuvastatin has also been shown to decrease both plaque volume and plaque ox-LDL content while increasing the expression of SOD1, CD36 and LXR-α, ABCA-1 and PPAR-γ. In another study by Holvoet et al. oxLDL was found to reduce the motility of macrophages and lead to state of chronic inflammation thus playing a role in the conversion of fatty streaks to more advanced atheromatous plaques [90]. Serum paraoxonase (PON 1) is a component of HDL and is anti-atherogenic through its ability to protect LDL against oxidation [85]. However even the most active variant of PON1 can be impacted by insults like smoking. Activities and concentration of PON1 were much lower in current smokers [107]. Ex-smokers who had quit in the last 3 months had levels comparable to current smokers. Concentration and activity returned to never smokers levels in approximately 2 years. Lower PON1 concentration and activity correlated with more severe coronary disease and decreased protection of LDL oxidation.

Mechanistically, oxLDL is involved in many pathways. In multiple epidemiological studies and cohort studies it was be found to be a very significant risk factor for CV disease. The Health ABC study included 3033 individuals aged 70–79 years and demonstrated that ox-LDL was elevated in persons with high predicted CHD risk by Framingham risk score and the third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III). Individuals in the highest quintile of ox-LDL were 3 times as likely to develop CHD, compared with those in the lowest quintile even after adjusting for age, gender, ethnicity, smoking, LDL-cholesterol (LDL-C) and C-reactive protein. Ox-LDL may be used to further risk stratify patients into a higher risk category [91]. In another cohort study by Holvoet patients with metabolic syndrome had a two-fold higher risk of myocardial infarction after adjustment for age, sex, ethnicity, and smoking status [83].

In a cohort of 504 patients pre-coronary angiography oxLDL was measured and reported as a ratio of oxidized phospholipid to apo B-100 [109]. The ratio showed a strong association with both diagnosis of CAD and severity of CAD. The highest quartile of the ratio had odds ratios for coronary artery disease of 3.12 ($P<0.001$) relative to the lowest quartile. The odds ratio was found to be significant in patients 60 years of age or younger as well. A cohort with 385 CHD patients and

1183 patients at high risk had ox LDL levels measured. Many of the high risk patients were not treated with statins and most of the CHD patients were treated. The oxLDL was found to be higher in the high risk patients without a diagnosis of CHD compared to those with known CHD on statin treatment [90]. This may help confirm some of the antioxidant and downstream effects of statins seen in cell line studies.

Research using antibodies against oxLDL have shown promise both as a marker for cardiovascular disease risk and to further elucidate mechanisms of atherosclerosis. Immunoglobulin G (IgG) that recognize epitopes of oxLDL can be found in atherosclerotic lesions in both humans and rabbits. [110] In a study including 123 patients with previous myocardial infarction serum IgM levels against malondialdehyde-modified LDL (MDA-LDL) were lower in the MI group. The serum IgM was thought to be lower because of increased uptake and higher levels found in vessel walls [111]. In the Epic Norfork study, a large case control study found that IgG and IgM antibodies against different forms of oxLDL were not independent predictors of CAD events [112]. However, they did find that higher levels of these antibodies may decrease the proatherogenic effect of oxLDL. In one study it was found that IgM oxLDL autoantibody levels were inversely associated with angiographically proven CAD, but IgG oxLDL autoantibodies were positively associated with CAD [112, 113]. However in another study looking at IgG autoantibodies offspring of the Framingham population did not find any correlation with CAD [114]. Thus far the role of antioxidants in the treatment as well as prevention of cardiovascular events has not been borne out in humans.

Cholesterol Crystals and Inflammation

Crystal-induced diseases have been well recognized as a cause of many illnesses. These conditions lead to crystal deposits that trigger inflammation. Crystalline substances, like monosodium urate crystals, can induce inflammation by stimulating a danger gene signal which results in production of interleukins and initiates an inflammatory response [115]. Crystal formation and deposition in tissues and confined spaces (i.e. plaque, joint, viscus) can trigger both local and systemic inflammation. Variable environmental conditions are known to facilitate this including local trauma, dehydration and saturation of proteins. Latz and Abela investigated the concept that cholesterol crystals might play a similar role in triggering inflammation in atherosclerosis [23].

Cholesterol crystal deposits are frequently noted in the necrotic core of advanced atherosclerotic lesions. Initially, due to their appearance in advanced atherosclerotic lesions, they were not considered to be primary inducers of inflammation. However, by using tissue processing techniques that excluded ethanol for scanning microscopy it was possible to detect the cholesterol crystals. That confirmed that cholesterol crystals are actually present very early in atherosclerosis as seen in diet induced atherosclerotic lesions in mice and rabbits [23, 116, 117]. Those coincided

with the initial appearance of inflammatory cells and serum inflammation biomarkers. The early formation of cholesterol crystals leads to activation of the NLRP3 inflammasome. This suggests that cholesterol crystals can trigger a danger signal within macrophages to initiate an inflammatory response that can eventually lead to macrophage apoptosis. This is the same inflammation pathway that is triggered by monosodium urate crystals in gout [115]. Also, cholesterol oxide (7-ketocholesterol) has been shown to form crystals in macrophages in a dose-dependent fashion, leading to moderate apoptosis [118]. Another study investigating the role of autophagy in atherosclerosis provided further evidence for the importance of inflammasome activation on cardiovascular disease progression [39]. These studies indicate that the inflammatory response caused by cholesterol crystal-induced NLRP3 inflammasome activation represents a major driving element in the development and progression of atherosclerosis.

Many studies have documented that LDL cholesterol can induce endothelial dysfunction, accelerate atherogenesis while lowering plasma LDL levels protects from cardiovascular events [119, 120]. Specifically, the 'vulnerable atherosclerotic plaque' has been defined as the primary pathological lesion that leads to cardiovascular events [121]. Histology of this plaque has typically been demonstrated to have a large lipid-rich necrotic core and a thin fibrous cap with weakened structural support by reduced smooth muscle cells and the presence of cellular inflammation [122]. However, the mechanism of plaque rupture has been elusive and not well identified until recently. The role of cholesterol crystals seems to help elucidate this process based on recent observations related to their presence, location in plaque and their effect on inducing inflammation. All these are major potential contributors to plaque rupture.

Liquid cholesterol is abundantly present in the necrotic core of the plaque [123]. When cholesterol crystallizes to a solid it occupies a greater volume expanding the necrotic core, stretches out the fibrous cap causing it to become thinner, and the sharp tipped crystals can then pierce the overlying cap and intima causing plaque rupture [124]. In post mortem studies of human coronary arteries, cholesterol crystals were found to be perforating the plaque caps and intima at sites of ruptured plaque only in patients who died with myocardial infarction. This was not present in those patients who had significant coronary artery disease but died of other causes [22]. Similarly, carotid arteries from patients who had neurological events had significantly greater amount of cholesterol crystals compared to those who had no symptoms but had high grade lumen stenosis ($\geq 70\%$) (Fig. 3.3).

Cholesterol crystals were found in both intra- and extracellular spaces within plaques at the submicron range, and when injected intraperitoneally, cholesterol crystals were found to induce acute inflammation [23]. Cholesterol crystal-mediated intimal injury with inflammatory response would be expected and the factors that reduce its formation can reduce this effect. Studies have demonstrated that statins, alcohol, and aspirin all have an ameliorative effect on cholesterol crystal formation while reducing inflammation and providing protective effects from acute events [125–127]. A study in atherosclerotic rabbits demonstrated that lowering the plaque burden with ezetimibe significantly reduced inflammation in arterial wall and serum [117]. This was accompanied by a significant reduction of plaque

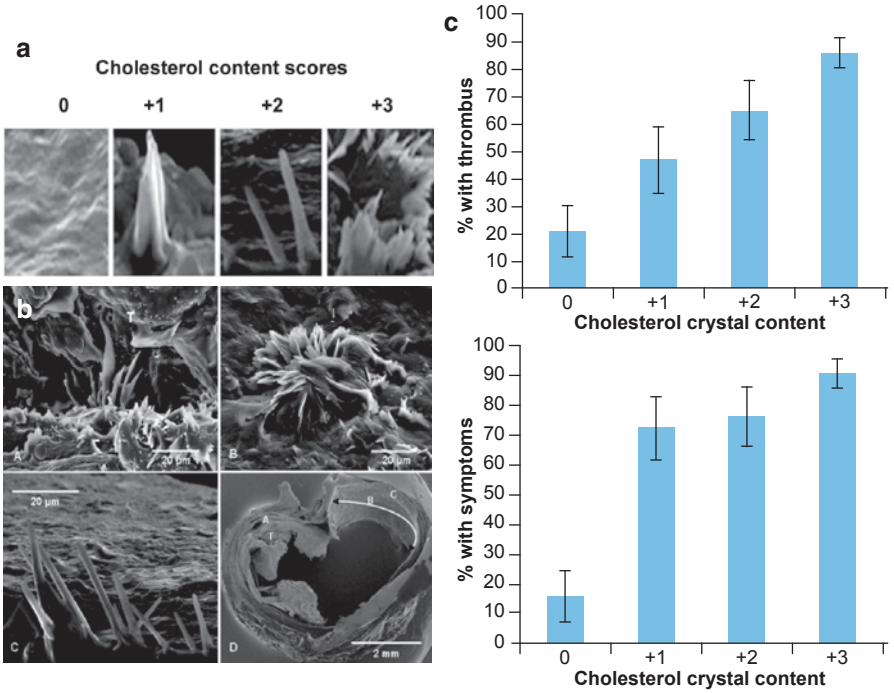

Fig. 3.3 The cholesterol crystal content is associated with more vascular injury and a greater amount of thrombosis. **a** Scanning electron micrographs of patients who had progressively more cholesterol crystals perforating the intimal surface of carotid plaques. **b** More examples of crystals perforating the intima. **c** The symptoms are greater in those patients who have a greater amount of crystals [22,146]

disruption and thrombosis following pharmacological triggering. Also, this was associated with a reduction in cholesterol crystal density.

The process of cholesterol crystallization and its role in inflammation and plaque rupture appears to occur in two stages (Fig. 3.4) [124]:

Stage I—Cholesterol Crystal-Induced Cellular Injury and Apoptosis Localized cellular inflammation results in the formation of the lipid-rich necrotic core, the major hallmark of a vulnerable plaque. As cholesterol builds up in macrophages, they are transformed into foam cells that lead to fatty streaks and eventually the development of a necrotic core. In the foam cells, cholesterol crystallizes triggering a danger signal that initiates a local inflammatory response via NLRP3 inflammasome. This process occurs very early in the development phase of atherosclerotic plaques. The intracellular crystals can lead to death of the foam cells, contributing to a local extracellular buildup of free cholesterol derived from both the cell membranes as well as their earlier imbibed cholesterol load [128]. These deposits in the extracellular space form a lipid pool and eventually the necrotic core. The necrotic core is an accumulation of an amorphous mixture of localized lipid intermixed with

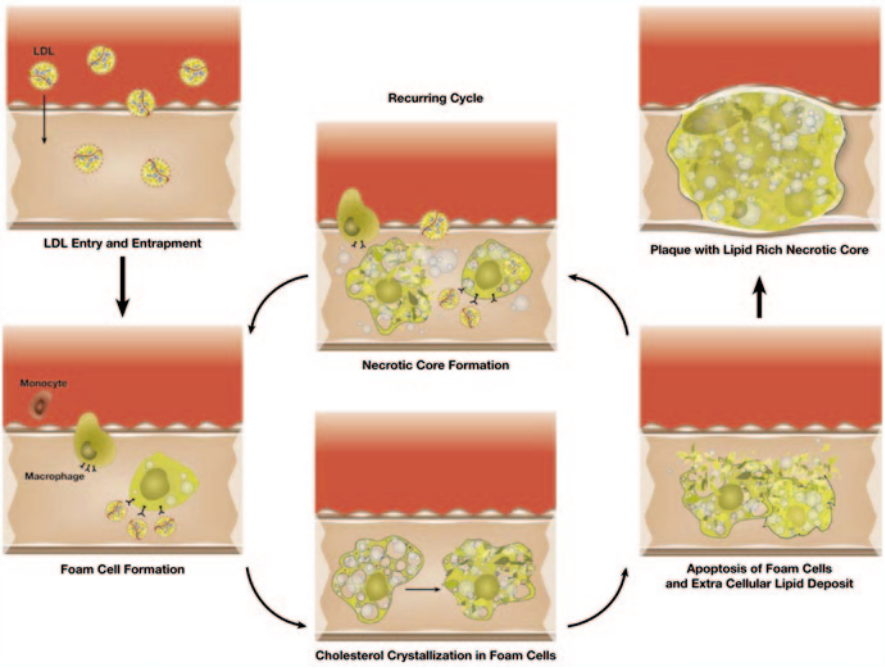

Fig. 3.4 A vicious cycle of inflammation within the arterial wall leading to the formation of the necrotic core and ultimately the vulnerable plaque. The initial step is the entry of LDL into the artery then its oxidation follow up monocyte entry and chemotaxis. This leads to the foam cells with an intracellular saturated cholesterol. Cholesterol crystals form within the foam cells leading to apoptosis and cell death releasing cholesterol, crystals and cell debris all contributing to the necrotic core. The positive feedback loop eventually leads to the vulnerable plaque that is ready to rupture [124]

cellular debris often referred to as "the gruel." As more macrophages are attracted to the site of inflammation by chemotactic signals, more lipids are taken up by those macrophages to form additional foam cells. As more macrophages die and release their content, signaling more macrophages to the site, a vicious cycle is set up that ultimately forms a lipid pool with saturated cholesterol. Cholesterol crystallization forms sharp tipped crystals that can cut their way through the vasa vasora releasing red blood cells (RBCs) into the necrotic core. RBC membranes from injured vasa vasora provide additional cholesterol that increases cholesterol concentration and saturation within the core. Moreover, the presence of free extracellular cholesterol in soft tissues has been identified as a cause of inflammation as well. Once the necrotic core is formed within the confined space of the arterial wall between the internal and external elastic lamina, it then becomes subject to the local physical and chemical forces. Consequently, saturation, temperature, pressure, and pH could individually or in combination trigger the crystallization of the cholesterol within the necrotic core [129]. Several of these physical factors have already been demonstrated in vitro to trigger cholesterol crystallization that can lead to plaque disrup-

tion. These observations also fit the unpredictable clinical presentations of acute cardiovascular syndromes that seem to increase with environmental stressors such as cold weather or physical exertion.

Stage II—Cholesterol Crystal-Induced Arterial Wall Injury and Plaque Rupture This stage is similar to Stage I but the process of cholesterol crystallization occurs predominantly in the extracellular space rather than with the cells. As crystals grow in the extracellular saturated lipid they expand the necrotic core and stretch out the fibrous cap thinning and penetrating it leading to erosion and/or rupture. This then can trigger a systemic inflammation. The local production of interleukin (IL)-6 molecules by lymphocytes occurs in response to intimal injury that then circulates to the liver and signals the production of hs-CRP, which is an acute phase reactant. Plaque rupture may then occur suddenly or slowly based of the size of the lipid pool in the necrotic core. Large pools would tend to produce sudden rupture by rapid expansion, which is often seen in men, whereas smaller pools would tend to produce a slower, more protracted event, causing erosion as seen more often in women (Fig. 3.5). Using magnetic resonance imaging, men have been shown to have larger necrotic cores than women [130]. Also, postmortem studies had previously demonstrated that women have more plaque erosion than men [131]. Moreover, in bench top studies, the amount of crystal volume and rate of expansion were linearly dependent on the amount of cholesterol content [116]. Erosion does eventually lead to thrombosis but its development is slower when compared with rupture. These observations seem to fit the clinical picture of gender differences with regards to presentation of females who have often have atypical symptoms with heart attacks compared with males who have a more dramatic and sudden onset of symptoms [132].

It was demonstrated in an atherosclerotic rabbit model that continuous feeding of cholesterol-enriched diet leads to a progressive increase in hs-CRP, IL-6, and plasminogen activator inhibitor-1 [81, 117]. However, once plaque rupture occurs, the biomarkers rise considerably higher. The model of cholesterol crystals causing the plaque rupture and systemic inflammation is supported by multiple studies [22, 117].

1. Cholesterol crystals were noted perforating the intima not only at the site of plaque rupture but in the adjacent arterial wall, indicating an active process involving locations beyond the rupture site.
2. Plaque disruption was found in multiple arterial sites other than coronary arteries. In vitro, studies have demonstrated that when a fibrous membrane is placed in the path of growing cholesterol crystals, the membrane is perforated and torn by sharp tipped crystals.
3. Histologically, the edges of ruptured fibrous plaque cap were found to be frayed, suggesting a dynamic snapping like a tear.
4. In an atherosclerotic rabbit model of plaque disruption and thrombosis, there was significant association between the amount of cholesterol crystals and thrombosis.
5. Patients who had severe atherosclerosis and died of noncardiac conditions did not have cholesterol crystals perforating the intimal surface and only those who

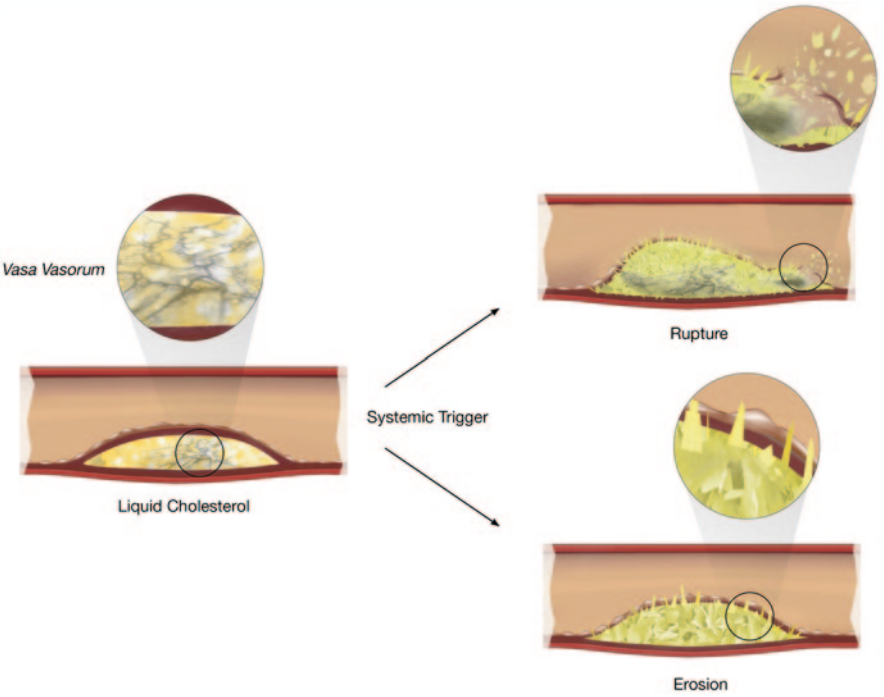

Fig. 3.5 The mechanism of plaque rupture and/or erosion by cholesterol is incurred by the expansion of the necrotic core by crystallizing cholesterol. A large necrotic core with a high content of cholesterol will expand more than a smaller core. Thus, men who usually have larger cores will tend to rupture the fibrous cap while women who have smaller cores will tend to erode. Also, the presence of sharp tipped cholesterol crystals can cut through the vasa vasorum in the plaque to induce hemorrhage [124]

died with acute coronary syndrome had evidence of cholesterol crystals perforating the intima.

Factors that Favor Cholesterol Crystallization Multiple sites of rupture in the same and other vascular beds occurred together suggesting a systemic triggering process. This has been reported using various imaging modalities including angiography, angioscopy, optical coherence tomography and intravascular ultrasound [133–136]. Cholesterol crystals were observed perforating the intima in several arterial beds in the same patient. This may be attributable to a systemic process that can lead to local environmental change such as pH rise, temperature drop (as in the early morning hours), cholesterol saturation, and hydration of the cholesterol molecules. These factors have all been tested and found to enhance cholesterol crystallization [129].

Statins, aspirin (ASA), and alcohol have all been found to be protective of acute cardiovascular events (Fig. 3.6) [137–139]. These agents were tested and found to be effective solvents of cholesterol crystals. Therefore, dissolving the cholesterol

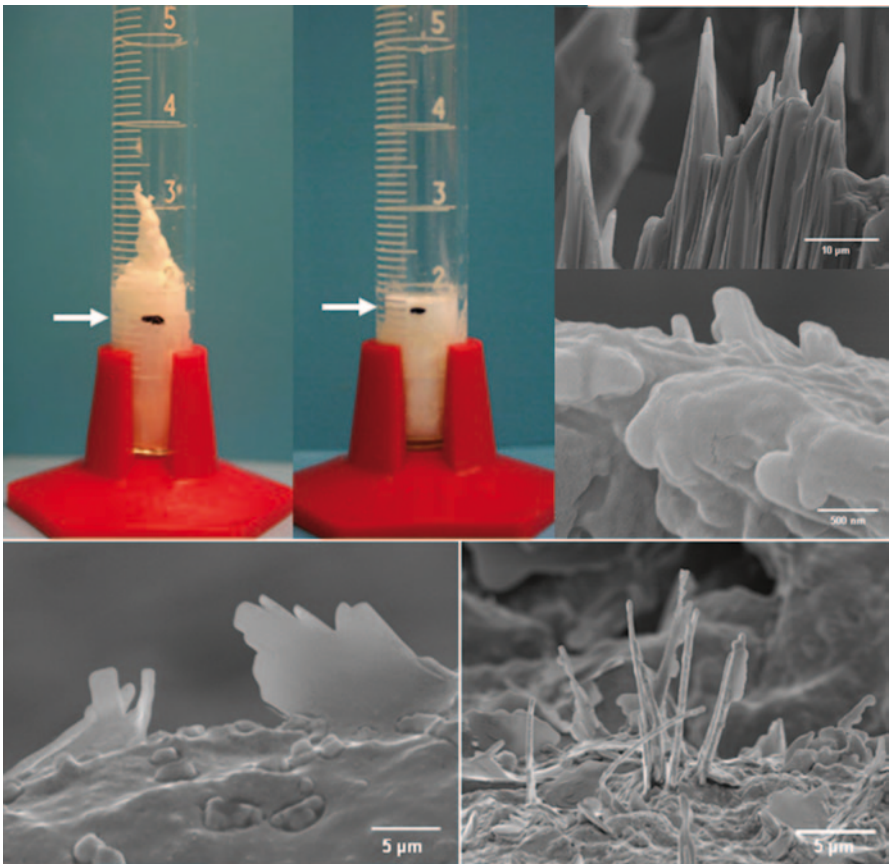

Fig. 3.6 (*Top* panel) Cylinders with pure cholestesterol (*left*) and cholesterol with pravastatin (50 mg) (*right*). After crystallization pure cholesterol expanded by 1.6 ml from the meniscus line and only 0.1 ml with pravastatin. Scanning electron micrographs of pure cholesterol crystals demonstrate pointed tips while with pravastatin there was complete absence of pointed tipped crystals and presence of melted forms. (*Bottom* panel)(*left*) Cholesterol crsytals perforating the intima from an endarterectomy specimen of a patient not on a statin prior to surgery and (*right*) dissolving crystals from a plaque of patient who was on atorvastatin prior to surgery. (Modified from [125])

crystals could explain some of the pleiotropic effects of statins and the dose relationship to enhanced effects beyond the scope of LDL lowering. Similarly, moderate ethanol consumption has been found to be protective, and it was demonstrated that ethanol dissolves cholesterol crystals. Alternatively, the cholesterol molecule may be altered chemically when combining with these compounds to change its crystallization characteristics.

These potential mechanisms could explain the pleiotropic benefits of these compounds as well as their early and quick action that has been described in acute cardiovascular syndrome [140]. The use of both statins and ASA has been shown to improve the immediate outcomes after interventional procedures especially dur-

ing acute cardiovascular events as well as during percutaneous procedures [141]. Also, both agents and ethanol have been shown to have anti-inflammatory properties that may be explained by the same process [142–145]. Furthermore, this may help explain how high-dose statins has been associated with a small-but-significant increase in hemorrhagic stroke because if cholesterol crystals are imbedded in the arterial wall at the site of the stroke and then dissolved, it could cause blood to leak at that site from "unplugged" holes in the arterial walls.

The current thinking is that plaque rupture occurs by weakening of the plaque cap from the release of metalloproteinases and collagenases that digest the fibrous cap. This is a potentially effective mechanism that contributes to the process of plaque rupture, yet a direct link between plaque rupture and this process has not been established. However, the recent findings regarding cholesterol crystallization and volume expansion of the necrotic provides a plausible mechanism responsible for the final stages that leads to plaque rupture.

Summary

Atherosclerosis is not only a cholesterol storage disease characterized by the collection of cholesterol and thrombotic debris in the artery wall, but also a complex condition involving inflammation. Many investigators have been extensively studying the association between lipid, inflammation and atherosclerosis. However, given the complexities of the pathways involving in vascular inflammation, clinical validation of the inflammation hypothesis of atherogenesis may require further testing to find a suitable spot for an intervention that mitigates the disease without undue impairment of host defenses. The findings related to cholesterol crystals provide new insights into the pathogenesis of atherosclerosis, inflammation and indicate new potential molecular targets for the therapy of this deadly disease.

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Chapter 4
Dyslipidemia: Relationship to Insulin Resistance, Fatty Liver, and Sub-Clinical Atherosclerosis

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Overview of Obesity Prevalence and Implications of Abdominal Adiposity

In the United States (U.S.) there is a high prevalence of overweight and obesity, affecting nearly 32 % of youth and 69 % of adults [1]. Compared to the national average, these prevalence estimates are even higher among minority groups where approximately 70 % of African American (AA) and Hispanic adults, but only 57 % of Caucasian adults are considered overweight and obese [1]. Across all segments of the population, obesity-associated diseases such as type 2 diabetes (T2D), cardiovascular disease (CVD), and non-alcoholic fatty liver disease (NAFLD) have contributed to $ 48–$ 66 billion a year in projected medical costs [2]. Perhaps more alarming is the fact that obesity rates are expected to increase to 51 % by 2030 [3]. Concurrent to national increases in obesity, minority children and adults are experiencing disproportionate risk for obesity-related diseases, where AAs and Hispanics are more affected by T2D and CVD than Caucasians and Asians [2, 4]. Of particular concern is the observation that overweight and obese children tend to become obese adults [5], highlighting the importance of understanding disease pathophysiology in order to prevent disease. In this regard, studies have identified specific patterns of fat distribution and ectopic fat as being linked with increased

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disease risk. Specifically, visceral and liver fat have been identified as significant risk factors for the development of T2D. Interestingly, at similar levels of obesity, AAs have lower levels of visceral and liver fat than Hispanics, ostensibly placing them at decreased risk for metabolic dysfunction. However, despite this more "protective" fat profile, AAs suffer from similar rates of T2D when compared to Hispanics. Additionally, early manifestation of ethnic disparities in cardiometabolic disease risk, highlight the need for a deeper understanding of the pathophysiology behind obesity-associated diseases.

In an effort to identify risk for developing diabetes and CVD [6–8], clinicians have relied on a constellation of measurements including blood pressure, fasting blood sugar, waist circumference, and plasma lipids, which have been used to identify metabolic syndrome (MetS) [9, 10]. All components of the MetS have been associated with increased risk of diabetes and CVD mortality [6–8]; however, hypertriglyceridemia, is the most prevalent risk factor affecting about 30% of U.S. adult population. Recently, sub-clinical states of glucose intolerance, such as prediabetes and insulin resistance, have been used to help identify risk of developing T2D and CVD [11]. Due to their ease of measurement in clinical and research settings, lipid profiles as well as markers of insulin resistance have become the primary focus of research aimed at identifying, understanding, and preventing the development of obestiy-associated diseases. Additionally, imaging techniques such as magnetic resonance imaging and arterial imaging have been used to assess risk factors such as ectopic fat deposition and plaque deposits.

To illustrate the importance of these areas of research and clinical care, we herein focus on how dyslipdemia and impaired insulin and glucose control interact with obesity, fatty acid metabolism, and ectopic fat accumation to increase risk for atherosclerosis, T2D, and fatty liver disease. We will also pay special attention to known ethnic differences in obesity-associated disease risk and possible therapeutic targets. These areas of clinical and translational research offer insight into novel detection and treatment strategies aimed at combating the higher prevalence of obesity and its associated metabolic complications.

Obesity, Insulin Resistance, Dyslipidemia, and Ectopic Fat

In order to begin to understand the pathophysiology behind obesity-associated disease risk, one must first grasp the key biological functions of adipose tissue. With excess energy intake, insulin stimulates adipose tissue to increase storage of free fatty acids (FFAs) as triglycerides (TAG) and decrease lipolysis [12]. This process results in increased total body fat, where the distribution of adipose tissue differs by sex and ethnicity [13, 14]. This is of particular importance since visceral adipose tissue (VAT), or fat surrounding the organs, has been shown to be more insulin resistant and have greater lipolytic activity when compared to subcutaneous adipose tissue (SAT). Adipose tissue continues to store excess energy until demands outweigh the ability of the tissue to expand and/or become adequately

vascularized [15]. Once this occurs, there is a marked increase in adipose tissue inflammation and insulin resistance. Adipose tissue insulin resistance is characterized by a decreased ability to take up FFAs and inhibit TAG lipolysis. One hypothesis suggests that adipose tissue dysfunction is the catalyst for systemic insulin resistance, where FFAs are increased in the plasma and delivered to other organs such as skeletal muscle, liver, and pancreas. This theory is supported by the observation that in obese patients, VAT releases FFAs and pro-inflammatory cytokines that are released into systemic circulation and reach the liver and pancreas via the portal vein.

Systemic insulin resistance is a hallmark of the MetS and is one of the primary factors affecting dyslipidemia. Once systemic insulin resistance ensues, there is a reduced ability of insulin to stimulate glucose uptake by muscle and fat as well as a decreased ability to suppress hepatic glucose production. As a result, excess FFAs are converted to TAG in the liver and stored in hepatocytes or carried into the bloodstream by VLDL. As liver fat increases, VLDL secretions result in high TAG and low HDL levels in the plasma, further increasing systemic insulin resistance [16]. Since insulin resistance, MetS, and fatty liver are interrelated, it is difficult to determine whether systemic insulin resistance causes fatty liver, or if liver fat contributes to the development of insulin resistance, or both. For example, an alternative hypothesis involves the causative role of diet in liver fat accumulation, which subsequently results in dyslipidemia and insulin resistance. For example, dietary fat and glucose contribute to liver fat accumulation and inflammation by increasing TAG delivery to the liver or acting as a substrate for de novo lipogenesis. Additionally, increased consumption of sugar sweetened beverages and fruit juice results in elevated exposure to fructose and high-fructose corn syrup, which has been linked to the obesity epidemic and insulin resistance [17–19]. Fructose is unique it that it is predominantly metabolized in the liver by fructokinase, which has no negative feedback system and directly contributes to increased liver fat and uric acid production [17, 20, 21]. It is through this mechanism, and possibly fructose induced endotoxemia [22], that increased fructose exposure likely contributes to the increased prevalence of fatty liver disease [23, 24].

Although the causal relationship between dyslipidemia and fatty liver has not been established, it is thought that prolonged exposure to plasma FFAs and pro-inflammatory adipokines leads to the development of NAFLD in insulin resistance patients. NAFLD is defined by a significant accumulation of fat in the liver, where liver fat fraction exceeds 5.5% [25]. Some patients with NAFLD develop steatohepatitis, which is characterized by inflammation, and can progress to cirrhosis and liver cancer [26]. Typically, NAFLD has been considered a hepatic manifestation of MetS [27]; however, recent studies suggest a causal role between NAFLD and insulin resistance and/or dyslipidemia. For example, one study observed that liver fat, independent of total body fat and intraabdominal obesity, was associated with insulin resistance, hyperinsulinemia, hypertriglyceridemia, low HDL, and increased blood pressure [28]. Additionally, a large multi-ethnic cohort study found that NAFLD was positively associated with dyslipidemia even after accounting insulin resistance [29]. Any discussion of NAFLD would be lacking if it failed to

acknowledge import ethnic/racial differences in the prevalence of NAFLD. Among children, adolescents, and adults, the prevalence of NAFLD has been shown to vary by ethnicity, where Hispanics suffer the highest rates of NAFLD, followed by Caucasians and AAs. Interestingly, although AAs have less VAT and liver fat compared to Hispanics, they have similar levels of insulin resistance and risk for diabetes, resulting in what has been referred to as the AA-Hispanic paradox. Observations such as these highlight the fact that the relationships between insulin resistance, dyslipidemia, MetS and fatty liver are likely affected by genetic differences among these groups. In this regard, genetic variants in the PNPLA3 gene have been shown to be associated with a two-fold higher liver fat content [30]. Interestingly, the occurrence of this variant is more frequent in Hispanics than any other population and has also been shown to be associated with lower HDL [31].

Recent studies have found that pancreatic fat correlates with metabolic dysfunction, however these findings are complicated by the fact the pancreatic fat is also related to increased hepatic steatosis [32, 33]. For example, among patients with NAFLD, there was a positive relationship between pancreatic and liver fat, suggesting that pancreatic fat affects the progression of NAFLD [33]. Despite this, pancreatic fat may directly interfere with insulin secretion since NAFLD and fatty pancreas were independently related to risk for diabetes in Chinese adults [34]. Similar to NAFLD, pancreatic fat has been shown to be associated with VAT, TAG, low-HDL, MetS, and impaired insulin response to glucose as early as adolescence [32]. Further complicating matters, there are well-documented ethnic differences in pancreatic fat accumulation and how it relates to dyslipidemia and insulin resistance. Among obese young adults, Hispanics have higher pancreatic fat accumulation than AAs and pancreatic fat has been shown to positively correlate with VAT, liver fat, increased plasma FFAs, and plasma marker of inflammation (PAI-1, MCP-1, IL-8, and HGF) [35]. Finally, data in adults suggest that pancreatic fat inhibits β-cell function in Hispanics but not whites or AAs [36]. Although it is difficult to tease apart the individual contributions of liver and pancreatic fat to disease risk, these studies confirm important ethnic differences in pancreatic fat accumulation and suggest that dyslipidemia and inflammation are related to fatty pancreas.

Preclinical Atherosclerosis

Atherosclerosis is considered the most common underlying pathological process of CVD and is likely to exist in a subclinical stage for many decades starting in early life [37–39]. For example, human autopsy studies have identified fatty streaks in at least one blood vessel in over 90 % of 204 autopised individuals aged 2–39 years of age [40]. In addition, lesions in the right coronary arteries were found to increase from 60 % in adolescents aged 15–19 years to almost 80 % in adults aged 30–34 years. Notably, the extent of fatty streaks were positively related to BMI and blood pressure [41, 42]. The results of these landmark studies suggest that atherosclerosis appears very early in life, hence detection of intima-media thickening at in early

stages, in conjunction with measurement of traditional risk factors such as dyslipidemias, could yield substantial utility to clinicians in CVD risk assessment.

Dysfunctional Endothelium: A Playground for LDL Accumulation

To fully appreciate obesity-related atherosclerosis progression, it is important to understand the vital functions of the endothelium and its resident cells. The endothelium is composed of three layers: the intima (which is directly exposed to blood flow), the media (composed of smooth muscle cells) and finally the adventitia (the outer structural layer composed of connective tissue). The three main functions of the endothelium are to serve an anatomical barrier, react to mechanical forces, and produce signals that prompt protective actions from toxic substances. Various hypotheses have been proposed with regard to failure of any of these endothelial functions as it has been thought to produce an ideal environment for atherosclerosis.

As an anatomical barrier, the endothelium acts to shield the inner elastic smooth muscle layer from any toxic substances circulating in the bloodstream. Mechanical and hemodynamic forces have been proposed to contribute to endothelial dysfunction with two main types: tensile stress and wall shear stress. Tensile stress is a radial and tangential outward force that is exerted on the vessel wall via blood pressure. Wall shear stress is a frictional force that is exerted parallel to the vessel wall and is directly related to the viscosity of blood [43]. Acute changes to these stresses cause alterations in vascular tone while chronic increases in these forces lead to modifications of the arterial walls. For example, tensile stress caused by hypertension is proposed to have an effect on arterial walls by increasing the thickness of the medial layer (as opposed to the endothelial layer). Studies have also shown that atherosclerosis affects the arteries in a site-specific manner, where inner wall curvatures and outer wall bifurcations [44] are thought to have a higher vulnerability to plaque accumulation due to damage to the endothelial layer [45].

In addition to acting as a barrier, the arterial endothelium also synthesizes and regulates various proteins that act as growth modulators and mediate vascular health [46]. For example, platelet-derived growth factor (PDGF) is a well-known protein that maintains an even surface area by reducing smooth muscle cell proliferation into the intima. Landmark studies by Ross & Glomset were the first to propose that mechanical, chemical, or immunologic injury to the endothelium would lead to platelet aggregation via PDGF [47, 48]. However, further studies found that PDGF may also contribute to endothelial damage through induction of hyperlipidemias, homocysteinemia, hypertension, infection, or other pro-inflammatory cytokines, such as IL-6 and TNF-α [44]. As illustrated by these findings, atherosclerosis risk involves highly complex processes, which likely require a balance of factors affecting the structural integrity of arterial walls and inflammation.

In addition to PDGF, nitric oxide, derived from arginine and oxygen by nitric oxide synthase (NOS), is another essential molecule synthesized by the endothelial cells [49]. Nitric oxide has three important functions: (1) it causes vasodilation of vascular smooth muscle, (2) mediates molecular signaling pathways that prevent platelet and leukocyte interaction and (3) inhibits vascular smooth muscle cell proliferation. Hence, the bioavailability of nitric oxide is important in vascular health, as the lack of nitric oxide has been shown to increase proinflammatory factors (e.g., IL-6, IL-1, TNF-α, and nuclear factor-κB) [50] and lead to the expression of leukocyte adhesion molecules via the up-regulation of vascular adhesion molecule-1 (VCAM-1), ultimately causing further development of foam cells and setting the foundation for the deposition of degenerative materials in the intima media known as atheromas.

Mechanisms of Insulin Resistance, Hyperglycemia, and Dyslipidemia in CVD and Atherosclerosis Progression

The strong link between T2D and CVD events has been demonstrated in numerous epidemiological studies [51–53]. The hypothesized physiological mechanisms underlying these relationships are multifactorial and include elevated FFAs, alterations in insulin signaling pathways, increased oxidative stress, advanced glycation end products (AGEs) and increased activity of proinflammatory factors including nuclear factor-κB [54–56]. These mechanisms are thought to have further molecular consequences on the availability of various endothelial factors, primarily the bioavailability of nitric oxide, which affects the vasodilation and inflammatory processes that ultimately lead to atherosclerosis.

Over the past several decades, large epidemiological studies and seminal clinical trials using insulin-sensitizing agents have helped us understand the specific link between insulin resistance, T2D, and detrimental changes in the arterial endothelium. Under normal, healthy conditions, insulin acts to trigger production of nitric oxide by endothelial cells through activation of the phospatidylinositol-3 kinase (PI3K) pathway. In insulin resistant subjects, this vasodilation is impaired due to reductions in the PI3K insulin signaling transduction pathway [57]. In this scenario, insulin resistance prevents the activation of NOS and decreases proper vasodilation, which in turn contributes to arterial thickening. As a means to counter CVD risk associated with obesity, insulin resistance, and inflammation, large clinical trials have used insulin-sensitizing drugs (e.g. pioglitazone) to improve insulin sensitivity and decrease risk of CVD events [58, 59]. Due to the fact that many of these drugs have been shown to affect adipose tissue differentiation, inflammation, and consequently insulin sensitivity [60–62], studies have began to focus on adipose tissue as another important organ contributing to CVD risk in obese populations [63, 64].

As previously mentioned, adipose tissue is an endocrine organ, which has been shown to contribute to whole body insulin sensitivity, immune activation, and

ectopic fat accumulation. Adipose tissue insulin resistance has been associated with increased lipolysis, elevated circulating FFAs, and hepatic insulin resistance [65, 66]. High levels of circulating plasma FFAs can also impair endothelial function by increasing production of free radicals and by activating protein kinase C (PKC). PKC has been shown to decrease insulin receptor substrate-1 in the PI3K pathway [67, 68], also resulting in decreased NOS activity, reduced production of nitric oxide, and decreased endothelial function. In addition, hepatic insulin resistance, thought to be caused by elevated FFAs, can stimulate hepatic gluconeogenesis and lead to hyperglycemia and glucose intolerance [69].

In conjunction with insulin resistance, resulting hyperglycemia contributes to increased CVD risk by increasing oxidative stress, which creates an imbalance between the production of reactive oxygen species (ROS) and the body's ability to repair any resulting damage. In diabetic animal models, the prominent ROS is superoxide anion, which is known to inactivate nitric oxide and initiate a cascade of events resulting inactivation of nitric oxide and the continuous production of free radicals [56]. Hyperglycemia is thought to initiate this chain of events by increasing superoxide anion production via the electron transport chain in the mitochondria [70]. Overproduction of these highly reactive free radicals has also been implicated in increased intracellular production of AGEs, which are modifications of lipids (or proteins) caused by non-enzymatic oxidation or glycation after contact with aldose sugars [71]. This is particularly important since the accumulation of AGEs can affect vascular cell function by modifying the extracellular matrix and interfering with hormonal and free radical function [72]. For example, in cholesterol samples from patients with and without diabetes, detrimental AGEs arise when they become cross-linked to lipids in LDL-cholesterol [73]. Glycated and/or oxidative LDL-cholesterol has also been shown to reduce production of nitric oxide and, when bound to receptors on endothelial cells, it suppresses the clearance of LDL-cholesterol [74].

As described above, CVD risk is multifactorial and the exact mechanisms are poorly understood. Despite this, it is clear that insulin resistance, dyslipidemia, and inflammation arising from excess fat accumulation contribute to CVD. In this regard, ethnic disparities in rates of insulin resistance, T2D, NAFLD, and fatty pancreas, will likely prove useful in teasing apart the exact behavioral, biological, and genetic risk factors for CVD. Specifically, data show that AAs, Asian Indians, and Filipinos have higher coronary heart disease compared to whites [75]. These differences may arise from ethnic/racial differences in dyslipidemia; however a reoccurring paradox emerges where, although AAs have a lower prevalence of high TAG and low HDL, they are not protected from CVD [75]. Again, perhaps genetics is an important contributing factor since genome-wide association studies have identified numerous genetic loci associated with plasma lipids, including TAG, LDL, and HDL. To date, most of these studies have been performed in European populations and it is only recently that researchers have begun to examine AAs and Hispanics [76, 77]. Findings from this work has identified mutations in genes coding for the LDL receptor or its ligand, which are more common in AAs compared Europeans and have been shown to result in sever hypercholesterolemia [78]. A more recent

study observed racial/ethnic differences in a genome-wide association study that identified three loci for TAG and four loci for HDL in Europeans but only a single locus for TAG in Mexicans [77]. Therefore, similar to patterns of fat deposition, risk for T2D and obesity-associated risk for CVD appear to be modified by racial/ethnic factors that warrant further investigation in order to elucidate important biological mechanisms that can be used as therapeutic targets.

Imaging of Early Atherosclerosis and Prediction of Coronary Events

The most logical place to begin our clinical examination of CVD risk progression is with the current methodologies used to quantify physiological changes within the arteries. In this regard, serial coronary angiography is considered the "gold standard" for measuring vascular disease progression, which allows visualization of the vessel lumen and is useful in predicting myocardial infarction, stroke, or death as clinical end points [79]. Due to the high cost and invasiveness of this procedure, this imaging technique is typically reserved for symptomatic patients. For this reason, non-invasive methods using ultrasonography and ultra-fast CT scanning are now extensively used for measurement of CIMT and coronary artery calcium (CAC) deposits.

Measurement of arterial wall intima-media thickness with high-resolution B-mode ultrasound imaging was first described on excised aortas during autopsy, demonstrating that ultrasound imaging used to measure arterial thickness yielded similar results to that obtained by microscopy [80]. Improvements in ultrasound imaging have resulted in computerized edge tracking-multi-frame image processing, which allows for more accurate measures of CIMT compared to other methodologies [81]. Despite this, due to the accuracy, feasibility, and relatively low-cost, measurement of ultrasonography for CIMT assessment has been a particularly useful method for clinical diagnosis and research aimed at studying risk for CVD [82].

Overall, CIMT and CAC scores are reliable predictors of coronary disease events, making their use clinically relevant. Several longitudinal studies have shown that elevated CIMT and CAC scores predict future clinical end points, such as myocardial infarction, coronary surgical procedures and/or cardiac death [83–88]. These populations were free of any previous cardiac events and 12 of the 13 studies were done in adults >40 years old. Only one study reported on a very large age range (19–90 years of age) confirming that CIMT independently predicted clinical endpoints. This same study also observed higher predictive values in the younger patients (<50 years old) than in the older patients (≥ 50 years old) [89]. Overall, each of these studies had a relatively short follow-up time (<5 years) with only a single measure of CIMT at baseline. CIMT and CAC scores are now recognized as valid surrogate markers for subclinical atherosclerosis and have become increasingly important for identifying and preventing future coronary disease events.

Non-Pharmacologic Treatments for Insulin Resistance, Dyslipidemia, MetS, and NAFLD

Given the inter-relationships between insulin resistance, dyslipidemia, MetS and NAFLD, it is not surprising that these conditions share similar treatment strategies. Current approaches for treatment include weight loss, dietary changes, exercise, as well as lipid lowering and insulin sensitizing drugs. Lifestyle interventions are likely the most effective treatment option for all comorbidities associated with obesity. Successful strategies include incorporation of exercise, weight loss, manipulation of dietary macronutrients, or the use of prebiotics and probiotics. For example, limiting consumption of carbohydrates, or increasing consumption of beneficial fatty acids has been shown to have therapeutic effects on these conditions [90, 91]. Some data suggests that supplementation with omega-3 polyunsaturated fatty acids can improve insulin resistance, hypertension, and dyslipidemia by decreasing TAG levels. These improvements are hypothesized to be mediated by decreases in systemic inflammation and could reduce the risk of CVD. Given that significant reductions in adiposity are difficult to achieve for most patients, surgical options have proven effective in aiding in weight loss and improving liver and systemic insulin sensitivity [92]. Recent data indicate that the largest improvements in blood sugar, LDL, and blood pressures arise from a combination of lifestyle interventions and Roux-en-Y gastric bypass surgery [93]. Studies have also shown that the gut microbiota is involved in obesity and related metabolic disorders, providing an avenue for future interventions [94]. Collectively, weight loss strategies, dietary interventions, prebiotics, and probiotics have all shown promise in regards to their ability to decrease inflammation, liver fat, and insulin resistance [95]. Finally, aside from weight loss, statins are recognized as the primary treatment for dyslipidemia by lowering levels of LDL, TAG, and inflammation as well as increasing HDL, thereby decreasing cardiovascular events [96–101]. However, studies have shown that statin therapy does not completely eliminate CVD risk, suggesting research into alternative therapies is needed as combination lipid therapies have failed to reduce CVD outcomes.

NAFLD is considered an emerging epidemic with nearly 30% of the general adult population in the Western world being affected by this chronic condition [102]. Perhaps of even greater importance in the large number of children being affected by NAFLD, presenting an increased risk for future development of nonalcoholic steatohepatitis (NASH) and cancer. For this reason, there is profound interest in NAFLD specific treatment options for both children and adults. Although a weight loss of 5% or more has been shown to decrease liver fat and improve cardiometabolic risk factors [103, 104], reductions in body weight are not feasible for most patients. Therefore, treatments that do not require weight loss are urgently needed. For example, dietary changes have been considered a viable treatment strategy for reducing liver fat. Although the specific dietary composition yielding the greatest improvements is unknown, data suggest that it likely includes dietary alterations that would decrease insulin resistance, hepatic FFA supply, and inflammation. As previously mentioned, excessive dietary fat and fructose consumption have been

shown to contribute to fatty liver, suggesting that decreases in these nutrients may benefit NAFLD patients even in the absence of weight loss. Treatment with insulin sensitizing drugs, such as metformin, have shown promise for not only regulating blood sugar but also improving liver fat, inflammation, fibrosis and ballooning. Finally, treatment with probiotics [105] or metformin therapy combined with vitamin E has shown promise in treating NAFLD and NASH [106]. Taken together, these findings suggest that future treatments will involve a multi-factorial treatment approach to NAFLD and NASH that does not necessarily involve weight loss.

Conclusions

Obesity-associated dyslipidemias may explain concomitant risk for atherosclerosis, T2D, and liver fat accumulation. Studies investigating differences in ectopic fat deposition have the potential to explain how dyslipidemia and insulin resistance may lead to increased cardiovascular and metabolic disease risk. Given that dyslipidemia, insulin resistance, and patterns of fat accumulation differ by ethnicity, future studies should examine these mechanisms in an ethnic-specific manner. Collectively, an improved understanding of obesity-associated dyslipidemias will help to guide specific behavioral and/or pharmacologic treatments needed to address differences in the underlying pathophysiology of cardiometabolic disease.

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Chapter 5
Is Low HDL Cholesterol a Marker or a Mediator of Cardiovascular Disease?

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Abbreviations

APOAI	Apolipoprotein A-I
ApoB	Apolipoprotein B
CVD	Cardiovascular disease
FH	Familial hyperlipidemia
HDL	High-density lipoprotein
IDL	Intermediate-density lipoprotein
LCAT	Lecithin-cholesterol acetyltransferase
LDL	Low-density lipoprotein
LDL-C	Low density lipoprotein- cholesterol
LDLR	Low density lipoprotein receptor
VLDL	Very-low-density lipoprotein

HDL Cholesterol and HDL Particles, an Important Distinction: It is important to understand that HDL particles carry HDL cholesterol, and that changes in HDL cholesterol may not indicate changes to HDL particle numbers. Indeed, some pharmacotherapies that increased HDL cholesterol (for example CETP inhibitors) increase the cholesterol content of HDL (by increasing the size of the particle) without increasing particle numbers. This distinction is important as we discuss below the many functions of HDL.

I. Reverse Cholesterol Transport

Reverse cholesterol transport was first defined by John Glomset [1] in the early 1960s as the capacity of HDL particles to transport cholesterol from the periphery to the liver for excretion. This concept of reverse cholesterol transport was mostly

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based on in vitro studies examining the ability of Apolipoprotein A-I to esterify cholesterol through LCAT and has been used to explain the protective properties of HDL. The idea gained wide spread acceptance with the publication of the initial Framingham study revealing that greater HDL cholesterol levels were associated with decreased CVD risk [2]. Over the last 50 years, several studies emerged supporting a different role for Apo A-I and HDL in cardiovascular disease. HDL forms after the secretion of disc-shaped apo A-I containing particle by hepatocytes and enterocytes, known prebeta-1 HDL. Apo A-I on this nascent HDL particle activates the adenosine triphosphate (ATP)–binding cassette (ABC) protein, ABCA1, on the surface of peripheral cells, including macrophages. Once activated, the ABCA 1 protein transports unesterified cholesterol and phospholipids from the cell onto the nascent HDL particle. On the surface of HDL particle, the cholesterol is esterified by lecithin-cholesterol acyl transferase (LCAT) and its cofactor, apo A-I. As it circulates, nascent HDL particles are transformed into a mature, spherical HDL particle that contains cholesteryl ester in its core. The current dogma suggests that HDL returns the majority of circulating cholesterol to the liver and thus acts as the good cholesterol favoring reverse cholesterol transport. Indeed, over the last 10 years, several large randomized control trials have been designed on this promise that raising HDL cholesterol lowers CVD risk. The results of the majority of these studies do not support the concept that raising HDL cholesterol reduces CVD risk. These recent findings have suggested the need to reexamine our understanding of HDL biology. In this chapter, I present evidence from both basic and clinical studies that suggest a *cholesterol shuttle* function for HDL particles.

II. HDL functions

1. **HDL particles shuttle cholesterol between lipoproteins and are a source for LDL cholesterol:** Emerging evidence suggests that HDL particles acquire and exchange a substantial amount of cholesterol from and to other lipoproteins and not just peripheral tissues [3, 4]. It has been shown that cellular unesterified cholesterol is initially taken up by lipid-poor (pre-beta-1 HDL) [5, 6]. However, the majority of this cholesterol is subsequently transferred to plasma LDL and only a small proportion (5%) of it is esterified on HDL before it reaches LDL [7]. This explains the higher amount of cholesterol that LDL particles carry. Moreover, patients with Tangier disease who have a defect in ABCA-1 and do not form HDL cholesterol have reduced levels (50% decrease) in their LDL cholesterol content [8].

2. **Prebeta-1 HDL particles are not exclusive acceptors of cellular cholesterol, and VLDL particles are critical for cholesterol return to the liver:** The HDL centric reverse cholesterol hypothesis is based on prebeta-1 HDL initially acquiring free cholesterol from peripheral tissues to be esterified and later circulated to the liver for excretion. Recent studies support a major role for LDL and VLDL as cholesterol acceptors in the fasting and post prandial state respectively. It has long been demonstrated that red blood cells are major storage sites of the circu-

lating cholesterol pool [9, 10]. Four to five hours after fatty meal, the efflux of un-esterified cholesterol from red blood cells (RBCs) increases; the most potent acceptor of RBC cholesterol are chylomicrons and VLDL, while low levels of HDL do not limit cholesterol efflux from the cells [11]. More recently, infusion of reconstituted HDL in humans was shown to "shuttle" cholesterol into VLDL particles, where VLDL metabolism became the primary mechanism for the ability of plasma to move cholesterol from cells to the liver [12]. In agreement, a kinetic study of lipoprotein metabolism demonstrated that the majority (70 %) of cholesterol ester transfers back to the liver on VLDL [13].

3. **LDL particles are a major storage site for cholesterol esters in plasma:** In fasting plasma, LDL un-esterified cholesterol was the only source of cholesterol for esterification. Based on these findings it appears that regardless whether the initial acceptors are prebeta1 HDL, chylomicrons or VLDL, cellular un-esterified cholesterol passes through LDL [3].

4. **HDL cholesterol sources and fates:** The HDL cholesterol pool is primarily a function of the ABCA-1, ABCG1, SR-BI transporters and CETP activity. ABCA-1 activity in the liver, intestine and adipose tissue [14] substantially contribute to the formation of lipidated Apo A-I and thus formation of HDL cholesterol. On the other hand, steroidogenic organs such as the ovaries and the adrenals expressing high levels of SR-BI transporter can exchange HDL cholesterol. In metabolic diseases such as obesity, CETP is a major factor in shuttling cholesterol between LDL and HDL particles. Importantly, macrophage cholesterol constitutes less than 5 % of HDL's cholesterol [15]. Thus, changes in HDL cholesterol levels may not imply that cholesterol is moving out of macrophages, and changes in macrophage cholesterol efflux may not result in increases in HDL cholesterol levels. Although HDL is involved in returning cholesterol to the liver, this likely constitutes a minor HDL function, and perhaps a minor mechanism for reverse cholesterol transport in humans. The increased ratio of phospholipid to cholesterol on the HDL surface suggests that HDL's role in cholesterol transport is through facilitate cholesterol delivery to the adrenals and steriodogenic organs to maintain steroid and sex hormone synthesis, perhaps through SR-BI receptors. One example is through a study by Vergeer et al [16] showing that carriers of a genetic defect in the SR-BI transporter with increased concentrations of HDL cholesterol have impaired adrenal and platelet functions.

5. **Apo A-I, the major HDL protein, protects against vascular inflammation and oxidation of LDL particles:** Infusing rabbits with increasing doses of Apo A-I reduces markers of vascular inflammation (ICAM and VCAM) [17], whereas infusion of recombinant HDL reduced both measures of atherosclerosis and these markers of inflammation [18]. There are human studies suggestive that low HDL-C is associated with increases in vascular measures of inflammation, and that HDL isolated from research participants following the ingestion of a polyunsaturated diet can reduce these markers of inflammation [19]. In addition to its capacity to reduce vascular inflammation, Apo A-1 or HDL appear

to reduce the susceptibility to LDL oxidation. Infusion of human apoA-I into human recipients results in LDLs becoming resistant to oxidation and being less effective in inducing monocyte chemotactic activity in a human artery wall co-culture [20]. However, it is important to note that studies infusing Apo-I or HDL and demonstrating beneficial clinical outcomes in humans are to date lacking.

6. **Complexity of the HDL proteome suggests pleiotropic HDL functions:** Recent proteomic studies suggest that HDL particles are highly complex [21–23]. For example, HDL enriched with complement proteins implicate functions related to the immune system. One particular example is illustrated by ApoL1. Patients lacking ApoL1 in their HDL's are at an increased risk of developing a Trypansomal infection [24]. Enrichment of HDL with SAA after inflammation assists in clearing of cellular cholesterol after macrophage induced cytotoxicity of microbial cells [25]. In addition, SAA enriched HDL has a strong affinity to SR-BI (highly expressed in the adrenals) [26] suggesting a mechanism for supplying cholesterol to assist with increased cortisol production during periods of stress.

Important Advances

A. **Lessons learned from CETP inhibition:**
CETP evolved with higher species (rabbits to primates) perhaps as a mechanism to accommodate an increase in cholesterol turnover by facilitating cholesterol exchange between HDL and VLDL particles. The rationale for inhibiting CETP was based on that CETP inhibition raised HDL cholesterol, lowered LDL cholesterol and that in some families loss of function mutations in CETP activity was associated with longevity. To date, interventional studies using three CETP inhibitors did not demonstrate improvements in cardiovascular risk. Three CETP inhibitor trials to date did not reveal CVD benefit, and in fact one of these trials revealed harm (Illuminate trial [27]). CETP inhibition represents the fallacy of good and bad cholesterol concept. As discussed before, a key kinetic study revealed that around 70% of cholesterol is returned to the liver via VLDL by CETP mediated transfer. Thus, inhibiting CETP will force reverse cholesterol transport through HDL, a mechanism that may not be very efficient with concomitant use of statins that are known to upregulate the liver LDL receptor (in anticipation for cholesterol getting returned to the liver on VLDL and LDL). Moreover, increasing HDL-C by CETP inhibition will favor SR-BI uptake not only in the liver, but also in the adrenals, or any tissues expressing high levels of SR-BI. Thus, it is not surprising that in the Illuminate trial [27] blood pressure was elevated in the Trocetrapib arm. This could represent an increase in HDL mediated activation of the steroidogenic aldosterone pathway in the adrenals that preferentially express SR-BI receptors.

B. **The LDL receptor and the ABCA-1 transporters in atherosclerosis:** Insights from Tangier's Disease and Familial Hyperlipidemia (FH) underpin important differences of HDL and LDL functions on atherosclerosis. FH is a common genetic disorder characterized by a defect in the LDL-R. Homozygous mutations lead to atherosclerosis in early childhood, whereas heterozygous mutations are more common and lead to atherosclerosis later childhood and into adulthood if untreated. The loss of the liver LDL receptor function is a bottle neck for reverse cholesterol transport as the majority of cholesterol esters are returned to the liver through the LDL-receptor. There is a futile increase in CETP activity in FH that compounds the defect in apoB clearance. In this situation, CETP activity favors increased HDL catabolism and explains the lower HDL-C levels seen in FH [28]. Thus, the liver LDL receptor is critical in LDL cholesterol re uptake for catabolism. Macrophages can take up LDL cholesterol without relying on the LDL receptor to (through scavenger receptor A and pinocytosis) [29]. The excess in circulating lipoproteins as a result of cholesterol clearance favors foam cell formation and atherosclerosis development. These events clearly demonstrate that excess cholesterol "exposure" as a function of defective liver clearance is atherogenic, and mechanisms to reduce atherosclerosis may depend on improving cholesterol clearance. In contrast to the importance of the liver LDL-receptor in atherosclerosis, the link between ABCA-1 activity and atherosclerosis is less clear. A recent study by the Parks Lab [30] suggested that ABCA-1 deletion in the liver did not accelerate atherosclerosis despite major reductions in HDL cholesterol content. This has implications on any strategy that raises HDL cholesterol through inhibiting its liver metabolism such as the newer anti-mir33b therapies. Mir-33 is a microRNA that inhibits ABCA-1 expression and regulates liver cholesterol metabolism [31]. Inhibiting mir-33 in the liver leads to increases in HDL cholesterol and decreases in triglycerides [32] and currently in the pipeline for human studies. However, it is not clear that increasing HDL cholesterol by making more liver HDL is atheroprotective, given the fact that HDL cholesterol exchanges its cholesterol with LDL without an overall effect that alters cholesterol excretion. Patients with Tangier's Disease have a complete loss-of-mutation in the ABCA-1 transporter, a critical membrane protein for lipidating Apo A-I and thus forming HDL. However, patients with Tangier have decreased LDL cholesterol reflecting the important concept that HDL-C substantially contributes to LDL-C content. Thus, the net effect is "less" exposure to cholesterol compared to the environment of FH. Clinically, patients with Tangier's Disease have a modest increase in the risk of premature CVD compared to FH, appearing later in life [8]. They have neurologic deficits and characteristic orange colored tonsils that likely reflect cholesterol accumulation in macrophages [8]. Moreover, the "less severe" loss-of-function mutations in ABCA-1 transporter that are associated with decreases in HDL-C are not associated with increased CVD risk [33] in one large prospective Danish population study. These findings suggest that changes in HDL cholesterol per se may not be critical to the development of atherosclerosis, and perhaps a marker of other diseases (such as metabolic diseases in case of increased CETP function).

C. **Is raising HDL cholesterol a good target for decreasing heart disease risk**?
To date, two randomized clinical trials involving CETP inhibitors [27, 34], and two niacin trials [35, 36] did not translate into decreased CVD outcomes. These findings raise an important question on the futility of raising HDL cholesterol. Indeed, deleting the major transporters known to contribute to macrophage cholesterol efflux and reverse cholesterol transport does not change HDL cholesterol levels. The macrophage cholesterol content is a very minor contributor to the HDL cholesterol pool [15, 37]. Another example of the discrepancy between HDL cholesterol levels and atherosclerosis is through studies involving the SR-BI receptor. SR-BI overexpression increases RCT through increased HDL particle uptake into the liver. In animal studies, the result is decreased atherosclerosis coupled with a decrease in HDL cholesterol [38]. Thus, mechanisms that increase HDL cholesterol by blocking its liver uptake may retard reverse cholesterol transport. The implications of the above mentioned studies are particularly relevant to niacin. Niacin increases HDL cholesterol, possibly by inhibiting HDL uptake into the liver, and to a lesser extent by increasing Apo A-I levels. This mechanism may not imply that cholesterol is being transported out of macrophages. However, niacin can lower LDL-C and triglyceride levels and these are potentially be atheroprotective changes. Recent clinical studies (AIM HIGH [35] and HPS-2 thrive [36]) with niacin added on top of statins highlight our knowledge gap in the role of raising HDL-C as a target for therapy to lessen the CVD burden. Recent studies have highlighted a potential role for cholesterol efflux as a metric of HDL functions representing a better index of reverse cholesterol transport than HDL cholesterol [39, 40]. However, the determinants of cholesterol efflux are not yet fully understood.

D. **Apo A-I raising therapies:**
An alternative strategy for raising HDL cholesterol is through increasing Apo A-I levels. As discussed above, animal and early clinical human studies clearly demonstrate that Apo A-I protects against atherosclerosis through its effect on modulation vascular inflammation and reducing LDL oxidation [17–20]. Over the last 10 years, Apo A-I and HDL infusion therapies [41–43] were shown to reduce the severity of atherosclerosis using intravascular ultrasound studies of coronary arteries (IVUS). In addition to Apo A-I infusion therapies, promoters of apoA-I synthesis (RX208) [44] and Apo A-I mimetic peptides were developed [45]. We are still awaiting randomized clinical trials of these Apo A-I therapies using clinical endpoints. The main barriers to the progression of these studies have been their toxicity profiles [46] or their methods of administration.

Summary

Raising HDL cholesterol as a sole target of therapy appears to be a futile strategy. Most of the cholesterol on HDL comes from the liver and intestine. Raising it adds more cholesterol into the system, and shuttling this cholesterol into LDL particles can be atherogenic. Macrophages do not store much cholesterol. Therapies that can increase cholesterol efflux out of macrophages are unlikely to change HDL cho-

lesterol unless they simultaneously move cholesterol out of the major cholesterol stores. One major gap in the field is a reliable marker of macrophage reverse cholesterol transport, and cholesterol efflux might represent such a metric. Development of such an index can help inform us on interventions favor an atheroprotective cholesterol transport. Thus, low HDL cholesterol is a marker and not a mediator of cardiovascular disease. Apo A-I has atheroprotective activities, but the developments of such therapies have been hampered with toxicity profiles or the methods of administration. It is likely that raising HDL cholesterol by increasing Apo A-I is atheroprotective, but more definitive answers await randomized clinical trials.

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Chapter 6
Approach to the Patient with Lipid Disorders

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Classification of Lipid Disorders

Historically, the primary dyslipidemias have been classified according to the defect in their metabolism of either excess production or impaired removal of lipoproteins (discussed in the Chap. 2). The Fredrickson Classification system for lipids separates diseases into clinical syndromes which affect the same lipoproteins and express a similar lipid pattern. These classifications have become somewhat less clinically useful over time, and we propose a new classification system below (Table 6.1) that is more practical for the practicing provider. This classification includes the genetic variations and associations that have been elucidated in the recent past, and their influence on CAD risk. There are 3 dyslipidemia categories that have shown consistent relation to increased CAD risk: (1) isolated LDL dyslipidemia (aka as Familial Hyperlipidemia or FH), (2) mixed dyslipidemia and (3) Lp(A) disorders. There are two other dyslipidemias that are not consistently associated with increased CVD risk (1) isolated hypertriglyceridemia, and isolated low HDL dyslipidemia.

Laboratory Evaluation

Cholesterol and triglyceride levels are classically measured using enzymatic or colorimetric approaches. HDL cholesterol is measured after LDL precipitation (using Phosphotungistic acid, MgCl2, Dextran, or polyethylene glycol). Triglyceride levels have been assessed in the laboratory using an enzymatic method

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Table 6.1 Classification of the common lipid disorders based on CVD risk

	Clinical picture	Lipoprotein abnormality	Incidence	Typical lipid level			Cardiac risk
				TG	Non-HDL-C	HDL-C	
Isolated hypertriglyceridemia	Generally asymptomatic, may have xanthomas, pancreatitis, secondary causes or familial disease	TG metabolism	1:300	↑↑	↔	↔	↔
Mixed hyperlipidemia	Obesity, metabolic syndrome, diabetes mellitus	Increased VLDL conversion to LDL	1:100	↑	↑	↓	↑
Isolated low HDL dyslipidemia	Asymptomatic	Genetic defects in HDL metabolism	1:400	↔	↔	↓↓	↔
Isolated LDL dyslipidemia (familial hyperlipidemia)	Planar, tuberous and tendon xanthomas	LDL receptor	1:500	↔	↑↑	↓	↑↑
Lp(a)	Asymptomatic Lp(a) > 95th percentile	Genetic defects in Lp(a) metabolism	Varies among ethnicities	↔	↑	↔	↑↑

following hydrolysis and release of glycerol [1]. Glycerol is either oxidized with sodium periodate to produce formaldehyde, or mixed with glycerol kinase in the presence of ATP to form glycerol-3-phosphage and ADP. Formaldehyde is then reacted with acetylacetone in the presence of ammonium ions which produced a yellow compound diacetyl dihydrolutidine which is then measured colorimetrically. Alternatively, glycerol-3-phosphate is oxidized by glycerol phosphate oxidase to dihydroxyacetone and hydrogen peroxide. Oxygen is released from hydrogen peroxide in the presence of peroxidase which oxidizes p-chlorophenol chromogen to form a colored compound which is then measured colormetrically [2]. Due to the reliance on the measurement of glycerol on the accuracy of triglyceride levels is influence by measurement of pre-existing glycerol in samples. There are a few situations where elevated glycerol levels in the sample can alter triglyceride concentrations. In normal individuals the plasma the glycerol concentrations are generally < 0.163 mmol/L, which is equivalent to a triglyceride concentration of

14 mg/dL. Interfering glycerol levels can be elevated in uncontrolled diabetes vigorous exercise, from contamination with stoppers of some blood collection tubes which use a glycerol lubricant, after ingestion of glycerol containing medications, or due to genetic hypertriglyceridemia as a result of a mutation in the glycerol kinase gene [3].

Newer methods include the VAP (Verticle Auto Profile) test which directly measures triglyceride levels as well as directly measures triglyceride-rich lipids, VLDL (1, 2, 3 and total). VAP generates a series of absorbance curves using density gradient ultracentrifugation from which proprietary software produces patterns of subclasses [4]. Alternative methods commercially available include Gradient Gel Electrophoresis where lipoproteins are separated in a gradient gel on the basis of their size and charge, Nuclear Magnetic Resonance (NMR) Spectroscopy which separates particles based on characteristic lipid methyl group NMR signals, and Ion –Mobility Analysis a newly developed method that measures the size and concentration of lipoprotein particles based on their gas-phase differential electric mobility [5]. The role of advanced testing in clinical practice is controversial and reserved to patients where the risk of cardiovascular disease is uncertain.

Triglyceride Metabolism Disorders

Hypertriglyceridemia as a dyslipidemia can be related to either a primary disorder or due to secondary causes. Secondary causes of hypertriglyceridemia include such processes as obesity, particularly central obesity, insulin resistance and type 2 diabetes, medications, alcohol, end stage renal failure, and HIV [6]. Many of the secondary causes of hypertriglyceridemia have been speculated to overlap or worsen an underlying genetic predisposition to dyslipidemia and its associated CAD risk. This potential connection to CAD becomes even more staggering when we consider that in 2010, and estimated 19.7 million Americans (8.3 % of the adult population) had diagnosed diabetes mellitus, with an estimated 8.2 million with undiagnosed diabetes mellitus and an additional 38.2 % with pre-diabetes [7]. As mentioned, the historic classification of lipids does not include the full spectrum of lipoprotein abnormalities and does not currently encompass the scope clinical disease [6]. For example, of these dyslipidemias familial combined hyperlipidemia, familial hypoalphalipoproteinemia and type 2 diabetes have been purported to account for up to 50 % of premature coronary artery disease (CAD) events [6].

Structurally Triglycerides are composed of three fatty acids and an ester component derived from glycerol. Triglycerides are a major source of the dietary lipids ingested by US adults, and are biologically important for several reasons [8]. Through enzymatic degradation these dietary lipids are made available to various tissues for energy production and storage. As mentioned clinically excess plasma TG has become progressively scrutinized for its epidemiologic connection to increased coronary heart disease and pancreatitis [9, 10]. It has also been implicated

as a marker in the assessment of metabolic syndrome together with risk factors such as hypertension, insulin resistance and prothrombolitc states [11]. The mean triglyceride level in a U.S. adult has been steadily increasing since the mid-1970s, and continues to trend upward, and with it a growing epidemic of obesity, type 2 diabetes, and metabolic syndrome [12].

As a clinical risk measurement plasma TG has been classified by the National Cholesterol Education Program as follows: 150–199 mg/dL, borderline high; 200–499 mg/dL, high; greater than or equal to 500 mg/dL, very high [12]. However, these classifications, particularly regarding borderline high and high levels may not be practical, as at elevated levels triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins) directly influence LDL and HDL composition and metabolism, which in turn may effect particle function and atherogenicity [12].

Hypertriglyceridemic Disorders

Hypertriglyceridemia has long been associated with risk of cardiovascular disease, but the relative contribution of TG as a direct promoter of CAD versus a marker of increased risk continues to be under debate [12]. Although the link between TG and CAD has been consistent, the effect size has been relatively modest compared to other lipoprotein abnormalities and parameters. Measurement of triglycerides has additionally been difficult to correlate with risk as there exists a high level of variability, and a lack of standardization of non-fasting TG levels, which in addition to fasting TG have been associated with heart disease [6]. Moreover, as evidenced by the genomic associations and overlap in the relationship with LDL, HDL-C and apoprotein AI, TG elevation rarely exists as an isolated association. There is a complex interaction between genetic risk, environmental stimulus and co-morbid diseases which are confounding factors in the evaluation of pathologic hypertriglycerideemia. The major clinical manifestations of hypertriglyceridemia have centered around two disease processes. Elevated levels have been associated with coronary artery disease and very elevated levels are a large risk factor for pancreatitis. Several landmark studies have evaluated the clinical utility of lowering triglyceride levels and the subsequent effects on the incidence of coronary heart disease.

Isolated Hypertriglyceridemia

This entity encompasses two groups of patients: (1) Familial Hypertriglyceridemia and (2) Secondary causes of Hypertriglyceridemia.

Familial Hypertriglyceridemia (FHTG)

A genetic variant where the risk of CVD is generally low, this is a relatively common autosomal dominant disorder [13]. Classified according the Fredrickson classification as Type 4 hyperlipidemia, the underlying pathophysiology is unclear but VLDL triglyceride production is increased in the setting of normal apo B production [3]. In this entity, the risk of CVD is not increased.

Secondary Causes of Hypertriglyceridemia

Causes of secondary hypertriglyceridemia include endocrine disorders, genetic disorders and medication side effects (Table 6.2). Hypothyroidism has been correlated with increased total cholesterol and triglyceride levels as well as increased non-HDL cholesterol levels. This in turn has been inconsistently linked with coronary heart disease. Changes in TSH have also been independently correlated with elevated triglycerides and risk of metabolic syndrome as coronary heart disease even within the euthyroid range [14]. The underlying mechanism behind these effects is unclear but has been linked to alterations in LDL receptors, diminished secretion of cholesterols into bile, reduced lipoprotein lipase activity and increased triglyceride synthesis. Chronic treatment with thyroid hormone has been shown to decrease lipid levels. T3 induced gene transcription in the liver in rodents was correlated with increased fatty acid oxidation and decreased steatohepatitis. Rare familial disorders of lipid metabolism are known to be associated with hypertriglyceridemia such as glycogen storage diseases in children, and Kobberling lipodystrophy (familial partial lipodystrophy type 1) which is associated with fat loss in the extremities, central obesity, and increased risk of pancreatitis and early cardiovascular disease [15]. Various other associated conditions and medications have been linked to hypertriglyceridemia, including acromegaly, PCOS, renal and hepatic disease, HIV and its treatment, pregnancy, oral estrogens, tamoxifen, glucocorticoids, and bile acid sequestrants [13]. The changes in acromegaly and PCOS, for example, are linked to increased insulin resistance and its changes as discussed below. Oral estrogens induce stimulation of secretion of TG rich lipoproteins, and glucocorticoids lead to increased fatty acid synthesis and decreased clearance of TG. HIV has been independently associated with hypertriglyceridemia, and decreased HDL. Increased inflammation and alterations in gut microbiology have been reported to cause increases in lipopolysaccharides, which have been shown to downregulate LPL and cause hypertriglyceridemia [16]. Protease inhibitors utilized in HIV therapy are associated with interactions that inhibit normal lipid metabolism by mechanisms such as interference with LDL receptor related protein and other lipid binding proteins [17]. Acute hepatitis has been liked with elevated triglycerides. Renal failure has been found to increase serum Tg by

Table 6.2 Secondary causes of hypertriglyceridemia

Acromegaly
Alcohol
Anorexia nervosa
Cholestasis
Chronic renal failure
Cushing's syndrome
Diabetes mellitus
Drugs
Cyclosporine
Glucocorticoids
Estrogens
Tamoxifen
Anabolic steroids
Antipsychotic drugs
Protease inhibitors
Retinoids
Thiazides
Beta blockers
Furosemide
Bile acid sequestrants
Hepatic disease
HIV/AIDS
Hypothyroidism
Metabolic syndrome
Myeloma
Obesity/high trans fat diet
Polycystic Ovarian Syndrome (PCOS)
Pregnancy
Sarcoidosis
Systemic Lupus Erythematous (SLE)
Sepsis

proposed mechanisms of decreased clearance and increased hepatic production of apo B containing lipoproteins [13]. Lipid lowering medications such as bile acid sequestrants bind bile acids in the intestine leading to decreased entero-hepatic recirculation of bile acids which ultimately decreases hepatocyte cholesterol content and LDL cholesterol concentrations. In some patients however, this can cause increased hepatic VLDL production and raise serum triglyceride levels. Use of these agents is contraindication in familial dysbetalipoproteinemia and with triglyceride levels >400 mg/dL, as well as relatively contraindicated with triglyceride levels >200 mg/dL [11].

Mixed Lipidemias

This entity makes up the majority of lipid disorders seen in clinic. The most common form of mixed dyslipidemia is Familial combined Hyperlipidemia.

Familial Combined Hyperlipidemia (FCHL)

Familial combined hyperlipidemia (FCHL) is associated with increased risk of coronary artery disease. Individuals, often also have associated risk factors such as central obesity, insulin resistance, hyperglycemia, and hypertension [18]. Fredrickson classification type 2B, these individuals can have simple hypercholesterolemia, simple hypertriglyceridemia or a mixed defect. Estimated to occur at frequency of 1 in 100 [3]. The specific gene defects are unclear and environmental factors likely play are role in this multifactorial disease, but obesity and diabetes are key elements of this lipid disorder. Dietary intake in particular of simple sugars such as those found in high carbohydrate meals (bread, pasta, rice, potatoes) or high fructose corn syrup containing meals, and particularly alcohol, increase the liver production of TG. Fat intake can also increase TG, particularly in those with plasma TG levels >500 mg/dL. Alcohol affects lipid metabolism by inducing de novo fatty acid synthesis and inhibiting fatty acid oxidation in the liver, with overproduction of VLDL and its remnant particles. Obesity itself strongly affects TG levels. In review of NHANES data collected between 1999 and 2004, 83 % of participants with TG levels ≥ 200 mg/dL were classified as obese with BMI ≥ 31 kg/m^2 [12]. Associations have also been seen between adipose tissue and visceral adiposity in individuals over age 50 and elevated TG levels [19]. Increased waist circumference >102 cm in men of >88 cm in women has been associated with increased cardiovascular risk [13].

In the insulin resistance state there exist an increased number of TG rich VLDL particles in the circulation as a result of increased hepatic production. Accumulation of these particles has been purposed to be a contributing factor in the increase risk of CAD in diabetes mellitus. Insulin itself has been shown to affect the normal suppression of hepatic VLDL production. The gene for insulin receptor substrate 1 (IRS1), which is one of the primary phosphorylation targets of the insulin receptor has been found in some individuals with type 2 diabetes mellitus to have a polymorphism placed in a non-coding region [20]. These individuals in genome wide association studies were found to have increased CAD compared to the general population [21]. Insulin resistance in type 2 diabetes mellitus also leads to loss of insulin's action of the degradation of apo B, as well as increases the free fatty acid flux to the liver and hepatic lipogenesis with increased triglyceride synthesis. LPL levels are reduced, and LDL particles are hypothesized to be more athrogenic due to smaller denser particles which are more susceptible to oxidative stress [22]. Apo C II deficiency creates a functional LPL deficiency, with similar phenotype. Whereas, elevated Apo C-III levels has been associated

with hyperinsulinemic patients and hypertrigiceridemia. Apo CIII inhibits LPL mediated TG hydrolysis, interfering with the effect of Apo C II and disrupting the ratio of Apo CII/Apo CIII which in turn modulates LPL activity [21]. Point mutations in Apo CIII have been shown to affect a transcription factor binding site associated with insulin response, leading to dysfunctional lack of response to insulin at the Apo CIII promotor, causing increased transcription of Apo CIII and subsequent increased levels of TG in plasma [3]. Insulin resistance also leads to increased levels of Apo CIII, which in turn may increase small dense LDL formation, and increase the risk of atherosclerosis. Increased Apo CIII levels have been seen in Hispanic and white non Hispanic populations over African American populations and elevated levels >14 mg/dL increase the risk of metabolic syndrome by more than 3 fold [23]. These increased Apo CIII levels have been shown in subjects with familial combined hyperlipidemia, obese subjects and those with metabolic syndrome [23].

Other genetic factors which affect glucose and TG metabolism include variants of the carbohydrate response element binding protein (CHREBP), encoded at MLXIPL locus. CHREBP is associated with lower TG concentrations, glycolytic enzymatic activation of GCK, and additionally fatty acid synthase, to alter dietary carbohydrate conversion to TG. These genetic susceptibilities underline the possible ethnic differences in, for example, the increase obesity, higher TG levels, increased prevalence of type 2 diabetes mellitus in the Hispanic population. Various other disorders such as chronic kidney disease, hyperbilirubinemia and thyroid dysfunction have also been shown to elevated Apo CIII levels [3]. Apo CIII levels are also affected by age, alcohol consumption and oral contraception use which all are associated with greater levels.

Dysbetalipoproteinemia/Type III Hyperlipidemia

Dysbetalipoproteinemia occurs when there is a genetic variant of ApoE resulting in decreased lipoprotein clearance, and a reduction in conversion of VLDL to IDL and LDL. The most common isoforms are E-3, E4 and E-2 [3]. Patients therefore have an elevated intermediate lipoprotein band on electrophoresis known as β-VLDL, as well as increased total cholesterol and triglyceride levels. Individuals have an increased risk of CAD and peripheral vascular disease. Prevalence is on the order of 1 in 100 persons for the homozygote Apo E2 isoform, but clinical expression occurs in about 1–5 out of 5000 persons [3, 24]. It is thought that secondary risks such as obesity, diabetes, hypothyroidism, etc. therefore are needed for full clinical expression of this disease. Patients generally present with symptoms of xanthomas, xanthoma striata palmaris, tuberous and tuberoeruptive xanthomas over the elbows, knees, and buttocks [24]. Premature CVD is common, along with hyperuricemia and glucose intolerance. Triglyceride and total cholesterol levels are generally in the range of 300–1000 mg/dL [13].

Isolated High LDL Dyslipidemias: Familial Hyperlipidemia

Disorders characterized by elevated LDL show a hefty association with cardiovascular risk, even with modestly elevated or normal triglyceride levels. Fredrickson classification Type 2A, individuals have increased vascular deposits of lipid, with premature CVD as well as corneal arcus, tendinous xanthomata and xanthelasma This increased LDL is the major metabolic abnormality Familial Hypercholesterolemia, characterized by defect in the LDL receptor gene. There are hundreds of genetic defects that have been implicated in the expression of these pheonotypes however the heterogeneity in clinical presentation varies greatly with environmental factors. Familial hypercholesterolemia (FH) for example occurs at a prevalence of approximately 1 in 500, and characteristically displays LDL levels 2–3 fold above the 50th percentile [3]. Homozygotes for FH have a 4–6 fold or higher increase in LDL and develop CVD in the 2nd or 3rd decade of life [24]. FH is discussed in greater detail in Chap. 2.

Isolated Low HDL Syndromes

HDL levels are influenced either by two process: (1) reduction in formation, or increases in catabolism in HDL proteins (mainly Apo A-I), or (2) by changes in HDL cholesterol content. HDL cholesterol content is commonly decreased in metabolic syndrome, but it is not clear if shuttling of cholesterol between the lipoprotein particles in the setting of hypertriglyceridemia is atherogenic per se. Isolated low HDL levels are thought to exert their effect through disruption of reverse cholesterol transport, which delivers excess cholesterol back to the liver for disposal as bile salts. Moreover, the risk of increased CVD in isolated HDL dyslipidemia depends on the mechanism for the low HDL. For example, heterozygote loss of function mutations in ABCA-1 transporter that is typically associated with decreased HDL-C (20–40 mg/dL range) is not associated with increased CVD risk [25]. Recently, genome wide association studies and observational biomarker data showed that isolated changes in HDL cholesterol do not necessarily confer increased CVD risk [26]. However, classic syndromes such as Familial Hypoalphalipoproteinemia, Apo A1 deficiency, Tangier Disease and Lecithin Cholesterol Acyltransferase Deficiency have been shown to have increased risk of CVD [18], but these conditions are uncommon and HDL cholesterol is less than 5 mg/dL.

Lp(a) Disorders

Lipoprotein(a) [Lp(a)] is a part of lipoprotein subclass made up of an LDL-like particle and the specific apolipoprotein(a) [apo(a)], which is covalently bound to the apoB of the LDL like particle. High concentrations of Lp(a) in blood is related to increased risk of coronary heart disease (CHD), cerebrovascular disease (CVD), atherosclerosis, thrombosis, and stroke [27]. Diagnosis is based on Lp(a) levels greater than the 95% percentile. Patients should be aggressively treated to lower their cholesterol levels. The genetic defects of this disorder are described in Chap. 2.

Approach to the Patient at Risk for Cardiovascular Disease

Treatment of Adults at Risk of Cardiovascular Disease

Evaluation of the patient should start with a fasting standard lipid profile, including cholesterol, triglycerides, LDL and HDL. For fasting analysis patients should be instructed to fast for at least 8–10 h prior to blood draw. The age at which this screening should take place varies according to the recommendations from various expert panels. The Unites States Preventive Task Force for instance recommends the following; screening men age 35 years and older, and women age 45 years and older. Younger individuals are recommended to be screened if they are at increased risk for coronary heart disease (ex. family history of male first degree relative with CAD less than age 50, or female relative with CAD less than age 60, a family history of hyperlipidemia, associated risks such as smoking, hypertension, diabetes mellitus). This screening for at risk individuals is recommended to start at age 20–35 in men, and age 20–45 in women. The frequency of repeat screening is debated but is generally recommended for patients to undergo repeat screening every 4–6 years or sooner if there are other metabolic concerns or high risk features [28]. Patients initiated on therapy should have repeat screening fasting lipid profile in 4–12 weeks, and ever 3–12 months thereafter [29]. Prior to starting medical therapy patients should undergo evaluation of liver enzymes, renal function, and HgbA1c if patient has an unknown diabetes mellitus status.

The American College of Cardiology (ACC) and American Heart Association (AHA) recently published new guidelines for the management of lipid disorders and cardiovascular health in 2013. Compared to the prior lipid management guidelines of ATP III, these newer recommendations forgo the classification of specific LDL and non HDL goals and instead focus on risk reduction of CAD [30]. Clinical atherosclerotic cardiovascular disease (ASCVD) is defined by a history of “acute coronary syndromes or MI, stable or unstable angina, coronoary or other arterial revascularization, stroke, TIA or peripheral arterial disease presumed to be of atherosclerotic origin” [30]. Risk evaluation by the ACC/AHA task force combines

lipid profile, sex, age, race, systolic blood pressure and its treatment, the presence of diabetes mellitus and smoking to determine a 10 year CVD risk (via the pooled cohort equation, at http://my.americanheart.org/cvriskcalculator).

The algorithm focuses on risk reduction in particular in adults with clinical atherosclerotic vascular disease, LDL ≥ 190 mg/dL, age 40–75 years of age with diabetes and LDL between 70 and 189, and those age 40–75 years of age with LDL between 70 and 189 mg/dL and an estimated 10 year atherosclerotic cardiovascular disease risk of 7.5% or higher [30]. Further evaluation for secondary causes of dyslipidemia is recommended in those individuals with LDL ≥ 190 mg/dL and triglyceride levels ≥ 500 mg/dL.

Statins are the focus and mainstay of therapy according to the ACC/AHA lipid guidelines. Doses and type of statin are segregated according to low, moderate, or high intensity therapy. Low dose statin therapy is defined as lowering LDL on average by < 30%, moderate intensity by 30–50%, and high intensity by ≥ 50% (see Table 6.3).

A suggested approach for managing hyperlipidemia or persons at risk for CVD is presented in Fig. 6.1. For those adults age 21–75 years with clinical ASCVD high intensity statin therapy is recommended. For those with ASCVD but over age 75 years moderate intensity statin therapy can be considered if the patient is not a candidate for a high intensity statin. Patients without ASCVD patients are broken down into the different risk categories mentioned above. If LDL is greater than or equal to 190 mg/dL, high intensity statin therapy is recommended. Diabetics both Type 1 and Type 2 aged 40–75 years with LDL between 70 and 189 mg/dL are recommended to be on at least moderate intensity statin, however if the estimated 10 year

Table 6.3 High- moderate- and low-intensity statin therapy. (ACC/AHA guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults [30])

High-intensity statin therapy	Moderate-intensity statin therapy	Low-intensity statin therapy
Daily dose lowers LDL-C on average, by approximately ≥ 50%	Daily dose lowers LDL-C on average, by approximately 30% to < 50%	Daily dose lowers LDL-C on average, by < 30%
Atorvastatin (40[a])–80 mg	Atorvastatin 10 (20) mg	Simvastatin 10 mg
Rosuvastatin 20 (40) mg	Rosuvastatin (5) 10 mg	Pravastatin 10–20 mg
	Simvastatin 20–40 mg[b]	Lovastatin 20 mg
	Pravastatin 40 (80) mg	Fluvastatin 20–40 mg
	Lovastatin 40 mg	Pitavastatin 1 mg
	Fluvastatin XL 80 mg	
	Fluvastatin 40 mg bid	
	Pitavastatin 2–4 mg	

[a]Evidence from 1 randomized controlled trial only: down-titration if unable to tolerate atorvastatin 80 mg in IDEAL

[b]Although simvastatin 80 mg was evaluated in randomized clinical trials, initiation of simvastatin 80 mg or titration to 80 mg is not recommended by the FDA due to the increased risk of myopathy, including rhabdomyolysis

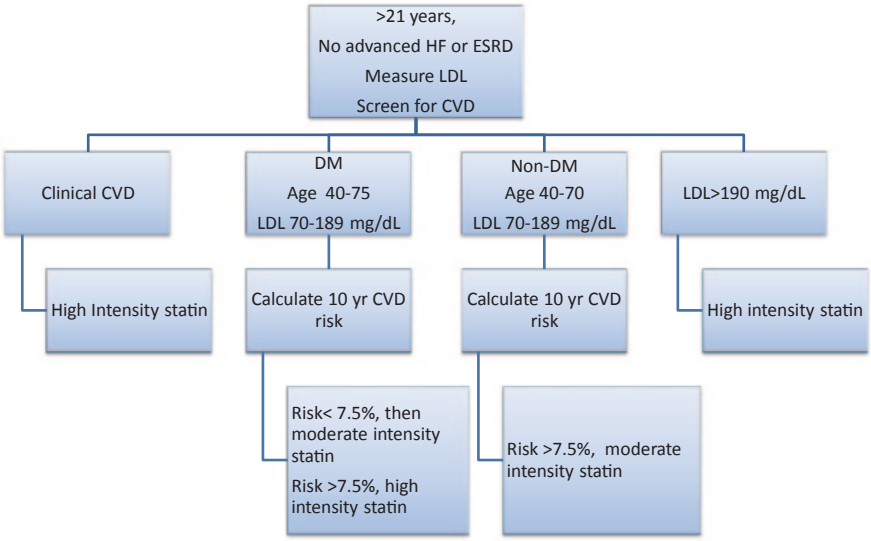

Fig. 6.1 A practical algorithm for starting statin therapy in persons at risk for cardiovascular disease (adapted for the AHA/ACC 2013 guidelines). The guidelines require using the Pooled Cohort Equations to calculate the expected 10-year ASCVD risk (http://tools.cardiosource.org/ASCVD-Risk-Estimator/). Few data were available to indicate an ASCVD event reduction benefit in primary prevention among individuals >75 years of age who do not have clinical ASCVD, with heart failure class II-IV or end stage kidney disease. Therefore, initiation of statins for primary prevention of ASCVD in individuals >75 years or of age or with HF or ESRD requires consideration of additional factors, including increasing comorbidities, safety considerations, and priorities of care. ASCVD: atherosclerotic cardiovascular disease, HF: (Heart failure New York Heart Association Class II-IV), ESRD: End stage renal disease, DM: diabetes mellitus.

ASCVD risk is greater than or equal to 7.5%, via the pooled cohort equation, high intensity statin is recommended. For all others age 40–75 years with an estimated 10 year ASCVD risk of greater than or equal to 7.5% either moderate or high intensity statin therapy is recommended. The guidelines also note that treatment with statin therapy can be considered with a 10 year ASCVD risk of over 5%. There are no recommendations made for those individuals with NYHA class II-IV ischemic heart failure or for those on chronic hemodialysis. Lifestyle modification and healthy diet remains an important component of any treatment and is recommended for all individuals. Additional non statin agents can be considered in those who do not have an anticipated therapeutic response from statin therapy, or are intolerant to statin therapy [29]. Additional agents such as fenofibrate therapy should be considered in those with persistent hypertriglyceridemia with TG ≥500 mg/dL and particularly those with TG >1000 mg/dL due to the increased risk of pancreatitis. A suggested approach for managing hypertriglyceridemia is presented in Fig. 6.2.

Alternative factors that may indicated increased CVD risk such as elevated C-reactive protein ≥2 mg/L, coronary artery calcium score ≥300 Agatston units or ≥75th percentile for age, sex, and ethnicity can be considered as adjunct factors

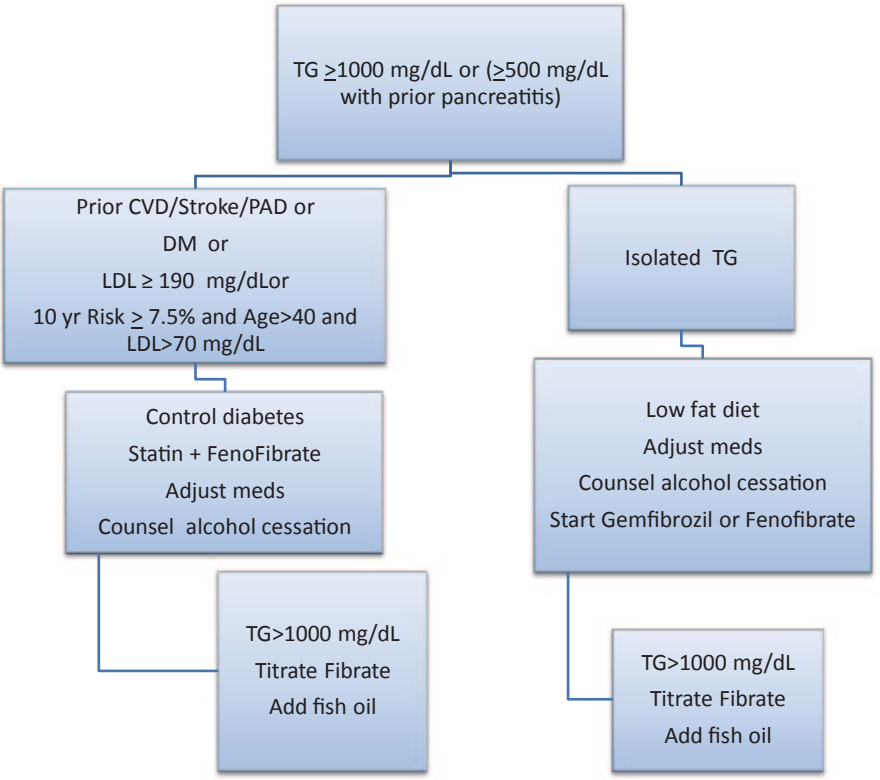

Fig. 6.2 A suggest algorithm for management of hypertriglyceridemia (TG). The preferred approach in hypertriglyceridemia is to estimate CVD risk and prevent of pancreatitis. Patients at increased ASCVD risk should be on concomitant statin treatment. DM: diabetes mellitus

in those individuals with indeterminate risk or who do not fit into the previously mentioned categories [29]. In the future, alternative measurements and more specific indicators of ASCVD may be used and more widely commercially available. Elevated levels and dysregulation of apolipoprotein B might be useful as a differentiation factor for the various dyslipoproteinemias [31]. Specific ratios of ceramides, waxy lipid molecules, found in cell membranes, cellular signaling, and in increased concentration in atherosclerotic plaques, may have better predictive potential for CAD mortality and may enable specific drug targeting. A few ceramide species have recently been shown to have a better predictive potential for CAD mortality with a test accuracy average of 0.66 with a 95% confidence interval, compared to classical markers such as LDL with a predictive CAD mortality of 0.55 [32].

Criticism of the new ACC/AHA guidelines and their variance from prior specific LDL targets has been focused on a few important considerations. The pooled cohort equation has not been specifically validated in the target population and many have expressed concerns over overestimated CVD risk in those who would otherwise

have minimal risk factors. Apprehensions have been expressed that without specific LDL goals for individual patients physicians may miss a chance for more individualized therapy and additional lipid modifying agents besides statins [29].

Referral to a lipid specialist may be considered in those with severe genetic dyslipidemia, those who fail conventional therapy or are intolerant to statin therapy, failure to lower triglyceride levels below 1000 mg/dL despite maximal therapies, or uncertainty as to whether or not a patient should be treated for dyslipidemia.

Treatment of Children and Adolescents at Risk of Cardiovascular Disease

This algorithm is adapted from Adapted from Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents [33]. Based on NHANES database, high and moderate risk classifications of at risk adolescents and children were defined as the following: High-level risk conditions are disease settings with clinical cardiac events before 30 years of age such as chronic kidney disease, post heart transplantation, diabetes mellitus, Kawasaki disease with persistent coronary artery aneurysms. Moderate risk conditions are disease settings with known pathophysiologic evidence of accelerated atherosclerosis such as chronic inflammatory disease (systemic lupus erythmatosus, juvenile inflammatory arthritis), HIV, nephrotic syndrome, Kawasaki disease with resolved coronary artery aneurysms). The following recommendations are for children > 10 years of age:

1. Initiate statin therapy if, after 6 months of lifestyle therapy, the fasting lipid profile shows LDL-C ≥ 190 mg/dL (based on average of 2 fasting lipid profiles).
2. Initiate statin therapy if, after 6 months of lifestyle therapy, if the fasting lipid profile shows LDL-C 160–189 mg/dL and one or more of the following: (1) a positive family history of premature cardiovascular disease (2) at least one high-level risk factor or risk condition (3) two or more moderate-level risk factors/risk conditions
3. Initiate statin therapy if, after 6 months of lifestyle therapy, if the fasting lipid profile show LDL-C ≥ 130–159 mg/dL and one or more of the following: (1) two or more high-level risk factors/risk conditions (2) one high-level risk factor or risk condition with at least two moderate-level risk factors/risk conditions (3) presence of clinical cardiovascular disease

Children aged < 10 years may be treated with lipid-lowering medication if they have a severe primary hyperlipidemia (LDL> 190 mg/dL) or a high-risk condition that is associated with serious medical condition.

Approach to Patients with Triglyceride Disorders

Do Triglyceride Reducing Therapies Reduce CVD?

Several key studies have evaluated the causation link between TG and CVD. In the Helinski Heart Study Gemfibrozil a fibric acid derivative was utilized to reduce levels of total and LDL cholesterol and triglycerides and raise the HDL cholesterol in middle aged men at high risk for coronary events due to hyperlipoproteinemia. A total of 4081 men were followed for 5 years and randomized to receive either gemfibrozil (2051) or placebo (2030). The treatment group showed a reduction in triglyceride levels by 43 %, as well as a reduction in total cholesterol by 11 %, LDL cholesterol by 10 % and non-HDL cholesterol by 14 %. HDL cholesterol also increased by approximately 10 %. The reduction in cardiac end points was not statistically significant in the first 2 years of therapy but thereafter the groups began to separate. Over a mean follow up period of 60.4 months, the gemfibrozil group showed an overall reduction in the frequency of cardiac end points (i.e. fatal and non-fatal myocardial infarction and cardiac death) by 34 % (95 % confidence interval, CI 8.2–52.6) compared to placebo. The greatest reduction in end points was in nonfatal myocardial infarction (221.9 per 1000 vs. 32 per 1000, $p < 0.02$. Adverse events of gastrointestinal upset were more common in the gemfibrozil group in the first 2 years. There were slightly more cases of eye surgery, mostly due to cataracts [34].

The VA High-Density Lipoprotein Intervention Trial (VA-HIT) evaluated the effect of gemfibrozil on 2531 men with known CHD. Treatment with gemfibrozil produced after 1 year an average increase in HDL-C of 6 %, and a decrease in triglycerides of 31 % and no change in LDL-C. All individuals in the VA-HIT study in contrast to prior studies had a low LDL-C ≤ 140/dL, and triglyceride levels ≤ 300 mg/dL, values representative of 75–80 % of men with CHD in the United States. At the end of 5 years, those men treated with placebo had an incidence of CHD events that was inversely related to HDL-C levels, but unrelated to their triglyceride and LDL-C levels. In those men treated with gemfibrozil, there was a reduction in CHD events compared with placebo in the second through fourth qunitiles of HDL-C levels ($p = 0.02$). The event rates in the gemfibrozil group did not differ with respect to triglyceride levels. For all levels of LDL-C, the gemfibrozil group had a lower CHD event rate. Only concentrations of HDL-C significantly predicted a CHD end point. The relative risk reduction in CHD end points for a 5 mg/dL increase in HDL-C with gemfibrozil was 11 %. In multivariable analysis triglyceride levels at baseline or after treatment did not predict CHD events. There was no independent benefit from triglyceride reduction. However, independently examining baseline triglyceride levels, particularly at the highest level of triglyceride levels, there was a significant relationship to the development of CHD. At the highest tertile level of baseline triglycerides treatment with gemfibrozil did result in a significant reduction in CHD events (RR of 28 %) [35].

The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial lipid arm evaluated middle aged and older individuals with type 2 diabetes at high risk for cardiovascular disease events and randomized them to receive simvastatin plus either fenofibrate or placebo. The lipid trials main aim was to examine whether the concurrent use of a fibrate with a reductase inhibitor (statin) in the setting of good glycemic control reduced the rate of cardiovascular events. The lipid trial included 5518 patients, whom had an estimated LDL-C off statin therapy of 60–180 mg/dL, and HDL-C < 55 mg/dL for women or African Americans, or HDL-C < 50 mg/dL for all other sex and age groups, and triglycerides < 750 mg/dL on no therapy or < 400 mg/dL on treatment with lipid lowering drugs. Patients were excluded if they had known hypersensitivity to lipid lower agents, on a medication which have reported interactions with statins or fibrates or a refusal to stop lipid lower drugs, history of pancreatitis, uncontrolled thyroid disease, breastfeeding, history of myopathy or pre-existing gallbladder disease. Over a mean 4.7 years of follow up there was no significant effect of fenofibrate on cardiovascular outcomes despite a significant reduction in triglycerides (42 vs. 16 mg/dL, mean triglyceride 162), increase in HDL and decrease in LDL [36]. As illustrated above, the addition of fenofibrates has not been shown to result in a significant benefit over statins alone in the reduction in non-fatal myocardial infarction or fatal coronary heart disease. Comparing the Helinski Heart Study and the VA HIT trial indicates that triglyceride lowering therapy may be effective to decrease CVD at the highest TG levels, however in the face of maximal statin therapy the addition of a fenofibrate to therapy has no clear additional benefit. Statin therapy therefore remains the first drug of choice to lower cholesterol levels in those with or at risk for coronary artery disease [6].

As mentioned above evaluation of triglyceride levels should start with a standard lipoprotein profile. Although increased post prandial levels of TG have been correlated with CAD, there is as yet no standardization regarding pathologic levels [6]. Risk and treatment plans are then stratified as mentioned according to degree of triglyceride elevation.

Pancreatitis Prevention and Treatment in Hypertriglyceridemia

Acute pancreatitis secondary to hyperlipidemia is generally characterized by abdominal pain, nausea and vomiting. Most commonly individuals are poorly controlled diabetics with hypertriglyceridemia. In a retrospective review of pancreatitis patients hospitalized in Augusta, Georgia, hypertriglyceridemia is implicated in the etiology of pancreatitis in 1.3–3.8 % of cases [37]. In a recent 15 year follow up of patients in Tayside, Scotland there was a significant dose-response relationship between triglyceride concentration and the incidence of acute pancreatitis. Hazard ratios increased with increasing triglyceride levels 1.04 [95 % CI, 1.02–1.05] for triglyceride levels between < 149 mg/dL, 1.50 [95 % CI 1.14–1.97] for triglycerides levels between 150–499 mg/dL, and 3.20 [95 % CI 1.99–5.16] for those with triglyceride levels >= 500 mg/dL. The risk of incident acute pancreatitis increased by

approximately 4 % for every 100 mg/dL increase in triglyceride concentration [10]. Acute pancreatitis is more likely to occur when triglycerides are > 1000 mg/dL.

Elevated triglyceride levels may cause their damage via the stimulation of amylase release due to excess free fatty acids within pancreatic acinar cells [38]. Autopsy studies show increased levels of intrapnacreatic fat content in patients with pancreatitis. Lipolysis of adiopocyte triglycerides by pancreatic lipase are proinflammatory, releasing intracellular calcium, inhibiting mitochondrial complexes and causing necrosis of the acinar cells [39].

With high levels > 500 mg/dL, triglyceride-lowering drugs (fibrate or nicotinic acid) are currently recommended as first line therapy, followed by statin therapy to lower LDL [11]. Because of the increased risk of pancreatits triglyceride levels > 2000 mg/dL are treated as a medical emergency. These high levels place patients at risk for chylomicronemia syndrome, which includes risks of eruptive skin xanthomas, lipemia retinalis, hepatic steatosis, mental status changes and acute pancreatitis [11].

Outpatient Management

With elevated triglyceride levels > 1000 mg/dL or 500 mg/dL and a prior hx of pancreatitis, the goal of treatment is to decrease the risk of pancreatitis and assessing the risk for cardiovascular therapy to assess the need for statin therapy. In the evolving medical treatment of hypertriglyceridemia, family history and risk factors for CAD have become increasingly emphasized. Chart 2 summarizes our suggestions for treating hypertriglyceridemia.

Lifestyle Modification

First line therapy is a change in lifestyle, with an emphasis on weight reduction for those with metabolic syndrome and an increase in physical activity. Goals of lifestyle changes include a healthy weight with goal BMI < 25 kg/m2, < 30 % total fat, and < 10 % saturated fat intake, at least three servings of vegetables per day, one serving of which being dark green or orange vegetable, two servings of fruit, and one serving of whole grain. In profound hypertriglyceridemia, restriction in fat to 10–15 g/day is sometimes required [24]. Smoking cessation is recommended to all patients. Excessive alcohol use in combination with elevated triglyceride levels has an increased risk or pancreatitis and should be discouraged. No more than two drinks a day in men and one drink a day in women is recommended. Moderate alcohol intake has inconsistently shown benefit to TG levels, with some studies showing no benefit, and other suggesting a slight decrease in TG levels [12]. Regular physical activity of moderate intensity (i.e 30 minutes daily on most days of the week) is recommended. Carbohydrate intake should be limited to no more than 60 % of total calories and 50 % of total calories in patients with metabolic syndrome.

Statins

In the pharmacologic management of hypertriglyceridemia, statins are the first line drug that should be offered particularly in mixed dyslipidemias given their consistent benefit to reduction of CAD. The response in triglyceride reduction is approximately 10–40 %. Side effects include muscular pain, tenderness, rhabdomyolysis, liver enzyme abnormalities, and can influence metabolism of drugs which are cleared via hepatic cytochrome P450 pathway. Statin use is discussed in detail in Chap. 8.

Fibric Acid Derivatives

Fibric acid derivatives gemfibrozil or fenofibrate reduce triglycerides by 40–60 %. Mechanistically they increase LPL synthesis, fatty acid oxidation and decrease APO CIII expression [40]. With triglyceride levels < 150 mg/dL statin effects on triglycerides are inconsistent. With levels > 200 mg/dL triglyceride levels fall in proportion to the decrease in LDL levels with statin use [40]. Fibrates particularly gemfibrozil can interact with any statin, and increase the risk of rhabdomyolysis. Patients can also experience diarrhea, gastritis, liver enzyme abnormalities, and increased risk of cholesterol gallstones. Fibrate treatment in meta-analysis has been shown to decrease cardiovascular events but has not shown a reduction in total mortality [6].

Nicotinic Acid

Nicotinic acid lowers cholesterol, LDL, triglyceride levels and raises HDL levels. Nicotinic acid is the most effective in reducing triglycerides, generally by 30–50 %. Contraindications to their use include hepatic dysfunction and hyperuricemia. There use is generally limited by side effects of flushing and GI distress. However, tolerance to these side effects can develop over time and can be minimized if aspirin is administered prior to taking the drug. High levels of the medication > 2 g/day and particularly > 3 g/day have been associated with decreased insulin sensitivity and worsening hyperglycemia in type 2 diabetics as well as increased risks of hepatotoxicity [11]. Niacin is contraindicated in those with peptic ulcer disease. Similarly to gemfibrozil, the independent benefit of niacin is seen in decreasing CAD rates, however no clear benefit has been shown when added as an adjunct therapy with statins [40].

Fish Oil

Fish oil (omega 3 PUFA) of more than 4 g/day is associated with a decrease in serum triglyceride concentrations by 25–30 %, as well as an increase of 5–10 % in

LDL and 1–3 % in HDL [12]. Omega 3 fatty acids are thought to exert their effect through decreased TG production by inhibiting free fatty acid and TG biosynthesis [24]. However, significant impact on decreased cardiovascular risk and mortality has not been consistently established in meta-analyses [6].

Inpatient Management

In those is acute pancreatitis as mentioned above, fibrate therapy is an option in those with TG levels ≥ 500. Patients with TG levels > 1000 mg/dL and symptoms are particularly concerning for acute pancreatits [11]. Patients should be given nothing by mouth and hydrated aggressively. Acutely, insulin therapy can also be used as it promotes the synthesis of fatty acids in the liver, inhibits adipose breakdown by inhibiting LPL and targets apo B100 for degradation. Insulin also regulates hepatic lipogenesis through its ability to increase gene expression of SREBP1-c a lipogenic transcription factor [41]. Glycemic agents such as thiazolidinediones can also lower TG levels by about 15–25 % through the action of PPARgamma, improving peripheral hepatic insulin sensitive and inhibition of lipolysis in adipose tissue.

Future Directions

Hypertriglyceridemia remains a clinical dilemma. Although common in the U.S. population and rising in prevalence, the clinical significance and treatment goals remain elusive. Measurement of TG levels is limited by no clear standardization of non-fasting levels. Indeed, as our understanding of lipid metabolism and signaling improves, our current markers for clinical intervention may become archaic and immaterial. Treatment based on genome analysis at this time remains in its infancy and until clearer ideas of the true benefit of modulating genetic risks are known treatment should focus on clinical endpoints (Fig. 6.1).

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Chapter 7
The Effects of Diet in Hyperlipidemia

Joseph Michael Allevato and Imelda Allevato

Introduction

When treating hyperlipidemia, the role of diet should not be underestimated. Unfortunately, changing peoples' diets is extremely challenging. Culture, habit, taste, food availability, and cost all have to be figured into the equation when we propose diet changes to patients. Approaches that emphasize what foods to avoid often fail to offer healthy replacements. When patients are instructed to eat a diet low in fat, for instance, they may increase their carbohydrate consumption, often offsetting any potential gains from the lower fat diet. Additionally, any diet proposed should be something patients can potentially follow for the rest of their lives. If diets are restrictive in taste or cost, long-term success will be difficult to obtain. Many studies have found short-term gains disappear over longer study periods. In clinical practice, we often see patients who lose weight because of dramatic changes to their diets, but later return to their baseline or worse due to lack of sustainability.

Recent evidence is pointing towards the adoption of the so-called Mediterranean Diet as an effective way to reduce the risk of cardiovascular disease and other major causes of mortality. The Mediterranean Diet was given its name after research done in the second half of the twentieth century discovered that people living in the Mediterranean area had lower cardiovascular mortality, despite coming from various different cultures in the area [1]. Researchers analyzed what Mediterranean people consumed and formed the basics principles which became the Mediterranean diet. These principles include having a diet with healthy fat (such as from fish, olive oil, and nuts), fruits, and vegetables, and emphasizes complex, whole-grain and whole-wheat carbohydrates along with mild to moderate consumption of alcohol. The tenants of the diet do not require that the dishes that people eat be specifically Mediterranean, but rather that the components of their meals match these basic principles.

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Clinical Studies

Dietary studies can be very challenging. Retrospective studies are wrought with many potential confounders, making it difficult to know if differences in peoples' diets truly cause the desired outcome. One of the sources of bias is adopting other healthy lifestyles that may lead to better health outcomes from other activities. In addition, randomized controlled trials rely heavily upon the adherence of the participants to a prescribed diet, with many patients not actually following the diet that was advised. Despite these issues, some well-designed trials have helped us to better understand what we can eat to reduce our cardiovascular risk.

Lyon Diet Heart Study

In the 1990s, the Lyon Diet Heart Study was the first major study to show the benefits of a Mediterranean diet approach for secondary prevention of cardiovascular disease [2, 3]. In this randomized control trial, the experimental group participated in a 1-h counseling session on the principles of the Mediterranean diet. They were advised to eat fish and poultry over beef, pork, and lamb. Fruit was to be consumed at least once daily. Olive oil and rapeseed oil were also recommended. The researchers supplied margarine to fully replace butter used by the treatment arm. The control group was given no instructions beyond what their routine care would dictate. At 27 months, the trial was halted due to a dramatic reduction in coronary events in the experimental group. Nonetheless, the researchers continued to follow the study participants and later reported, with an average follow-up of 46 months, that the patients randomized to the Mediterranean diet had a 47–72% reduction in adjusted risk for composite outcomes of (1) cardiac death and nonfatal myocardial infarction, (2) unstable angina, heart failure, embolic events, or stroke, and (3) any event requiring hospitalization. The striking finding that the patients in the Mediterranean diet group had sustained long-term adherence to the diet and continued to see health benefits, even 4 years after the start of the study, proves that the Mediterranean diet is a sustainable diet.

After the Lyon Diet Heart Study, researchers did population studies, applying various scoring systems to measure adherence to the Mediterranean diet. Increased adherence of the study population to the principles of the Mediterranean diet resulted in greater reduction in CVD events and other causes of mortality.

Women's Health Initiative Randomized Controlled Dietary Modification Trial

In 2006, the Women's Health Initiative Randomized Controlled Dietary Modification Trial was published[4]. This large trial randomized 48,835 women to either a low-fat diet (40%) or a "free-living" setting (60%). The low-fat group

decreased their energy intake from fat by a median level of 8.2 %, with decreases in saturated, monounsaturated, and polyunsaturated fat. After an average follow up of 8.1 years, there was no difference in the rates of cardiovascular disease by following a low-fat diet.

Primary Prevention of Cardiovascular Disease with a Mediterranean Diet (PREDIMED)

The PREDIMED study was concluded in 2013 and brought the Mediterranean diet back into the national spotlight [5]. This critical study was a multicenter randomized-controlled trial performed in Spain, involving 7447 adults ranging from 55 to 80 years old were randomized to one of 3 groups: a control arm in which subjects were instructed to reduce their dietary fat consumption, a second arm in which participants had a Mediterranean diet supplemented with extra-virgin olive oil., and a third arm in which people were assigned to a Mediterranean diet supplemented with mixed nuts. Participants were followed for a median length of time of 4.8 years. Striking differences were found between the 2 groups that consumed a Mediterranean diet supplemented by either nuts or olive oil compared to the control group. There was a relative risk reduction of 30 % for cardiovascular disease end points in both study groups compared to the control group. Some critics have noted that the study took place in a Mediterranean country in which study participants may have already been following a Mediterranean diet. Additionally, there was not a large reduction in calories from fat in the control group, suggesting that the control group didn't adopt the low-fat diet that was recommended. However, considering that achieving reductions in fat consumption in the Women's Health Initiative study did not improve cardiovascular risk, it is unlikely that the failure to reduce consumption of fats in the control arm of PREDIMED had any impact.

Food Components

In understanding diets effects on lipids, it is useful to understand the role of various micronutrients.

Fatty Acids

Fatty acids were viewed as deleterious to our health in the twentiethcentury and by the 1950s, a reduction in total fat intake was being recommended. Having a high total fat diet does lead to increased total cholesterol, HDL cholesterol, and LDL cholesterol, but low-fat diets have not been shown to decrease mortality. Low-fat diets are difficult to adhere to as well as seen in the Women's Heath Initiative study.

As science regarding the different types of fatty acids became available, it became clear that not all fats were created equal, with critical differences between fats that change their impact on health.

Fatty acids all have a carboxylic head and a tail that is made up of hydrogen and carbon. These fats are called "saturated" if there are no carbon-carbon double bonds (therefore, the carbons are maximally saturated with hydrogen). If the fatty acid does contain a carbon-carbon double bond, it is called an "unsaturated" fatty acid. Monounsaturated fats contain a single carbon-carbon double bond, while polyunsaturated fatty acids have 2 or more carbon-carbon double bonds. The presence of these bonds is important since they create kinks in the molecular structure of the fatty acid. Saturated fatty acids are straight, allowing for tight packing of molecules and, hence, a relatively high melting point. As the number of carbon-carbon double bonds increases, the melting point of the fatty acid tends to drop as well, making mono- and polyunsaturated fats more likely to be liquid at room temperature

Saturated Fats are mostly obtained from animal sources. They are known to increase LDL cholesterol compared to their mono- and polyunsaturated counterparts. Simply decreasing the consumption of saturated fats, however, may not be ideal. Data from research has not shown any difference in cardiovascular disease in people assigned to a low saturated fat diet [4]. The reason for that, however, may be due to what people eat in lieu of those saturated fats. If people increase their consumption of carbohydrates to replace the lost calories from saturated fat, for instance, the deleterious effects of a high carbohydrate diet can offset any gain from reducing the intake of saturated fats.

Mono- and polyunsaturated fats, on the other hand, are derived from fish and plant sources. Monounsaturated fats have been shown to decrease triglycerides and increase the HDL/LDL cholesterol ratio. Oleic acid is a commonly consumed monounsaturated fat. Polyunsaturated fats are known to decrease LDL cholesterol and may decrease HDL as well to a lesser degree. The polyunsaturated fats are further separated into different categories depending on how far away their carbon-carbon double bond is from the last carbon on their tail (the "omega" carbon). Examples are the omega-3 fatty acids (with carbon-carbon double bond 3 atoms away from the final carbon), such as docosahexaeoic acid (DHA) and eicosapentaenoic acid (EPA), and the omega-6 fatty acids such as alpha-linoleic acid (ALA). Interestingly, omega-3 fatty acids have been shown to reduce coronary heart disease mortality without decreasing nonfatal myocardial infarctions [6]. This has lead investigators to believe that omega-3 fatty acids have intrinsic antiarrhythmic qualities that are preventing sudden death [6]. That hypothesis is supported by the fact that the reduction in death is achieved soon after the start of trials that increased their consumption, before any improvement in coronary plaque could be achieved. In 2013, research published in the Annals of Internal Medicine in which they measured the circulating levels of DHA and EPA in older adults (average age of 74 ± 5 years) [7]. It was discovered that higher circulating levels of omega-3 fatty acids were associated with 27% lower total mortality, especially death from coronary heart disease. People who had the highest circulating levels of omega-3 fatty acids were found to live 2.2 years longer than those with lower levels.

Trans fatty acids (also called "trans-fats") are yet another category of fatty acid. They have gained particular notoriety in recent years, as their deleterious effects have become known. Unsaturated fatty acids in nature almost always have their carbon-carbon double bonds in a *cis* configuration, which gives the molecule a kink or bend. Trans-fats have their double bonds in the *trans* configuration, which maintains the linear structure of the fatty acid. Trans-fats were introduced into foods unintentionally in an effort to convert unsaturated fats into saturated fats (a process called hydrogenation) so that they would be solid and more manageable at room temperature. Unfortunately, these new fats increased total cholesterol and decreased HDL and have been linked to increased cardiovascular disease morbidity and mortality risk. As this fact became common knowledge, strong public outcry encouraged many food manufacturers to remove trans-fats from the foods they produce.

Cholesterol

Contrary to what would seem intuitively obvious, dietary cholesterol doesn't have much impact on serum cholesterol levels.

Carbohydrates

Carbohydrates are another type of food component that affect lipid levels. Simple carbohydrates can lead to higher triglyceride levels and decrease HDL cholesterol. This problem is complicated in people with insulin resistance, who get increases in LDL particle concentration as well. This likely explains why low-fat diets that replace the calories from fats with calories from carbohydrates do not improve cardiovascular disease risk. Complex carbohydrates, however, can improve postprandial triglyceride levels, especially when combined with omega-3 fatty acids. Fiber, which is a type of indigestible carbohydrate, has been shown to decrease LDL cholesterol by 9%. In fact, observational studies suggest that the quantity of fiber in a diet is more important that the total or saturated fat content.

Foods

Although discussing micronutrients can be informative, people eat macronutrient food. In most cases, it is critical to know how those foods affect health. In fact, most of the information gained about diet was based on the macronutrients people consumed and then investigation into the micronutrients that are likely to contribute to their beneficial or deleterious nature. Sadly, most attempts to separate the micronutrients from the macronutrients are not effective. In the end, we must eat healthy foods.

Foods High in Omega-3 Fatty Acids

Omega-3 fatty acids are found in fish. Longer lifespans and lower risk of coronary heart disease have long been observed in populations that have a high intake of fish. Unfortunately, fish is not a very commonly consumed food in the United States. Simply adding fish to one's diet appears to have marked benefits. By increasing fish consumption from 0 to 1 serving per week, CHD risk is estimated to be reduced 15%. More servings per week reduce the risk by 18%. In 2006, a clinical review of fish intake published in the Journal of the American Medical Association showed that consuming 1–2 servings of fish per week reduced CHD risk by 36% and reduced total mortality by 17% [8]. The American Heart Association now recommends that people eat 2 servings of fish (particularly fatty fish) every week. Fish that have particularly high levels of omega-3 fatty acids are herring, mackerel, salmon, sardines, lake trout, and tuna. It may be prudent to avoid regular eating of some types of fish due to potentially dangerous levels of mercury. This tends to be more concerning when eating fish that prey on other smaller fish, such as sharks, swordfish, king mackerel, and tilefish.

Something that has gained popularity in recent years has been the consumption of fish oil or omega-3 fatty acid supplements. The hope is that, by taking such supplements, the same benefits realized by people who consume fish can be achieved by people who do not. Disappointingly, a large meta-analysis failed to show any benefit from supplementation with these fatty acids for the primary prevention of CHD [9]. It can be hypothesized that the failure of supplementation may be that the supplements do not replace other foods in the diet. People taking these supplements are likely to be consuming the same quantities of other fats as they did prior to the supplementation, with no reduction in saturated fat intake. Another possibility is that these fatty acids simply don't have an equal effect when they are separated from their macronutrient context. Supplementation has a role however, especially in certain patient populations. Patients with high triglyceride levels have been shown to have a 20–50% reduction in their triglycerides with fish oil supplements. This could potentially help reduce the risk of pancreatitis in such patients. Patients with a prior history of CHD may also benefit from these supplements, as research has shown reduction in cardiac death, especially sudden cardiac death, in this population.

Foods High in Omega 6 Fatty Acids

Olive oil, nuts (particularly walnuts), flaxseed, canola oil, and soybean oil all are excellent sources of omega-6 fatty acids such as alpha-linoleic acid. In the PREDIMED study, both olive oil and nuts can provide an impressive reduction in CHD risk. In 2013, researchers observed that men and women in the Nurses' Health Study and Health Professionals Follow-up Study that consumed nuts even less than once per week had a reduction in total and cause-specific mortality by 7% [10]. Consumption of nuts once per week, two to four times per week, five to six times

per week, and seven or more times per week, resulted in reductions mortality of 11, 13, 15, and 20% respectively. These reductions were independent of other predictors of death.

Alcohol

Excessive intake of alcohol is clearly dangerous and addictive. It can negatively impact one's health, family life, and work. It often leads to other risky behaviors as well as fatal and nonfatal accidents. Moderate consumption of alcohol, however, can have some health benefits. It has been associated with increasing levels of HDL cholesterol. People who drink no alcohol at all have higher risks of CHD. The lowest risk of CHD appears to be in people who consume two to three drinks per day [11]. Excessive alcohol, however, can wipe out these benefits. Stroke mortality is lowest in people who consume less than one drink of alcohol per day. In patients that drank six or more drinks of alcohol per day, stroke risk was markedly increased. Drinking in moderation but not excess, therefore, is likely to be of benefit in reducing cardiovascular disease risk.

Mediterranean Diet

As discussed above, a diet that combines many of these beneficial foods is the Mediterranean diet. Ample fish, nuts, olive oil, and moderate alcohol intake have all been shown to help reduce risk. Researchers have developed tools to track this diet pattern as a whole in observational studies. Scores are given to participants in these studies based on how many of eight different Mediterranean diet principals they followed (with zero points indicating no adherence to the Mediterranean diet and eight points being full adherence). A 2010 meta-analysis showed that, for every two points scored on this scale, overall mortality was reduced by 8% and cardiovascular disease incidence dropped by 10% [12].

We suggest the following "plate" Fig. 7.1, as an example of a sustainable healthy dietary approach that we provide to our patients in clinic. It is based on increasing the amount of vegetables per meal, choosing healthy fats, and limiting excessive carbohydrate intake.

Summary

The composition of our diets is an important determinant of our cardiovascular health. Rather than focusing on restricting foods, it may be more beneficial to discuss what healthy foods should be included in our diets. We recommend the "Mediterranean" diet approach based on ample scientific evidence of its benefits. By advising our patients to adhere to a Mediterranean diet, we can beneficially affect their

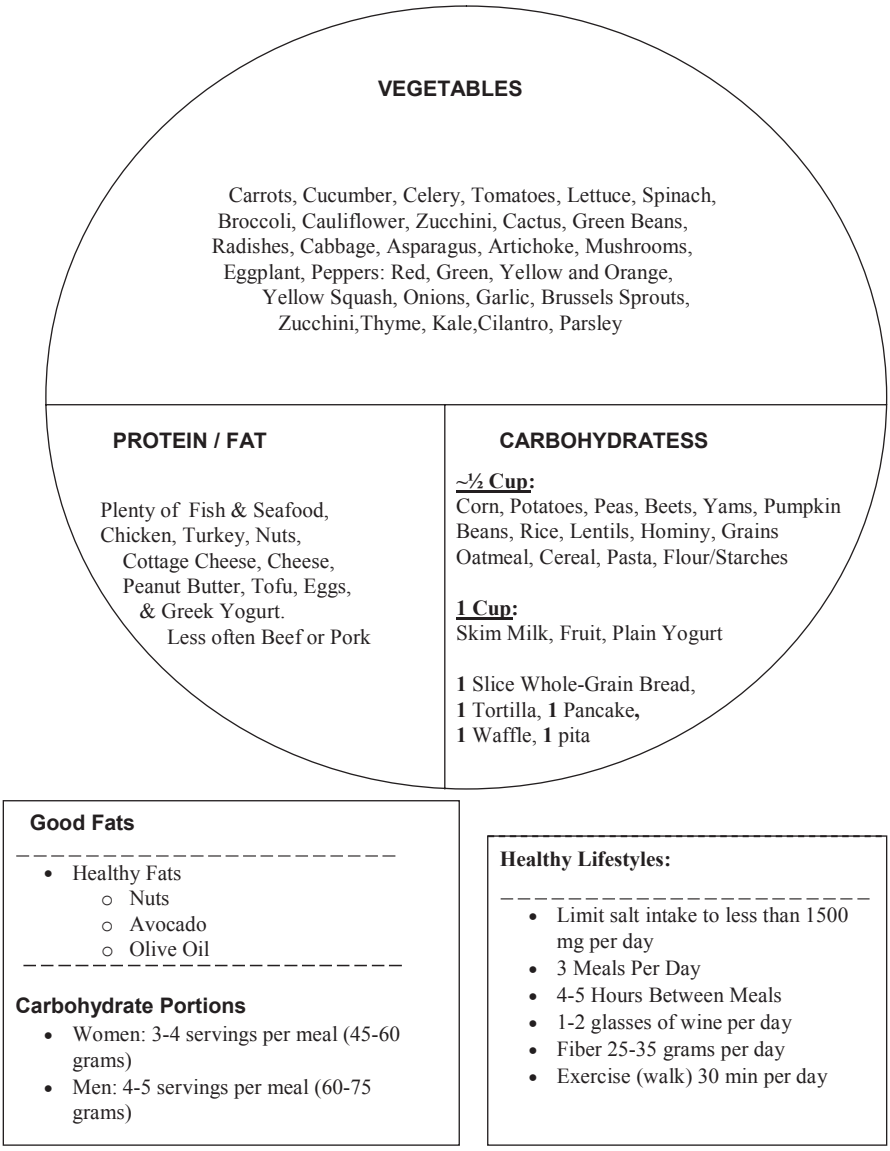

Fig. 7.1 A suggested plate providing a sustainable healthy dietary approach that we provide to our patients in clinic. It is based on increasing the amount of vegetables per meal, choosing healthy fats, and limiting excessive carbohydrate intake

lipids and, more importantly, dramatically improve their outcomes. Importantly, it is not an all or nothing diet. A few small changes using the Mediterranean diet can provide marked improvements to health. In combination with other treatment approaches, such as statins, these principals can be powerful weapons in our battle with hyperlipidemia and cardiovascular disease.

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Chapter 8
Statins

Marija Stojanova Jordanov and Hiba Abou Assi

Abbreviations

ACC/AHA	American College of Cardiology and the American Heart Association
AFCAPS/TexCAPS	The Air Force/Texas Coronary Atherosclerosis Prevention Study
ALF	Acute liver failure
ALLHAT-LLT	Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial
ALT	Alanine aminotransferase
ANA	Antinuclear antibody
ASCOT-LLA	Anglo-Scandinavia Cardiac outcome Trial- Lipid Lowering Arm
ASCVD	Atherosclerotic cardiovascular disease
ATP	Adenosine triphosphate
AUC	Area under the curve
CABG	Coronary artery bypass grafting
CAD	Coronary artery disease
CARDS	Collaborative Atorvastatin Diabetes Study
CARE	Cholesterol and Recurrent Events
CHD	Coronary heart disease
CK	Creatine Kinase
CPK	Creatine phosphokinase

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CRP	C—reactive protein
CVD	Cardio vascular disease
CYP450	Cytochromes P450
DILI	Drug-induced liver injury
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
HDL	High-density lipoprotein
HMG-CoA	(3-hydroxy−3-methylglutaryl-coenzyme A)
HMGR	HMG-CoA reductase
HPS	Heart Protection Study
Jupiter	Justification for the use of statins in prevention: an intervention trial evaluating rosuvastatin
LDL	Low-density lipoprotein
LDL-C	Low density lipoprotein- cholesterol
LIPID	Long-term intervention with Pravastatin in Ischemic Disease
MEGA	Management of elevated cholesterol in the primary prevention group of adult Japanese
Mg	Milligrams
mg/dl	Milligrams/deciliter
MI	Myocardial infarction
Ml	Milliliter
mmol/l	Milimol per litre
NCEP ATP III	National Cholesterol Education Program Adult Treatment Panel III guidelines
NLA	National Lipid Association
NSAIDs	nonsteroidal anti-inflammatory drugs
OAT3	organic anion transporter 3
OATP	Organic anion transporting polypeptides
OATP1B1	Organic anion-transporting polypeptide 1B1 inhibitors
P-gp	P-glycoprotein
PONs	Paraoxonases are a group of enzymes involved in the hydrolysis of organophosphates
PRIMO	Prediction of Muscular Risk in Observational conditions
RRR	Relative risk reduction
SLC	Solute carrier
SLCO	Solute carrier organic
SLCO1B1	Solute carrier organic anion transporter family member 1B1
TSH	Thyroid stimulating hormone
UDP	Uridine diphosphate
UGT	UDP-glucuronosyltransferase
ULN	Upper limit of normal
WOSCOPS	West Of Scotland Coronary Prevention Study

Statin Types and Classes

Statin drugs are a group of lipid lowering medications. They are taken by millions to lower cholesterol, especially designed to lower LDL (low-density lipoprotein) cholesterol. In addition to lowering LDL, statin drugs can lower inflammation. Statins lower cholesterol levels by inhibiting the enzyme HMG-CoA reductase, which plays a central role in the production of cholesterol in the liver, producing about 70% of total cholesterol in the body.

Types of Statins

The essential structural components of all statins are a dihydroxyheptanoic acid unit and a ring system with different substituents. The statin pharmacophore is modified hydroxyglutaric acid component, which is structurally similar to the endogenous substrate HMG CoA and the mevaldyl CoA transition state intermediate. The statin pharmacophore binds to the same active site as the substrate HMG-CoA and inhibits the HMGR enzyme. It has also been shown that the HMGR is stereoselective and as a result all statins need to have the required 3R, 5R stereochemistry [1].

The ideal statin should have the following properties [2]:

- High affinity for the enzyme active site
- Marked selectivity of uptake into hepatic cells compared with non-hepatic cells
- Low systemic availability of active inhibitory equivalents
- Relatively prolonged duration of effect.

One way to classify statins is by their manufacture. Some are derived from microorganisms through biotechnology. These are called fermentation-dericed or Type 1.

Others are made through chemical synthesis (no living organisms involved). These are synthetic, or Type 2 statins. It is common for pharmaceuticals to be made through fermentation and through chemical synthesis. (Table 8.1)

The Type 1 drugs have chemical structures similar to mevastatin which is naturally occurring compound, found in red yeast. In the 1970s the Japanese microbiologist Akira Endo first discovered natural products with a powerful inhibitory effect on HMGR in a fermentation broth of *Penicillium citrinum*, during his search for antimicrobial agents. The first product was named compactin (ML236B or mevastatin) [3].

Table 8.1 Classification by manufacture method

Manufacture method	Type	Medicines
Fermentation	Type 1	Lovastatin, simvastatin, pravastatin
Synthetic	Type 2	Fluvastatin, atorvastatin, rosuvastatin, pitavastatin, cerivastatin

Table 8.2 Classification by solubility

Classification by solubility	Medicines
Water soluble (hydrophilic)	Pravastatin, pitavastatin, and rosuvastatin
Fat soluble (lipophilic)	Atorvastatin, fluvastatin, lovastatin and simvastatin.

Table 8.3 Combination therapy

Simvastatin + Ezetimibe	Combination therapy
Lovastatin + Niacin extended-release	Combination therapy
Atorvastatin + Amlodipine Besylate	Combination therapy—Cholesterol + Blood Pressure
Simvastatin + Niacin extended-release	Combination therapy

Other way to classify statins is by their solubility. Some are water soluble (hydrophilic) and other are fat soluble (lipophilic).

Like most chemicals, statins are soluble in both aqueous environments and oily environments. The solubility levels differ enough that it is possible to classify some as hydrophilic (better solubiity in water) or lipophilic (better solubility in fats). Very generally speaking, the hydrophilic statins are excreted from the body largely unmetabolized by the liver. Lipophilic statins are broken down in the liver by the cytochrome P450 (CYP450) system. Hydrophilic statins tend to have fewer interactions with other drugs. (Table 8.2, 8.3)

Statin Trials

It has been known for quite some time that there is a continuous positive correlation between plasma cholesterol levels and coronary heart disease [4]. One of the earliest trials of statins in secondary prevention of cardiovascular disease (CVD) was the Scandinavian Simvastatin Survival Study (4 S) that randomized 4444 patients with coronary heart disease and hyperlipidemia with a mean age of 58 years and mean low density lipoprotein- cholesterol (LDL-C) of 188 mg/dl, into simvastatin 20 mg daily versus placebo. The study's primary end point was total mortality. The DSMB suggested the study be stopped after the third and final interim analysis as there was 30% reduction in total mortality. The median follow up time was 5.5 years. Over the whole course of the study the mean change in LDL-C from baselines was −35% in the simvastatin group versus +1% in the placebo group. The Kaplan Meier probability of survival over 6 years was 91.3% in the simvastatin group compared to 87.7% in the placebo group with 42% reduction in risk of coronary death accounting for improvement in survival. The relative risk of a major coronary event was 0.66 (96% CI: 0.59–0.75), of undergoing CABG or angioplasty was 0.63(95%

CI: 0.54–0.74), of fatal plus nonfatal cerebrovascular event was 0.70 (95% CI: 0.52–0.96). This was the first trial showing that a cholesterol lowering treatment decreased total mortality in any population and decreased major coronary events in women [5].

The Cholesterol And Recurrent Events (CARE) trial randomized 4159 patients with prior MI but no history of hyperlipidemia to receive pravastatin 40 mg daily versus placebo. The participants' mean age was 59 years and mean LDL – C was 139 mg/dl. The primary end point of the trial was death from coronary heart disease or symptomatic nonfatal MI. There was 24% lower risk with pravastatin. LDL-C was 28% lower in the pravastatin group versus placebo. In the pravastatin group there was 23% reduction in nonfatal MI, 20% reduction in death from coronary heart disease (but P value was 0.10), 26% lower rate of CABG and 31% lower incidence of stroke [6].

The Long Term Intervention with Pravastatin in Ischemic Heart Disease (LIPID) trial randomized 9014 patients with history of MI or unstable angina, mean age 62 years and median LDL-C 150 mg/dl to receive either pravastatin 40 mg daily versus placebo. The primary outcome of the study was death from coronary heart disease. The reduction in risk was 24% in the pravastatin group. The mean duration of the trial was 6.1 years. There was 25% LDL –C reduction over an average of 5 years of follow up. All secondary end points were significantly reduced in the pravastatin group compared to placebo, including risk of MI, stroke, CABG, angioplasty, hospitalization for unstable angina and length of stay. This study differs from 4 S and CARE trials by inclusion of unstable angina, but it does extend the findings of 4 S to patients with lower total cholesterol and confirms benefit in terms of mortality from coronary heart disease and overall mortality that was found in CARE [7].

One of the largest trials came afterwards, the Heart Protection Study (HPS) which can be considered both primary and secondary prevention trial. 20,536 patients ages 40–80 years, with either prior coronary artery disease or other risk factors and mean LDL –C of 131 mg/dl (3500 participants had LDL < 100 mg/dl), were randomized to simvastatin 40 mg versus placebo. 35% of the participants had no history of prior coronary artery disease but had either cerebrovascular accident, peripheral arterial disease or diabetes. The mean follow up was 5 years. The primary end point was death from all causes, from coronary heart disease and from all other causes. There was 13% reduction in risk of any death and 17% reduction in any vascular death. As far as secondary end points: there was 38% reduction in incidence rate of first nonfatal MI, 27% reduction in nonfatal MI or coronary death, 25% reduction in incidence rate of first stroke and 24% reduction in incidence rate of first revascularization procedure.

HPS showed that reducing LDL cholesterol to targets below those of NCEP ATP III is safe and still provided a reduction in risk [8]. This paved the way to treat to lower goals in patients at higher risk [9].

One of the earliest trials in primary prevention was the West Of Scotland Coronary Prevention Study (WOSCOPS) during which 6595 men with moderate hyperlipidemia and no history of MI were randomized to pravastatin 40 mg versus placebo. The mean age of participants was 55.3 years and mean LDL- C was 192 mg/dl.

The primary outcome was nonfatal MI or death from coronary heart disease. There was 31% risk reduction in the primary outcome with pravastatin. There was 31% risk reduction in definite nonfatal MI and 32% in death from all cardiovascular causes [10].

The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) was designed to find out whether the benefit in reduction of LDL-C in patients without coronary heart disease (i.e. findings from WOSCOPS) can be extended to older persons, women and those with average serum cholesterol levels). 6605 patients, mean age 57.5 years (men) and 62.5 years (women) without coronary heart disease were randomized to lovastatin 20 mg daily versus placebo. The mean LDL-C was 150 mg/dl. Primary end point was incidence of first major acute coronary event (fatal or nonfatal MI, unstable angina or sudden death). There was 37% reduction in the lovastatin group and the difference between the 2 intervention groups appeared as early as 1 year. There was reduction in risk of revascularization by 33%, unstable angina by 32%, and fatal and nonfatal MI by 40%. Treatment effects were similar for men and women. DSMB recommended early termination for efficacy after the second interim analysis. This trial was very important in being the first primary prevention trial to show benefit from lipid lowering in a healthy population of men and women without history of cardiovascular disease or hyperlipidemia. Interestingly there was no threshold to benefit observed in the LDL and HDL cholesterol ranges that were studied [11].

The Antihypertensive and Lipid- Lowering Treatment to prevent Heart Attack trial (ALLHAT-LLT) did not show statistically significant reduction in all-cause mortality or coronary heart disease in the pravastatin arm compared to the usual care arm in older patient population with well controlled hypertension and moderately high LDL cholesterol, although it showed a favorable trend [12]. The Anglo-Scandinavia Cardiac outcome Trial- Lipid Lowering Arm (ASCOT-LLA) confirmed those results. In ASCOT-LLA 10,305 men and women, mean age 63.1 years with at least 3 cardiovascular disease risk factors and mean LDL-C of 131 mg/dl were randomized to receive atorvastatin 10 mg daily versus placebo. The primary end point was combined end point of nonfatal MI, including silent MI and fatal CHD. The Lipid lowering arm of ASCOT was terminated early because atorvastatin resulted in a highly significant (36%) reduction in the primary end point. At 1 year follow up there was 35% reduction in LDL –C in the atorvastatin group. There was 27% reduction in fatal and nonfatal stroke, and 21% reduction in total cardiovascular events and procedures [13].

The Collaborative Atorvastatin Diabetes Study (CARDS) trial randomized 2838 patient with type 2 diabetes ages 40–75 years with mean LDL- C of 117 mg/dl to atorvastatin 10 mg daily versus placebo. Participants were diagnosed with type 2 diabetes at least 6 months before study entry and had one or more of the following: history of hypertension, retinopathy, microalbuminurea or macroalbuminurea or active smoking. There was 37% reduction in the primary end point which was the first of the following: acute CHD event, coronary revascularization procedure or stroke. The trial was terminated 2 years early due to the large beneficial effect of atorvastatin and raised level of awareness that lipids

in patients with diabetes should receive as much attention as glycemic and blood pressure control [14].

MEGA (Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese) randomized 7832 participants between ages 40 to 70 years old without CVD and with total cholesterol between 220–270 mg/dl to pravastatin 10–20 mg daily versus placebo. Mean follow up was 5.3 years. Primary end point was first occurrence of coronary heart disease which was significantly lower in the pravastatin group (RRR of 34%) [15].

The Jupiter (Justification for the Use of Statins in prevention: an Intervention trial Evaluating Rosuvastatin) trial randomized 17,802 patients without CVD and with LDL-C less than 130 mg/dl (median LDL-C 108 mg/dl) and CRP more than 2 mg/dl (median CRP 4.25 mg/dl) to receive rosuvastatin 20 mg daily versus placebo. The primary outcome was occurrence of first major cardiovascular event (nonfatal MI, nonfatal stroke, hospitalization for unstable angina, arterial revascularization procedure, or confirmed death from cardiovascular causes). There was 44% reduction in primary outcome, 47% reduction in MI, stroke or death from cardiovascular causes, 47% reduction in revascularization or hospitalization for unstable angina and 20% reduction in death from any cause. At months of follow up there was 50% reduction in LDL-C in the rosuvastatin group compared to placebo [16].

The findings from these and other studies leave little doubt about the cardiovascular benefit from statins in primary and secondary prevention. As noticed above the studies have not been designed to target a certain LDL-C goal, hence the shift away from treating to an LDL-C goal with the new ACC/AHA guidelines [17]. Those guidelines identified 4 statin benefit groups: Patients with ASCVD, patients with diabetes between ages 40 to 75 years, patients with LDL-C more than 190 mg/dl and patients with more than 7.5% estimated 10-year ASCVD risk between ages 40 and 75 years. With the application of the new ACC-AHA guidelines, about 13 million more US adults will be eligible for statin therapy [18].

Pharmacokinetic Properties of Statins and Statin Dose Comparison

Pharmacokinetic Properties of Statins

The pharmacokinetic properties of the statins are orchestrated by several factors, including their active or lactone form, their lipophilic/hydrophilic rate, and their absorption and metabolism. The percentage of absorption is between 30 and 98% and the time to reach peak plasma concentration (T_{max}) is within 4 h after administration [19–22]. The daily absorption may vary according to the time of administration [21] and food intake [23]. Because the liver is the target organ of statins, an efficient first-pass uptake may be more important than high bioavailability to achieve the statin effect. An extensive first-pass extraction implies a low systemic bioavailability;

The solubility profile is a fundamental characteristic that governs the hepatoselectivity of the statins and their inhibitory effect on HMG-CoA reductase. Lipophilic statins enter the hepatocytes by passive diffusion, whereas hydrophilic statin uptake is carrier-mediated [24, 25]. Lipophilic statins show an efficient activity at both hepatic and extrahepatic sites, whereas hydrophilic statins are more hepatoselective [24].

Cytochrome P450-Mediated Metabolism of Statins

In the liver, statin lactones are hydrolyzed to their open acid forms chemically or enzymatically by esterases or paraoxonases (PONs) [26]. The open acid form is converted to its corresponding lactone via a CoA-dependent pathway and via glucuronidation by UDP-glucuronosyl oxidation and glucuronidation processes, statins as lactone forms rapidly undergo oxidation through the transferase (UGT). Both acyl glucuronide and acyl CoA derivatives may return to statin acids by hydrolysis. In addition, whereas statin open acids are irreversibly cleared by β-oxidation and glucuronidation processes statins as lactone forms rapidly undergo oxidation through the microsomal cytochrome P450 (P450) family of enzyme [27]. The **CYP3A4** isoenzyme is the major microsomal enzyme that metabolizes many statins, including lovastatin, simvastatin, atorvastatin, and cerivastatin, into active derivates responsible for HMG-CoA reductase inhibition [28]. On the other hand, the metabolism of pravastatin in the liver cytosol is not enzyme depended [29]. Metabolism of fluvastatin, predominantly occurring through the isoenzyme CYP2C9 (50–80 %) [30] and metabolisam of rovastatin thought CYP2C9 and CYP2C19.

Statin Excretion

Liver and kidney are involved in the elimination of statins from the systemic circulation via the bile into the feces. The hepatic elimination of the statins is limited by their uptake and controlled by the transporters on the basolateral membrane of the liver. Canalicular efflux transporters P-glycoprotein (P-gp) and multidrug resistance-associated protein 2 are two of the major ATP-dependent efflux pumps for statin excretion into the bile.

On the other hand, the urinary excretion of statins, except for pravastatin, is quite low. Unlike other statins, up to 60 % of intravenously administered pravastatin is excreted in the urine in humans [31]. Tubular secretion is the main mechanism involved in the renal excretion of pravastatin and is primarily mediated by the OAT3 transporter. However, when renal elimination is low, the exposure of statins in the liver depends only on the sequestration clearance and is independent of the uptake activity. Instead, when statins, such as pravastatin, undergo significant renal elimination, the increase in the AUC of the plasma concentration does not compensate the reduced hepatic uptake activity, resulting in a weaker pharmacological effect. The half-life elimination of all statins, except atorvastatin and

pitavastatin, is very short (0.5–3 h), and drugs do not accumulate in plasma after repeated administrations.

Factors That May Affect Statin Metabolism

Other factors may influence the statin metabolism. These factors including race or ethnicity, food intake, age and sex, and concomitant diseases may affect the pharmacokinetic and pharmacodynamic profile of the statins.

Concomitant administration of statins with food may alter their pharmacokinetic and pharmacodynamic profile. It has been reported that consumption of pectin or oat bran soluble fiber together with lovastatin reduces its absorption [32] whereas alcohol intake does not affect the efficacy and safety of fluvastatin treatment. [33] Moreover, olive oil, consumed in a Mediterranean-style diet, can increase the cholesterol-lowering effect of simvastatin compared with sunflower oil. In contrast, the consumption of polyunsaturated rich oils, through the cytochrome P450 activation, could decrease the half-life of some statins and therefore their cholesterol-lowering effects [34]. Age and sex related differences do not require modification of dosage regimens, because statin plasma concentrations are not necessarily related to their efficacy [35]. Statin treatment is required in patients affected by renal and hepatic diseases [36]. However, in pathological conditions of severe renal dysfunction, the elimination kinetic of statins seems to be altered. In patients receiving long-term dialysis, plasma concentrations of cerivastatin and its metabolites are higher (up to 50%) than in healthy subjects.

With regard to hepatic diseases, the steady-state pharmacokinetics of rosuvastatin and its lactone, after the administration of a single dose, are very similar in male patients with liver cirrhosis and male volunteers without liver disease. In contrast, these patients showed increased pitavastatin plasma concentration after administration [37]. (Table 8.4)

Statin Dose Comparison

Table 8.4 This table shows statin doses that provide similar LDL-lowering effect

Dose [milligrams]						% reduction	
Atorvastatin	Simvastatin	Lovastatin	Pravastatin	Fluvastatin	Cerivastatin	TC	LDL-C
	10	20	20	40	0.2	22	27
10	20	40	40	80*	0.4	27	34
20	40	80			0.8	32	41
40	80					37	48
80						42	55

*Extended release

LDL-C low density lipoprotein cholesterol; TC—total cholesterol

Data from New Statins and New Doses of Older Statins by Evan A. Stein MD PhD [38]

Clinically Relevant Drug-Drug Interactions with HMG-CoA Reductase Inhibitors

Drug interactions involving statins have been studied since 2001, when the first case of fatal rhabdomyolysis after cerivastatin and gemfibrozil coadministration was reported [39]. The inhibition or induction of P450 isoenzymes, involved in the metabolism of more than 50% of the drugs currently available in clinical practice, is the mechanism responsible for many drug-drug interactions [40].

Statins and CYP3A4 Inhibitors

Most of the drug interactions with statins result from the inhibition of CYP3A4 enzyme. Indeed, statin binding and thereby its metabolism could be blocked by drugs with a higher affinity for CYP3A4 enzyme. The co-administration of the CYP3A4 inhibitor itraconazole with simvastatin and lovastatin increases their mean peak concentration and the AUC, causing rhabdomyolysis; [41] this effect is lower on atorvastatin metabolism [42].

Statins and Calcium Channel Blockers

The effect of calcium channel antagonists on the pharmacokinetics of statins, by inhibition of CYP3A4 and/or P-gp, has been widely reported [43]. The coadministration of verapamil, a calcium blocker, substrate of both P-gp and CYP3A4, [44] with lovastatin or Simvastatin [45] as well as atorvastatin [46] increased their plasma concentrations. Diltiazem, another calcium channel-antagonist, in combination with simvastatin, lovastatin and pravastatin, [47] Fluvastatin [48] and atorvastatin therapy, [49] increases plasma levels of the statins and the risk of associated rhabdomyolysis and hepatitis [50]. Amlodipine can increase the risk for myopathy/rhabdomyolysis due to decreased metabolism of simvastatin.

Statins and Macrolides/Ketolide Antibiotics

Several macrolides/ketolide antibiotics, including erythromycin, clarithromycin, and azithromycin, are potent inhibitors of CYP3A4 isoenzymes and consequently can increase the plasma concentrations of coadministered CYP3A4-dependent statins [51].

Statins and Protease Inhibitors

Several interactions of statins with the protease inhibitors have been described. As an example, coadministration of nelfinavir increases the concentration of simvastatin by more than 500 % and consequently the associated risk of skeletal muscle damage. On the contrary, the effect of nelfinavir is moderate on atorvastatin.

Statins and Organic Anion-Transporting Polypeptide 1B1 Inhibitors (OATP1B1)

Uptake transporters of the OATP (SLCO) family are new additional regulators of drug disposition, [52] including fexofenadine, digoxin, rifampicin, methotrexate, nonsteroidal anti-inflammatory drugs (NSAIDs), and HMG-CoA reductase inhibitors. In particular, Pravastatin [53] and cerivastatin are substrates of OATP1B1 (SLCO21A6), a liver-specific uptake transporter. The increase of cerivastatin systemic concentrations with cyclosporin A occurs through the inhibition of the hepatic uptake transporter OATP1B1 rather than inhibition of CYP3A4- or CYP2C8-mediated metabolism. A similar mechanism of statin interaction occurs with some oral antidiabetic drugs and has been reported to be responsible for diabetes-related cardiovascular disease. In particular, repaglinide, rosiglitazone, and metformin influence the transport of pravastatin by inhibition of OATP1B1 [54].

Statin Interactions With Cytochrome P450 Inducers

Coadministration of drugs that are enzyme inducers with statins reduced statin plasma concentrations and therefore decreased their cholesterol-lowering effects. As an example, when coadministered with rifampicin or with carbamazepine, the plasma AUC of simvastatin and its metabolite are reduced, through the induction of CYP3A4 [55, 56].

Other Interactions

Interactions between statins and coumarin anticoagulants such as warfarin, fluindione, phenprocoumon, and acenocoumarol have been reported. Reduced clearance of both warfarin enantiomers (10–20 %) after coadministration of simvastatin or lovastatin have been reported, [57] through CYP3A4 oxidation.

As a result of statin glucuronidation inhibition, the coadministration of gemfibrozil with statins generally increases the statin AUC, with the exception of simvastatin, pravastatin, atorvastatin, and rosuvastatin.

It is noteworthy that grapefruit juice intake has been described to inhibit simvastatin metabolism. Indeed, its active ingredient, bergamottin, has been shown to increase serum concentrations of lovastatin and its active metabolite, [58] as well as that of simvastatin and its active metabolite simvastatin acid, [59] by inhibition of CYP3A4 in the small intestine.

Histopathological studies revealed that ginger reduces liver lesions induced by atorvastatin. Therefore, a combination of ginger with low dose of statins could be useful for the treatment of patients with hypercholesterolemia who are susceptible to liver function abnormalities [60]. (Table 8.5)

Adverse Effects

Statins are the revolutionary drugs in the cardiovascular pharmacotherapy. But they also possess several adverse effects. The most common adverse side effects are raised liver enzymes and muscle problems.

Statins and Muscles Injury

Problems with muscles are reported by 10–15 % of people who take statins. Some people on statin therapy report myalgias [46] and muscle cramps [61]. Rare reactions include myositis and myopathy, with the potential for rhabdomyolysis (a significant breakdown of skeletal muscle) possibly leading to acute renal failure. The mechanism of statin induced myopathy is not very well understood. Proposed hypothesis include: cell membrane lysis of skeletal myocytes due to decreased cholesterol content, apoptosis due to depletion of isoprenoids, depletion of coQ10 which leads to mitochondrial dysfunction [62]. Coenzyme Q10 (ubiquinone) levels are decreased in statin use; [63] CoQ10 supplements are sometimes tried in statin-associated myopathy, but thus far there is not conclusive evidence of their effectiveness despite their ability to raise the circulating levels of CoQ10 in blood plasma. The gene SLCO1B1 (Solute carrier organic anion transporter family member 1B1) codes for an organic anion-transporting polypeptide that is involved in the regulation of the absorption of statins. A common variation in this gene was found in 2008 to significantly increase the risk of myopathy [64]. Graham *et al.* (2004) reviewed records of over 250,000 patients treated from 1998 to 2001 with the statin drugs atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin, and simvastatin [65]. The incidence of rhabdomyolyis was 0.44 per 10,000 patients treated with statins other than cerivastatin. However, the risk was over 10-fold greater if cerivastatin was used, or if the standard statins (atorvastatin, fluvastatin, lovastatin, pravastatin, or simvastatin) were combined with fibrate (fenofibrate or gemfibrozil) treatment. Cerivastatin was withdrawn by its manufacturer in 2001. Some researchers have suggested hydrophilic statins, such

Table 8.5 Clinically significant statin drug interactions

Drug or food interaction		Mechanism of interaction and side effects	Statins and their major metabolic enzymes			
			Atorvastatin CYP3A4	Fluvastatin CYP2C9	Lovastatin CYP3A4	Simvastatin CYP3A4
1	CYP3A4 inhibitors	CYP3A4 inhibition > to decreased metabolism of statins > myopathy/rhabdomyolysis	++		++	++
2	Calcium Channel Blockers	CYP3A4 and/or P-gp inhibition > to decreased metabolism of statins > myopathy/rhabdomyolysis and hepatitis	+		++	++
3	Macrolide Antibiotics	CYP3A4 inhibition > to decreased metabolism of statins > myopathy/rhabdomyolysis	++		++	++
4	Protease Inhibitors	CYP3A4 inhibition > to decreased metabolism of statins > myopathy/rhabdomyolysis	++		++	++
5	OATP1B1 inchibitors	Inhibition of the hepatic uptake transporter OATP1B1 and CYP3A4 enzyme	+			+
6	Cytochrome P450 inducers	CYP3A4 induction > decrease in TG-lowering effect of statins	++		++	++
7	Coumarin anticoagulants	CYP3A4 oxidation > reduced clearance of coumarin anticoagulants > increased INR	+	+	+	+
8	Fibrate acid derivate	Inhibition ofstatin glucuronidation in liver and CYP2C9 inhibition > myopathy/rhabdomyolysis	++	++	++	++
9	Grapefruit juice	CYP3A4 and P-gp inhibition > to decreased metabolism of statins > myopathy/rhabdomyolysis	+	+	+	+

as fluvastatin, rosuvastatin, and pravastatin, are less toxic than lipophilic statins, such as atorvastatin, lovastatin, and simvastatin, but other studies have not found a connection [66].

The PRIMO (Prediction of Muscular Risk in Observational conditions) was an observational survey that included close to 8000 patients on high dose statins. Muscular symptoms were reported in about 10.5% of patients most commonly as pain in the lower limbs but about 25% reported tendon pain. Pain prevented moderate exertion in about 38% of those patient and 4% were bed ridden. There was a temporal association between symptoms and initiation of statins or titration to a higher dose. Risk factors associated with myalgias during high dose statin therapy from the PRIMO included: History of myalgias with another lipid lowering agent (OR 10.12), Unexplained cramps: (OR 4.14), History of elevated creatine kinase (CK) (OR 2.04), family history of muscular symptoms (OR 1.93), family history of muscular symptoms with lipid lowering agent (OR 1.89), hypothyroidism (OR 1.71), type of statin: Atorvastatin (OR 1.28, simvastatin OR 1.78) compared to high dose pravastatin, whereas fluvastatin XL was associated with significantly lower muscular symptoms (OR 0.33 $P < 0.0001$). Duration of statin treatment more than 3 months and concomitant use of antidepressant were associated with significantly lower prevalence of muscular symptoms with OR 0.28 and 0.51 respectively [67].

Recommendations Regarding Statin and Muscle Safety

While changes in CK levels rarely correlate with myopathic symptoms, the National Lipid Association (NLA) recommends the following: providers should obtain baseline CK in high-risk patients (renal dysfunction, liver disease, polypharmacy)[121]. Routine baseline CPK is not recommended in asymptomatic patients. CK determination should be considered in patients with muscle-related symptoms. Rule out other etiologies in symptomatic patients or those with elevated CPK levels (hypothyroidism, trauma, seizures, infection, strenuous physical activity). Exacerbating factors, such as concomitant medications and herbal remedies, should be considered. If intolerable muscle symptoms develop, discontinue statin regardless of CK levels and re-challenge only after the patient becomes asymptomatic. If muscle symptoms are tolerable and CK elevation is mildly elevated (< 3 times the upper limit of normal) < 3 times baseline CK then the statin may be continued and muscle symptoms can be used as a guide to stop or continue treatment. If muscle symptoms are intolerable or if CK elevation is moderate to severe, then discontinue statin therapy and weigh the risks and benefits. For patients in whom muscle symptoms are absent or present and CK elevation is associated with elevated creatinine or a need for intravenous hydration, then discontinue therapy.

Statins and Liver Injury

Clinical trials have shown that statin use has been associated with elevations in serum alanine aminotransferase (ALT) levels in approximately 3 % of persons who take the drugs. Such elevations are not clinically significant in the great majority of cases; indeed, ALT levels greater than 3 times the upper limit of normal (ULN) are seen in only a small minority of patients. With continued use, the mild elevations of serum aminotransferases generally resolve. This phenomenon, which has been observed for a number of drugs, is not well understood but has been called adaptation. Patterns of liver abnormalities seen with statins include: (1) asymptomatic elevations of ALT: usually transient and mild (ALT < 3 × ULN), as already described; (2) hepatitis: with ALT > 3 × ULN and clinical symptoms of liver disease; (3) cholestatic or mixed hepatitis: with development of jaundice; and (4) autoantibody-associated DILI with the presence of antinuclear antibody (ANA) and antismooth muscle antibody or antimitochondrial antibody with or without plasma cells on liver biopsy. Acute liver failure (ALF) develops in a very small minority of persons who are taking statins; indeed, the incidence is not different from that in the general population [51]. The overall risk of DILI with statin use is estimated to be approximately 1 in 100,000 with the estimated risk of ALF being approximately 1 in 1,000,000 [68].

Recommendations Regarding Statin and Liver Safety

The FDA now recommends that clinicians test liver enzymes in their patients before prescribing statin treatment and as clinically indicated thereafter, rather than routinely monitoring liver enzymes every 3 mounts as was recommended previously. Statin treatment should be interrupted in patients who develop serious liver injury with clinical symptoms and/or hyperbilirubinemia or jaundice, and drug therapy should not resume unless an alternate cause is found for the hepatic dysfunction.

Statins and Diabetes Mellitus

Careful review of findings from many trials combined does show that statins can modestly raise blood sugars, and more patients who are on statin therapy are diagnosed with diabetes mellitus compared with those not on statins. Statins may increase the risk of diabetes by 9 %, [69] with higher doses appearing to have a larger effect [70]. A meta-analysis in 2010 of 13 trials (n = 91,140) showed 9 % increased risk of diabetes over a mean of 4 years, the risk was highest in trials with older participants [71]. A meta- analysis in 2011 of 5 trials (n = 32,752) showed a 12 % increased risk of diabetes with intensive statin therapy compared to moderate—dose statin over a mean of 4.9 years [72]. A subgroup analysis of JUPITER stratified participants based on presence of risk factors for developing diabetes. In the group without risk factors for diabetes, there were no new cases of diabetes detected. In

the group with one or more risk factors for diabetes there was a 28% higher risk of developing diabetes with rosuvastatin use compared to placebo, however there was 39% reduction in the primary end point. In this group a total of 134 vascular events or deaths were prevented for every 54 new cases of diabetes diagnosed [73].

Hence statin benefits usually outweigh the risk of developing new diabetes when they are used in the appropriate patient population. Dormuth CR et al [74] have reported that there is moderate increase in the risk of new diabetes among patients with cardiovascular diseases treated with high potency statins as atorvastatin, rosuvastatin and simvastatin compared to low potency statins for secondary prevention of cardiovascular diseases.

Statins and Cancer Association

Statins do not appear to be associated with cancer [75, 71]. Although there have been concerns that they might increase risk, [74] several meta-analyses have found no relationship [76, 77].

They may reduce the risk of esophageal cancer, [78] colorectal cancer, [79] gastric cancer, [66, 80] hepatocellular carcinoma, [81] and possibly prostate cancer [82, 83]. They appear to have no effect on the risk of lung cancer, [84] kidney cancer, [85] breast cancer, [86] pancreatic cancer, [87] or bladder cancer [88].

Statins and Acute Kidney Injury

Evidence has indicated that statin use could lead to unintended adverse renal effects [89–91]. Various data sources have pointed to a possible harmful effect of statins on the kidney. Prescription of high potency statins ($\geq$10 mg rosuvastatin, $\geq$ 20 mg atorvastatin, $\geq$ 40 mg simvastatin) is associated with an increased rate of hospital admission for acute kidney injury, compared with lower potency statin. In patients with non-chronic kidney disease, current users of high potency statins were 34% more likely to be hospitalized with acute kidney injury. Increased risk of admission occurs early after starting statin treatment, it seems to be strongest in the first 120 days after initiation of statin treatment and remains elevated for at least two years [92].

Statins and Sleep Problems

Sleep has not generally been reported as an adverse event in large efficacy trials, although case series and smaller trials have indicated insomnia, nightmares and other sleep disturbances may be more common with some statins. Simvastatin modestly but significantly reduced sleep quality and increased sleep problems compared

with pravastatin, according to a large study of subjective sleep measures reported at the American Heart Association meeting. Simvastatin is the most lipophilic of the statins and this may allow the drug to more readily cross the blood-brain barrier and impact serotonin or other sleep-related factors compared with statins such as pravastatin, which is the most hydrophilic [93].

Statins and Memory Loss

FDA has been investigating reports of cognitive impairment from statin use for several years. The reports about memory loss, forgetfulness and confusion span all statin products and all age groups. In general, the symptoms were not serious and were reversible within a few weeks after the patient stopped using the statin. Some people affected in this way had been taking the medicine for a day; others had been taking it for years.

Statins and Erectile Dysfunction

Erectile dysfunction (ED) is commonly associated with atherosclerosis [94–96]. Vascular ED can therefore be exacerbated by many of the risk factors which cause atherosclerosis, but it is also well documented that control of these risk factors with statin therapy, routinely recommended for cardiovascular disease, has been associated with worsen or even precipitate erectile function.

Some researchers have looked at the possibility that the statins' inhibition of cholesterol synthesis may interfere with the production of testosterone, which depends on a supply of cholesterol. The statins may disrupt the body's feedback mechanism to instruct it to make more testosterone.

A second hypothesis by which statin therapy may worsen erectile function is that it may interact with other agents that are also implicated in the causation of impotence such as age, smoking and diabetes [97]. However it is impossible to delineate whether severity of atherosclerotic disease, drug doses or drug interactions are responsible for ED.

Management of Statin Intolerance

The first step in the strategy to manage statin intolerance is to rule out extraneous factors that may increase the risk of myopathy/rhabdomyolysis or elevate hepatic transaminases. Other strategies used to manage statin intolerance are switching therapy, alternate day dosing, non-statin lipid-lowering drugs, lipid lowering nutraceuticals, and specific pharmacotherapies.

Switching Therapy

This strategy of switching therapy is effective in only some patients since the criteria to select the new statins are not clearly delineated [98, 99]. Switching from (1) Mild to high lipophilic statin (2) from cytochrome P450 metabolised to non-cytochrome P450 metabolised statin, and (3) to a lower dosage of a more potent statin have been utilized.

Alternate Day Dosing

The best lipid lowering agent for patients with history of statin intolerance remains a statin. In fact studies have shown that most patients with history of statin intolerance, tolerate subsequent challenge with a statin [100, 101]. As recommended by the Statin Intolerance Expert Panel, clinicians should make every attempt to maintain some form of statin therapy in every case of statin intolerance and to clarify and differentiate statin intolerance from "Drug Allergy" which have implications on statin rechallenge [102]. When suspicion for statin intolerance arises, the first step would be to discontinue the statin and check CPK and TSH after detailed history and physical exam. Usually statin related muscle symptoms resolve partially or completely within 2 months. If symptoms resolve rechallenge with lower dose of the same or a different statin and continue this cycle of dechallenge/rechallenge with low dose or alternate-day dosing until all statins have been tried [122].

Statins with longer half-life maintain lipid lowering effect over a longer period of time, enabling alternate day dosing strategy with statin. Atorvastatin with a mean half-life of 14 h is metabolised into two active metabolites-orthohydroxy and para-hydroxy forms. Both these active metabolites contribute to 70% activity of atorvastatin and have a half-life of 20 to 30 h [103]. This pharmacokinetic parameter of atorvastatin makes it suitable for an alternate-day dosage regimen and continues its lipid lowering activity for considerably a longer period of time. Evidence for use of intermittent statin dosing with tolerability ranging between 80–100% and LDL-C reduction between 20–39% comes from some case reports and case series with rosuvastatin [104–106] and from retrospective studies of rosuvastatin monotherapy LDL-C reduction 23–34.5% and tolerability 72.5–89%. [103, 107–109]

Rosuvastatin, a third generation statin, possess a long half-life period of around 19 h. In 2008, Gadarla *et al*., [107] reported use of rosuvastatin (5 and 10 mg), two-times a week (on the first and fourth day of the week) for a period longer than 3 weeks in patients aged 62–70 years who developed myopathy due to other lipid-lowering therapy [108]. The rosuvastatin dosage regimen was well accepted by 80% of the patients with significant 26% LDL-C reduction from the baseline. In another study, eight patients who were intolerant to daily statin responded well with once-weekly dosage of rosuvastatin (5–20 mg) and reported a mean LDL-C reduction of 29% [109]. The apparent reasons for weekly statin regimen tolerance could be due to either lowering of overall plasma concentration of statins or psychological

reasons. However, this alternate day dosing strategy has some limitations, including less reduction of LDL-C and the fact that the alternate-day dosing strategy has not been established through clinical trials. Although there are many areas of uncertainty especially with the lack of large scale clinical outcome trials, intermittent statin dosing (particularly with atorvastatin and rosuvastatin) seems to be a very useful strategy in patients with history of statin intolerance [110]. Initiating once a week rosuvastatin or atorvastatin and slowly increasing the frequency as tolerated to every other day and potentially adding ezetimibe or a bile acid sequestrant is a reasonable approach that will provide statin intolerant patients with some benefit from statins with improved tolerability and LDL-C lowering that will hopefully translate into cardiovascular risk reduction [110].

Non Statin Lipid-Lowering Drugs

Non-statin lipid lowering drugs include a bile acid sequestrant (colesevelam), an intestinal cholesterol absorption inhibitor (ezetimibe), fibrates, and niacin which may be either used alone or in combination. These drugs have been considered in cases of statin intolerance. Co-administration of ezetimibe and bile acid sequestrants (colesevelam, colestipol, or cholestyramine) yields additional reduction of LDL-C levels without any adverse effects in comparison to stable bile acid sequestrant regimen alone. Addition of ezetimibe to nicotinic acid lowers LDL-C levels without modifying nicotinic acid-induced increase of HDL-C. The triple therapy i.e. bile acid sequestrant, statin, and ezetimibe or nicotinic acid further reduces LDL-C levels. However, no clinical outcome studies with these combinations have been performed. Functional food containing phytosterols or plant sterol containing tablets have been reported to reduce LDL-C levels up to 5–10% in patients taking a stable dose of a statin. This combination of plant sterol and statins was have been reported as well tolerated and safe [111, 112]. Since no clinical trials with combination of plant sterols and other lipid-lowering drugs have been established for CVD outcomes, their efficacy in CVD risk reduction remains speculative.

Use of Lipid Lowering Nutraceuticals

Various dietary interventions, including foods low in saturated fat and high in viscous fibers (e.g., oats and barley), plant sterols, vegetable protein foods (soy), and nuts (e.g., almonds) have been used in patients who cannot tolerate statins. The efficacy of these dietary interventions was further strengthened by addition of nutraceuticals such as red yeast [113]. In 2003, Jenkins and colleagues reported that dietary portfolio of cholesterol-lowering foods caused cholesterol lowering effect comparable to a statin. But due to its limitation of palatability, dietary supplements were used as an alternative option [114]. Chinese red yeast rice is a dietary supplement made by fermenting the yeast, *Monascus purpureus*, over rice. Monascus

yeast produces a family of substances called monacolins capable of inhibiting the enzyme HMG-CoA reductase and also contains unsaturated fatty acids and phytosterols. Red yeast rice offers only modest LDL-C lowering (up to 20 %) and has been prescribed to only low-risk individuals or in whom LDL-C level is not far from the target [115].

Specific Pharmacotherapies

Presently, there is a lack of consensus on the use of specific pharmacotherapy for statin-induced myopathy. Coenzyme Q10 (CoQ10) deficiency has been correlated with the development of myopathy. Various studies have reported significant improvement in statin-induced adverse effects-myopathy, myalgia, peripheral neuropathy, fatigue, dyspnoea, and memory loss if coenzyme Q10 was given as a co-therapy with statins [116–119, 70]. In 2009, Kalra *et al*., [99] reported that coenzyme Q10 (200 mg/day) supplementation in statin-treated patients would help in preventing statin-induced adverse effects, leading to low statin intolerance and maximal benefits of statin.

Vitamin deficiency has been associated with myalgia and poor muscle function and its supplementation have shown ameliorative effects in statin induced myopathy. A recent trial has shown that 92 % of patients become myalgia free after three months of vitamin D supplementation. [120]. However, the trial was not a randomized clinical trial and more definitive studies are needed before making Vitamin D supplementation recommendations.

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Chapter 9
Non-Alcoholic Fatty Liver Disease (NAFLD): The Lipid Disease of the Liver and the Effect of Statins

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Diagnosis is performed by ruling out other causes of elevation of liver enzymes and performing imaging studies. Liver biopsy is still the gold standard to differentiate NAFLD (or simple steatosis) from NASH and to stage the disease. New magnetic resonance imaging (MRI) techniques have been shown to be very promising in quantifying fat (MR Proton Density Fat Fraction) (MR-PDFF) and in detect fibrosis (MRE elastography). There is currently no Food and Drug Administration-approved treatment. Weight loss and exercise are generally the first recommended approach. Vitamin E and pioglitazone have been shown to improve liver enzymes and histology; however, the long-term effects are unknown. Finally, statins have been shown to be safe and helpful in NAFLD and NASH patients. Statins are recommended for the treatment of dyslipidemia in these patients. Randomized controlled trials are needed to assess the effects of statins on NAFLD/NASH.

Introduction

Non-alcoholic fatty liver disease (NAFLD) has emerged as a major health problem in the last decade in parallel with the increasing epidemic of obesity [1]. It has been recognized as the hepatic manifestation of metabolic syndrome and is the most

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common reason for referral to hepatologists today [2]. The disease has a variable histological course, with some patients only accumulating fat in the liver and not progressing beyond simple steatosis while a subpopulation of patients progress to the more advanced stage of nonalcoholic steatohepatitis (NASH) in which inflammation and cell injury occur [3]. NASH can lead to liver cirrhosis and hepatocellular carcinoma (HCC) and is expected to be the leading cause of liver transplant and liver-associated mortality in the coming decade [4]. It is not surprising that the liver is the major affected organ with the epidemic of obesity as it plays a central role in lipid metabolism through the synthesis of apoproteins and lipoproteins as well as de novo lipogenesis [5]. Since the liver regulates lipid metabolism and secretion, the disruption of normal physiologic lipid regulation can lead to fat accumulation in the liver and subsequently liver injury. In this chapter we highlight the different aspects of the disease, including natural history, epidemiology, pathophysiology, diagnosis and treatment. In addition, we discuss the role of statins and whether they may be harmful or beneficial in NAFLD patients.

Epidemiology and Natural History

NAFLD has become the most common cause of asymptomatic elevation of liver enzymes and the most common reason for referral to liver clinics [1, 6]. While initially thought to be exclusively a disease of adults, it has become the most common liver disease among adolescents in the United States, with older age often being predictive of more advanced disease [7–9]. It is estimated that one in three adult Americans is afflicted with NAFLD, with a higher prevalence in Hispanic populations likely due to the higher prevalence of obesity and insulin resistance in this ethnic group [1]. The importance of genetic and epigenetic changes in the etiology and pathogenesis of NAFLD has been increasingly recognized. Genome-wide association studies have led to increased understanding of genomic variations of NAFLD. Patatin-like phospholipase domain containing family member A3 (PNPLA3, SNP rs738409, encoding I148M), also termed adiponutrin, may be of particular importance [10]. A series of studies has validated that PNPLA3 is associated with increased hepatic fat levels and hepatic inflammation [11]. This allele is most common in Hispanics, with hepatic fat content being more than two-fold higher in G homozygous subjects than in non-carriers. G allele frequency is lower in people of European descent and is lowest in African Americans who constitute the population least likely to have hepatic fat accumulation [11].

Around 10–20 % of patients with simple steatosis progress to NASH; of those, 10–20 % progress to cirrhosis over 10–20 years [12, 13]. HCC may develop in those who progress to cirrhosis; however, the incidence rate is still unknown [14]. In addition, many reports have described cases of HCC in NASH patients that have developed without underlying cirrhosis [14]. Epidemiologic risk factors associated with NAFLD and NASH include obesity, type 2 diabetes and hyperlipidemia [15]. Metabolic syndrome has been shown to increase the risk of NASH and advanced

fibrosis, in particular if there is coexisting diabetes [15]. Cardiovascular diseases have been shown to play a significant role in the natural history, morbidity and mortality of NAFLD [16, 17]. Indeed, cardiovascular events have been thought to be the leading cause of death in NAFLD [18, 19].

Pathogenesis of NAFLD/NASH

A two-hit model has been proposed to explain the progression of NAFLD. The role of hyperinsulinemia and insulin resistance in lipid accumulation in the liver is essential in the disease process [20]. The disease develops with the abnormal hepatic accumulation of triglycerides (TG), which can progress to NASH in some patients. Factors that promote the progression from steatosis to NASH in humans are incompletely understood but include genetic and behavioral factors [21].

Development of Hepatic Steatosis

Increased Fatty Acid Synthesis, Increased Triglyceride Storage and Impaired Secretion

Lipid metabolism is imbalanced in the liver in the setting of obesity and insulin resistance, leading to accumulation of triglycerides in the liver. This process is usually due to increased free fatty acid (FFA) flux from adipose tissue to the liver, increased caloric intake, and increased de novo lipogenesis in the liver [22]. As the adipose tissue is increased with obesity, there is increased hormone-sensitive lipase (HSL) activity and accelerated release of FFA from adipose cells into circulation. FFA uptake by the liver is increased proportionally to the increase in FFA in the blood circulation [22, 23]. The fate of FFA in the liver is either metabolism via oxidation to generate ATP through β-oxidation in the mitochondria or esterification to produce triglycerides. These triglycerides (TG) are either packaged into very-low-density lipoproteins (VLDL) for export or are used for the production of lipids such as phospholipids [22]. These processes have been shown to be impaired in NAFLD, leading to imbalance between the uptake and metabolism of FFA which, in turn, leads to TG accumulation in the liver [22]. Furthermore, when there is increased caloric intake, glucose gets converted to pyruvate which enters the Krebs cycle in the mitochondria. Acetyl-CoA is formed from pyruvate by pyruvate dehydrogenase in the mitochondria. Acetyl-CoA produced in the mitochondria is condensed with oxaloacetate by citrate synthase to form citrate. In the presence of ATP and Coenzyme A, citrate lyase catalyzes the cleavage of citrate to yield acetyl CoA, oxaloacetate, ADP, and orthophosphate. Acetyl-CoA carboxylase (ACC), a biotin-dependent enzyme, catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA through its two catalytic activities, biotin carboxylase (BC)

and carboxyltransferase. Malonyl-CoA is utilized in fatty acid biosynthesis by the enzyme malonyl coenzyme A:acyl carrier protein transacylase (MCAT). MCAT serves to transfer malonate from malonyl-CoA to the terminal thiol of holo-acyl carrier protein (ACP). Malonyl-CoA also converts to palmitic acid via fatty acid synthase (FAS) [21, 24]. Subsequently, the enzymes stearyl-CoA desaturase (SCD) and long chain fatty acid elongase are used to create other fatty acids such as palmitoleic acid (C16:1), stearic acid (C18:0), or oleic acid (C18:1) [21, 24]. Ultimately these fatty acids form triglycerides.

Along with increased FFA flux into the liver and increased fatty acid formation due to increased caloric intake, de novo lipogenesis is augmented [22, 25]. In the normal state, de novo lipogenesis contributes to less than 5% of fatty acid, TG, and VLDL synthesis [26]. However, in NAFLD patients this process is upregulated, contributing to synthesis of up to 26% of fatty acids, TG, and VLDL [25, 27]. Hyperglycemia stimulates carbohydrate response element-binding protein (ChREBP), which transcriptionally stimulates the liver-type pyruvate kinase (L-PK), a key enzyme in glycolysis. LPK stimulates the entry of pyruvate into the mitochondria and its conversion into citrate, which forms acetyl-CoA and hence increases fatty acid synthesis [28, 29]. On the other hand, hyperinsulinemia leads to activation of a membrane-bound transcription factor, sterol regulatory element-binding protein-1c (SREBP-1c), which triggers all lipogenesis genes and thus increases de novo fatty acid synthesis [30]. One of the important effects of increased fatty acid synthesis is increased malonyl-CoA which inhibits carnitine palmitoyl transferase-1 (CPT-1), the protein responsible for fatty acid transport into the mitochondria [31]. TG synthesis has also been shown to be affected by increasing levels of glycerol-3-phosphate acyltransferase (GPAT); and VLDL secretion is impaired by decreasing expression of microsomal transfer protein [32]. Gluconeogenesis is also diminished secondary to SREBP-1c inhibition of phosphoenolpyruvate carboxykinase (PEPCK) [33]. The net result is increased de novo lipogenesis and impaired VLDL packaging and secretion [34].

The Role of PPAR, LXR and FXR Receptors

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors, regulating the expression of genes and playing essential roles in lipid metabolism and hepatic steatosis. There are three PPAR isotypes, including PPARα, PPARβ, and PPARγ. PPARα is mainly expressed in the liver where it increases the use of fatty acids [35]. It induces the transcription of genes for movement of fatty acids into the cell and mitochondria including fatty acid transport protein and CPT1. The result of PPARα activation is increased fatty acid uptake and oxidation, lipolysis, and clearance of ApoB-containing lipoprotein [36]. The role of PPARβ in hepatic steatosis is not completely understood but it is thought to play a role in fatty acid transportation and oxidation. PPARγ is mainly located in adipose tissue but it is also formed to a lesser extent in skeletal muscle, liver, pancreatic beta cells, myeloid dendritic cells, and macrophages. PPARγ agonists

(thiazolidinediones and others now being studied or in development) have been shown to act on adipocyte tissue in a way that increases fatty acid uptake and storage and increases insulin sensitivity [36]. This results in redistribution of fat from the liver into the subcutaneous fat. PPARγ also increases production of adiponectin which has significant effects on fatty acid oxidation and insulin sensitivity [37].

A recent role for the liver X receptor (LXR), a member of the nuclear receptor family of transcription factors, has been postulated [38]. The LXR has many similarities to PPARα as both are transcription factors that belong to class II nuclear receptors [39]. LXRα is found mostly in hepatocytes, adipose tissue, and macrophages, whereas LXRβ is more widespread [40]. LXRs induce the key enzymes in the de novo lipogenesis pathway including acetyl-CoA carboxylase 1 (ACC1), FAS, and stearoyl CoA desaturase 1 (SCD1) [41, 42]. Both SREBP -1c and ChREBP have been shown to be target genes of LXRs. LXRs mainly increase hepatic lipogenesis by upregulating the expression of SREBP-1c and to a lesser extent by activating ChREBP [42]. A recent study has shown the role of the LXR-lysophosphatidyl acyltransferase 3 (Lpcat3) pathway in modulating phospholipid metabolism, ER stress and inflammation [38]. Lpcat3 catalyzes the formation of phosphatidylcholine (PC) from saturated lysophosphatidylcholines (LysoPC) and unsaturated fatty acyl-CoAs, with PC containing unsaturated fatty acids preferentially synthesized by this enzyme [43, 44]. It has been shown that increased levels of saturated fatty acids lead to changes in ER membrane composition and induce ER stress [45]. LXR activates Lpcat3 leading to formation of polyunsaturated phospholipids, which decreases membrane saturation. This membrane remodeling leads to decreased ER stress in liver cells. Moreover, the LXR-Lpcat3 pathway decreases hepatic inflammation through a c-Jun NH2-terminal kinase (JNK) pathway-mediated mechanism.

The farnesoid X receptor (FXR) is expressed mainly in the liver, intestine, adrenal glands and kidneys. It has also been shown to be expressed in lower levels in the heart, adipose tissue and vasculature [46, 47]. FXR inhibits SREBP-1c and FAS leading to reduced lipogenesis [48]. It also affects glucose metabolism in the liver by reducing gluconeogenesis via the downregulation of PEPCK and glucose-6-phosphatase (G6Pase). FXR reduces conversion of cholesterol to bile acids by inhibiting enzymes involved in bile acid synthesis such as cytochrome P450 7A1 (CYP7A1) and CYP8B1 [49]. Prior to secretion into the bile, bile acids are conjugated to either glycine or taurine. FXR enhances bile acid conjugation and stimulates the transport of bile acids to the gallbladder [50]. It also decreases bile acid absorption in the small intestine and stimulates reabsorption recycling of bile acids to the liver. FXR reduces hepatic uptake of bile acids and promotes the release of fibroblast growth factor 15 (FGF15) and FGF19 from the intestine [51]. FGF15 and FGF19 circulate to the liver and reduce CYP7A1 expression, thus repressing bile acid synthesis [51]. Recently, bile acids have been shown to play a significant role in glucose homeostasis. They regulate cholesterol, glucose, and metabolic homeostasis in addition to regulating their own synthesis [50]. FXR knockout mice have been shown to have elevated plasma triglycerides and cholesterol levels, impaired glucose hemostasis and decreased insulin sensitivity [52]. FXR agonists inhibit hepatic gluconeogenesis and stimulate glycogen synthesis and storage, resulting in an

overall low glucose level [53]. A recent trial in NAFLD patients has shown that an FXR agonist reduces liver inflammation and fibrosis markers in addition to improving insulin sensitivity [54].

Another mediator that has been shown to play a role in metabolism is adenosine monophosphate-activated protein kinase (AMPK). AMPK stimulates fatty acid oxidation and glucose transport. In the liver, it augments fatty acid oxidation and decreases glucose output and cholesterol and triglyceride synthesis, metabolic effects that result in lowered blood glucose levels in hyperglycemic individuals [55]. Two types of oral antihyperglycemic drugs, the biguanidines and thiazolidinediones, have been shown to work in part by directly or indirectly activating AMPK [55]. For example, metformin is known to activate AMPK [56]. Once energy in increased AMP accumulates, it stimulates AMPK and leads to formation of adenosine triphosphate. AMPK inhibits ACC, decreases expression of SREBP-1 and stimulates deactivation of ChREBP. It also increases β-oxidation [57].

The Emerging Role of Gut Microbiota

A relationship between gut microbiota, the collective term for the 100 trillion bacteria that inhabit the GI tract. and the development of NAFLD has been demonstrated in mice and humans. Transplantation of normal cecal microbiota into germ-free mice induced a 60% increase in body fat and a twofold increase in hepatic fat [58]. One of the first observations of the relationship between gut microbiota and hepatic steatosis was in the 1980s when steatosis, NASH and bacterial overgrowth were seen to develop after intestinal bypass [59]. Interestingly, steatosis was reversed by metronidazole, suggesting a causative role of the microbiota in fatty liver disease and antibiotics as potential candidates for treatment [59].

There are several potential mechanisms through which gut microbiota may cause hepatic steatosis and NASH. These may include stimulation of obesity, increased gut permeability, inflammation and altered immune balance, modulation of dietary choline metabolism increasing ethanol production by the bacteria, and regulation of bile acid metabolism [60]. Bile acids damage bacterial cell membranes by interacting with membrane phospholipids which results in bactericidal activity. Conversely, the gut microbiota modulates bile acid metabolism through FXR stimulation. Bile acids are ligands for a G-protein coupled receptor (TGR5/Gpbar-1) and activate FXR. Therefore, through bile acid metabolism and FXR/TGR5 signaling, gut flora could contribute indirectly to the development of NAFLD [61].

Progression from Simple Steatosis to NASH

While increased storage of circulating FFA, increased de novo lipogenesis and impaired β-oxidation and TG secretion may explain the significant triglyceride accumulation in simple steatosis, a “second hit” or more precisely “multiple hits”

are thought to be required to promote inflammation, cell death, and fibrosis and the resultant progression to NASH. There are many potential candidates for the additional hits which may play a role in the shift from steatosis to NASH, including oxidative stress, iron, endotoxins, cytokines, mitochondrial dysfunction and induction of the cytochrome P450 system. Lipotoxicity and oxidative stress are key drivers of disease progression. Increased reactive oxygen species (ROS) production has been shown to play a major role in progression to NASH [22]. Sources of increased ROS production include proinflammatory cytokines (such as TNF-α and IL6), iron overload, overburdened and dysfunctional mitochondria, CYPs, and peroxisomes [21]. The role of mitochondria in NASH development has been shown to be essential. In normal conditions fatty acids get oxidized mainly by the mitochondria via β-oxidation and then get transported to the mitochondrial respiratory chain (MRC), leading to production of ATP and generation of CO_2 and water. A small portion of oxygen is not utilized, leading to formation of ROS including superoxide, hydrogen peroxide and the hydroxyl radical species [34, 62]. In the setting of increased free fatty acids flux the mitochondria exhaust and fatty acids are then metabolized at other sites in hepatocytes including peroxisomes (β-oxidation) and the CYP enzymes of the smooth endoplasmic reticulum (ω-oxidation) [62, 63]. In the mitochondria, long-chain fatty acids are oxidized and transported using the carnitine shuttle enzymes carnitine palmitoyltransferase I (CPT-I) and carnitine palmitoyltransferase II (CPT-II). This leads to formation of shorter acyl-CoA moieties, acetyl-CoA. This oxidation process is associated with the reduction of oxidized NAD+ and FAD to NADH and FADH2, which produces electrons that transfer to the MRC. These partially reduced oxygen molecules (ROS) lead to oxidant stress as the mitochondria are overwhelmed [62, 64]. CYP2E1 then oxidizes the rest of the excess free fatty acids which further increases ROS production within hepatocytes. Using immunostaining, CYP2E1 has been shown to be increased in NASH patients [65, 66]. Other excess free fatty acids undergo oxidation in the peroxisomes in which electrons from FADH2 and NADH are transferred directly to oxygen leading to further formation of ROS. These overwhelming processes in the mitochondria result in mitochondrial dysfunction manifested by depletion of ATP, and decreased mitochondrial DNA levels and proteins produced by mitochondrial genes [62]. Crystalline inclusions within the mitochondrial matrix seen by electron microscopy and megamitochondria detected by microscopy have been observed in NASH patients [62, 64, 67].

FFAs can also lead to lipotoxicity in an apoptosis process due to translocation into lysosomes resulting in release of lysosomal enzymes and subsequently activation of nuclear factor (NF)-κB activation and TNF-α overexpression in the liver. TNF-α activates two pathways including (NF)-κB and JNK [68]. JNK leads to insulin resistance by phosphorylation of insulin receptor substrate 1 (IRS-1). The (NF)-κB pathway leads to production of proinflammatory cytokines. These pathways have been shown to be activated in NASH patients [68, 69]. The results of the previous process with a central role of the mitochondria collectively lead to NASH progression [70]. Other possible etiologies have been considered in the last few years including the roles of dietary fructose, toll-like receptors (TLRs),

nucleotide-binding oligomerization domain receptors (NOD-like receptors), and the hedgehog signaling pathway [71–84].

Fructose consumption has gained significant attention as a possible cause of NAFLD. High-fructose corn syrup has been shown to increase endoplasmic reticulum stress, activate JNK, induce mitochondrial dysfunction, and increase apoptotis in hepatocytes [80, 85, 86]. In addition, dietary fructose intake has been found to have close association with gut-derived endotoxemia, toll-like receptor 4 and NAFLD [87]. Human studies have shown correlation between high fructose consumption and NAFLD [83, 88].

TLRs and NOD-like receptors (NLRs) are pattern recognition signal receptors involved in activation of the innate immune system [89]. In general, activation of NLRs and TLRs induces pro-inflammatory cytokine production, as well as recruitment in the liver of immune cells, including macrophages and T cells, resulting in chronic low-grade inflammation that promotes insulin resistance and contributes to development of fatty liver [90]. In response to pathogens, TLR signaling induces proinflammatory cytokines in immune cells [91]. With the increased intestinal permeability in NASH patients, intestine-derived pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS), translocate to the liver and activate TLR signaling cascades [92]. The activation of TLR2, TLR4 and TLR9 induce production of various cytokines, including transforming growth factor-beta (TGF-β), interleukin 1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α), which in turn stimulate hepatic stellate cells (HSC), leading to lipid accumulation and apoptosis in liver cells [91, 93–95]. Moreover, apoptotic hepatocytes activate Kuppfer cells via TLRs and produce inflammatory cytokines such as interleukin 6 (IL-6). TLR2, TLR4, and TLR9 have been reported to be associated with steatohepatitis [94, 96, 97]. In experimental NASH, TLR4 and TLR9 have been shown to promote hepatic inflammation and fibrosis [94, 95], while inactivation of TLR4 has been shown to lead to attenuation of steatosis and NASH [87, 98]. It has also been reported that TLR2 and palmitic acid cooperatively contribute to the development of NASH through inflammasome activation [91]. NLR activation leads to assembly of the caspase 1-containing inflammasome, resulting in inflammation and apoptosis [90]. NOD1 and NOD2 have both been associated with many inflammatory diseases, and both NOD1 and NOD2 mRNA and protein have been shown to be highly expressed in hepatocytes [99]

The Hedgehog pathway is one of the complex signaling cascades that are important for the immune response [71]. Studies in mice have shown that the development of fibrosis and steatohepatitis correlate with the intensity and duration of Hedgehog pathway activation that develops during fatty liver injury [100]. This pathway is essential in embryogenesis and can be triggered in adult life in the setting of tissue regeneration [101]. It has been shown that hepatocyte injury in an environment of lipotoxicity can produce Hedgehog pathway activation which in turn stimulates inflammatory cells and, in particular, natural killer T (NKT) cells. It also promotes growth and hepatocyte differentiation but at the same time activates stellate cells leading to fibrosis [72, 102, 103]. It has been hypothesized that differences in Hedgehog pathway activity may contribute to the varying outcomes of fatty liver

injury in NAFLD patients. In a study of a large cohort of NAFLD patients, it was found that the level of Hedgehog activity paralleled the severity of liver damage (hepatocyte ballooning, portal inflammation and liver fibrosis) [71]. The researchers suggested that development of non-invasive tests that quantify Hedgehog pathway activity might help identify patients developing tissue damage related to metabolic syndrome before irreparable end-organ damage occurs.

Diagnosis

For diagnosis of NAFLD, clinical history and laboratory and radiological investigations are the first step to exclude other causes of liver disease [104]. History of alcohol intake should be taken carefully to rule out alcoholic liver disease which shares many common findings with NAFLD [104]. Imaging studies are needed to assess hepatic steatosis, with ultrasound being the most widely used method [104]. However, ultrasound and computed tomography (CT) scan lack sensitivity and specificity to detect steatosis [105]. MRI techniques have been shown to be highly accurate in detecting liver fat [106]. MR spectroscopy (MRS) has been shown to be highly accurate in detecting liver fat and in quantifying it. MRS has been used for longitudinal follow up in clinical trials in NASH [106] and has become a reference standard. However, it has been mainly used as a research tool since it requires a special coil and special software and is time consuming. New MRI techniques such as MRI-Proton Density fat fraction have been shown to be highly precise in quantifying liver fat and are easier to use than MRS [107]. While fat can be detected by imaging, liver biopsy remains the gold standard for the accurate diagnosis of NASH and can differentiate simple fatty liver without inflammation, cell injury or fibrosis from NASH [104, 108, 109]. Metabolic syndrome is a strong predictor for the presence of steatohepatitis in NAFLD patients [15, 110, 111]. Histological scoring systems have been proposed to stage and grade the disease. The most widely used scoring system was described by Kleiner et al. from the NASH Clinical Research Network (CRN) established by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) [108, 112, 113]. This scoring system created a numeric score called NAFLD activity score (NAS) for grading activity and for use in clinical trials [108]. NAS consists of three key histological elements in NASH: steatosis, lobular inflammation and ballooning. Validation studies showed that an NAS score of 5–8 correlates with definitive NASH while a score of 1–2 correlates with definitive exclusion of NASH [114, 115]. However, other important histological findings that are seen in NASH such as portal inflammation and megamitochondria suggest that this score can be improved. Indeed, portal inflammation was later found to be associated with clinically and histologically advanced NAFLD in children and adults [116]. Children have two types of histological presentation. One of these types resembles adults where there is zone 3 prominence of steatosis. On the other hand, the most common type consists of either zone 1 prominence of steatosis, or panacinar steatosis [117]. Ballooning has been found to be uncommon in both

types. More recently, a study from the NASH CRN has shown that the elderly (defined as >65 years of age) have more azonal distribution of steatosis and are more likely to have NASH and advanced fibrosis [7].

Non-invasive serum and imaging markers as well as predictive scores of NASH and advanced fibrosis have emerged but are not yet widely utilized. Some of the biomarkers that have been investigated include C-reactive protein, hyaluronic acid (HA), tumor necrosis factor-α, leptin, interleukin-6, ferritin, resistin and adiponectin [118, 119]. Blood levels of cytokeratin 18 have been shown to be promising in predicting NASH but this method is not yet commercially available [120]. The NAFLD fibrosis score has been shown to be a good predictor of fibrosis and cirrhosis [121]. Other scores that have been used include ELF score, modified ELF score, BARD (body mass index, alanine aminotransferase/aspartate aminotransferase ratio, and presence of diabetes) and BAAT (body mass index, alanine aminotransferase, and triglycerides) [122]. Many of these scores have achieved an excellent area under the receiver operating characteristic curve (AUROC). The details of these biomarkers are beyond the scope of this chapter but can be found in reviews [122]. Other evolving imaging techniques such as transient elastography and MR elastography are now being investigated in assessing fibrosis in NASH patients [123–125].

Treatment

Weight loss and exercise are the recommended treatments for NAFLD and NASH by the Food and Drug Administration (FDA). It is thought that at least 7% reduction of body weight is needed for histological improvement in NASH patients [126]. Because it is often difficult for patients to maintain these lifestyle changes over the long term therapeutic agents have been investigated. However, no pharmaceutical agent has yet been approved [126, 127]. The current focus is to find treatments for NASH. Treatment for simple steatosis has not been a priority since the long term outcome is unknown. Small, mostly uncontrolled studies have been conducted showing limited benefit, if any, of the use of ursodeoxycholic acid, metformin, betaine, N-acetyl cysteine, and orlistat [128–135]. In a trial that randomized 166 NASH patients to ursodeoxycholic acid (13–15 mg/kg daily) or placebo, liver biopsies were performed before and after 2 years of treatment [128]. There was no difference in liver enzymes or histological changes between the two groups. Other clinical trials including high dose ursodeoxycholic acid were unsuccessful in showing a significant effect on NASH and in particular on histology [136, 137].

Metformin has been studied as potential agent for NASH treatment. Pilot trials have shown limited improvement in liver enzymes and less in histology; the beneficial effect was thought to be due to the weight loss effect of metformin [131, 138]. Metformin has also been shown to improve insulin resistance, prevent diabetic complications, and play a role in hepatocellular carcinoma chemoprevention, all of which coexist in NASH [139]. Therefore, although metformin has minimal

effect on NASH itself, long-term studies with either metformin monotherapy or with combination therapies that include metformin are needed. In a pilot study of 10 NASH patients, betaine was shown to be a promising agent for treatment of NASH as patients had improvement in aminotransferases and histology [132].

Betaine is required for the generation of methionine from homocysteine, a reaction that is central to the recycling of S-adenosyl-L-methionine (SAMe). Betaine has been shown to increase SAMe levels and protect against steatosis in animal models [140], while alteration of enzymes in the SAMe cycle has been shown to lead to NASH and hepatocellular carcinoma [141]. More recently, a human study suggested a role of methionine adenosyl methyltransferase 1 A (MAT1 A), one of the enzymes in the SAMe cycle in NASH [142]. Although a randomized clinical trial showed that betaine may protect against worsening of steatosis but may not play a role in improvement of the other histological features of NASH, of the study's initial 55 patients, only 34 patients were available for the exit biopsy [132]; additional research is needed.

Pentoxifylline has been shown to have a possible beneficial effect in improving serum aminotransferase and histology in NASH patients. In one study in which 55 patients were randomized to either pentoxifylline (400 mg, three times daily) or placebo for 1 year, pentoxifylline led to histological improvement of the NAFLD activity score (NAS) in 38.5 % of patients compared to 13.8 % in those given placebo [143]. Larger randomized controlled trials are needed to assess the effect of pentoxifylline.

Thiazolidinediones have been studied extensively in NASH patients. The two most commonly used agents have been rosiglitazone [144] and pioglitazone [145]. Rosiglitazone has led to improvement in steatosis and liver enzymes but not other histological parameters [144]. On the other hand, pioglitazone has been shown to be beneficial in improving liver enzymes and histology [145]. However, weight gain led to less enthusiasm by patients and hepatologists for its use [145–147]. The American Association for the Study of Liver Diseases (AASLD) guidelines state that pioglitazone can be used in biopsy-proved NASH. However, the guidelines highlight the fact that most trials have been carried out with non-diabetics and that the long term effects are unknown [104].

The PIVEN and TONIC trials using vitamin E have shown benefits in the treatment of NASH in adults and children [147, 148]. Although vitamin E has not yet been widely used in clinical practice, the AASLD has recommended vitamin E (d-alpha-tocopherol) administered at a daily dose of 800 IU for non-diabetic adults with biopsy-proven NASH as a first-line pharmacotherapy [104]. The long-term effects of vitamin E therapy in NASH patients have not been determined. Further therapies for NAFLD and NASH are still under investigation. The FXR agonist obeticholic acid is under investigation. Obeticholic acid has been shown to increase insulin sensitivity and decrease markers of inflammation and fibrosis [54]. Bariatric surgery has been shown to improve histology including fibrosis in NASH patients. However randomized clinical trials are needed to confirm benefits and for now a surgical approach is not recommended [149]. Many trials have looked at statins in NAFLD/NASH patients and shown some benefits.

Statins in NAFLD/NASH

A growing body of evidence is beginning to elucidate the extent to which alcoholic liver disease, chronic hepatitis C, and NAFLD raise a patient's risk of a significant cardiovascular event. Many of the factors mediating this increased cardiovascular risk include disruption of lipid metabolism resulting in unfavorable lipid profiles, insulin resistance, and features of metabolic syndrome. Thus, the use of lipid-regulating agents such as HMG-CoA reductase inhibitors (statins) may play an important role to help mitigate this pro-atherogenic profile seen in liver disease [150]. However, statins have been previously thought to be a common cause of abnormal liver enzymes, a major concern in the setting of already present liver disease. Of note, liver disease from chronic hepatitis B infection is associated with a far more favorable lipid profile, including decreased total cholesterol and decreased triglyceride levels, less steatosis, far less association with insulin resistance and type 2 diabetes, and in keeping with these factors lower cardiovascular risk [151]. Chronic HCV on the other hand has a unique constellation of findings. On the one hand, it is associated with decreased levels of total cholesterol, LDL, and triglycerides. Yet it has been demonstrated to have a significant association with hepatic steatosis, increased visceral fat and insulin resistance, and excess type 2 diabetes risk. HCV infection has been shown to be an independent predictor of angiographically detected coronary artery stenosis as well as increased carotid intimal thickness [152, 153]. While there has been an argument that statins may have limited value in the setting of decreased cholesterol and LDL, they have been shown to independently lower AST and ALT levels [154]. In conjunction with interferon alpha and ribavirin, statins may also increase rates of rapid virologic response (RVR), early virological response, and sustained virological response (SVR) [155]. Significant alcohol intake has been demonstrated to be a common cause of hyperlipidemia [156]. Patients with chronic alcoholic liver diseases have been demonstrated to have elevations in serum levels of triglycerides, chylomicrons, and VLDL. These lipid derangements in the setting of alcohol-induced pro-inflammatory and pro-thrombotic changes, insulin resistance, and features of metabolic syndrome yield a definite increase in cardiovascular risk for patients with alcoholic liver disease [5]. Patients with NAFLD/NASH arguably have the worst lipid profile with a combination of elevated triglyceride levels along with a significantly decreased HDL. LDL is not different in NAFLD patients; however, higher levels of small, dense LDL particles (nontype A), which are more atherogenic than type A LDL particles, are seen in these patients [16, 157]. The mechanisms for these changes are not completely understood but involve overproduction of the very-low-density lipoprotein (VLDL) particles and abnormal clearance of various lipoproteins. Thus it comes as no surprise that there is a significant increase in cardiovascular biomarkers (such as coronary calcium score or carotid artery intima-media thickness) in NAFLD patients. Indeed, cardiovascular events have been proved to be the leading cause of death in NAFLD patients [18, 19, 158].

Statins have been demonstrated to significantly mitigate the risk of cardiovascular events and provide a benefit to patients with these underlying causes of liver disease. However, because statins are cleared by the liver and are known to cause elevations of liver enzymes, there was much concern that patients with underlying liver disease may be at increased risk for statin-induced hepatotoxicity. This, however, has proven not to be the case. Two retrospective cohort studies have served to alleviate this concern. In patients with underlying liver disease and abnormal liver biochemistries who were treated for 6–12 months with statins there was no significant increase in the frequency of liver biochemistry abnormalities nor in severe liver disease when compared with patients who had normal liver biochemistries at baseline and received the same treatment [159, 160].

Are Statins Harmful in NAFLD/NASH?

The current evidence points toward no harmful effect of statins on NAFLD/NASH patients and a possible beneficial effect. In a long-term study of 86 patients who were followed for up to 16 years, 17 patients were on statins. Patients on statins had higher BMI and more sever hepatic steatosis at baseline [161]. Patients who were on statins had a decrease in their histological steatosis. However, there was a slight increase in fibrosis progression in the statin group. This was attributed to possibly more severe lipotoxicity ay baseline in the statins subgroup and more rapid fibrosis progression despite therapeutic measures such as statins [161]. In a prospective study of high-dose pravastatin therapy of patients with chronic liver diseases, including 64 % with NAFLD, there was a reduction in LDL cholesterol, without a significant change in aminotransferase elevation [162]. There is growing evidence that statins are safe in patients with NAFLD/NASH and may have histological benefit.

Are Statins Useful in NAFLD/NASH?

Treatment modalities for the spectrum of NAFLD remain controversial. However, one common cause of morbidity and mortality that is of great concern in these patients is the significantly atherogenic lipid profile that may play a role in the progression of the disease as well as contribute to the increased cardiovascular disease risk. One possible NAFLD treatment that is being explored is the use of statins. In Table 9.2, eight studies are outlined which assessed the effect of statin therapy in patients with NAFLD. While the dosing and length of treatment varied, all of the studies demonstrated a significant and persistent improvement of aminotransferase levels after the treatment period. Athyros et al demonstrated complete normalization of aminotransferases in all of their patients using atorvastatin; other smaller studies confirmed this improvement in liver enzymes [163–166]. In a non-randomized trial in which rosuvastatin was used for approximately 8 months in NAFLD

Table 9.1 NASH CRN scoring system for non-alcoholic steatohepatitis

Steatosis grade (%)	Lobular inflammation	Ballooning
0: <5	0: None	0: None
1: 5–33	1: <2	1: Few ballooned cells
2: 34–66	2: 2–4	2: Many ballooned cells
3: >66	3: >4	

patients, there was complete resolution of biochemical and ultasonographic evidence of NAFLD in 67% of patients [163]. Multiple retrospective studies which have looked at the effects of simvastatin and pravastatin in NAFLD patients have shown improvement in aminotransferases [161, 167, 168]. A post-hoc analysis of the GREek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study demonstrated that atorvastatin improves liver enzymes and cardiac outcomes in patients with increased liver enzymes most likely due to NAFLD [169]. Further studies demonstrating histological improvement of NAFLD with statin treatment may bolster their use in treating fatty liver, especially in those with abnormal lipid panels (Table 9.1).

With the evidence of benefit seen with statin use in NAFLD, it would stand to reason that there may also be benefits from their use in NASH. The majority of studies of statin use in biopsy-proven NASH echo the findings of improved aminotransferases seen with NAFLD (Table 9.3). The first evidence came from a pilot study of 7 biopsy-proven NASH patients. Although there was no statistically significant improvement of aminotransferase after 12 months of atorvastatin, there were improvements in both steatosis and inflammation. In a prospective non-randomized trial, a total of 44 biopsy-proven NASH patients were enrolled in the study. Patients without dyslipidemia ($n=17$) were given ursodeoxycholic acid (UDCA) while patients with dyslipidemia ($n=27$) were given atorvastatin 10 mg daily for 6 months [170]. There was more significant improvement in liver enzymes in the atorvastatin group compared to the UDCA group. There was also improvement in steatosis measured by CT in the atorvastatin group which was not seen in the UDCA group. Other small studies with different durations of treatment have confirmed the beneficial effect of atorvastatin on histology in NASH patients.

Pitvastatin and simvastatin have been studied less extensively, with a beneficial effect on aminotransferases shown with pitvastatin but no such effect shown with simvastatin [171, 172]. Both medications failed to show a significant effect on improving histology in NASH patients. Because randomized clinical trials haven't been performed to examine the effect of statins in NASH patients, they have not been recommended by the AASLD as a treatment for NASH. However, statins are recommended to address the dyslipidemia that is estimated to occur in from 20–80% of NAFLD/NASH patients [121, 173–175]. Further studies may advance our understanding of the possible value of statins in the slowing the progression of NASH to end stage liver disease.

Table 9.2 Statins in NAFLD

Study	*N*	Design	Treatment	Duration	Aminotransferases	Imaging	Histology
Hatzitolios et al. 2004 [165]	28	Prospective, non-randomized, uncontrolled	Atorvastatin 20 mg/ daily	6 months	Improvement	Improvement (US)	NA
Athyros et al. 2006 [164]	*63*	*Prospective, open-label, randomized*	*Atorvastatin 20 mg/ day*	*13.5 months*	*Improvement*	*Improvement (US)*	*NA*
Antonopoulos et al. 2006 [163]	23	Prospective, uncontrolled	Rosuvastatin 10 mg/ day	8 months	Improvement	NA	NA
Gomez-Dominguez et al. 2006 [166]	*22*	*Prospective, uncontrolled*	*Atorvastatin 10–80 mg daily*	*6 or 12 months*	*Improvement*	*No sig change (US)*	*NA*
Ekstedt et al. 2007 [161]	17	Retrospective, cohort, non-randomized	Simvastatin, Pravastatin, or Atorvastatin	6 years	Improvement	NA	↓steatosis ≈ inflammation ≈ fibrosis
Riley et al. 2008 [168]	*26*	*Retrospective*	*Pravastatin*	*11 months*	*Improvement*	*NA*	*NA*
Abel et al. 2009 [167]	26	Retrospective	Simvastatin 20 mg/ daily	6 months	Improvement	NA	NA
Athyros et al. 2010 [169]	*227*	*Post-hoc prospective, controlled, randomized*	*Atorvastatin 24 mg/ day*	*36 months*	*Improvement*	*NA*	*NA*
Total	*432*						

Table 9.3 Statins In NASH

Study	Population (*n*=)	Design	Treatment	Duration	Aminotransferases	Imaging	Histology
Harlander et al. 2001 [180]	7	Prospective, uncontrolled	Atorvastatin variable dosing (10–30 mg)	12 months	No sig change	NA	↓steatosis ↓inflammation ≈fibrosis
Kiyici et al. 2003 [170]	*27*	*Prospective, nonrandomized, uncontrolled*	*Atorvastatin 10 mg/day*	*6 months*	*Improvement*	*Improvement (CT)*	*No follow Up biopsy*
Rallidis et al. 2004 [176]	5	Prospective, uncontrolled	Pravastatin 20 mg/day	6 months	Improvement	NA	↓steatosis ↓inflammation ≈fibrosis
Georgescu et al. 2007 [177]	*10*	*Prospective, nonrandomized, uncontrolled*	*Atorvastatin 20 mg/day*	*7.5 months*	*Improvement*	*NA*	*↓steatosis* *↓inflammation* *≈fibrosis*
Hyogo et al. 2008 [172]	31	Prospective, open label, uncontrolled	Atorvastatin 10 mg/day	24 months	Improvement	Improvement (CT)	↓steatosis ↓inflammation ≈fibrosis
Nelson et al. 2009 [171]	*10*	*Prospective, randomized, double-blind, controlled*	*Simvastatin 40 mg/day*	*12 months*	*No sig change*	*NA*	*≈steatosis* *≈inflammation* *≈fibrosis*
Kimura et al. 2010 [178]	43	Prospective, open label, uncontrolled	Atorvastatin 10 mg/day	12 months	Improvement	Improvement (CT)	↓steatosis ↓inflammation ≈fibrosis
Hyogo et al. 2011 [179]	*20*	*Prospective, uncontrolled*	*Pitavastatin 2 mg/day*	*12 months*	*Improvement*	*NA*	*≈steatosis* *≈inflammation* *≈fibrosis*
Total	*153*						

Summary

NAFLD is the most common liver disease in western countries and is very prevalent today. It is thought to be benign unless it progresses to NASH. NASH can lead to cirrhosis and liver morbidity and mortality. Metabolic syndrome and, in particular, type 2 diabetes are thought to be risk factors for developing NASH. Thus, special attention should be paid to NAFLD patients with diabetes. Liver biopsy should be considered for staging. New imaging techniques have evolved to quantify liver fat and to assess fibrosis, including MRI-PDFF and MR elastography. There is currently no FDA-approved treatment for NASH but vitamin E and pioglitazone have been shown to be helpful; the long-term effects for these are unknown. Statins have not been shown to be harmful in NAFLD and NASH patients and may be beneficial. Larger studies and randomized trials are needed to explore the effect of statins on NAFLD/NASH patients, especially in those with dyslipidemia.

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Chapter 10
The Role of Statins in Heart Failure

Allen Kuo and Michael Fong

Introduction

Statins have been a primary tool in the physician's armamentarium in treating hyperlipidemia, coronary artery disease and other atherosclerotic processes. They have been shown to be effective in reducing cardiovascular events and death in patients with coronary artery disease [1, 2]. Furthermore, they have also been shown to be effective in the setting of primary prevention for the development of coronary artery disease [3, 4]. In treating one of the primary disease processes responsible for ischemic heart disease, a secondary benefit has been a reduction in the incidence of heart failure [1, 5].

However, the benefit of statins in heart failure not linked to ischemic heart disease remains controversial. The data for their use in this setting remains less robust as heart failure has been an exclusion criterion in most large scale cardiovascular statin studies. Additionally, until recently, the data has been limited by the retrospective and post-hoc nature of the analyses, the variability in statin dosing and type, and small sample size in prospective studies. While there have been some theories and data to show that statins may be harmful, the majority of the retrospective and post-hoc analyses have suggested that statins are beneficial through a variety of effects. Nevertheless, the latest series of large randomized controlled trials have failed to show a benefit in mortality [6–8].

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As a result, the guidelines by the ACC/AHA have given statins a Level III (no benefit) recommendation (Level of Evidence A) when "statins are used as an adjunctive therapy when prescribed solely for the diagnosis of Heart failure *in the absence of other indications for their use*" [9]. In this chapter, we will review the literature and data regarding the possible mechanisms of effect, outcomes data, and future directions for research and clinical practice.

Mechanisms for Harm

Low cholesterol levels have been shown to be an important poor prognostic indicator in advanced heart failure [10]. These levels are typically associated with markers such as low albumin, worsening liver function and lower body mass index; signs of poor nutrition and/or cardiac cachexia. Conversely, it has been shown that total cholesterol greater than 200 mg/dL may actually be a predictor of better prognosis in heart failure patients, and that low cholesterol is associated with increased mortality independent of other heart failure risk factors [11]. Because of this data, it has been postulated that cholesterol may have a beneficial role in congestive heart failure (CHF) and that attempting treatment with statins may have a harmful effect.

One of the earliest theories of how cholesterol lowering may cause harm is the endotoxin-lipoprotein hypothesis by Rauchhaus et al. [12]. Central to the theory is that circulating bacterial lipopolysaccharides, known as endotoxins, are important stimuli of pro-inflammatory cytokine production. This pro-inflammatory cascade has been shown to be a poor prognostic indicator, and negatively impact cardiac function by increasing myocardial fibrosis, apoptosis, endothelial atherosclerosis, and promoting further cardiac cachexia [13]. Inflammation has also been shown to change the composition of LDL, which may increase atherogenicity and cause a lower HDL [14]. Niebauer and colleagues demonstrated that patients with heart failure have elevated endotoxin and cytokine levels in edematous states [15]. Because the lipoprotein portions of cholesterol can bind and neutralize endotoxins [16], it was postulated that statins may worsen heart failure by reducing the endotoxin-neutralizing ability of lipoproteins.

An alternative leading hypothesis involves ubiquinone or Coenzyme Q10(CoQ10). CoQ10 is an essential cofactor for mitochondria in the production of adenosine triphosphate. Like LDL, it is a product of the melavonic acid pathway, which statins target by inhibiting the HMG CoA reductase enzyme. As such, it has been shown that statins can reduce both serum LDL and CoQ10 levels by up to 50% [17]. By decreasing the energy supply to an already compromised system, it has been suggested that statins may cause harm by potentiating further dysfunction in myopathic cardiac muscle. Furthermore, low CoQ10 levels have been shown to be a predictor of cardiac mortality [18], and meta-analyses have shown that supplementation of CoQ10 has improved heart failure outcomes [19].

Mechanisms for Benefit

Separate from their effects on LDL, and despite the above hypotheses, statins have been shown to have many pleiotropic effects that may benefit patients with heart failure.

Inflammation

Statin therapy has been shown to be anti-inflammatory in patients with normal ejection fraction in the setting of acute coronary syndrome [20]. In addition, patients with an elevated white blood cell count at the time of myocardial infarction have been shown to have better outcomes when they receive statin therapy [21]. The anti-inflammatory effect of statins against macrophages is thought to be one of the key elements in cholesterol plaque stabilization and the reduction of coronary events [22]. As inflammation in CHF has been shown to be a poor prognostic indicator [13], it is postulated that statins could benefit heart failure patients through their anti-inflammatory effect, in contrast to the endotoxin lipoprotein hypothesis.

Sola et al. was able to show in a prospective, non-randomized study of 446 patients with heart failure and a left ventricular ejection fraction (LVEF) $\leq 35\%$, that treatment with statins resulted in an overall reduction of multiple inflammatory cytokine markers including C reactive protein (CRP), interleukin-6 and tumor necrosis factor-alpha receptor II. Statin use was also associated with a highly significant 18% absolute risk reduction in all-cause mortality ($p < 0.005$), as well as a significant reduction in hospitalizations for heart failure and nonfatal myocardial infarction over two years in this population [23].

Endothelial Function

Endothelial dysfunction has been demonstrated on both a micro and macrosvascular level in patients with idiopathic dilated cardiomyopathy [24]. In fact, research has shown that endothelial dysfunction is present at the very early onset of disease. This dysfunction results in progressive myocardial dilatation, decreased contractile strength and worsening heart failure [25].

Statins can improve endothelial dysfunction independent of their cholesterol effects, primarily through improvement in nitric oxide availability [26]. Additionally, statins have shown improvement in endothelial function tests likely due to a corresponding decrease in inflammatory markers [27].

Autonomic Function

It has been suggested that the pleotropic effect of statins on the sympathetic nervous system and renin-angiotensin-aldosterone system are linked to improvements

in heart failure severity and mortality. Statins have been shown in the animal model to normalize autonomic neural control by decreasing renal sympathetic nerve activity, normalizing baroreflex function and decreasing serum norepinephrine [28]. Additionally, statins have been demonstrated in humans to have a beneficial effect on frequency domain heart rate variability measures, suggesting an impact on the sympathetic system, while having no effect on time domain heart rate variability indices, suggesting a lesser to no effect on parasympathetic tone [29].

These findings might help to explain the favorable interaction between statins and beta blockers seen in the CIBIS II trial, in which post hoc analysis suggested that patients receiving both statins and bisoprolol had decreased cardiovascular death and sudden death compared to those on bisoprolol, statin, or placebo alone [30]. However, this theory has been called into question by a recent small double-blind, placebo-controlled trial which did not show changes in sympathetic nervous system activity by microneurography with statins in nonischemic cardiomyopathy patients with an LVEF $\leq 35\%$ [31].

Left Ventricular Remodeling

Left ventricular (LV) function has long been demonstrated to correlate with mortality in patients with heart failure, and it has been suggested that statins may have beneficial effects on LV remodeling and LV function through their pleotropic effects.

LV mass and dimensions have been shown to improve favorably after administration of statins [32]. Furthermore, studies have shown that statins can increase LV function and improve New York Heart Association (NYHA) functional class compared with those taking placebo [33]. Statins have also been shown to have a positive effect on remodeling in patients after myocardial infarction by preserving ejection fraction [34].

Arrhythmia Burden and Sudden Cardiac Death

Sudden cardiac death is one of the main causes of mortality in heart failure patients. Statins have been associated with improved outcomes in relation to ventricular arrhythmic events. This effect is thought to be secondary to the medicine's effect on ischemic processes, but is also postulated to be mediated by an antiarrhythmic effect though membrane-stabilizing and anti-inflammatory properties.

Post hoc analysis of many of the large implanted cardioverter-defibrillator (ICD) and heart failure trials have suggested that statins may have a role in decreasing ventricular arrhythmias and arrhythmic death. In the AVID trial, patients who received statins had lower risk of ventricular arrhythmias [35]. MADIT-II also demonstrated overall less therapies provided by ICDs for ventricular tachycardia/ventricular fibrillation in those that received statins [36]. The SCD-HeFT trial showed a significant mortality benefit in both ischemic and nonischemic cardiomyopathy

with statins [37]. Furthermore, analysis of MADIT-CRT showed that there was a 77% less risk of death and a 46% reduction in the risk of appropriate ICD shocks in those on statin therapy [38].

Randomized Controlled Trial Data

To date, there have been two large scale randomized controlled trials and one moderate sized trial which have driven much of the recommendations in the current guidelines. These are the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA) and Groppo Italiano per lo Studio della Sopravvivenza nell'Insufficienza Cardiaca (GISSI-HF) trials, and the Pitavastatin Heart Failure (PEARL) trial. Prior to this, there were many small prospective studies, retrospective analyses and post-hoc analyses that suggested a benefit in heart failure, but which were cautiously regarded due to the nature of their analyses.

CORONA

The CORONA trial was the first study to evaluate statins directly in the systolic heart failure population [7].

From September 15, 2003 to April 21, 2005 a total of 5459 patients were entered into the study over 371 sites in 19 European countries, Russia and South Africa. Two thousand five hundred and fourteen people were ultimately assigned to receive 10 mg of rosuvastatin and 2497 were assigned to receive placebo. The mean follow up time was 32.8 months with approximately 6290 patient-years accumulated in the rosuvastatin group and 6219 in the placebo group.

Patients included were those that were greater than 60 years of age, and those that had chronic NYHA functional class II-IV symptoms of ischemic cause with an LVEF no more than 40% (and no more than 30% in those NYHA class II). Patients had to be on stable, optimal treatment for at least two weeks prior to randomization.

In the trial, the LDL dropped approximately 43.8%, HDL increased by 4.2% and triglycerides decreased 22.5% in the rosuvastatin group ($p < 0.001$ in all three), and did not change significantly in the placebo group. However, the primary outcome which was a composite of death, myocardial infarction, and non-fatal stroke was not significantly different between the two groups (Hazard Ratio 0.92, confidence interval (CI) 0.83–1.02, $p = 0.12$). For secondary outcomes, there was a significant decrease in number of hospitalizations, 4074 in the placebo group versus 3695 in the rosuvastatin group ($p = 0.007$).

Post-hoc analyses suggests that those with lower brain natriuretic peptide (BNP) and higher CRP levels may benefit from rosuvastatin therapy, as reflected by fewer atherothrombotic events and sudden death with rosuvastatin [39, 40]. Additionally, contrary to previous hypotheses, CoQ10 was not an independent prognostic variable

in heart failure, and even in patients with low baseline CoQ10, use of rosuvastatin did not portend a significantly worse outcome [41].

Lastly, an economic evaluation of rosuvastatin treatment suggested that there was a reduction in associated costs for major cardiovascular events, however this did not offset the cost for drug and overall there were significantly higher total costs for the rosuvastatin group (1769 British pounds in placebo group vs. 2072 British pounds in the rosuvastatin group; difference of 303 British pounds, CI 138–468, $p < 0.001$) [42].

GISSI-HF

The GISSI-HF trial was also a randomized, double blind, placebo-controlled multicenter study involving 326 cardiology and 31 internal medicine centers in Italy [8]. Its rationale was similar to the CORONA trial, and the patients were enrolled between August 6, 2002 and February 2, 2005.

This trial enrolled patients 18 years or older with chronic heart failure, NYHA class II-IV, irrespective of cause and LVEF, and randomly assigned 2285 patients to receive rosuvastatin 10 mg daily, and 2289 patients to receive placebo. Patients were followed for a median of 3.9 years.

Primary endpoints were time to death, and time to death or admission to hospital for cardiovascular events. Both of the primary endpoints were found to be non-significant between the groups (adjusted Hazard Ratio 1.00 [95.5% CI 0.898–1.122] $p = 0.943$). Also, contrary to the CORONA trial, there was no difference in hospitalization (adjusted Hazard Ratio 1.01 [99% CI 0.908–1.112] $p = 0.903$).

PEARL

The PEARL study was performed in Japan due to the results of the GISSI-HF and CORONA trials [43]. The investigators postulated that using a lipophilic statin in patients with heart failure might demonstrate a benefit over the previously studied hydrophilic drug, rosuvastatin.

From June 2006 to June 2008, 577 NYHA functional class II-III patients with LVEF $\leq$ 45% and mild hypercholesterolemia (total cholesterol $\leq$250 mg/dL or LDL $\leq$ 170 mg/dL) were recruited in over 160 centers in Japan. Patients were excluded for receiving treatment with a statin prior to randomization, history of acute myocardial infarction within 3 months prior to randomization, percutaneous intervention, coronary artery bypass, cardiac resynchronization therapy pacemaker or defibrillator implanted within 3 months, malignancy, serious renal or hepatic dysfunction, collagen disease, pregnancy and lack of informed consent.

A total of 577 patients were randomized wherein 288 were assigned to the pitavastatin group and 289 were assigned to the control group. The two groups were well matched at clinical baseline and the primary outcome measure was cardiac

death and hospitalization for worsening heart failure. The mean duration of follow up was 35.5 months.

In this trial, there was no significant difference between the two groups for the primary outcome. Additionally, none of the secondary outcomes including all-cause death, cardiac death, hospitalization due to worsening heart failure, myocardial infarction or unstable angina, stroke, percutaneous coronary intervention or surgical therapy for heart failure showed any significant differences.

However, when the results were broken down by subgroups based on LVEF, they found that those taking pitavastatin with a LVEF >30% had a significant mortality benefit (adjusted Hazard Ratio 0.525, CI 0.308–0.86, $p=0.018$).

Meta-analysis Data

Recent meta-analyses have raised the question of whether differences in outcome may be related to the lipophilic or hydrophilic nature of the statins used. Uptake studies in rats have shown that concentrations of hydrophilic statins, such as rosuvastatin and pravastatin, are extremely low in the heart whereas simvastatin, a lipophilic statin, was found in much higher concentrations in the heart cells [44]. As such, meta-analysis has suggested that atorvastatin, a lipophilic statin, may have stronger advantages in mortality, hospitalization, and left ventricular function compared to rosuvastatin [45]. The results must be interpreted with caution however, given the discrepancy in the number of patients analyzed (471 atorvastatin patients vs. 9670 rosuvastatin patients in all-cause mortality categories). The smaller number of patients in the atorvastatin studies could have resulted in a magnified effect that may otherwise not have been seen in a larger cohort of patients.

Nevertheless, the most recent PEARL study suggests that the lipophilic theory may not pan out, although further studies with atorvastatin should be considered as the findings may not be a class effect.

Non-Statin Cholesterol Targeted Therapies

Interestingly, Omega-3 polyunsaturated fatty acid (PUFA) supplementation, another long examined treatment for hyperlipidemia, has been associated with significant improvement in heart failure outcomes.

Some of the earliest data showing this compound's promise originated from the GISSI Prevenzione trial [46]. In this study, there was a 20% reduction of fatal events and a 30% reduction in cardiovascular death in patients surviving a recent myocardial infarction that were randomized to treatment with 1 g of PUFA supplementation daily. A post-hoc analysis of the trial showed that PUFA supplementation reduced mortality similarly in patients with and without systolic dysfunction, but that there was a greater reduction in sudden death in those with systolic dysfunction

[47]. This led to the GISSI-HF trial, a randomized, double-blind, placebo-controlled trial which set out to test the effect of PUFA supplementation in 6975 NYHA class II–IV patients with chronic heart failure of any cause [48]. Patients were followed for a median of 3.9 years, and the primary end points were time to death, and time to death or hospital admission for cardiovascular reasons. The trial demonstrated a small but significant benefit of PUFA supplementation, with one life saved for every 56 patients treated for 3.9 years, and the avoidance of one death or hospitalization for a cardiovascular cause for every 44 patients treated.

As such, the 2013 ACC/AHA guidelines have given a Class IIa (Level of Evidence B) recommendation in that it is reasonable to use PUFA as adjunctive therapy in patients with NYHA class II-IV symptoms, unless contraindicated, to reduce mortality and cardiovascular hospitalizations [9]. One important caution is that the amount of eicosapentaenoic acid and docosahexaenoic acid in commercially available preparations of Omega-3 is variable, and no set standard has been endorsed or currently exists.

Conclusions

The literature on statins in heart failure to date has included many observational, small prospective studies, and post-hoc analyses that have suggested that statins may improve outcomes and quality of life in patients with heart failure of all etiologies. However, the largest, best designed, double-blind, placebo-controlled trials to date have shown that there is no convincing evidence that statins are beneficial for mortality in heart failure despite the literature preceding it. Additionally, in the trials that may have shown benefit, cost effectiveness was not demonstrated. Meta-analyses have identified atorvastatin as a theoretical target drug to be researched further in the future, and it may have benefit in part due to its lipophilic nature. Lastly, certain subgroups of patients such as those with lower BNP, higher CRP, and LVEF > 30 % may have an increased benefit from therapy with statins.

The guideline recommendations for statin therapy remains appropriate given the current evidence, and statins should not be initiated for heart failure alone. However, if there is another indication for statin use, clinicians can be reassured that statins are safe, well tolerated and do not increase mortality in the heart failure population.

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Chapter 11
Statins in Chronic Kidney Disease

Sahar H. Koubar

List of Abbreviations

4D	Die Deutsche Diabetes Dialyse Studie
ALERT	Assessment of Lescol in Renal Transplantation
AURORA	A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events
CKD	Chronic Kidney Disease
CPK	Creatine Phosphokinase
CUA	Calcific Uremic Arteriopathy
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
ESRD	End Stage Renal Disease
GFR	Glomerular Filtration Rate
IDL	Intermediate Density Lipoprotein
KDIGO	Kidney Disease Improving Global Outcomes
LDL	Low Density Lipoprotein
Lp(a)	Lipoprotein a
LVH	Left Ventricular Hypertrophy
MI	Myocardial Infarction
PTH	Parathyroid Hormone
RR	Risk Ratio
RCT	Randomized Controlled Trial
RRT	Renal Replacement Therapy
SHARP	Study of Heart and Renal Protection
TG	Triglycerides
UK-Harp-II	Second United Kingdom Heart and Renal Protection
VLDL	Very Low Density Lipoprotein

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Introduction

Cardiovascular disease is the leading cause of death in the chronic kidney disease population which is by itself a heterogeneous population when it comes to cardiovascular risk. According to KDIGO guidelines [1], chronic kidney disease (CKD) is defined as abnormalities of kidney structure or function, present for >3months, with implications for health. It is classified into five stages based on glomerular Filtration rate (GFR) (Table 11.1).

CKD whether manifested by decreased GFR, proteinuria or both is an independent risk factor for CVD. Reduced GFR is associated with increased prevalence of left ventricular hypertrophy, coronary artery disease, diabetes mellitus, heart failure, more severe hypertension and dyslipidemia [2–5].It is an independent risk factor for CVD outcomes and all cause mortality in the high risk population (defined as those already having CVD, other vascular disease, or surrogates of CVD such as LVH or DM) [6, 7]; this risk is evident even with mild reduction in kidney function [8–10].

ESRD patients face a great risk of premature death. It is estimated that there is a 19–25% increased risk of death/year while on dialysis [11].The renal transplant population is a particular population with a unique set of risk factors. Mortality rates in kidney transplant recipients are higher than the general population but still less than age-matched patients on dialysis.

Most large studies that have assessed statins included people with mild stages of CKD (most had GFR >50 ml/min/1.73 m^2 or Creatinine <1.4 mg/dL) (Table 11.2). All of these studies have shown that statins reduce the risk of MI, stroke, cardiovascular mortality and all cause-mortality. It is not until 2005 when studies were conducted in the advanced stages of CKD and in the ESRD population (Table 11.3).

Epidemiology and Risk Factors of CVD in the CKD population

The CKD population shares the conventional risk factors for atherosclerotic disease including older age, hypertension, LVH, dyslipidemia, smoking, sedentary life style and Diabetes Mellitus. It also has its unique set of risk factors brought on by kidney failure itself; these are divided into uremia specific risk factors including anemia,

Table 11.1 Stages of chronic kidney disease

Stage 1	Normal or increased GFR with signs of kidney damage*		≥90 ml/min/1.73 m^2
Stage 2	Mildly decreased GFR		89–60 ml/min/1.73 m^2
Stage 3	3a	Mildly to moderately decreased GFR	59–45 ml/min/1.73 m^2
	3b	Moderately to severely decreased GFR	44–30 ml/min/1.73 m^2
Stage 4	Severely decreased GFR		29–15 ml/min/1.73 m^2
Stage 5	Kidney failure		<15 ml/min/1.73 m^2

*Kidney damage includes abnormalities in urine sediment (hematuria, proteinuria, pyuria, casts...), renal pathology or imaging studies.

Table 11.2 RCTs Evaluating statins in the general population with CKD subgroups

Study	Year	Region	Statin	CKD (%)	Mean baseline eGFR (ml/min/m^2) or SCr (mg/dl)	Primary end point
CARE, LIPID, WOSCOPS	2005	International	Pravastatin	23.7	eGFR 57	CV mortality, CV events, or need for revascularization
LIPS	2005	International	Lovastatin	19.9	SCr 1.3	No data
PREVENT IT	2005	Netherlands	Pravastatin	10	SCr 1	CV mortality or morbidity
4S	2007	Scandinavia	Simvastatin	60.2	eGFR 65.2	All-cause mortality
ALLHAT	2008	International	Pravastatin	15	eGFR 50.8	No data
TNT	2008	International	Atorvastatin	32.2	I: eGFR 53 C:eGFR 52.8	Major CV event
ALLIANCE	2009	United States	Atorvastatin	23.7	eGFR 51.3	MACE
CARDS	2009	UK and Ireland	Atorvastatin	34.2	I: eGFR 53.5 C:eGFR 54.1	Cardiac events, revascularization, or stroke
MEGA	2009	Japan	Pravastatin	41.4	I: eGFR 52.6 C:eGFR 52.5	MACE
AFCAPS TexCAPS	2010	United States	Lovastatin	4.6	SCr 1.4	First Major CV event
JUPITER	2010	International	Rosuvastatin	18.37	eGFR 56	CV Mortality, CV events, revascularization, and stroke

4S Scandinavian Simvastatin Survival Study; *AFCAPS/TexCAPS* Air Force Coronary Atherosclerosis Prevention study/Texas Coronary Atherosclerosis Prevention Study; *ALLHAT* Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial; *ALLIANCE* Aggressive Lipid-Lowering to Alleviate New Cardiovascular Endpoints; *C* comparator group; *CARDS* Collaborative Atorvastatin Diabetes Study; *CARE* Cholesterol and Recurrent Events; *CHF* congestive heart failure; *CKD* chronic kidney disease; *CrCl* creatinine clearance; *CV* cardiovascular; *CVD* cardiovascular disease; *e GFR* estimated glomerular filtration rate; *ESRD* end stage renal disease; *I* intervention group; *JUPITER* Justification for the Use of Statins in Primary Prevention: An intervention Trial Evaluating Rosuvastatin; *LDL* low-density lipoprotein; *LIPID* Long-term Intervention with Pravastatin in Ischemic Disease; *LIPS* Lescol Intervention Prevention Study; *MACE* major adverse cardiac event; *MEGA* Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese; *PREVEND IT* Prevention of Renal and Vascular End-Stage Disease Intervention Trial; *SCr* serum creatinine; *TNT* Treating to New Targets; *UK* United Kingdom; *US* United States; *WOSCOPS* West of Scotland Coronary Prevention Study

Table 11.3 Major RCTs of lipid-lowering therapy in patients with CKD

Study (Reference)	Year	Region	Population	Intervention	Size	Mean age (Yr)
ALERT [39]	2003	Europe and Canada	Kidney transplant recipients	Fluvastatin	I: 1050 C:1052	50
4D [40]	2005	Germany	HD recipients	Atorvastatin	I:619 C:636	66
UK-HARP-II [41]	2006	UK	Stage 3–5 CKD/HD and PD recipients	Simvastatin plus Ezetimibe	I:102 C:101	66
AURORA [42]	2009	Europe, Canada, Mexico, Brazil, Australia and South Korea	HD recipients	Rosuvastatin	I:1389 C:1384	64
SHARP [43]	2011	Europe, North America, Australia, New Zealand, China, Thailand and Malaysia	Stage 3–5 CKD/HD and PD recipients	Simvastatin plus Ezetimibe	I:4650 C:4620	62

ALERT Assessment of Lescol in Renal Transplantation; *4D* Die Deutsche Diabetes Dialyse Studie; *UK-Harp-II* Second United Kingdom Heart and Renal Protection; *AURORA* A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events; *SHARP* Study of Heart and Renal Protection; *I* Intervention group; *C* Comparator group

phosphate retention, hyperparathyroidism, vascular calcification, hyperhomocysteinemia and volume overload and novel risk factors including: carbamylation of proteins, endothelial dysfunction, sympathetic activity, inflammation, oxidative stress and wasting.

Disordered Lipid Metabolism in Patients with Renal Failure

Based on animal studies, among the different lipoproteins, LDL is the one responsible for the pathogenesis of atherosclerotic plaques. LDL size seems to be an important factor with small dense LDL particles the most incriminated in the process.

Lipid Profile of the CKD Population

Patients with CKD tend to have increased Triglycerides (VLDL and IDL), apolipoprotein B and oxidized LDL, abnormalities in LDL particle size and decreased HDL

and apolipoprotein A1. People with CKD seem to have a more atherogenic lipid profile even in the absence of dyslipidemia.

People receiving renal replacement therapy in the form of hemodialysis are known to have high triglyceride, low HDL, and normal total Cholesterol and LDL levels [11]. This "normal" LDL level may not accurately represent the relative increase in the more atherogenic oxidized form. People receiving peritoneal dialysis seem to have a more dyslipidemic profile with increased LDL, TG and Lp(a) and decreased HDL. Lp(a) is clearly associated with coronary heart disease and in one meta-analysis, elevated Lp(a) increased the 10-year risk of a coronary event by 70% [12].

Role of Oxidized LDL

There are increased amounts of oxidized LDL in patients with kidney disease. The heme moieties in patients receiving hemodialysis and peritoneal dialysis increase the susceptibility to LDL oxidation [13]. Oxidized LDL enhances the expression of pro-inflammatory markers which may by themselves induce glomerular injury either to the vascular cells forming the capillary wall or to the mesangial cells forming the matrix.

Oxidized LDL enhances accumulation of LDL particles inside macrophages transforming them into foam cells. It was also shown to enhance macrophages motility and chemotactic activity. Foam cells can cause vascular injury through three mechanisms: direct toxic effect, inducing apoptosis and altering vascular homeostasis through interfering with Nitric Oxide pathway [13].

Oxidized LDL particles are strongly immunogenic. Antibody titers against oxidized LDL correlate with the severity of atherosclerosis and the rate of progression of the atherosclerotic plaques.

Disordered Mineral Metabolism in Patients with CKD Leading to Accelerated Atherosclerosis

Disordered mineral metabolism is a unique complication in patients with CKD. This complication accelerates with the progression of CKD and is especially manifested in end stage renal disease patients requiring renal replacement therapy.

As the GFR decreases, the ability of the kidney to excrete phosphorus decreases as well. This results in hyperphosphatemia which by itself exerts a positive feedback on the parathyroid gland to increase secretion of PTH which has a phosphaturic effect. Indeed, hyperparathyroidism is one of the earliest biomarkers of disturbed bone mineral metabolism in patients with CKD. It appears as early as stage 3 CKD.

The earlier rise in PTH is protective and aims to keep phosphorus within the normal range. Though PTH increases as early as stage 3 CKD, significant

hyperphosphatemia is not observed until stage 4 CKD. Hyperphosphatemia stimulates diffuse hyperplasia of the parathyroid gland (Secondary hyperparathyroidism). With worsening GFR and worsening hyperphosphatemia, the diffuse hyperplasia of the parathyroid gland transforms into monoclonal nodular hyperplasia which is responsible for autonomous unregulated increased PTH secretion (tertiary hyperparathyroidism).

As the renal failure continues to progress, the kidney loses its ability to activate 25-hydroxy vitamin D into its active form 1-25 di-hydroxy vitamin D. This usually becomes manifested at stage 4-5 CKD. Hyperparathyroidism in earlier stages of CKD helps to maintain calcium in the normal range as 1-25 di-hydroxy vitamin D level starts to decline.

Disordered mineral metabolism results in accelerated vascular calcification. This can be intimal and is usually seen in atherosclerosis or medial which is usually seen with diabetes mellitus and renal failure. These can only be differentiated based on biopsy.

Vascular calcification is an active process similar to bone resorption. Mesenchymal cells within the vessels acquire an osteoblastic phenotype and lay down hydroxy apatite matrix (similar to bone matrix) causing vascular calcifications. There is evidence that Phosphorus stimulates the change of mesenchymal cells into osteoblasts. This effect is concentration dependent and it has been shown in vitro studies with phosphorus concentration $\geq$6.2 mg/dl [14, 15]. In epidemiological studies, hyperphosphatemia even mild (4.5–5 mg/dl) has been associated with increased risk for non-fatal cardiovascular events, cardiovascular mortality and all cause mortality [16, 17]. The use of the phosphate binder sevelamer (Renagel©) has been shown to attenuate the progression of vascular calcification [18, 19]. It seems that calcium plays a synergistic effect inducing mineralization [15] while PTH is actually protective and inhibits vascular calcification. In ESRD population, vascular calcification leads to increased vascular stiffness and increased peripheral vascular resistance which subsequently leads to increased left ventricular mass index [20, 21].

Studies have also shown that ESRD population may have decreased levels of inhibitors of vascular calcifications namely Matrix G1a protein and Fetuin A [22, 23]. The data about vitamin D and vascular calcification is quite limited.

Disordered mineral metabolism leads to a unique complication known as calciphylaxis or calcific uremic arteriopathy (CUA). It is an ischemic vasculopathy that occurs primarily in the CKD and ESRD population. It mainly affects the skin leading to severe painful necrosis. The pathogenesis is not quite clear but has been attributed to high PTH levels, treatment with vitamin D analogues and calcium based phosphate binders, insufficient activation of inhibitors of calcification and hypercoagulable states [24].

Both coronary artery calcification and calcific uremic arteriopathy (CUA) are prototypes of arterial calcifications that are associated with disordered phosphate metabolism.

Proteinuria is a Risk Factor for Atherosclerosis in CKD

Microalbuminuria is an independent risk factor for CKD in diabetics [25, 26] and in non-diabetics [27–29]. Diabetics with proteinuria (>1 g/day) have increased coronary artery calcification scores as compared to age matched diabetics without proteinuria [30, 31]. People with nephrotic range proteinuria (>3.5 g/day) are at a particular risk for accelerated atherosclerosis [32]. This has been demonstrated in autopsies of children and young adults [33].

There are several explanations as to why albuminuria is a risk factor for atherosclerosis in CKD. It might denote a more damaged endothelium. It is sometimes preceded by nocturnal non-dipping pattern in blood pressure and it is associated with more inflammatory and hypercoagulable states [34, 35].

CVD in the Transplant Population

CVD is responsible for 35–50% of all-cause mortality in kidney transplant recipients [36, 37]. The transplant population shares the traditional risk factors for CVD and the non-traditional risk factors associated with low GFR. It also has its unique risk factors that are attributed to immunosuppression medications and episodes of rejection. Medications used for maintenance Immunosuppression are known to cause post-transplant DM (tacrolimus, cyclosporine, sirolimus, prednisone) and post -transplant dyslipidemia(sirolimus, cyclosporine, prednisone) [38].

Treatment of Dyslipidemia in CKD and ESRD

Lipid management starts with life style modifications including weight loss, smoking cessation and exercise. Few randomized controlled trials have evaluated the use of statins in CKD and ESRD patients (Table 11.3).

The SHARP Trial (Study of Heart and Renal Protection) was an international randomized double blinded trial conducted in 2011 and compared simvastatin 20 mg plus ezitimibe 10 mg daily versus a matching placebo [43]. It included CKD patients stage 3–5 and ESRD receiving renal replacement therapy. Two thirds of the study group was not receiving renal replacement therapy. One third was receiving either hemodialysis or peritoneal dialysis (Table 11.4). The mean GFR in both the treatment and the placebo groups was 26.6 ml/min/m^2 (Table 11.5).

The SHARP trial showed a 17% reduction in major atherosclerotic events in the treatment study groups (95% CI 16–26%; p value = 0.0021). The major reduction in LDL with Simvastatin and ezetimibe occurred in the 1st year.

Table 11.4 Subgroups of the CKD population in the SHARP study

	Study drug	Placebo
On dialysis	1533 (33%)	1490 (32%)
HD	1275 (27%)	1252 (27%)
PD	258 (6%)	238 (5%)
Not on dialysis	3117 (67%)	3130 (68%)

CKD Chronic Kidney Disease; *SHARP* Study of heart and renal protection; *HD* Hemodialysis; *PD* Peritoneal dialysis

Table 11.5 Subgroups of the CKD population not on dialysis in the SHARP study

GFR (ml/min/m^2)	Study drug	Placebo
Mean (SD)	26.6(12.9)	26.6(13.1)
>=60	1%	1%
30–60	37%	35%
15–29	41%	44%
<15	20%	20%

CKD Chronic Kidney Disease; *SHARP* Study of heart and renal protection

Upon subgroup analysis, the beneficial effect on major atherosclerotic events was statistically significant in the CKD group not receiving dialysis (RR 0.78 with a 95% CI of 0.67–0.91) but it was not statistically significant in the dialysis population (RR 0.9 with a 95%CI of 0.75–1.08). The SHARP trial did not show any beneficial effect on cause-specific and overall mortality. It also did not show any difference in cancer incidence, cancer mortality or side effects profile.

The AURORA Trial (A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events) was an international RCT done on patients with ESRD on hemodialysis [42]. The study included patients aged 50–80 years and examined the effect of rosuvastatin 10 mg versus placebo on major cardiovascular events (non-fatal MI, non-fatal stroke, death from cardiovascular events). In spite of showing a statistically significant 42.9% reduction in LDL level at 3 months, the AURORA trial failed to show a beneficial effect on dialysis patients receiving statins (RR 0.96 with a 95% CI of 0.84–1.11; *p* value 0.59).The study excluded people already receiving a statin.

The results of the AURORA study was consistent with the 4D study (Die Deutsche Diabetes Dialyse Studie) which also failed to show a significant reduction in composite primary cardiovascular endpoints in the Hemodialysis population with type 2 diabetes Mellitus in spite of 42% reduction in LDL levels [40].

In 2012, a group lead by Palmer conducted a meta-analysis to assess the benefits and harms of statin therapy in the CKD population including those receiving dialysis [44]. This meta-analysis concluded that there is a clear and significant beneficial effect for statins on the CKD population not receiving dialysis. This beneficial effect was in terms of all cause mortality, CV mortality, major cardiovascular events, fatal

and nonfatal MI and fatal and non-fatal stroke. It did not show any significant beneficial effect on the dialysis population [44]. Of note, subgroup analysis of the group receiving dialysis had an increased number of fatal or non-fatal strokes. The level of evidence was high for the CKD population not receiving dialysis, moderate for the dialysis population and low for Kidney transplant recipients. The lack of beneficial effect on the dialysis population might reflect the different epidemiology of cardiovascular death in that population with arrhythmia, sudden cardiac death and cardiomyopathy being more frequent causes of death than atherosclerotic heart disease. Statins were safe with no increase in side effects between the statin and the placebo groups.

The KDIGO guidelines recommend measuring a lipid profile in each patient with newly diagnosed CKD. Each Patient with stage 1–5 CKD aged above 50 years should be started on a statin regardless of his/her LDL level. Patients aged < 50 years of age should be addressed according to their cardiovascular risk rather than their absolute LDL value since the association between LDL and adverse outcomes is weak in the CKD population. LDL level per se is not enough to identify CKD patients with high risk for CVD. Some studies have shown that LDL level and the risk of MI decrease with reduction in GFR but these results are rather misleading. The lower LDL level with advanced CKD rather reflects the poor nutritional status imposed with worsening kidney function and doesn't correlate with cardiovascular risk. Besides, increased amounts of LDL in the CKD population occur in the oxidized more atherogenic form. The cardiovascular risk in the CKD population is worse with age and MI fatality is higher in the CKD population as compared to an age matched control.

In contrary to the general practice, there is no set target for LDL cholesterol in the CKD population and thus a follow up LDL cholesterol level is not indicated expect in instances where it will change treatment plans. These instances include change in RRT modality, concern about secondary causes of dyslipidemia or change in cardiovascular risk. A lipid profile might be helpful to determine compliance with treatment. Cardiovascular risk should be assessed yearly in the CKD population.

The CKD population is at a higher risk of medication-induced side effects; this is likely related to poly-pharmacy, decreased drug clearance and frequent co-morbidities. Therefore, lower doses are recommended for the CKD population. The recommended doses are those used in the major trials (Table 11.6). These doses are well tolerated and there is no statistically significant difference in adverse events including myalgias, elevation in CPK or increase in liver enzymes [45].

Table 11.6 Statins used in major trials

Trial	Statin	Dose
SHARP	Simvastatin + Ezitimibe	20 + 10 mg/day
4D	Atorvastatin	20 mg/day
AURORA	Rosuvastatin	10 mg/day
ALERT	Fluvastatin	10 mg/day

ALERT Assessment of Lescol in Renal Transplantation; *4D* Die Deutsche Diabetes Dialyse Studie; *AURORA* A Study to Evaluate the Use of Rosuvastatin in Subjects on Regular Hemodialysis: An Assessment of Survival and Cardiovascular Events; *SHARP* Study of Heart and Renal Protection

There was a concern that rosuvastatin in doses >80 mg daily increases the risk of proteinuria. This dose is twice the dose approved by FDA for rosuvastatin and 8 times the dose used in the AURORA trial (10 mg/day). The proposed mechanism is by inhibition of tubular reabsorption of protein [46]. Whether statins decrease the rate of progression of CKD as well as proteinuria is still controversial.

Baseline transaminases levels should be obtained before commencing therapy. Baseline CPK level is not recommended in the CKD population. It should be checked if patients develop symptoms of myopathy. Concomitant fibrates carry higher risk of transaminitis and rhabdomyolysis in the CKD population and should be avoided. Statins are considered category X in Pregnancy and are not safe with breast feeding as well. They should not be given to patients with active liver disease or baseline transaminase level 3 times above normal limits.

Summary of KDIGO Clinical Practice Guidelines 2013 for Lipid Management in CKD[1]

Assessment of Lipid Status in Adults with CKD

1. In adults with newly identified CKD (including those treated with chronic dialysis or kidney transplantation), evaluation with a lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) is recommended. (1C)
2. In adults with CKD (including those treated with chronic dialysis or kidney transplantation), follow up measurement of lipid profile is not required for the majority of patients. (Not Graded)

Pharmacological Cholesterol-Lowering Therapy in Adults

1. In adults aged ≥50 years with an eGFR <60 ml/min/1.73 m^2 but not treated with dialysis or kidney transplantation, treatment with a statin or a statin/ezetimibe combination is recommended. (1A)
2. In adults aged ≥50 years with an eGFR ≥60 ml/min/1.73 m^2, treatment with a statin is recommended. (1B)
3. In adults aged 18–49 years with CKD but not treated with chronic dialysis or Kidney transplantation, statin treatment is recommended in people with one or more of the following: (2A)
   - known coronary artery disease (myocardial infarction or coronary revascularization)
   - diabetes mellitus
   - Prior ischemic stroke
   - estimated 10-year incidence of coronary death or non-fatal myocardial infarction >10%

4. In adults with dialysis-dependent CKD, statins or statin/ezetimibe combination should not be initiated. (2A)
5. In adults already receiving statins or statin/ezetimibe combination at the time of dialysis initiation, these agents can be continued. (2C)
6. In adult kidney transplant recipients, treatment with a statin is suggested. (2B)

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Chapter 12
Non-Statin Treatment of Dyslipidemia

Stanley J. Goldberg

Niacin

Niacin is the oldest of lipid altering medications currently in used for therapy. The first report regarding niacin occurred in 1955 [1]. The report was a letter to the editor regarding the effect of immediate release niacin on total cholesterol lowering in psychiatric patients. This report was followed by several more formal studies demonstrating similar findings [2–4], but most of these studies regarded reduction of total serum cholesterol and triglycerides since HDL-C was not frequently analyzed at that time and LDL-C was not measured or calculated in these studies. Further, niacin was also used by practitioners who theorized that the cutaneous flushing effects might also be present for cerebral circulation. Thus, it was used for some stroke patients although no formal reports or studies were published to confirm or deny effects on cerebral circulation.

Lipid Effects of Niacin Niacin therapy decreases VLDL, triglycerides, LDL-C, lipoprotein (a), LDL particle number, and non-HDL. Niacin also increases HDL-C and changes smaller, denser LDL and HDL particles into larger particles [5]. Epidemiologic studies suggest that these changes in lipids would be beneficial [6].

Mechanism of Niacin Effect Niacin, which is also known as vitamin B3, imparts its lipid alterations by decreasing the mobilization of free fatty acids from adipose tissue, thereby decreasing hepatic synthesis of VLDL-C and subsequent triglyceride levels. Niacin also reduces VLDL-C level by inhibiting the synthesis of apolipoprotein B-100 [7]. Since niacin decreases triglycerides, it also affects the ratio of cholesterol esters and triglycerides in lipoprotein particles. This ratio change is associated with changing smaller, denser HDL and LDL into larger, less dense particles. Niacin has also been shown to increase the metabolic breakdown of VLDL-C through its stimulatory affect on lipoprotein lipase [8, 9]. A metanalysis conducted

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by Bruckert et al. observed that dosages of niacin of 1–3 mg/day caused desirable effects on cardiovascular events and on the evolution of atherosclerosis [7]. Increased HDL-C is mainly secondary to increasing duration of HDL in the circulation circulatory.

Hepatic niacin metabolism includes 2 pathways: conjugation and oxidation-reduction reaction [10]. In the first pathway, niacin is conjugated with glycine to produce nicotinuric acid and other metabolites that create the flushing side effect. Conjugation is a low affinity, high capacity pathway. In the second pathway, niacin goes through a complicated redox reaction to create nicotinamide and pyrimidine metabolites. Niacin creates a flushing side effect because it quickly saturates the second pathway, and uses the high capacity conjugation pathway for metabolism and, as a consequence, produces metabolites that result in flushing.

Available Niacin Preparations Niacin is produced as a white powder which is formed into tablets. These tablets are known as immediate release niacin because of their very rapid metabolism. Ingestion of niacin, in most patients taking larger doses, may cause the cutaneous reaction called flushing. The flushing effect is best described as a sensation of heat or a sensation of mild cutaneous stinging or, occasionally, itching. This is usually confined to the head, arms, and upper torso but may extend throughout the body. Individuals usually develop a tolerance to flushing by increasing the niacin dosage slowly over time. The tolerance to flushing is expressed as flushing effect becoming very minimal or disappearing completely. In order to reduce or eliminate flushing, numerous preparations have been developed to create a slower release of niacin. The mechanism that allows the sustained release is frequently proprietary but may include pockets of niacin in a wax preparation that slowly dissolves, coated beads of niacin with different dissolving properties or other slow dissolution techniques. As a generality, niacin is available in the immediate release form, another form that has a dissolution time of about six hours that is frequently referred to as "extended release" and very long dissolution forms. Examples of the immediate release form are Niacor (Upshire-Smith) or a preparation by Darby pharmaceuticals (now a division of Watson pharmaceuticals) that has a product number of 0536-4078-108 for 500 mg tablets and 0536-4076-01-2 for 100 mg tablets. Examples of the extended release niacin are Niaspan (Abbott Laboratories) or Slo-Niacin (Upshire Smith). These two extended release niacins are not necessarily equivalent. An example of long acting niacin is Enduracin (Endur Products). Many health food stores sell various niacin products as dietary supplements under the label of "sustained release" with no additional information regarding the duration of action. Another preparation is sold as " no flush" niacin but this compound is inositol hexanicotinate which has no effect on lipids, but is often recommended in health food stores and pharmacies because it does not cause a flush. In general, the shorter the release of niacin, the greater the flushing potential and the less hepatic toxic it is. The longer the niacin release, the greater the possibility of hepatic toxicity and the less the flushing.

Niaspan became a prescription medication approximately 13 years ago and has gained favor with many physicians because of its once nightly dosage and presumed decrease in flushing [11]. However, in the author's experience, many patients using

this extended release form have flushing late after taking this medication. The immediate release form is seldom used today.

Randomized, Controlled Trials with Niacin The Coronary Drug Project, begun in 1966 [12] and concluded in 1974, was initiated as a study to evaluate the outcomes of patients treated with immediate release niacin and several other medications in the six arms of the study to determine whether niacin or the other medications had any significant effect in reducing cardiovascular outcomes and/or total mortality. The other medications in this trial included 2 different doses of estrogen, dextrothyroxine, a placebo and clofibrate. Groups for both doses of estrogen and dextro thyroxine were terminated early because of adverse results. The niacin, placebo and clofibrate arms of the study were continued. The Coronary Drug Project was a double blind, randomized, placebo-controlled, male only, secondary prevention study. The outcome was a statistically significant lower incidence of definite nonfatal myocardial infarction for the niacin group compared to placebo group. The study had a duration of 5 years and most participants completed 5 years. Side effects recorded for the niacin group included cutaneous effects such as flushing, itching or occasionally a rash. Other significant side effects were gastrointestinal irritation, and elevations of liver enzymes, uric acid and plasma glucose. The niacin group also had a higher incidence of atrial fibrillation and other cardiac arrhythmias as compared to the placebo.

Although there was a decrease in definite, nonfatal myocardial infarction, there was no demonstrated efficacy of niacin with regard to decreasing total mortality or cause specific mortality. A follow-up study with nearly complete ascertainment of the original niacin and placebo groups at 15 years demonstrated a significant 11 % decrease in mortality for the niacin group compared to placebo group even though the niacin group was no longer treated after conclusion of the original study [13]. The Coronary Drug Project did not include a lipid profile, but rather evaluated total cholesterol and triglycerides. Because total cholesterol was quite elevated, it can be presumed that LDL was also high. In summary, niacin did have a significant outcome regarding nonfatal myocardial infarction but important side effects were noted. No subsequent monotherapy niacin outcome study has been performed. Despite these somewhat salutary results, niacin has not been widely used because of side effects.

Niacin has been demonstrated over the course of subsequent studies to increase HDL-C, and decrease LDL-C, LDL particle number, apoprotein B, triglycerides, and lipoprotein (a) [5]. In this regard, studies more recent than the Coronary Drug Project using a form of extended release niacin have demonstrated positive results in clinical trials for reductions in plaque and cardiovascular events [14, 15]. Side effects that continued to be reported after the Coronary Drug Project included mild increases fasting glucose and in uric acid [3, 4], increased atrial fibrillation, and gastrointestinal effects such as abdominal pain, diarrhea, and decreased appetite [5].

Clinical Trials of the Combination of Niacin and Statins Forms of longer release niacins have been combined with statins in 3 randomized clinical outcome trials and one surrogate outcome study.

The HATS trial was first reported in 2001 [14] as a 3-year, double-blind trial of 160 patients with coronary disease, low HDL-C and modestly elevated LDL-C. Patients were randomly assigned to receive (1) simvastatin + niacin, (2) antioxidants, (3) simvastatin + niacin + antioxidants, or (4) a placebo which contained 50 mg of immediate release niacin to produce flushing for the purpose of masking the trial pharmaceutical preparation to allow a double-blind study. This study also included coronary arteriography. Endpoints were cardiovascular death, myocardial infarction, stroke, or revascularization. Mean dosage of simvastatin was only 13 mg/d and a niacin dosage of 2.4 g/d. Simvastatin was used in variable doses attempting to achieve an LDL < 90 mg/dl. The niacin form for this study was Slo-Niacin (Upshire-Smith) but immediate release niacin (niacor also manufactured by Upshire Smith) was used if HDL-C did not meet pre-designated targets with Slo-Niacin. Antioxidants included 800 IU of vitamin E, 1000 mg of vitamin C, 25 mg of beta-carotene, and 100 μg of selenium. Baseline LDL-C (all expressed as mg) was 127 for the placebo group, 132 for the simvastatin + niacin group and 124 for the simvastatin + niacin + antioxidants group. Baseline HDL-C (all expressed as mg/dl) was 32 for the placebo group 31 for the simvastatin + niacin group and 34 for the simvastatin + niacin + antioxidants group. Niacin compliance varied from 80 to 83% for the two niacin groups. Simvastatin compliance varied from 88 to 92% compliance. Results of this study showed that antioxidants did not affect lipid levels except for a significant 15% decrease in HDL 2. Accordingly, one conclusion of the study was that the antioxidants tested reduced the rise in HDL-C induced by niacin. LDL-C and triglycerides decreased by 42 and 36% respectively with simvastatin + niacin therapy and with the combination of simvastatin + niacin + antioxidants. HDL-C increased 26% in the simvastatin + niacin group but only by 18% when antioxidants were added to simvastatin + niacin. With respect to severity of proximal coronary stenoses, these stenoses increased 3.9% for the placebo group, increased 1.8% for the antioxidant therapy and decreased significantly 0.4% for the simvastatin + niacin therapy group. For the simvastatin + niacin + antioxidant group, proximal stenosis increased significantly by 0.7%. With respect to pre-designated outcome measures, risk significantly decreased by 90% in the simvastatin + niacin group compared to the placebo group. Utilizing Kaplan Meyer curves, the decrease in outcomes was 60% for the simvastatin niacin group. Risk for other groups did not change significantly. It should be noted that most studies using statins alone show a reduction of 30–35% in outcomes. It remains unclear why this study demonstrated a much greater improvement in outcome.

The AIM-HIGH study [5] was a secondary prevention study with 1696 subjects in the placebo + simvastatin group and 1718 subjects in the extended release niacin + simvastatin group. Simvastatin was utilized in dosages of 40–80 mg and ezetimibe 10 mg was added as needed to maintain LDL-C in a range of 40–80 mg/dl. For the niacin + simvastatin group, extended release niacin was used. A matching placebo contained 50 mg of immediate release niacin to produce flushing was used for the simvastatin monotherapy group. The pre-specified primary endpoint was the first event of (1) death from coronary artery disease, (2) nonfatal myocardial infarction, (3) ischemic stroke, (4) hospitalization for acute coronary syndrome, or (5) symptom driven coronary or cerebral revascularization. Baseline mean LDL-C was 74 mg/dl, triglycerides 163–167.5 mg/dl, HDL-C approximately 35 mg/dl for

the two groups consisting of (1) placebo + statin and (2) extended release niacin + statin. At the end of 3 years mean LDL-C was 68.3 mg/dl and 65.2 mg/dl in the two groups respectively, mean triglycerides were 152 and 120 mg/dl respectively and HDL was 39.1 and 44.1 mg/dl respectively. Accordingly, LDL-C was very low initially and at the end of the study. Triglycerides fell minimally in the statin only group and more in statin plus niacin group. HDL-C rose approximately 5 mg/dl in the statin monotherapy group and 9 mg/dl in the simvastatin + niacin group. The study was terminated at the end of 3 years as there was no incremental benefit in outcome from the group that treated with niacin + simvastatin as compared to the simvastatin monotherapy treatment. With respect to adverse events, flushing or itching requiring a reduction in study dosage occurred in approximate 1.4% of the placebo + statin group and 3.3% in the niacin + statin group. Increased glucose level occurred in 0.3% of the statin + placebo group and 0.6% in niacin + statin group, and gastrointestinal issues occurred in only 0.2% of both groups. Discontinuation of the study drug(s) was required in 2.5% of the placebo + statin group and 6.1% of the niacin + statin group. Gastrointestinal problems occurred in 0.7% of the placebo + statin group and 1.5% of the statin + niacin group. These data demonstrate that adverse reactions to medications were relatively low in that study but occurred more frequently in the group that contained niacin therapy.

HPS-2-THRIVE [16] was a large randomized, placebo controlled, double-blind study which sought to answer the question of whether adding extended release niacin to 40 mg of simvastatin would improve major cardiovascular outcomes. The study also used a new preparation, laropiprant, to reduce or eliminate flushing from the niacin preparation. Laropiprant is a specific prostaglandin D inhibitor with a subtype DP1 inhibitor. It is the DP1 receptor that is specifically in the pathway of niacin flushing. Thus, the intent of this study was to provide an inhibitor for niacin flushing and to use approximate 2 g of extended release niacin in combination with 40 mg of simvastatin to determine outcome. Some patients also received ezetimibe to further lower LDL. This study involved 25,673 patients who were randomized between simvastatin alone and simvastatin + extended release niacin + laropiprant. The study was conducted in Scandinavia, the United Kingdom, and in China. European subjects constituted 14,741 participants and Chinese participants numbered 10,932. Both arms of the study had essentially equal patient numbers. Patients were followed for a median of 3.9 years. The study demonstrated no significant difference in the 2 arms study for the pre-specified question of whether adding niacin would improve major cardiovascular outcomes. Baseline LDL-C was very low at 62 and HDL-C was relatively high at approximately 43 mg/dl respectively. Equally importantly, the niacin + simvastatin group demonstrated increased myopathy with a highly significant risk ratio of 4.4, and slightly higher rhabdomyolysis rate of occurrence. Myopathy issues were particularly present in Chinese subjects. Other side effects in the niacin arm included an excess of 2.1% for gastrointestinal issues, a 0.5% excess of diabetes, and a 0.8% excess of musculoskeletal symptomatology. All of the aforementioned side effects were highly significant when comparing results of the niacin arm to the simvastatin monotherapy arm (with or without ezetimibe). The conclusion of this study was that niacin added to simvastatin produced no significant outcome benefit but did produce significant side effects.

The ARBITER 6-HALTS [15] was a clinical trial which used carotid intimal medial thickness (CIMT) as a surrogate endpoint. This trial involved secondary prevention patients treated with statin therapy which decreased LDL-C to < 100 mg/dl. HDL-C was < 50 mg/dl (men) or < 55 mg/dl (women). Additions to this therapy were ezetimibe 10 mg daily or extended release niacin 2000 mg daily. The niacin arm demonstrated a mean decrease in CIMT of 10 μm which was statistically significant. The ezetimibe arm demonstrated no significant CIMT change. The study was interpreted as niacin demonstrating superiority to ezetimibe in reducing CIMT in patients already treated with statins.

Contradictory conclusions are contained in these clinical trials. The Coronary Drug Project demonstrated that niacin decreased cardiovascular events and, eventually, decreased total mortality. The HATS [14] trial demonstrated a marked decrease in cardiovascular events with the combination of niacin and simvastatin. The ARBITER 6-HALTS demonstrated a decrease in CIMT, a surrogate endpoint, with the combination of a statin and niacin as compared to statin + ezetimibe. On the other hand, AIM-HIGH and HPS-2-THRIVE demonstrated no improvement when adding niacin to a statin. One significant difference between the trials relates to the baseline level of LDL-C. AIM-HIGH and HPS-2-THRIVE both had very low LDL-C at the start, 74 and 62 mg/dl respectively. Although LDL-C was not measured in the Coronary Drug Project, total serum cholesterol was quite high suggesting LDL-C was also quite high. In the HATS trial [14], LDL-C was significantly elevated. A recent study [17] demonstrated that niacin does not lower LDL-C below a threshold of about 125 mg/dl but thereafter decreases LDL-C by approximately 35%. Perhaps the beneficial effect of niacin is related to lowering LDL-C. The rise in HDL-C created by niacin may or may not be as important. Raising HDL-C by pharmaceutical means has not yet been proven to be of significant clinical benefit [18, 19] even though epidemiologic studies show a definite relationship between cardiovascular events and low HDL-C. Much more needs to be learned about raising HDL by pharmaceutical means.

Niacin Dosing and Utilization The dosage of niacin depends on the type of niacin used. As a general rule, the longer the action of niacin, the lower the safe dose is. Large doses of longer acting niacins may cause an increase in transaminases and possibly other liver issues. Extended release niacin is usually used at a peak dose of 2 g/day given just before bedtime. Longer release niacin are safest at a peak dose of 1.5 g/day in divided doses.

Although some reports have used immediate release niacin as high as 4 g/day, fewer side effects occur if the peak dose is limited to 3 g/day. The method for initiating therapy with immediate release niacin is probably quite individual depending upon the prescriber. The author has utilized a protocol for many years in which the patient starts with 50 mg three times a day with meals. The patient is asked to take an 81 mg chewable aspirin prior to the meal to block a prostaglandin mediated flush. The patient then eats the meal which creates volume in the stomach to slow the absorption of the niacin, and at the end of the meal the patient takes 50 mg of niacin. This protocol continues for one week and then the patient continues the second week with the 50 mg of niacin but does not take the chewable aspirin during unless flushing occurs. The niacin dose is doubled every 2 weeks and the weekly

protocol including or not including the chewable aspirin is continued up to a dose of 200 mg. At this point, the dose is increased to 500 mg following each meal with aspirin for one week and without aspirin on the second week. At this point a lipid profile, fasting glucose and liver function tests are obtained to determine if the patient has reached the goal level on the lipid profile. If the goal has been achieved, the patient will be maintained on 1500 mg immediate release niacin each day. If the goal has not been achieved, the dose is doubled again but not raised beyond 3000 mg/day. If patients are going to have significant flushing, they usually do it at about the 200 mg level although the flush is usually just occasional and relatively mild. Patients are advised that if they have a severe flush they should chew 3 of the 81 mg aspirins and swallow them with a large glass of water. With this treatment the flush will usually disappear within 10 min. Aspirin is stopped when patients are no longer flushing. Most patients develop sufficient tolerance with this protocol that they are able to take immediate release niacin with only very rare or no further flushing. However, very rare patients will flush at 50 mg with or without the 81 mg aspirin, and usually these patients are not able to tolerate immediate release niacin. Liver enzymes are almost never elevated with immediate release niacin. Fasting glucose for patients on monotherapy niacin increased about 2% but occasional patients will increase early morning fasting glucose about 10% [18] but niacin does not ordinarily change HbA-1C.

Bile Acid Sequestrants

Bile acid sequestrants have been used to reduce LDL-C, particularly in the pre-statin era. However, the more recent bile acid sequestrant, colesevelam, has resurrected the use of bile acid sequestrants.

Lipid Effects of Bile Acid Sequestrants The principal effect of bile acid sequestrants is lowering LDL-C. A secondary effect of 2 of the bile acid sequestrants, cholestyramine and cholestipol, is to raise triglycerides.

Mechanism of Bile Acid Sequestrant Effects Hepatocytes synthesize bile acids from cholesterol as a result of 7 α-hydroxylase activity. The purpose of bile acids is to emulsify cholesterol and lipids in the biliary and gastrointestinal tracts. If the quantity of bile acids is reduced, 7 α-hydroxylase is upregulated and hepatocytes convert more cholesterol into bile acids. This action depletes cholesterol in hepatocytes. Accordingly, these hepatocytes upregulate LDL receptors which increases clearance of LDL particles from the circulation. The mechanism of action of bile acid sequestrants is to bind bile acids in the gastrointestinal tract to prevent enterohepatinic reabsorption of these bile acids. Bile acids are bound in a resin matrix and excreted in stool. These resins are not absorbed in the gastrointestinal tract.

Available Preparations of Bile Acid Sequestrants Several bile acid sequestrants have been used in clinical practice and include cholestipol, cholestyramine, and colesevelam.

Cholestipol, although still available, is rarely utilized today because of interactions with cardiovascular drugs and intestinal tract binding with commonly prescribed cardiovascular drugs and vitamin K. The usual adult dose is 2–16 g of tablets administered once or twice daily or 5–30 g of granules once daily or 4 times daily.

Cholestyramine, which is closely related to cholestipol, is usually administered in doses of 4 to 8 g to a maximum dose of 24 g/day. Cholestyramine comes as a powder which can be dissolved in liquids and has also been produced as a pill. It has a non-linear effect on LDL-C so that larger doses do not cause a proportional fall in LDL-C compared to smaller doses. It is taken with meals. Twice-daily administration is usually recommended although single-dose administration with the evening meal does provide may be as effective as twice-daily dosing [20]. Most patients prefer to take cholestyramine with a flavored drink to mask the taste. Since it is a gritty material, some patients prefer to take it with a pulpy orange juice or tomato juice to decrease the grittiness. Major issues include interference with absorption of warfarin, levothyroxine, some diuretics and other medications and some vitamins. Thus, these medications need to be taken 1 h prior to cholestyramine or 6 h thereafter. Another major issue is that cholestyramine increases triglycerides and is not recommended for individuals with significant hypertriglyceridemia.

Colesevelam tablets have a single dosage of 625 mg and the usual dose is 4–6 tablets/day. Tablets are physically large and this may cause problems for certain individuals. However, individual packets of granules are available containing 1.875 or 3.75 g of colesevelam. It should be noted that colesevelam contains a small amount of phenylalanine which could be important for patients who have phenylketonuria.

Colesevelam shares some similar properties with cholestyramine but considerable differences also exist. It is less constipating than cholestyramine and thus somewhat better tolerated. Nonetheless, some patients still report constipation with Colesevelam. Another important difference is that it does not absorb fat-soluble vitamins. Similar to cholestyramine, it is not absorbed in the intestinal tract [21].

Clinical Trials with Bile Acid Sequestrants The use of cholestyramine was tested in a randomized clinical trial, Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT) first reported in 1984 [22]. This trial was a double-blind study of 3806 asymptomatic men with familial hyperlipidemia, and the study had a mean duration of 7.4 years. Dietary intervention was used in both the placebo and cholestyramine arms. LDL-C decreased 20.3% which was 12.6% greater than LDL-C reduction in the placebo group. Further, HDL rose trivially. The cholestyramine group had a significant 19% reduction in risk of the primary endpoint of coronary heart disease death or nonfatal myocardial infarctions. An important feature of the LRC-CPPT clinical trial was that it was the first trial to establish that reduction of LDL-C reduced cardiovascular events.

Although cholestyramine demonstrated a significant reduction in coronary heart disease in a randomized clinical control, it remains a difficult drug to utilize because of gastrointestinal side effects which include bloating, abdominal discomfort, and, particularly, constipation. Cholestyramine is rarely used today but it can be considered for treatment of high LDL-C in patients who cannot tolerate statins. Further,

for many years the American Academy of Pediatrics recommended cholestyramine for use in children with familial hyperlipidemia since it was not absorbed, but more recent recommendations have been for statins due to the difficult tolerability of cholestyramine.

No outcome studies have been performed for colesevelam, but studies have shown it to be effective as monotherapy for lowering LDL-C [21, 23]. LDL-C is lowered in a dose dependent manner by usually 13–20 %. Colesevelam has also been reported to further lower LDL-C when used in combination with statins [24, 25]. A further interesting lipid effect is that colesevelam does not significantly raise triglycerides [23, 24].

Cholestyramine raises HDL-C a very small amount but colesevelam increases HDL-C by 7–12 % for doses exceeding 3 g/day. The mechanism for increasing HDL appears to involve the farnesoid X receptor (FXR) which is down regulated by decreased bile acids and the downregulation causes less inhibition of the liver X receptor (LXR) and subsequently upregulation of the gene encoding adenosine triphosphate-binding cassette transporter protein A1. This enhances reverse cholesterol transport and thereby increases HDL-C [26].

An interesting non-statin combination of ezetimibe and colesevelam has been reported to reduce LDL-C a mean of 32–42 % [27, 28]. This type of therapy is important for patients who are severely statin intolerant.

An important additional factor regarding colesevelam is that it has an approved FDA indication for treatment of type II diabetes. It lowers fasting glucose to some extent and lowers hemoglobin A-1 C by about 5 %. It may be combined with other diabetic medications to improve diabetes control. This glucose lowering appears to be a class of fact of bile acid sequestrants although colesevelam has a greater effect in this regard than cholestyramine. The exact mechanism of its action in glucose metabolism remains is beyond the scope of this chapter but remains unclear as numerous studies have conflicting data. It seems probable that this effect is mediated by FXR, but more research needs to be done in this area.

Most common side effects of colesevelam include mild constipation; nausea, vomiting, upset stomach, gas, indigestion, runny nose, sore throat, flu symptoms, runny nose, sore throat, flu symptoms, weakness or fatigue. Although not commonly reported, occasional patients complain of muscle pain, even with monotherapy Colesevelam. Whether these side effects are secondary to the medication or a result of other factors remains uncertain since less than 1 % of colesevelam is absorbed into the circulation [21].

Fibric Acids

Fibric acids are a class of pharmaceuticals that have been utilized for almost 40 years to treat lipid disorders. Five different fibrates have been used in human therapeutics and include clofibrate, gemfibrozil, fenofibrate, benzafibrate, ciprofibrate. Currently, 2 of these fibric acids, gemfibrozil and fenofibrate, are available and

approved for use in the United States. Ciprofibrate and, to a lesser extent, benafibrate are available and used in Europe and Asia.

Lipid Effects The major use of fibric acids is to decrease triglycerides and, to a lesser extent, to raise HDL-C.

Mechanism of Effect All fibric acids have a somewhat similar chemical structure and all are peroxisome proliferator activated receptor (PPAR) ligands of the alpha class. Their common ability is to increase lipoprotein lipase and decrease apoprotein CIII. The action of fibric acids on LDL is somewhat variable with the different forms of fibric acids. If a patient has elevated triglycerides, fibric acids may change the LDL particle from small and dense to larger particles which contain more cholesterol. Accordingly, in the latter instance, LDL-C may increase a bit. In other cases, LDL-C may fall modestly.

An important issue in the pharmacokinetics of gemfibrozil is that gemfibrozil is a significant inhibitor of CYP2C8, statin glucuronidation, and AOTP2 [29]. On the other hand, fenofibrate is not an inhibitor of CYP2C8. This becomes important because a number of drugs commonly used for patients with cardiovascular disease are substrates for the CYP2C8 system. Fenofibrate, on the other hand, is a substrate or CYP 2C8 and has few drug interactions. Accordingly, fenofibrate can be utilized with statins to lower triglycerides and raise HDL whereas gemfibrozil does not influence the area under the curve for fluvastatin and pitavastatin but has significant changes in the area under the curve for the other statins. The combination of gemfibrozil and statins other than fluvastatin or pitavastatin probably accounted for a number of the cases of rhabdomyolysis in the past. The combination of cerivistatin and gemfibrozil was probably the most dangerous with respect to rhabdomyolysis.

Clinical Trials Clofibrate (atromid-S) was the first fibric acid product which was approved by the FDA in 1967. Clofibrate was tested in the Coronary Drug Project and demonstrated no beneficial effect on cardiovascular events [12]. Clofibrate increased activity of lipoprotein lipase which increased lipolysis of triglycerides and decreased synthesis of apoprotein B. Clofibrate usage in the Coronary Drug Project was associated with a significant increased incidence of cholelithiasis compared to placebo. Further, compared to placebo, it had an increased incidence of pulmonary embolism. Clofibrate production was discontinued in 2002.

Gemfibrozil was tested in 2 outcome trials: Helsinki Heart Study (HHS) and Veterans Administration HDL intervention trial (VA-HIT). Historically, gemfibrozil was the 2nd fibric acid to be approved for use following clofibrate. The first experimental use occurred in 1975. The drug was approved by the FDA in 1981 in first marketed in the United States in 1982 [30]. Gemfibrozil lowers VLDL and triglycerides by increasing activity of lipoprotein lipase and acting as an activator of the PPAR-alpha receptor in a manner generally similar to that of clofibrate. At the beginning of the statin era, gemfibrozil accounted for approximately 29 % of all lipid lowering drug prescriptions, but its usage has declined steadily in recent years. A major reason for its declining use is its general incompatibility with statins other than fluvastatin and pitavastatin. Further, more effective medications are currently

available for decreasing cardiovascular risk. For statins other than pitavastatin and fluvastatin, gemfibrozil interferes with glucuronidation of the statin and increases the blood level of the statin which can lead to adverse effects of statins. However, it is compatible with niacin, ezetimibe, and bile acid resins.

The Helsinki Heart Study (HHS) trial [31–32] was a 5 year primary prevention study which was a randomized, double-blind, placebo-controlled, primary prevention study. The study included 4081 male patients, ages 40–45, who had non-HDL-C of >200 mg/dl. None of these patients had a history of coronary heart disease. The treatment group received 600 mg of gemfibrozil twice daily and the control group received a placebo. The composition of the population studied in HHS is quite interesting. In both the experimental and placebo group, 63% had Fredrickson type IIa familial hyperlipidemia, 27% had Fredrickson type IIb and 8% had Fredrickson type 1V. Accordingly, this was a very high risk group. In the HHS, HDL-C initially rose about 15% but by the end of the 5 year period, the HDL-C increase over baseline was approximately 9%. LDL-C initially fell 10% and by the end of the 5-year period LDL-C was down 9% and non-HDL-C decreased 14%. Triglycerides initially decreased about 42% but by the end of the study triglycerides compared to baseline decreased about 35%. LDL-C was not significantly change during the study.

Although the HHS has been viewed as one in which HDL-C was raised, the investigators of the study clearly indicate that this occurred in the presence of a significant decrease in non-HDL-C. It remains unclear whether the result of this study was secondary to raising HDL-C or lowering non-HDL-C, or perhaps a combination of both. The result was a significant 1.4% absolute reduction in sudden cardiac death and/or fatal and nonfatal myocardial infarction. This amounted to a 34% relative decrease compared to placebo. Further, there was a 37% relative reduction in nonfatal myocardial infarction compared to placebo. Death from any cause was not statistically significant. The primary benefit appeared to occur principally in patients with the highest triglycerides.

The Veterans Administration HDL Intervention Trial (VA-HIT) [33], in contrast to the Helsinki Heart Study, was a secondary prevention trial. The design of this trial was predicated on the basis of a prior study by Rubins et al. [34] demonstrating that approximately 25% of patients with coronary heart disease have low HDL-C in the absence of high LDL-C. VA-HIT was a placebo controlled, randomized trial of 2531 individuals who had HDL-C <39 mg/dl and LDL-C less than 140 mg/dl. 61% of these patients had a history of prior myocardial infarction. The group had a mean age of 64 years, a mean BMI of 29, a 57% prevalence of hypertension and 25% prevalence of diabetes. The pre-designated primary endpoint was decreased combined incidence of non-fatal myocardial infarction and coronary artery disease death. For the primary endpoint, gemfibrozil resulted in a 22% relative risk reduction and a 4.4% absolute risk reduction. Further, there were significant reductions in transient ischemic attacks (−59%) carotid endarterectomy (−65%) hospitalization for congestive heart failure (−22%) but only a trend toward stroke reduction (−29%). Although there was a slight decrease in cancer and all-cause mortality, neither were significant when comparing the gemfibrozil and placebo groups.

In VA HIT, gemfibrozil increased HDL-C by only 6% but triglycerides were decreased by 31%. Accordingly, non-HDL-C was significantly decreased. Further, in light of current knowledge, an LDL-C criterion of 140 mg/dl or actual mean value of 111 mg/dl would still be considered an elevated LDL-C. Therefore it remains uncertain whether the change in HDL-C or the reduction in non-HDL was the factor that permitted the reduction in primary end points. Gemfibrozil also affects LDL particle number and size by changing smaller particles into larger particles and the larger particles have increased cholesterol content [35]. Although LDL-C did not change significantly in this study, particle composition did. To make the issue even more complex, some statins also increase HDL-C in the similar range of 6%. Further, it is established that gemfibrozil has some plieotrophic properties including altering clotting factors, decreasing platelet aggregation, and plasminogen activator inhibitor-1 activity. The exact factor or factors that accounted for the primary endpoint significant decreases remain uncertain.

Clinical Trials with Fenofibrate Fenofibrate has lipid effects generally similar to those of gemfibrozil. However, the metabolism is somewhat different and fenofibrate can be used with all current statins. A major question is whether fenofibrate reduces cardiovascular events. In the Diabetes Atherosclerosis Intervention Study (DAIS) [36] 418 subjects with type II diabetes and documented coronary artery disease were studied. The study was not a clinical outcome study but it did demonstrate that coronary atherosclerosis progressed less in the fenofibrate group than in the placebo group over a duration of 3 years.

The major clinical outcome trial for fenofibrate was FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) [37]. FIELD was designed as a monotherapy study utilizing fenofibrate in a randomized trial of 9795 type 2 diabetic patients ages 50–75 years. Of these patients, 2131 had previous evidence of cardiovascular disease and 7664 had no detected evidence of cardiovascular disease at the start of the study. Patients were treated with micronized fenofibrate 200 mg/day. 4900 patients were treated with placebo and 4895 were treated with fenofibrate. The pre-designated primary outcome was coronary heart disease death or nonfatal myocardial infarction. Lipid effects in FIELD after 5 years: total cholesterol was reduced by 13%, LDL-C was reduced 15%, HDL-C increased only 2% and triglycerides decreased by 27%. It is interesting to note that the change in HDL-C and triglyceride was somewhat less than that for gemfibrozil and the decrease in LDL-C was greater than that for gemfibrozil.

The outcome findings were conflicted and difficult to interpret because primary health providers started some study patients on statins outside of the study design. Results showed a reduction in the fenofibrate group of 11% for coronary events but this was not statistically significant. There was a significant 24% reduction in nonfatal myocardial infarction and a significant reduction in coronary and all revascularization procedures. Further, the fenofibrate group showed significantly less albuminuria progression and less retinopathy but a significantly greater incidence of pancreatitis and pulmonary embolism. Interestingly, the statin therapy introduction by primary care providers was greater in the placebo group than in the fenofibrate

group. This difference in statin initiation in the placebo group could have significantly affected the outcome and biased the study toward non-significant outcome.

Analysis of fenofibrate tolerability and safety in the FIELD trial showed that fenofibrate was well tolerated. Only 0.5% patients on the placebo and 0.8% taking fenofibrate were considered to have had possible adverse drug reactions. Rhabdomyolysis occurred in 1 patient in the placebo group and 3 in the fenofibrate group. None of the 4 rhabdomyolysis patients were using statin therapy. Fenofibrate caused a mild reduction in creatinine and a mild increase in homocysteine. Importantly, body weight was unchanged. Hemoglobin A-1c showed no significant change.

Unfortunately, whether monotherapy fenofibrate will reduce cardiovascular events significantly will probably never be determined in the future because it would now be unethical not treat diabetic patients with a statin and the addition of fenofibrate to a statin might not give an accurate assessment of what fenofibrate might do as monotherapy.

Clinical Trial with Benzafibrate Benzafibrate is a medication that has not been approved by the FDA in United States.

The Bezafibrate Infarction Prevention Study (BIP) [38] was a double-blind, secondary prevention, randomized, mainly male (91%), nondiabetic, study of patients, ages 45–74 years with previous myocardial infarction or stable angina. Total subject number was 3090 and these subjects were divided approximately equally between the bezafibrate and control groups. Participants were required to have a total cholesterol of 180–250 mg/dl, triglycerides <300 mg/dl and HDL-C less than 45 mg/dl. Subjects were randomized either 400 mg of bezafibrate daily, or a placebo. Study duration was 6.2 years. The predesignated primary endpoint was fatal or nonfatal myocardial infarction or sudden death. Use of other lipid lowering drugs was an exclusion criterion. Mean lipid effects demonstrated a reduction of triglycerides by 21%, and 18% increase in HDL-C and an LDL-C reduction of 6.5%. The bezafibrate group demonstrated a 13.6% occurrence in primary endpoint events and the placebo group demonstrated an occurrence of 15% ($p=0.24$). A post-hoc evaluation using Kaplan Meyer analysis showed that patients with baseline triglycerides >200 mg/dl had a significantly decreased primary event rate ($p=0.02$). Patients with baseline triglycerides <200 had a Kaplan Meyer diagrams with results of the placebo and treated groups completely overlying one another. However, the predesignated endpoints for the entire bezafibrate group compared to the control group showed no statistical significance.

An extension of the BIP trial for patients whose bezafibrate medication was terminated at 6.2 years and who did not take additional lipid lowering medications was conducted for an additional 2 years, and mean follow up duration of 8.2 years [39]. The results of this extension study demonstrated that the bezafibrate monotherapy group showed a 17% reduction in events as compared to the control group ($p=0.03$). It is necessary to indicate that many patients following the original BIP study received lipid lowering drugs and this was significantly more common in patients who were in the control group. Analysis of the entire group including those taking other lipid lowering drugs showed no significant difference between the

control and bezafibrate arms of the study. Thus, the extended study showed that patients taking bezafibrate monotherapy compared to controls not taking lipid drugs demonstrated a significant result in terms of prespecified endpoints, but when other lipid lowering drugs were added to members of both groups, no significant difference was found.

Available Preparations and Pharmacology Gemfibrozil is available as a tablet at a single dosage of 600 mg which is ordinarily given twice a day with food. Fenofibrate comes in many doses as a tablet and is usually given as a single daily dose. Absorption of fibrates taken with food is >90 %. Absorption is poorer without food. Fibric acids are extensively bound to albumin. An important issue is that other drugs depending on albumin binding might find less albumin for binding purposes. This is particularly true for warfarin use combined with a fibric acid. Patients utilizing this combination might demonstrate decreased INR. Approximately 60–90 % of most fibric acids are excreted by the kidney. Accordingly, caution should be utilized in individuals with kidney disease who are taking fibric acids. The effect of fibric acids given during pregnancy is uncertain, but, if possible, they should be avoided. Smalley et al. reported that gemfibrozil was effective and well tolerated in children with elevated triglycerides [40].

Omega-3 Fatty Acids (Fish Oil)

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 fatty acids which have long been utilized in treatment of hypertriglyceridemia. EPA is usually obtained from eating fish or taking a fish oil supplement, but fish do not actively produce EPA. Fish obtain EPA from consumption of algae. Humans can convert ingested alpha linoleic acid into EPA, but the process is inefficient and only a very small portion of alpha linoleic acid is actually converted into EPA. Further, EPA can be metabolized into DHA. DHA is found in brain, retina and human breast milk.

Lipid Effects The major lipid effect of EPA and DHA is to reduce elevated triglycerides. DHA raises LDL-C but EPA has no significant LDL-C effects.

Mechanisms of Action The mechanisms by which EPA and DHA decrease hypertriglyceridemia is less understood than mechanisms by which other lipid lowering drugs act. It is clear that these two omega-3 compounds reduce hepatic production of VLDL, perhaps by inhibition of DGAT (1 and 2). DGAT catalyzes the formation of triglycerides from diacylglycerol and Acyl-CoA. This step is necessary in order to form triglycerides and also adipose tissue.

The basic process of triglyceride reduction is that EPA and DHA change fatty acid metabolism away from triglyceride storage by increasing fatty acid oxidation in the hepatocyte. Also, apoprotein B degradation is increased. Since each VLDL contains an apoprotein B, this reduction in apoprotein B reduces hepatic secretion

of VLDL. EPA and DHA may also increase lipoprotein lipase activity and increase chylomicron clearance. Further, EPA and DHA may counter intracellular adipocyte lipolysis and thus suppress adipose tissue inflammation. Through some or all of these multiple mechanisms, EPA and DHA decrease the production of VLDL and thus the amount of triglycerides.

Current Recommendations for Triglyceride Control Adult Treatment Panel III recommendations for treatment of high triglycerides (>500 mg/dl) included treatment of triglycerides to prevent pancreatitis at levels greater than 500 mg/dl with a fibric acid, nicotinic acid, or fish oil. Adult Treatment Panel IV carried through these recommendations unchanged. The usual dosage of fish oil to accomplish these changes in high or very high triglycerides is 3–4 g/day.

Available Preparations Currently, there are two approved prescription fish oils, Lovaza and Vascepa. Each one-gram capsule of Lovaza contains 465 mg of EPA and 375 mg of DHA. Each 1 g capsule of Vascepa contains 96% icosapent ethyl which is EPA only. Either of these two prescription medications may be utilized for patients with high or very high triglycerides after dietary and exercise therapy has been instituted. Additionally, medications that exacerbate hypertriglyceridemia (beta blockers, thiazides, estrogens, and retinoic acid) should be discontinued, if possible, prior to starting a fish oil to lower triglycerides. Triglycerides are frequently normally elevated in pregnant women. A potential issue is that the safety of these medications for individuals who have fish or seafood allergy has not been determined.

Clinical Trial Results Lovaza has been studied in several randomized placebo-controlled double-blind studies and two will be reviewed. The first [41] was a monotherapy study with 84 patients, half treated with 4 g Lovaza and half treated with a placebo. Pretreatment triglycerides ranged from 500 to 2000 mg/dl and median triglycerides were 792 mg/dl and median LDL was 100 mg/dl Lovaza, compared to the placebo, decrease triglycerides 52%, non-HDL-C 10%, total cholesterol 8%, VLDL-C 41% and increased HDL-C 9% and increased LDL-C 49%.

In a combined therapy study [42] 254 patients with a mean age of 59.8 were randomized in a double-blind study to receive either simvastatin 40 mg + 4 g of Lovaza or simvastatin 40 mg plus a placebo. Pretreatment triglycerides in this population ranged from 200 to 500 mg/dL. Comparison of the groups showed that the addition of 4 g of Lovaza and 40 mg of simvastatin, as compared to the placebo plus simvastatin 40 mg demonstrated a decrease in non-HDL-C of 9 vs 2.2%, a 29.5 vs 6.3% decrease in triglycerides, and a decrease in VLDL-C, a 27.5 vs 7.2%. All of the % changes mentioned were statistically significant.

Vascepa clinical trial information: two clinical trials have been completed for Vascepa. The MARINE study [43] was a double-blind, randomized investigation of 229 diet stable patients with fasting triglycerides ranging from 500 to 2000 mg/dl. Dosage was 4, 2 g/day or placebo. Baseline triglyceride levels were 680, 657 and 702 mg/dl for the three groups. Each group contained 76 or 77 patients. Approximately 97% of patients completed a 4 week study and of these 96, 92, and 93%

completed 12 weeks. In the 4 g/per day group, corrected for placebo, triglyceride levels decreased by 33 and 20% in 2 g/day group. For the 4 g/day group, VLDL decreased 29%, apoprotein B decreased 8.5%, total cholesterol decreased 16% and VLDL decreased 26%. For the 2 g/day group triglycerides decreased 20% non-HDL 8% VLDL 15%. Changes in apoprotein B, and HDL for the 2 g/day group were not significant. Importantly, the placebo corrected level of LDL-C in both groups did not show a significant change.

ANCHOR [44] was a phase 3, multicenter, placebo-controlled, randomized, double-blind 12 week study in high risk statin treated patients with triglycerides ranging from 200 and 500 mg/dl. 702 patients were randomized to Vascepa 4 or 2 g/day or a placebo. Vascepa 4 g/day decreased triglycerides 21.5%, LDL-C 6%, non-HDL 14%, VLDL 24% apoprotein B 9%, total cholesterol 12% and hsCRP 22%. All of these decreases were significant. For the 2 g/day group, triglycerides decreased 10%, non-HDL-C 5.5% VLDL-C 10.5%, apoprotein B 4%, total cholesterol 5%. Other changes were not statistically significant. The significance of this study was the it again demonstrated that EPA did not increase LDL-C as demonstrated by the combination of EPA + DHA and that further triglyceride lowering occurred with baseline triglyceride levels between 200 and 500 mg/dl. However, this was not an outcome study. An outcome study for Vascepa is currently underway named REDUCE-IT.

Clinical Trials of Fish Oils to Reduce Mortality The JELIS trial (Japan Eicosapentaenoic Acid Lipid Interventional Study) [45] was a 5 year prospective, randomized trial of statins + EPA. Dosage of EPA was 1800 mg/day given to Japanese patients with hypercholesterolemia. The study was to a designed as a prospective, randomized, open label, blinded endpoint trial. Participants were men age 40 to 75 and postmenopausal women aged 75 years or older. Total serum cholesterol was >250 mg/dl. 18,645 subjects were recruited with a mean age of 61 years. Subjects were randomized to pravastatin 10 mg/day + EPA 1800 mg/day or for the control group simvastatin 5 mg/day. Baseline LDL-C mean levels were 180 mg/dL and total cholesterol 275 mg/dL. Primary endpoints were pre-designated as sudden cardiac death, fatal or nonfatal myocardial infarction, unstable angina, coronary artery bypass surgery or percutaneous coronary intervention. The outcome of the trial was a significant decrease ($p=0.01$) of major coronary events and a significant 24% decrease in unstable angina. No significant difference occurred in all cause mortality. Sudden cardiac death and coronary death did not differ between groups. In patients with a history of coronary disease, major coronary events were reduced by 19% ($p=0.048$). To put the results of this trial into perspective, it is important to point out that Japanese eat large amounts of fish, far larger than is common in the United States or Europe. The dose of EPA was substantial and given to individuals who probably already have high levels of EPA and DHA. It is not certain whether a similar dose in Western population would produce similar results. Further, the EPA was combined with low dose statins and results might have been different with EPA monotherapy. Finally, the doses of statins and type of statin in the control and experimental group were not precisely matched.

The GISSI-P [46] study was an open controlled clinical trial with randomization of patients following a 2 × 2 factorial design to fish oil, vitamin E, both or neither. The trial was significantly powered and included 11,324 patients recruited following a myocardial infarction 3 months or less prior to enrollment. Subjects with elevated cholesterol levels were subsequently randomized to receive pravastatin 6 months after the cardiovascular event. All patients were treated with the then current (years 1992–1995) maximal post myocardial infarctions therapy (antiplatelet drugs, beta blockers, ACE inhibitors) plus the trial medications. Absolute risk reductions were 2.1, 2 and 1.6 % for overall mortality, cardiac mortality and sudden cardiac death, respectively. Benefits were seen within the first 4 months of treatment and the major benefit appeared to be reduction in ventricular arrhythmias. Vitamin E did not demonstrate a significant outcome.

The ORIGIN clinical trial [47] was a randomized, international, multicenter study which compared results of the population of 12,536 dysglycemic subjects divided equally into two groups in which half of the group received 900 mg of EPA + DHA and the other half received an equal volumetric amount of olive oil. The primary outcome measure was death from cardiovascular causes. Secondary outcomes were the composite outcome of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke; death from any cause; and death from arrhythmia (which included sudden unexpected death, death from documented arrhythmia, unwitnessed death, and resuscitated cardiac arrest). Other outcomes included all myocardial infarctions, all strokes, revascularizations, heart failure, angina, limb amputation for ischemia, and hospitalization for cardiovascular causes. Participant criteria were an age of at least 50 years; a diagnosis of diabetes with receipt of no more than one oral glucose-lowering drug, impaired glucose tolerance (after a 75-g oral glucose load), or impaired fasting glucose 110 mg/dl, or a fasting glucose > 126 mg/dl, a history of myocardial infarction, stroke, or revascularization; angina with documented ischemia; a ratio of urinary albumin to creatinine of more than 30 mg per gram; left ventricular hypertrophy; 50 % or more stenosis of a coronary, carotid, or lower-limb artery on angiography; or an ankle-brachial index of less than 0.9. At baseline 79 % of patients were hypertensive, 59 % had a prior myocardial infarction stroke or revascularization, mean LDL was 112 mg/dl, HDL was 46 mg/dl, and triglycerides were within the normal range. Patients were extensively treated at the baseline of the study as 69 % were taking an ACE inhibitor or a ARB2, antiplatelet therapy in 69 %, beta blockers in 52 %, calcium channel blockers in 28 % and statins in 54 %. Triglyceride lowering was not object the study. Subjects were not encouraged to eat a high content fish diet or to use additional supplements of fish oils. The result at 6 years showed no significant difference in primary or secondary outcomes.

The results of these several studies utilizing omega-3 fatty acids to improve cardiovascular outcomes do not allow reaching a single conclusion. The populations were different and the JELIS population utilized a different dose and different preparation, EPA only, than the other two. The question regarding supplementation with fish oils to improve cardiovascular outcome remains unresolved as results of one of the studies does not necessarily override results of the others.

Ezetimibe

Ezetimibe (Zetia) was approved by the FDA in 2002.

Lipid Effects Ezetimibe decreases LDL-C, non-HDL-C, and total cholesterol by acting the small intestine to decrease cholesterol absorption. It also decreases elevated sitosterol and campesterol. It is usually taken orally as a 10 mg tablet once daily with or without food. It has been utilized as monotherapy and for individuals who cannot tolerate statins or in combination with statins or possibly other lipid medications

Mechanism of Action The mechanism of action for reducing cholesterol absorption occurs at the brush border of the small intestine. Ezetimibe inhibits cholesterol absorption from the intestine by binding to a protein product of the Nieman-Pick C1-like 1 gene. This gene appears to control or partially control absorption of cholesterol at the intestinal brush border [48, 49]. As might be anticipated, decreasing cholesterol absorption also secondarily up regulates LDL receptors. Pooled clinical data indicates that ezetimibe decreases LDL-C a mean of 18% [50]. Data regarding decrease in LDL-C is approximately equal for both monotherapy and combined therapy.

Metabolism of Ezetimibe Ezetimibe is metabolized principally in the liver and small intestine by glucuronide conjugation. Ezetimibe is not metabolized by cytochrome P-450 isoenzymes. It has an active metabolite and both the active metabolite and ezetimibe are metabolized in approximately 20 h. It has a few possibly important drug interaction with cyclosporine and fibric acids. In patients taking warfarin, INR should be followed closely. It is not recommended for use in individuals with severe hepatic dysfunction. However, dosage adjustment is unnecessary for individuals with renal impairment [50].

Clinical Trials The SHARP clinical trial [51] was a randomized, double-blind trial including 9270 patients with chronic kidney disease. 33% of these patients were on dialysis. None of these patients had prior history of myocardial infarction or coronary revascularization. Patients were randomly assigned to simvastatin 20 mg and ezetimibe 10 mg daily or simvastatin 20 mg and a matching placebo. Primary outcome was the occurrence of a nonfatal myocardial infarction, coronary death, a nonhemorrhagic stroke or arterial revascularization procedure. 4650 patients received simvastatin plus ezetimibe and the remaining 4620 received placebo. Follow-up averaged 4.9 years. The simvastatin-ezetimibe group had 526 atherosclerotic events compared to 619 for the placebo group. Thus the combination of simvastatin and ezetimibe demonstrated a 17% reduction in such events. There was a significant reduction of non-hemorrhagic stroke with 131 in the treatment arm and 174 in the placebo group ($p=0.01$). Further, arterial revascularization procedures were performed in 284 patients in the treatment arm and 352 in the placebo arm and the difference was significant ($p=.0036$). Although fewer patients in the treatment arm

had a nonfatal myocardial infarction or coronary heart death (213) than in the placebo arm (230) the difference was not significant. The study showed no significant difference in events for patients on dialysis therapy compared to participants not on dialysis. Importantly, there was no signal for excess risk of cholelithiasis, cancer, hepatitis, myopathy, or death from non-vascular causes.

Adverse reactions include steatorrhea as a result of having more cholesterol in the intestinal tract. Headaches are also reported as well as myalgias. Hypersensitivity reactions may occur. Rarely, pancreatitis, hepatitis or cholecystitis have been reported. The possibility of rhabdomyolysis exists although it remains uncertain whether it was due to the ezetimibe, or the combination of statins and ezetimibe.

The ENHANCE clinical trial [52] was a study of patients with familial hyperlipidemia treated with either simvastatin 80 mg or simvastatin 80 mg plus ezetimibe 10 mg. This trial used a surrogate endpoint of carotid IMT measurements, carotid plaque and femoral artery plaque. In the simvastatin + placebo arm there were 256 patients who finished the study and in the simvastatin plus ezetimibe arm there were 281 patients completing the study. Study duration was approximately 2 years. The predefined primary outcome was change in ultrasound measurement of mean carotid IMT. Secondary outcomes were (1) the proportion of patients with new carotid artery plaques of >1.3 mm, (2) a change from baseline in mean maximal carotid artery IMT and (3) the change from baseline in the average IMT of the carotid and common femoral arteries. The predefined primary outcome was not significantly different between the two groups. With respect to baseline LDL-C, mean LDL-C of the simvastatin + placebo group was 319 mg/dl and for the combination therapy group 317 mg/dl. At study termination, LDL-C was 192.7 mg/dl in the simvastatin arm and 141.3 mg/dl in the combined therapy group. The LDL-C difference was statistically significant ($p < .01$). A slight but statistically significant increase in mean IMT of 9.5 μm occurred in the simvastatin only group and 12.1 μm in the combined therapy group ($p < 0.01$). Mean regression of carotid IMT was not statistically significant between groups. Plaque formation which the authors defined as an IMT >1.3 mm was not statistically different between groups. Interestingly, the starting carotid IMT measurement would be considered normal in many laboratories. This brings up the question of whether one can expect treatment to decrease carotid IMT if the starting IMT measurement falls within the normal range.

In the ENHANCE study adverse events that were considered related to treatment were similar in the 2 groups. Adverse events included discontinuation due to consecutive elevations of ALT and AST, increase in creatine kinase >10 times normal upper,, myopathy, and changes in vital signs or echocardiography.

If one subscribes to the proposition that lowering LDL-C by any means decreases cardiovascular events, ezetimibe added to simvastatin in the SHARP study appeared to improve outcomes. On the other hand, if one points to the ENHANCE study, ezetimibe did not “enhance” the outcome in patients with familial hyperlipidemia who started with mean carotid IMT values within the normal range. Accordingly, these two outcome studies have somewhat divergent results. The question of whether ezetimibe just lowers LDL-C or whether it improves outcomes remains open.

Hegele et al. [53] reported that 12.5% of individuals in their studies lacked the NPC1L1 common haplotype 1735C-25342A-27677T. Individuals who lacked this haplotype had significantly greater LDL reduction with ezetimibe. Specifically, about one subject in eight lacked the common NPC1L1 haplotype 1735C-25342A-27677T and these subjects had a significantly greater reduction in plasma LDL cholesterol with ezetimibe than subjects with at least one copy of this haplotype (−35.9+4.0 versus −23.6+1.6% reduction, $p=0.0054$). The authors demonstrated that monotherapy ezetimibe in patients without the common haplotype can reduce LDL by as much as 65%. Thus, ezetimibe is not restricted in this haplotype population to merely lowering LDL-C by 18% [50]. This finding probably has significance for this select group of patients.

Lomidipide

The Problem of Homozygous Familial Hyperlipidemia One concept for reducing LDL-C for individuals who do not respond adequately to statins by increasing LDL receptors would be to decrease production of LDL-C. One particular patient group that faces this problem consists of those with homozygous familial hyperlipidemia. Although that particular group may have a very limited capability to generate functional LDL receptors, they cannot produce enough LDL receptors to lower LDL-C to safer levels. These patients are characterized by early cardiovascular disease, considerable xanthomas, and, frequently early myocardial infarctions and subsequent death. This group of patients may respond to some extent to ezetimibe, statins, intestinal fat absorption inhibitors or fibric acids but these patients often require apheresis or even liver transplant. Thus, the goal would be to institute a medical therapy to reduce or eliminate the need for these more extensive measures. One possibility might be to decrease lipid input into lipoproteins. Theoretically, this could be accomplished by blocking microsomal triglyceride transfer protein (usually abbreviated MTP but probably better abbreviated MTTP).

Lipid Effects of Lomidipide Lomidipide decreases the production of apoprotein B lipoproteins by partially blocking MTTP. Dr Daniel Rader has pioneered this potential therapeutic area. A major impediment is that if lipids are not transferred into lipoproteins, those lipids will accumulate in the liver causing an increase in liver fat. While this problem of increased liver fat will probably not be completely compensated, the potential clinical importance of an MTTP inhibitor needed a clinical trial to determine the risk-benefit ratio of such a medication. One such MTTP inhibitor is lomitapide.

Clinical Trials of Lomidipide Lomitapide was tested in a phase 2 clinical trial in patients with homozygous familial hyperlipidemia and results warranted a phase 3 trial. The lomidipide phase 3 study was an open label, single arm, nonrandomized study of 78 weeks duration to test the safety and efficacy of this medication for

lowering LDL-C in patients with homozygous familial hyperlipidemia. The prespecified endpoint in this clinical trial was a significant reduction of LDL-C. The study enrolled 29 subjects, aged 18 years or older from Italy, South Africa, the United States and Canada. Diagnosis was based upon an untreated LDL-C of greater than 503 mg/dl and triglycerides >272 mg/dl and both parents having a history of untreated total cholesterol >251 mg/dl or documented mutations in both alleles of the LDL receptor or other genes known to affect LDL receptor function. Excluded patients included those with congestive heart failure, history of liver disease or transaminases greater than twice the upper normal limits, evidence of kidney disease, recent malignancy, alcohol or drug abuse, chronic lung disease or bowel disease such as malabsorption. Mean starting LDL-C was 336 mg/dl with patients already taking a variety of other medications or apheresis or both. At baseline most patients were treated with statins and ezetimibe. Additional treatments included Niacin (3), fibrates (1), and a bile acid sequestrant (1). Treatment with these medications was allowed to continue. Apheresis was used in 18 patients and this also continued as needed. The decrease in LDL-C with lomidipide was gradual during the first 18 weeks of the study and then stabilized. Over the full 78 week lomitapide protocol, LDL-C decreased 38%. Triglycerides decreased 31%. HDL-C, lipoprotein a, and apoprotein A-1 showed no statistical change at the end of the trial. Lomitipide dosing was increased incrementally during the study to improve tolerability from a beginning dose of 5 mg to the finally tolerated dose or a maximum of 60 mg. Median dose for the 23 patients who completed the study was 40 mg daily. Lomitipide dosage was adjusted depending on transaminase elevations or symptomatology. Liver lipid content was assessed by nuclear magnetic resonance spectroscopy at baseline and at 6-month intervals. Mean hepatic fat increased from baseline and stabilized at less than 10% by week 26 and then did not significantly further increase. 23/29 patients completed the full study. Six patients who did not complete the study terminated as a result of gastrointestinal problems (3), headache (1), noncompliance (1), and personal reasons (1). Mean ALT and AST increased immediately upon treatment with Lomitipide and then stabilized at approximately twice normal or less. At least one adverse effects occurred in approximately 92% patients and most were considered mild to moderate intensity. Gastrointestinal issues predominated as the adverse effect. Three of the original 29 patients had serious adverse effects including an acute coronary syndrome, angina, or lower respiratory infection. All of the three serious side effects were probably unrelated to therapy with lomitapide. Although 10 subjects had elevations of ALT and AST or both, no patient was discontinued from the study for this reason. Significant transaminase elevations were treated by decreasing the dose or temporarily discontinuing the medication. Three of four who had significant elevations were consuming alcohol at amounts greater than allowed by the protocol.

Based upon this phase 3 clinical trial, the FDA approved lomitapide in December 2012 for treatment of homozygous familial hyperlipidemia. Several different clinical perspectives can be derived from the lomidipide clinical trial. First, the differentiation line between heterozygous and homozygous familial hyperlipidemia

became somewhat blurred. Although most patients with homozygous familial hyperlipidemia have LDL-C in a very high range, one patient in this phase 3 trial had had genetically demonstrated homozygous familial hyperlipidemia with an LDL-C of approximately 180 mg/dl. Thus, clinical differentiation between the two forms is less possible than previously thought.

Another clinical perspective is that although lomidipide (like mipomersen) increases liver fat, the accumulation percentage in the lomidipide study did not significantly increase between 26 and 78 weeks of therapy, but the hepatic fat accumulation did occur as predicted on theoretical grounds. The importance and prognosis of this increase in liver fat over a much longer duration must be investigated. Finally, on the positive side, lomidipide provides an additional therapy for a very difficult disease to treat. Although side effects were considerable, most patients were able to complete the 78 week study. Accordingly, lomitapide provides a possible way to treat homozygous familial hyperliidpemia, although this therapy is very expensive and prone to side effects.

Mipomersen

Mipromersen (Kynamro) was approved by the FDA January 2013 for treatment of homozygous familial hyperlipidemia. This was the first anti-sense medication approved for any purpose in the United States.

Lipid Effects Mipomersen is an anti-sense oligonucleotide which interferes with apoprotein B synthesis. Accordingly, it decreases apoprotein B containing particles including LDL, lipoprotein a, and to a lesser extent, triglycerides.

Mechanism of Action Mipromersen, like lomidipide, is another drug for homozygous familial hyperlipidemia. Mipromersen works through a different mechanism that lomidipide, but both interfere with apoprotein B synthesis. The mechanism of action of mipomersen is to bind to messenger RNA to prevent apoprotein B synthesis and thus inhibit the synthesis of apoprotein B containing lipoproteins.

Clinical Trial Data Mipromersen was tested in a randomized, double-blind, placebo-controlled, multicenter trial involving 58 homozygous familial hyperlipidemia patients who were >18 years of age. Two reports of the clinical trial are available containing somewhat different data. The first was a publication in Lancet [54] which appears to be earlier results and the latter was published 6 months later and appears to have extended results [55]. Data from the latter will be presented here. The pre-specified endpoint was a significant decrease in LDL-C. These patients were all maintained during the trial on maximally tolerated LDL-C lowering therapy. Apheresis patients were excluded from this trial. Patients were treated with weekly subcutaneous injection of mipomersen 200 mg ($n=39$) or a placebo ($n=19$) for 26 weeks. For the placebo group, LDL increased 12.5%, apoprotein B

increased 11.4%, HDL increased 3.2%, triglycerides increased 26.6% and lipoprotein (a) fell 1.5%. For the mipomersen arm of the study the result was a 36% decrease in LDL-C, a similar % decrease in apoprotein B, HDL-C increased 5.8%. A mild decrease of 9.7% occurred for triglycerides and lipoprotein (a) decreased 32.7%. All of these changes reached statistical significance for the mipomersen group as compared to the control group except for the change in HDL-C. Although LDL-C in the mipomersen group fell quite significantly by the end of the study, 28% of mipomersen patients still met apheresis indications.

Adverse Effects of Mipomersen All mipomersen patients experienced at least one adverse event. Injection site reactions constituted 79% of these adverse reactions and included pain (59%), erythema (56%) and itching (33%). Mild to moderate flulike symptoms occurred 59 times in patients receiving mipomersen and 4 times in controls. Other less common adverse events included ALT elevations, hepatic steatosis, angina, and one acute myocardial infarction. Some degree of hepatic steatosis is expected since lipids that would ordinarily be incorporated into apoprotein B containing particles would mainly remain in the liver. Whether the observed cardiac symptomatology was secondary to mipomersen or the underlying homozygous familial hyperlipidemia is uncertain.

Although mipomersen had numerous side effects and did not bring 28% of subjects out of the range of apheresis, it did lower LDL-C 36% in a life-threatening condition. Although not discussed in the phase 3 study, subsequent discussions by the investigators indicated that many xanthomonas disappeared with treatment, and some of the injection site reactions and flulike symptoms were decreased or eliminated with anti-inflammatory medications given before the injection. It is clear that mipomersen is not an ideal medication, but it was certainly beneficial for most treated patients with familial homozygous hyperlipidemia. As this medication has moved from clinical trial to practical use, it is probable that much more will be learned about the efficacy and side effects.

Medications Showing Promise but Not yet FDA Approved Thus far in this chapter all pharmaceuticals have been approved by the FDA in the United States and are available for approved indications. Other medications are of considerable interest but still in clinical trials. In most instances, data is sparse regarding results since most of these preparations are in phase 3 clinical trials. Data will be presented regarding the current state of knowledge at the time of this writing.

Medications Showing Promise but Not yet FDA Approved Thus far in this chapter all pharmaceuticals have been approved by the FDA in the United States and are available for approved indications. Other medications are of considerable interest but still in clinical trials. In most instances, data is sparse regarding results since most of these preparations are in phase 3 clinical trials. Data will be presented regarding the current state of knowledge at the time of this writing.

Cholesterol Ester Transfer Protein Inhibitors

Epidemiologic data has demonstrated that low HDL-C is a risk factor for coronary artery disease [6]. An unanswered question is whether raising HDL-C by pharmaceutical means will decrease cardiovascular risk. This question remains unanswered even though several trials that raised HDL-C also changed non-HDL-C [12, 31–33]. Genetic mutations in cholesterol ester transfer protein (CETP) [56, 57] have been associated with elevated HDL-C. Accordingly, investigations were made to determine if raising HDL-C by CETP inhibitors would reduce risk. The first trial, ILLUMINATE [18], evaluated the outcome effect in 15,067 subjects treated with torcetrapib 60 mg + atorvastatin as compared to atorvastatin alone. Primary endpoints were cardiovascular outcome measures included death from coronary heart disease, nonfatal myocardial infarction, stroke, or hospitalization for unstable angina. The trial was stopped prematurely because of increased risk of death and cardiac events in patients taking torcetrapib. It is unclear whether the increased risk of death and cardiac events resulted from the use of the CEPT drug or from an off-target increase of 5.4 mm Hg in systolic blood pressure, a decrease in serum potassium, and increases in serum sodium, bicarbonate, and aldosterone. Investigation with torcetrapib has been terminated. Another CETP inhibitor, dalcetrapib, lacked the off target effects of torcetrapib. In the dalcetrapib clinical trial, patients who recently had an acute coronary syndrome were randomized to either 600 mg daily of dalcetrapib + standard medical care or a placebo and standard medical care. This trial was stopped prematurely because of lack of reduction in cardiovascular events [19] in the dalcetrapib arm of the study. It was unclear whether the result of the dalcetrapib trial was due to an increase of only approximately 25 % in HDL-C or whether raising HDL-C by a CETP inhibitor was ineffective in reducing cardiovascular outcomes. Currently, clinical trials are in progress utilizing two additional CETP inhibitors, evocetrapib and anacetrapib. The results of those two trials will be of interest but, currently, no data are available regarding those results.

PCSK9 Antibodies

Approximately a decade ago a loss of function of both alleles for PCSK9 (proprotein convertase subtilisin/kexin type 9), was discovered in the Dallas Heart Study which was associated with very low LDL-C. This began investigations regarding PCSK9 and it was learned that this protein attaches to LDL particles and when the LDL particle-PCSK9 combination attaches to the LDL receptor, the combined particle does not allow recycling of the LDL receptor. This process is further complicated by the fact that statins increase PCSK9 and this rise in PCSK9 probably accounts for the fact that the statin dose response for increasing LDL receptors is somewhat muted. Accordingly, the magnitude of PCSK9 causes fewer LDL eventual receptors and thus influences the statin effect on LDL-C. Since PCSK9 is a

protein, antibodies can be made against it. The last several years has witnessed the rise of numerous pharmaceutical companies developing antibodies against PCSK9 and subsequent clinical trials. Many initial clinical trials have confirmed the efficacy and safety of injecting these antibodies every two or four weeks. Phase 2 trials have demonstrated that these antibodies can lower LDL-C as monotherapy or in combination with a statin between 40–70 %. Currently, multiple phase 3 trials by pharmaceutical companies are in progress which will hopefully evaluate whether the use of PCSK9 will improve clinical outcomes and/or all cause mortality. These trials are not due to report on clinical outcomes for several years. Efficacy trials for lowering LDL-C have demonstrated marked increase in ability to lower LDL-C to < 100 mg/dl (or even < 70 mg/dl) even in patients with heterozygous familial hyperlipidemia. Also, the use of PCSK9 antibodies may become very important in treating patients who have adverse reactions to statins. Thus, injection of PCSK9 antibodies will probably change techniques for managing patients with excessive LDL-C. In most of these early clinical trials, subcutaneous injection in varying doses has yielded a dose response and the duration of effect appears to be 2–4 weeks. Most adverse reactions reported thus far are infections which occurred in approximately the same frequency in the experimental and placebo groups. The frequency of mild injection site reactions has been quite low. Currently, few phase 2 or early phase 3 studies have been published in peer-reviewed form, but presentations of results have occurred at meetings. Examples of presentations at the American College of Cardiology 2014 regarding Mendel-2, Rutherford-2, Laplace-2, Gauss-2, and descartes, all using [58] evolocumab as monotherapy or as combined therapy with a statin. LDL-C decreased 55–70 % respectively in those studies and lipoprotein (a) was also significantly decreased [59–61]. Further, other pharmaceutical companies are producing other antibody products which are early phase trials. Preliminary results and commentary are available on some of the pharmaceutical company websites. An example utilizing SAR236553, produced generally similar LDL-C results as evolocumab when used in combination with atorvastatin [62]. The outlook for this type of treatment appears promising. However, long-term outcome results and adverse reactions are extremely important and not yet available. Further, statins, niacin, and probably fibric acids have pleiotropic effects which monotherapy with PCSK9 will probably not provide. It seems likely that PCSK9 antibodies may substantially improve therapy for LDL-C in the future.

Medication for Cholesterol Ester Storage Disease (Sebelipase Alfa)

Cholesterol ester storage disease (CESD) is a rare recessive disease caused by mutation in the LIPA gene which encodes the enzyme lysosomal acid lipase (LAL). CESD is characterized by deficiency of LAL. In the past this condition had no approved therapy although cholesterol-lowering medications have been used with

definite lipid results, but these did not appear to substantially alter the outcome. CESD was characterized by increased LDL-C, increased triglycerides, elevated transaminases, hepatomegaly and frequently splenomegaly. CESD occurs (1) in infancy as a rapidly progressing condition called Wolmans disease and (2) a much slower progressing disease that is usually found in later childhood or in adults. Accumulation of cholesterol ester in hepatocyte lysosomes produces microvascular steatosis which may progress to cirrhosis and all of the complications of cirrhosis. Further, as a result of high LDL-C, cardiovascular complications also occur. Recently, a recombinant human LAL has been developed which is called sebelipase alfa. Sebelipase alfa is designed the infused slowly intravenously. The frequency of infusion is under investigation. A phase 2 study [63] demonstrated an early decrease in transaminases. Although LDL-C and triglycerides initially rose, they later fell below baseline levels. HDL-C was generally unaffected. Ferritin also fell with therapy. Adverse effects of the infusion were minimal. This phase 2 study of only nine patients was promising. A substantially longer study with a larger population is currently being conducted. Clearly, a long term outcome study is necessary to determine if sebelipase alfa can change the course of the liver disease as demonstrated by biopsy and sufficiently alter lipids to reverse or keep atherosclerotic changes at the entry level.

Apoprotein Clll Antisense Drug

Familial Chylomicronemia patients are characterized by extremely high levels of triglycerides and are subject to pancreatitis. No approved current treatment for this condition exists. This is a recessive rare condition estimated to have a frequency of approximately 1–2/million persons. The problem is that these individuals have defective apoprotein Cll, an activating cofactor for lipoprotein lipase, defective lipoprotein lipase, or excessive apoprotein C-lll. Apoprotein C-lll is an inhibitor of lipoprotein lipase. A recent open label phase 2 study was performed utilizing an anti-sense preparation of apoprotein C-lll for 3 subjects with fasting triglycerides >1400 mg/dl [64]. Results were a mean reduction of 69% for chylomicrons-triglycerides. Apoprotein C-lll reduction was 81% and mean HDL-C increased 78%. Adverse effects were limited to injection site reactions which were characterized as mild. In contrast to anti-sense injection of mipromersen, there was no flulike syndrome, significant elevation of liver enzymes or alterations in renal function.

Although familial chylomicronemia syndrome is quite rare, treatment for this condition would definitely meet a need. Patients with this condition frequently have multiple hospital admissions for pancreatitis. Lomidipide is another possibility for treating this condition, but no report studies have been performed to validate the possibility. It will be necessary to await the phase 3 study with the anti-sense preparation to determine more fully the efficacy and safety of this treatment.

Conclusions Regarding Non-Statin Therapy for Dyslipidemia As of this writing, almost 60 years have passed since the first report that niacin alters total serum cholesterol. During this time many reports have been written and many clinical trials accomplished. Perhaps the most consistent finding is that lowering LDL-C and/or non-HDL-C improves cardiovascular outcomes. This has been accomplished with cholestyramine, statins, apheresis and probably with niacin and gemfibrozil. Newer ways of reducing LDL-C have recently been approved with lomidipide and mipromersen for use in special situations. PCSK9 inhibitors show great promise for lowering LDL-C and even lipoprotein (a). There is still no definite proof that raising HDL-C by pharmaceutical means promotes improved outcomes. New approaches to very difficult genetic problems such as LAL deficiency and chylomicronemia are on the horizon. The future seems bright for further therapeutic advances.

A summary of non-statin lipid therapies is provided in Table 12.1.

Table 12.1 Summary of the major non-statin lipid therapies

Drug	Trade name	Dose in mg	common side effects
NIACIN			
Immediate release	Niacor	1000–3000	Flushing, itching, increases in glucose and uric acid
Extended release	Niaspan	500–2000	Flushing, itching, increases in glucose and uric acid
Long release	Enduracin	1500	ALT and AST elevation
BILE ACID SEQUESTRANTS			
cholestyramine	Questran	8–12 g twice daily	Constipation, loss of fat soluble vitamins
Colesevelam	Welchol	3750	Constipation, myalgia
FIBRIC ACIDS			
Gemfibrozil	Lopid	600 bid	Gastrointestinal symptoms, myalgia with statins
Fenofibrate	(several such as Tricor)	40–200	Liver enzyme increases, Gastrointestinal symptoms
OMEGA 3 FATTY ACIDS			
EPA + DHA	Lovaza	2000–4000	Mild gastrointestinal symptoms, antiplatelet effects
EPA	Vascepa	2000–4000	Arthralgia
EZETIMIBE	Zetia	10	Diarrhea, arthralgia, muscle pain
LOMIDIPIDE	Juxtipid	up to 60	Gastrointestinal symptoms, ALT and AST elevation, back pain
MIPOMERSEN	Kynamro	200 SQ weekly	Flu symptoms, ALT and AST elevation, headache

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Chapter 13
LDL Apheresis

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Introduction and Background

Low density lipoprotein (LDL) apheresis is a procedure used primarily to treat patients with Familial Hypercholesterolemia (FH). Patients with this disease generally have mutations to the LDLR gene, which codes for the LDL receptor protein. The LDLR gene is located on the short arm of chromosome 19 (19p13.1-13.3). This gene codes for a protein which normally aids in the removal of LDL from the blood stream. LDL levels are inversely proportional to the activity of the LDL receptor protein. Some patients may have a mutation in the ApoB gene, which codes for Apo lipoprotein B, a part of the LDL molecule that acts as a ligand to the LDL receptor protein. This gene is located on the second chromosome (2p24-p23) [1]. The incidence of heterozygous FH is 200/100,000/year, and homozygous incidence is 1/1,000,000/year. High levels of LDL and Apo lipoprotein B are known to be high risk markers for atherosclerotic disease. Patients with a single mutation may have elevated LDL levels and increased risk of atherosclerotic disease by the fourth to fifth decade. Those with homozygous mutations are at high risk for cardiovascular events as early as childhood [2]. Generally, heterozygous patients tend to respond to medical treatment with medication and dietary changes. While homozygous patients may benefit from high doses of cholesterol lowering agents and dietary regulation is standard, their response to treatment is often poor.

LDL apheresis has been found to be useful in the treatment of these homozygous FH patients and patients with severe heterozygous FH. The U.S. Food and Drug Administration (FDA) approved indications for LDL apheresis include all patients with homozygous FH. In addition, patients with heterozygous FH who have failed a 6-month trial of dietary modification and maximal drug therapy with LDL levels >300 mg/dL, or who have LDL levels >200 mg/dL with

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documented coronary artery disease meet FDA criteria for indicated treatment [3]. The American Society for Apheresis (ASFA) puts LDL apheresis for homozygous FH in category I, “Disorders for which apheresis is accepted as first-line therapy, either as a primary stand alone treatment or in conjunction with other modes of treatment”; recommendation grade 1A, “Strong recommendation, high-quality evidence”. Heterozygotes are placed in category II, “Disorders for which apheresis is accepted as second line therapy, either as a stand alone treatment or in conjunction with other modes of treatment”, also with a recommendation grade of 1A [4].

Diagnostic criteria of FH (Simon Broome Register Group definition) includes: total cholesterol of > 290 mg/dL in adults or > 260 mg/dL in children under 16 years of age; LDL cholesterol of > 190 mg/dL; tendon xanthomas in patient or close relative; or DNA evidence of LDL receptor mutation or ApoB-100 familial dysfunction [1]. Untreated homozygous FH patients may have cholesterol in the range of 650–1,000 mg/dL, xanthomas by the age of 4 years and death from coronary artery disease by age 20. Heterozygotes have a similar, but delayed course, with cholesterol levels in the range of 250–550 mg/dL, xanthomas by age 20, and atherosclerosis by age 30. All patients with cholesterol concentrations greater than 200 mg/dL are at increased risk for coronary artery disease. The risk doubles when the value rises to between 200–250 mg/dL, and increases fourfold at values of 250–300 mg/dL. Those patients with FH, both homozygous and heterozygous, are at significantly higher risk than the general population [5].

The first trials using apheresis for FH were carried out in France in the late 1960s by DeGennes. During the 1980s and 1990s, evidence that plasma exchange could lower LDL cholesterol levels and even cause atherosclerotic plaques to regress in homozygous and severe heterozygous FH patients was presented. In the early days of apheresis for treatment of FH patients, it took the form of plasma exchange. By the late 1990s, double-membrane (cascade) filtration was introduced with the advantage of avoiding albumin and other plasma protein loss. Concurrently, methods were being developed to specifically remove LDL cholesterol by adsorbtion. In 1975 Lupien in Canada, introduced a batch adsorbtion system using heparin agarose beads in a plastic bag. Several iterations of the adsorbtion principal were developed in the following decade, culminating in the introduction of the anti-LDL antibody column developed by Stoffel and carried out by Borberg in 1988. Pokrovsky added a mono-clonal antibody to his column in 1995, enhancing specificity of LDL adsorbtion [6]. Currently, two lipid apheresis systems have received approval from the FDA for marketing. In February 1996, a dextran sulfate device “Liposorber LA-15® System” (Kaneka Pharma, New York City, NY) was approved, and in September 2007, a heparin-induced extracorporeal LDL precipitation “HELP® System” (B. Braun, Melsungen, Germany) was added [7].

Methods of LDL Apheresis

The systems available for LDL apheresis in the United States are the heparin extracorporeal LDL precipitation (HELP), and the dextran sulfate (DS) adsorption methods. Each system reliably reduces LDL cholesterol by approximately 60–70 %, and triglycerides by 40 % per treatment [1]. The heparin precipitation method is based on the concept that apoB containing lipoproteins, including LDL cholesterol (LDL-C) and Lp(a), are precipitated by heparin in an acidic environment, while the dextran sulfate adsorption method takes advantage of the ability of negatively charged ligands to bind the positively charged apoB containing lipoproteins. Based on volume of plasma treated, the HELP system is slightly more efficient than the DS method; HELP results in LDL-C reduction of 25 %/L, DS in 21 %/L [8].

The HELP system is manufactured by the German company Braun-Melsungen. Plasma is initially separated from whole blood by filtration then mixed with heparin at a pH less than 5. The LDL cholesterol precipitates and is subsequently removed by a second filtration step. Any remaining heparin as well as the acidic buffer solution are removed by heparin adsorption and dialysis with a bicarbonate solution. The treated plasma is returned to the patient, along with the cells from the initial separation step. Each treatment is performed using a disposable kit containing the filters, heparin adsorber and dialyzer. In addition to reductions in LDL cholesterol, HELP results in up to a 15 % reduction in HDL. HELP also removes fibrinogen, and weekly treatment has been reported to lead to a more than 50 % decrease in fibrinogen concentration. Despite the observed reduction in fibrinogen, bleeding complications have not been observed [9]. Nevertheless, it is recommended that treatment be limited to 3 L of plasma, to avoid further loss of fibrinogen. It has also been shown that HELP reduces circulating levels of other prothrombotic and pro-inflammatory molecules, including tissue factor, homocysteine, c-reactive protein and soluble vascular adhesion molecule [1]. It has been suggested that the reduction in these plasma constituents might contribute to the positive effects of lipid apheresis on progression of coronary artery disease. Additional studies are required to explore these relationships [10].

The dextran sulfate system, manufactured by Kaneka and marketed as the Liposorber LA 15, consists of two parallel columns containing dextran sulfate (DS) attached to cellulose beads. Following separation of plasma by filtration from the cellular components of whole blood, 500 mls of plasma is passed over one column. Cholesterol containing compounds are bound to dextran sulfate with high affinity, and saturation occurs after exposure to 500 ml of plasma. Patient plasma is then routed to the second column while the first column is rinsed with hypertonic saline followed by lactated ringer's solution to regenerate its lipid binding capacity. Treated plasma is mixed with the previously separated blood cells and returned to the patient. The amount of LDL absorbed is related to the amount of plasma treated, and generally at least one plasma volume is treated at each session. Patients undergoing LDL apheresis with the Liposorber must not take an angiotensin converting enzyme inhibitor (ACEi) drug for at least 24 h prior to treatment. ACE inhibitors

also inhibit the enzymes necessary for catabolism of bradykinin that accumulates as a result of activation of the kinin system as plasma passes over the DS column. Unopposed bradykinin results in flushing, bradycardia, hypotension and dyspnea.

Both systems have demonstrated efficacy in LDL lowering, and have excellent safety profiles, however minor adverse events have been reported in 3–10 % of patients, most commonly bleeding after the procedure, vomiting, hypoglycemia and hypotension [11]. Our institution has experience with both operating systems, and while the DS method is associated with more unpleasant side effects for a minority of patients (abdominal discomfort, flushing), it is easier to operate and ultimately preferred by our nurses. Both systems require anticoagulation with heparin, and patients with sensitivity to heparin cannot be treated safely with either method.

Vascular Access

Vascular access is often challenging, especially in the newly diagnosed homozygous FH patient, who is typically a young child. The peripheral veins in a small child may not be large enough to accommodate needles of the size necessary to sustain apheresis blood flow rates. In addition, a small child is often not able to cooperate for the full duration of the treatment due to fear, discomfort, or maturity. For young children and small adolescents, a central venous catheter is generally required. A 7 French size is suitable for small children and a 9 French may be used in larger children. Placements in the subclavian, internal jugular, or femoral site as well as insertion technique (percutaneous or tunneled) considerations are similar to those encountered in adults. Adults and adolescents will vary according to age, gender, and size. However, many are able to tolerate peripheral vascular access via the antecubital veins. A 17-guage or larger needle is required for the draw line, and a 19-guage or larger is needed for the return [12]. Many patients are able to sustain use of peripheral veins every 2 weeks for years. Arterio-venous fistula is a consideration for patients whose veins are not adequate for peripheral access. Fistula construction is generally only considered for adolescents and older due to the small size of younger children. As with all apheresis procedures, the central venous catheter carries the majority of the risk to the patient. Therefore, avoidance of the catheter when possible is preferred.

Frequency of Treatment

Currently the most widely accepted and practiced frequency for LDLa is every 2 weeks. However, ASFA recommends a frequency of once every 1–2 weeks, adjusted to reduce the time averaged LDL cholesterol by ≥60 % [4] (see below). In addition, studies have shown that selective removal of LDL on a weekly schedule can reduce or at least stabilize the progression of atherosclerosis [13], therefore patients may benefit from more frequent sessions.

Efficacy of Treatment

Nearly 40 years ago, Thompson et al described the successful use of plasma exchange to treat homozygous Familial Hypercholesterolemia (FH) and its beneficial effects in atherosclerotic disease [14, 15]. They reported regression of cutaneous and tendinous xanthomas as well as slower rate of progression of atherosclerotic disease. While there were no randomized controlled studies, a small study did examine efficacy of plasma exchange by comparing individuals undergoing plasma exchange to siblings who did not receive treatments. Plasma exchange was undertaken in five patients with homozygous familial hypercholesterolaemia at intervals of two weeks for a mean of 8.4 years. These patients had survived an average of 5.5 years longer than their five respective homozygous siblings ($p = 0.3$), each of whom were presumed to have a matching genetic defect but who died untreated. The 37% decrease in peak serum cholesterol concentrations maintained by plasma exchange presumably reduced progression of atherosclerosis in the treated patients and thus lessened their risk of premature death [16], however they did not include specific data regarding cardiovascular status of each patient.

As previously mentioned, over the last three decades, selective methods for removing LDL have been developed ranging from precipitation of LDL through addition of heparin to plasma to dextran sulphate cellulose adsorption (DSA) columns [17, 18]. LDL apheresis has now largely replaced plasma exchange as a means of treating patients with drug-refractory hypercholesterolaemia, many of whom have homozygous FH, and is now recognized as the treatment of choice for the latter disorder [19]. However, guidelines regarding the level of plasma cholesterol which need to be achieved to prevent cardiovascular disease in such patients are lacking. In addition, while (LDL) apheresis effectively lowers LDL cholesterol in the short term, there is little published information on the long-term efficacy of this treatment.

Two separate groups have reported their experiences with long-term LDL apheresis in children with FH [20, 21]. Hudgins et al. analyzed effects of LDL apheresis in 29 children over an 11 year period and showed that the procedure was well tolerated and systemic adverse events were uncommon. While they were able to effectively lower LDL cholesterol per session, they were not able to achieve predicted target LDL levels, and 30% of patients progressed to more severe symptomatic disease, including some patients who did not have baseline atherosclerotic disease [21]. A different study completed by a French group also demonstrated adequate LDL cholesterol reduction per session (72+/−10%) and disappearance or regression of tendinous xanthomas in 62% of the children. However, over 18% of the children did have a cardiovascular event during the course of LDL apheresis treatment [20].

Similar trends have been seen in adults. A multicenter study of 19 patients (12 males; 7 females; aged 53.8+/−9.3 years), regularly treated on average every 10.1+/−2.6 days, did show significant decrease in LDL per session. In 5.5% coronary artery disease recurred despite treatment with LDL apheresis, however in 94.5% of the patients the lesions were stable over 3.1+/−2.7 years [13].

These studies show that LDL apheresis is well tolerated and effectively lowers LDL cholesterol, however some patients will show progression of cardiovascular disease. In order to best determine the efficacy of LDL pheresis, fluctuations in lipid levels between treatment sessions must be considered. In 2000 Kroon et al. devised an equation to calculate the interval mean of LDL levels between sessions. The interval mean LDL concentration (C) was calculated using equation; Cmean = Cmin + K(Cmax-Cmin) [22], where K is the rebound coefficient K. The initial coefficient developed was for heterozygotes and likely not applicable to homozygotes and could possibly lead to an overestimation of the interval mean and underestimation of the efficacy of LDL apheresis in homozygous population. As the a result, Thompson et al [23] further analyzed Kroon's equation and considered differences between FH heterozygotes and homozygotes to estimate rebound coefficient K (homozygous = 0.65 and heterozygous = 0.71), which allows for more accurate assessment of interval LDL mean in each patient population.

The American Society for Apheresis recommends a goal of > 60 % reduction in time averaged (interval mean) LDL from the pre apheresis level [4]. However, even with the alteration made to the rebound coefficient K, in our experience we have not been able to achieve the predicted decrease in interval mean. Over the last 10 years, we have treated 14 patients with LDL apheresis and while we observed the predicted per session reduction in LDL concentration of 70 % on average in our patients, we did not achieve the recommended goal of 60 % reduction of the interval mean LDL concentration in our FH patients. We suggest that this may be due to the observation that pre treatment LDL concentration did not decrease over time and only by achieving a marked reduction in pre treatment LDL concentration will the desired time averaged reduction in LDL be attainable. Nonetheless, LDL levels are decreased appropriately per session and our patients have been clinically stable during the course of LDL apheresis. Therefore, perhaps the equation developed by Kroon et al. may be flawed by using rebound coefficients that don't take into account variations in patient populations. While challenging, daily or every other day measurements of LDL levels between apheresis sessions is the best way to determine interval mean LDL concentrations. In addition, as mentioned above more frequent or weekly LDL apheresis may be the most reliable means to reduce pre treatment LDL or interval mean LDL concentration in these patients if other strategies such as medication, diet and lifestyle modifications are ineffective.

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Chapter 14
Dyslipidemia in HIV

Michael P. Dubé

Introduction

Dyslipidemia is a common problem affecting a large proportion of patients infected with the human immunodeficiency virus (HIV). Antiretroviral therapy (ART), particularly with certain implicated agents, may cause or considerably worsen pre-existing dyslipidemia. Improvements in ART have resulted in continual improvements in the long-term prognosis persons infected with HIV. There have been marked reductions in opportunistic infections and improved overall survival where ART is available. However, ultimately these individuals are at increased risk for other diseases that are associated with aging, such as cardiovascular disease (CVD) and diabetes mellitus, in addition to HIV-related and treatment-related issues. Evaluation and treatment of lipid disorders and management of other cardiovascular risk factors has become increasingly important during clinical care of patients living with HIV.

Increased Cardiovascular Disease in HIV

Both HIV infection itself [1, 2] and ART [3–6] contribute to the increase in CVD events among HIV-infected individuals. Treatment-induced lipid disorders [5] are partially responsible for this greater risk, although other mechanisms may also be involved [7] including endothelial dysfunction [8, 9] and heightened inflammation and immune activation [10–16]. Other risk factors for CVD which are also more prevalent among HIV-infected patients than in the general population include

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insulin resistance [17], diabetes mellitus [18], and cigarette smoking [19]. More recent data, however, suggest that CVD risk is decreasing among the HIV infected population [20]. This may be due to greater recognition of the problem, use of more metabolically friendly ART regimens, and better application of interventions to reduce CVD risk [21]. In general, most experts recommend that guidelines developed for CVD prevention and evaluation and treatment of lipid disorders in the general population should also be applied to HIV-infected individuals [22]. Importantly, drug-drug interactions are a critical consideration when prescribing lipid-lowering drugs to patients with HIV who are receiving ART.

Lipid Disorders in Untreated HIV Infection

Prior to the availability of effective ART, the presence of multiple lipid abnormalities in untreated HIV-infected patients were well-described [23, 24]. Increased serum triglycerides along with reduced total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol levels characterized more advanced HIV disease and greater degrees of immunosuppression [24]. In addition, there is a tendency for the lipoprotein particle composition to be more atherogenic, with higher proportions of small, dense LDL particles [25]. Following HIV seroconversion, there are considerable decreases in total, HDL, and LDL cholesterol [26]. The mechanism of these generalized decreases in cholesterol fractions, as well as the increases seen in triglycerides levels with disease progression, is presumed to be due to a generalized inflammatory state associated with chronic viral infection and is associated with immune activation [24, 27–29].

Low levels of HDL cholesterol are particularly prevalent during HIV infection [29–31]. Treatment of HIV with effective ART generally results in modest improvement in low HDL cholesterol levels, regardless of which regimen or agents are chosen [32–34]. Increases in total and LDL cholesterol also occur [26, 30, 35] but these levels generally remain lower, or comparable to, HIV uninfected controls. In part, this represents a general return-to-health phenomenon, but many ART drugs have direct effects on lipid metabolism (see below HIV TREATMENT-ASSOCIATED LIPID DISORDERS). The greatest increases in HDL cholesterol after ART initiation tend to occur with use of certain non-nucleoside reverse transcriptase inhibitors (NNRTIs) [36]. However, HDL cholesterol levels typically do not return to normal levels even with prolonged ART [30] and are associated with increased CVD risk [31].

HIV Treatment-Associated Lipid Disorders

It is critical to recognize that different antiretroviral agents and different combination regimens may have much different effects on lipids. As such, it becomes difficult to correctly assign a “class effect” for any particular ART drug class. Instead

Table 14.1 Lipid changes commonly associated with antiretroviral drugs

Increased triglycerides
Increased total cholesterol
Increased VLDL cholesterol
Increased LDL cholesterol (less common)
Increased proportion of small, dense LDL particles
Increased HDL cholesterol (but levels do not normalize)

it is often more appropriate to consider each individual agent within a class as a unique entity (see sections on individual drug classes). Lipid disorders that commonly experienced in general with ART use are shown in Table 14.1.

Protease Inhibitors

Many, but not all, studies implicate protease inhibitor (PI) use as increasing cardiovascular risk [5, 37–39]. However, PI-associated dyslipidemia explains only part of this increased risk [5] and other PI-related factors such as endothelial dysfunction due to reductions in NO availability [7, 40] and increased macrophage cholesterol uptake [41] may contribute.

PI-associated dyslipidemia is multifactorial and has been associated with multiple hepatocyte, adipocyte, and endothelial enzyme abnormalities [41–46]. Increased triglyceride and VLDL cholesterol levels as a direct result of PI use tend to predominate over increased LDL cholesterol levels [33]. Importantly, although infrequently used clinically, combinations of PIs and NNRTIs are additive with regards to cholesterol increases [33, 47]. Isolated elevations of LDL cholesterol levels, however, appear to be no more prevalent in HIV than in the general population [30]. HDL cholesterol levels do tend to increase in patients who initiate treatment with PIs, but generally these levels do not normalize [32, 33]. HDL cholesterol increases tend to be lesser with the PI drugs than with the NNRTI class (see section below on non-nucleoside reverse transcriptase inhibitors).

Hypertriglyceridemia is very common with certain PIs, particularly with full doses of ritonavir [48, 49]. However, this is a drug that currently is used only at much lower doses solely as a pharmacokinetic enhancing agent to boost the levels of a concurrently administered PI. When used in these low doses, ritonavir itself contributes only modestly to hypertriglyceridemia [50]. Currently used PIs vary in their tendency to induce hypertriglyceridemia. Tipranavir-ritonavir [51], lopinavir-ritonavir [33, 51], fosamprenavir-ritonavir [51] have the greatest effects on lipids. Indinavir-ritonavir [52], and nelfinavir [32] (a PI that is used alone and not boosted with ritonavir) tend to have intermediate effects. Saquinavir-ritonavir [52, 53] and the newer and recommended first line PIs atazanavir-ritonavir [54] and darunavir-ritonavir [53, 55] appear to have relatively little direct effect on lipid concentrations. These latter drugs have frequently been substituted for older PIs when dyslipidemia

Table 14.2 Relative tendency for commonly used individual ART drugs to induce dyslipidemia

	Protease inhibitors	Nucleoside reverse transcriptase inhibitors	Non-nucleoside reverse transcriptase inhibitors
High	Fosamprenavir[a]-ritonavir Lopinavir-ritonavir Tipranavir-ritonavir	Stavudine	–
Intermediate	Indinavir[a]-ritonavir Nelfinavir[b]	Didanosine Zidovudine[c]	Efavirenz[g]
Low	Atazanavir*-ritonavir Darunavir-ritonavir Saquinavir-ritonavir	Abacavir[d] Tenofovir[e] Emtricitabine[e] Lamivudine[f]	Nevirapine Rilpivirine[h]

[a] These drugs are usually, but not always, prescribed with ritonavir boosting.
[b] Nelfinavir is not administered with ritonavir boosting.
[c] Also a component in the combination pills Combivir (zidovudine-lamivudine fixed dose combination) and Trizivir (zidovudine-lamivudine-abacavir fixed dose combination).
[d] Also a component in the combination pills Trizivir and Epzicom (lamivudine-abacavir fixed dose combination).
[e] Also a component in the combination pills Atripla (efavirenz-tenofovir-emtricitabine fixed dose combination), Truvada (efavirenz-tenofovir-emtricitabine fixed dose combination), and Stribild (elvitegravir-cobicistat-tenofovir-emtricitabine fixed dose combination).
[f] Also a component in the combination pills Trizivir, Epzicom, and Combivir.
[g] Also a component in the combination pill Atripla.
[h] Also a component in the combination pill Complera (rilpivirine-tenofovir-emtricitabine fixed dose combination)

is a problem [56–58]. The general tendencies of different PIs to perturb lipid levels are shown in Table 14.2.

The use of lopinavir-ritonavir and the infrequently-used PI indinavir are associated with an increased incidence of myocardial infarction, even after controlling for lipid levels [59]. There was no such increase with the PIs saquinavir, nelfinavir [59], or atazanavir [60]. There is yet insufficient data with darunavir-ritonavir in order to evaluate its independent contribution to CVD risk. It is reasonable to avoid the use of PIs that have been shown to increase myocardial infarction risk (indinavir and lopinavir-ritonavir) in those individuals with multiple other CVD risk factors—but only when other potent alternative ART regimens exist.

Nucleoside Reverse Transcriptase Inhibitors

The nucleoside reverse transcriptase inhibitor (NRTI) stavudine tends to result in higher lipid levels than regimens without it [32, 61]. The NRTI tenofovir has few adverse lipid effects [61] and may have a modest lipid-lowering effect in itself. The NRTI abacavir has slightly more adverse lipid effects than tenofovir [62, 63]. The use of the NRTIs abacavir and didanosine has been associated with increased myocardial infarction risk [4], but this is likely due to their non-lipid effects such as endothelial dysfunction [64] and abnormal leukocyte-endothelial cell interactions [65, 66]. Lamivudine and emtricitabine also appear to have relatively few effects on lipid levels. The general tendencies of different NRTIs to perturb lipid levels are shown in Table 14.2.

HIV lipodystrophy is a prevalent condition (present in up to 50% of treated patients in some cohorts) that is associated with use of certain older NRTIs, primarily the thymidine analogs stavudine and zidovudine (reviewed in [67]). Both subcutaneous fat loss (lipoatrophy) and central fat accumulation (lipohypertorphy) may complicate treatment for HIV infection but these two conditions are not thought to be linked [68]. Patients with lipodystrophy tend to have greater dyslipidemia, insulin resistance, and glucose intolerance [18, 69] and the presence of lipodystrophy alone may increase the risk of myocardial infarction [4] and is associated with endothelial dysfunction [70]. Therefore, individuals with lipodystrophy often have a cluster of metabolic abnormalities which closely resembles the metabolic syndrome, and thus this group of patients may require particular attention to intervening for cardiovascular risk factors. Despite their close association with lipoatrophy, use of the thymidine analogs stavudine and zidovudine has not, however, been linked to an increased risk of myocardial infarction [4]. Fortunately, with more contemporary regimens in the current treatment era where thymidine analog use is no longer recommended for initial therapy [71], lipoatrophy has become distinctly less common [72].

Non-nucleoside Reverse Transcriptase Inhibitors

The non-nucleoside reverse transcriptase inhibitors (NNRTIs) efavirenz and nevirapine result in substantial increases in HDL cholesterol levels, often in the range of 8–10 mg/dL [36]. The mechanism of HDL cholesterol increase with these drugs is via increased apolipoprotein A-I production without affecting HDL catabolism [73]. Despite modest increases in LDL cholesterol and non-HDL cholesterol levels with NNRTIs, the resultant total/HDL cholesterol ratios either do not increase or decrease [32, 36]. Thus, the overall impression has been that use of the NNRTI class does not result in a more atherogenic lipid profile nor has their use been associated with an increased incidence of myocardial infarction [5]. The newer NNRTI rilpivirine has lesser lipid effects than efavirenz but has a greater risk of virologic failure in patients with high HIV RNA levels [74]. The general tendencies of different NNRTIs to perturb lipid levels are shown in Table 14.2.

Other Antiretroviral Agents: Integrase Inhibitors, CCR5 Antagonists, HIV Entry Inhibitors

Most drugs from the newer drug classes that are increasingly used to treat HIV are all relatively lipid neutral. These include the HIV integrase inhibitors raltegravir (Isentress), elvitegravir (included in the combination tablet Stribild, pharmacologically boosted by the non-PI drug cobicistat and also containing tenofovir-emtricitibine), and dolutegravir (Tivicay) as well as the CCR5 chemokine receptor blocker maraviroc (Selzentry).

Substituting one of these drugs can be expected to improve the lipid profile in patients who are currently receiving other drugs such as PIs having less favorable lipid changes [75, 76]. Such a change should only be done in consultation with an experienced HIV clinician with detailed knowledge of the patient's past HIV treatment history and HIV resistance profiles because this type of ART substitution can lead to virologic failure [75]. The effects of such a change on risk of cardiovascular events is unknown however, so there is currently no evidence that substituting one of these agents for older drugs in a regimen will be of any CVD benefit. Indeed, switching from efavirenz to the integrase inhibitor raltegravir did not improve endothelial function and led to increased levels of soluble CD163 [77], a marker of monocyte activation that is closely associated with atherosclerosis in patients with HIV [78].

Evaluation and Therapeutic Options

Evaluation of CVD risk and therapy for increased risk among HIV-infected patients should generally follow guidelines as recently proposed by the American College of Cardiology/American Heart Association Task Force on Practice Guidelines [79, 80]. However, there are important caveats regarding choice of lipid-lowering drugs (see below) [81]. In the current ACC/AHA guidelines, there is consideration of certain factors that may increase the likelihood that a statin will be of benefit, including elevated hsCRP, family history of CVD, abnormal ankle-brachial index, and a significant level of coronary calcium [79]. Although the evidence basis for statin benefit is not as strong in this clinical setting, HIV infection may represent a chronic inflammatory condition whose presence can be considered as an additional CVD risk factor that could tip the balance towards statin treatment (author opinion). An HIV-specific 5-year risk calculator has been developed [82] (available at http://www.cphiv.dk/TOOLS/DADRiskEquations/tabid/437/Default.aspx). Whilst this equation may perform better than the Framingham risk equation in patients with HIV [82], the role of this calculator within the new ACC/AHA construct is not established.

Fasting lipid profiles are essential because of the high prevalence of hypertriglyceridemia in this population. Lipids should be measured before and again within 6 months of initiating ART [22, 83]. Diet and exercise interventions are also likely

to be effective in patients with HIV [84–88]. In general, lipid lowering drugs work as effectively, or only slightly less effectively, for patients with HIV compared to the general population [89].

Switching Antiviral Therapies

Switch strategies have the potential advantage of avoiding pharmacologic intervention to address lipid elevations. In patients with a favorable treatment history as determined by an experienced HIV clinician, switching from a potentially lipid-raising PI to nevirapine [90], abacavir [91], or the lipid-friendly PI atazanavir [56, 63], or the integrase inhibitor raltegravir [76, 77] may be preferable to using a lipid-lowering drug. In practice, however, many patients will have pre-existing drug resistance that limits these switch options. Although lipid values will improve, there is an increased risk of virologic failure when the integrase inhibitor raltegravir is substituted for ongoing successful PI therapy with lopinavir-ritonavir [75]. This unexpected study finding emphasizes the risk and potential complexity of switch strategies. Experienced HIV specialists must always balance the risks of new treatment-related toxicities and the possibility of virologic relapse when switching ART to the risks of drug interactions and new toxicities from lipid-lowering agents.

Choice of Statin

Because many antiretroviral drugs are either inducers (NNRTIs) or suppressors (PIs, cobicistat) of CYP 3A4 and other hepatic drug metabolizing enzymes, pravastatin is often preferred in HIV-infected patients [81]. When a more potent statin is needed, atorvastatin, rosuvastatin, and pitavastatin can be considered. Simvastatin and lovastatin are options only when HIV PIs are not being used. Because of modest drug interactions, lower doses of atorvastatin should be used ($\leq$20 mg/day) when it is used with PIs [81, 92]. Rosuvastatin is not metabolized by CYP 3A4, but major interactions may still occur [93–95] (Table). The newest agent pitavastatin is also not metabolized by CYP 3A4, but drug-drug interaction are limited while data presented in abstract form appearing promising in HIV infected subjects receiving the maximum approved dosage [96]. There is no evidence that statin-related skeletal muscle toxicity tends to be more frequent in HIV-infected patients. However, simvastatin and lovastatin are absolutely contraindicated with PIs due to markedly increased statin blood levels [97] and risk of rhabdomyolysis when coadministered with PIs [98]. Statin-PI interactions are summarized in Table 14.3.

Suggested statin doses that correspond to the high, moderate, and low intensity treatment categories recommended for the general population [80] are shown in Table 14.4. Because of the variability of statin-PI drug interactions, it may be wise for initial therapy to choose a dose in the moderate intensity category before escalating

Table 14.3 Selected statin interactions with antiretroviral drugs

Simvastatin	Extensive CYP3A4 metabolism, marked ↑ levels with PIs and is contraindicated; ↓ 60% levels with efavirenz
Rosuvastatin	Not CYP3A4 metabolized but 2–3 × ↑ levels with lopinavir-ritonavir and atazanavir-ritonavir, use care if exceeding 10 mg; 1.5 × ↑ levels with darunavir-ritonavir. Low starting doses (5 mg) recommended with PIs
Fluvastatin	Metabolized by CYP2C9, possible interactions with nelfinavir and efavirenz
Atorvastatin	Some CYP3A4 metabolism, 3–6 × ↑ levels with PI including darunavir-, lopinavir-, or saquinavir-ritonavir; 1.5 × ↑ with fosamprenavir; ↓ 35% with efavirenz. Maximum dose of 20 mg/d when used with PIs
Pravastatin	No significant p450 interactions, primarily renal excretion *but* 50%↓ with lopinavir-ritonavir; 45% ↓ with nelfinavir; 80% ↑ with darunavir-ritonavir; ↓ 40% with efavirenz
Pitavastatin	No significant p450 interactions; levels ↑ 31% with unboosted atazanavir; 26% ↓ with darunavir-ritonavir; 20% ↓ with lopinavir-ritonavir. No data with atazanavir-ritonavir or efavirenz

Table 14.4 Suggested intensity of statin therapy in HIV while receiving ART

High	Moderate	Low
PI-or cobicistat containing regimens		
Atorvastatin 20 mg	Atorvastatin 10 mg	Pravastatin 10–20 mg
Rosuvastatin 10–20 mg	Rosuvastatin 5 mg	Fluvastatin 20–40 mg
	Pravastatin 40–80 mg*	Pitavastatin 1 mg
	Pitavastatin 2–4 mg	
NOTE: *Simvastatin and lovastatin are absolutely contraindicated in patients receiving PIs or cobicistat* *With darunavir, pravastatin dose should be 20–40 mg		
NNRTI, raltegravir or dolutegravir-based regimens (only those regimens that do not *contain a PI or cobicistat*)		
Atorvastatin 40–80 mg	Atorvastatin 10–20 mg	Pravastatin 10–20 mg Fluvastatin
Rosuvastatin 20 mg	Rosuvastatin 10 mg	20–40 mg
	Pravastatin 40–80 mg	Pitavastatin 1 mg
	Pitavastatin 2–4 mg	Lovastatin 20 mg
	Lovastatin 40 mg	Simvastatin 10 mg
	Simvastatin 20–40 mg	

to a high intensity statin dose. Although LDL cholesterol goal directed statin treatment is no longer recommended [80], for patients with HIV receiving CYP3A4 inhibitors such as PIs and cobicistat, it may be reasonable to adjust an individual's statin dose to achieve a target LDL cholesterol reduction that falls within the high (~50% reduction), moderate (30 to <50% reduction), or low intensity treatment categories.

Non-Statin Drugs

The new ACC/AHA guidelines do not recommend non-statin therapies for routine use in CVD prevention [80]. It is reasonable to follow these recommendations in patients with HIV. Data with non-statin therapies do exist in the HIV infected population, and these can be considered if statins are contraindicated or poorly tolerated. Ezetimibe appears to be effective for LDL cholesterol lowering in HIV-infected patients [99–101]. Extended-release niacin is generally well-tolerated and effective in patients with HIV [102–105]. Significant increases in HDL cholesterol, large HDL particles, and decreases in triglycerides and apolipoprotein B levels occur, while adverse effects on insulin sensitivity and glycemia are transient [102]. Bile acid binding resins have generally been avoided in those with HIV infection due to their unknown effects on antiretroviral drug absorption, but with the availability of once-daily antiretroviral drug regimens and once-daily bile acid binders such as colesevelam, it is probably safe to administer these agents well apart in time. The prescribing information for colesevalam states that for drugs with a narrow therapeutic index, these should also be administered at least 4 hours prior to colesevalam dosing [106].

Triglyceride Lowering Drugs

Fish oils [107, 108], fenofibrate [107, 109, 110], gemfibrozil [89, 109, 111, 112] and extended-release niacin [102] are all safe and effective at lowering triglycerides in HIV-infected patients. However, as compared to the general population, in patients receiving PIs gemfibrozil was somewhat less efficacious [89]. Fish oil and fenofibrate appear to be additive for treating triglyceride elevations [107]. For 3rd line therapy, a combination of a fibrate-fish oils-niacin should be considered. However, in the current treatment era such severe hypertriglyceridemia has become uncommon.

Summary

In spite of improvements in ART and lesser dyslipidemia with modern drug regimens, dyslipidemia remains a prevalent problem in persons infected with HIV. Because of greatly reduced risk of encountering fatal complications directly due to immunosuppression and AIDS and greater risk of CVD, management of risk factors such as dyslipidemia have become increasingly important in routine clinical care of HIV infection. This chapter has outlined an approach to managing HIV-infected patients that is consistent with the latest guidelines for the general population, but with important caveats regarding the choice and dosage of lipid-lowering drugs.

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Chapter 15
New and Emerging Therapies for Hyperlipidemia

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The need for new therapies to lower LDLC (low-density-lipoprotein cholesterol has been well documented. Statins provide the greatest reduction of LDL when compared to bile acid sequestrants, the cholesterol absorption inhibitor, and niacin. However, many patients are unable to tolerate statins because of mild to moderate muscular symptoms. The PRIMO study [1] revealed that muscular symptoms were reported by 832 out of 7924 hyperlipidemic patients (10.5%) within 1 month following statin initiation. Muscular pain prevented even moderate exertion during everyday activities in 315 patients (38%) while 31 (4%) were confined to bed or unable to work.

Familial hypercholesterolemia (FH) is difficult to treat well with maximally tolerated statins and other lipid-lowering therapy. Conventional medical therapy can result in an approximately 20–30% reduction in LDLC when baseline is greater than 400 mg/dL in homozygous FH (hoFH), and 200–400 mg/dL in heterozygous FH (heFH) [2]. Many hoFH and heFH patients have benefited with longer-than-expected survival, but almost all hoFH and a smaller portion of heFH patients continue to have dangerously high LDL levels [3]. Vishwanath and Hemphill said that the 315 patients with hoFH in the United States receiving maximally tolerated lipid-lowering therapy and who are eligible for apheresis may be a low estimate [4]. They also find that approximately 15,000 of the potential 625,000 patients with HeFH in the United States would be still be eligible for apheresis. Approximately 1600 loss-of-function polymorphisms have been found in patients who are compound heterozygotes [2]. Treatment options other than a 3 h plus apheresis every 1–2 weeks would be desirable for these patients.

This chapter will review new technologies that can lower LDL cholesterol through non-statin mechanisms. These new methods involve inhibiting a genetic allele, invoking an anti-sense block of mRNA (messenger ribonucleic acid) translation, and inhibiting the function of microsomal triglyceride transfer protein (MTP).

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PCSK9 Inhibition

Abifedel and colleagues in 2003 reported two French families in whom selected missense mutations in PCSK9 (proprotein convertase subtilisin/kexin type 9) caused a new form of autosomal dominant hypercholesterolemia [5]. Gain-of-function was considered the mechanism with its interaction with LDL receptor.

In 2005 Hobbes and colleagues sequenced the coding region of PCSK9 in African Americans with low LDLC [6]. They found two nonsense mutations (Y142X and C679X) that were associated with a 40% reduction in LDLC. This was explained by a loss-of-function mechanism. These alleles occur in approximately 2–2.6% of the African American population [7].

Subsequently other PCSK9 alleles associated with low LDLC have been identified in whites in the U.S., Danes (R46 L) [8, 9, 10], Swedes (R46I) [11], and Japanese (474 V) [12].

PCSK9, synthesized primarily in the liver, regulates the surface expression of the LDL receptor by targeting it for lysosomal degradation [13]. Loss-of-function PCSK9 mutations lead to decreased LDL-receptor degradation, resulting in more LDL receptors residing on the surface of the liver and lower LDL plasma concentrations.

LDLC was lowered by 15–28% and ischemic heart disease incidence was reduced by 47–88% by PCSK9 loss-of-function carriers, compared with those lacking such mutations [8]. Hobbs and colleagues found an average LDLC of 63 in 33 African Americans carrying loss-of-function alleles [6]. African Americans who carry these alleles have 80–90% decrease in cardiovascular (CV) risk. One patient, 32, had no immunodetectable circulating PCSK9 and an LDLC of only 14 mg/dL. This patient was described as "an apparently healthy, fertile, normotensive, college-educated woman with normal renal and liver function tests (including urinalysis) who works as an aerobic instructor."

Cariou and colleagues reported a white 49-year-old French male with an LDLC of 16 mg/dL who carried a double mutation, R104C/V114A [14]. He had no detectable circulating PCSK9. He was free of microvascular and macrovascular diabetes-related complications, had normal liver function tests, but moderate liver steatosis on ultrasonography. His mother died at 66 from dementia, but his father was a healthy 79. His grandparents died at the ages of 79, 87, 91 and 94, suggesting familial longevity.

Unlike other Mendelian forms of severe hypocholesterolemia (e.g., abetalipoproteinemia, homozygous hypobetalipoproteinemia) which are associated with malnutrition, steatorrhea, hepatic steatosis, night blindness and vibratory and proprioception defects, individuals heterozygous for nonsense mutation in PCSK9 do not have those signs or symptoms and do not exhibit any detectable increase in hepatic triglyceride content [5].

Several pharmaceutical companies have completed phase 1, 2, and 3 studies using monoclonal antibodies targeting inhibition of PCSK9 with the intent of markedly reducing LDLC.

Regeneron Pharmaceuticals and Sanofi created REGN727/SAR236553 (REGN727/alirocumab), a fully human monoclonal antibody that is highly specific

for human PCSK9 and blocks its interaction with the LDL receptor. AMG145/evolocumab from Amgen, also a fully human monoclonal antibody against PCSK9, behaves similarly. Both are administered subcutaneously.

A randomized, single-blind, placebo-controlled phase 1 trial of 32 healthy patients with alirocumab revealed a 70% reduction in circulating PCSK9 plasma protein ($p < 0.0001$) and a mean reduction in LDLC from baseline relative to placebo ($p < 0.0001$) [15]. A phase 2 double-blind parallel-group, placebo-controlled trial randomized 183 patients with LDLC equal to or greater than 100 on stable doses of atorvastatin 10, 20 or 40 mg for equal to or greater than 6 weeks. Alirocumab further reduced LDLC by 40–72% [16].

Another phase 2 study of 92 patients, double-blinded, placebo-controlled trial studied two different doses of atorvastatin, 10 mg and 80 mg [17]. Adding alirocumab to both doses of atorvastatin showed no significant difference from baseline. Both groups had LDLC lowered about 70%. One explanation for the lack of difference in the two doses is that the higher dose of atorvastatin increased PCSK9 level [18]. Other statins (pitavastin, rosuvastatin, pravastatin) also raise PCSK9 levels [19, 20]). These observations explain the "rule of 6%" for statins, which indicates that each doubling of the statin dose results in only an approximate 6% additional reduction in LDLC level.

Fenofibrate [21] and ezetimide [22] have also been found to increase PCSK9 levels.

Side effects reported with alirocumab include diarrhea reported in 7.1% (0% in placebo), as well as injection site (IS) erythema up to 9%, IS pruritus 9.7%, IS swelling up to 6.5%, IS hematoma up to 6.7%, and IS rash up to 6.7%, and none in IS placebo. There were no significant changes in liver function tests, troponin, cytokines, C-reactive protein.

AMG 145/evolocumab was studied in three phase 2 studies. Koren et al. [23] reported a phase 2 trial of 406 patients with hypercholesterolemia treatment-naïve patients who had 40–50% LDLC reduction in all groups ($P < .001$ for all doses of evolocumab vs. placebo or vs. 10 mg of ezetimibe). None had significantly increased liver function or creatine kinase. Injection site skin reactions were rare and mild.

Giugliano, et al. [24] found in a phase 2 trial of 631 patients with hypercholesterolemia who were receiving statins with or without ezetimibe, a 42–50% reduction in LDLC ($P < .0001$ for each of six escalating statin doses vs. placebo) for 12 weeks. Adverse events with evolocumab and placebo were similar (8% and 7%, respectively), and none were severe or life-threatening.

Robinson et al. [25] reported a phase 2 12-week trial of 2067 patients who were randomized to daily atorvastatin 10 mg, simvastatin 40 mg, rosuvastatin 5 mg, atorvastatin 80 mg, and rosuvastatin 40 mg. Ezetimibe (10 mg or placebo) was added only to the atorvastatin patients after a 4-week stabilization period. For the lower-intensity statin groups, evolocumab every 2 weeks reduced LDLC from a baseline mean of 115–124 mg/dL to an on-treatment mean of 39–49 mg/dL; monthly evolocumab reduced LDLC from a baseline mean of 123–126 mg/dL to an on-treatment mean of 43–48 mg/dL. For the high-statin groups, evolocumab every 2 weeks reduced LDLC from a baseline mean of 89–94 mg/dL to an on-treatment

mean of 35–38 mg/dL; monthly evolocumab reduced LDLC from a baseline mean of 89–94 mg/dl to an on-treatment mean of 33–35 mg/dL.

In the ezetimibe/atorvastatin patients an LDLC less than 70 mg/dL was achieved in 17–20% by the lower-dose statin group and by 51–62% in the high-dose statin group. In contrast an LDLC level less than 70 mg/dL in the atorvastatin-only group was achieved by 86–94% with added evolocumab.

The DESCARTES trial evaluated the effect of evolocumab on 901 patients taking atorvastatin 10 mg only, 80 mg only, or atorvastatin 80 with ezetimibe 10 mg. The lowest mean LDLC of 44.7 mg/dL was present in the atorvastatin 10 mg only group that was also the group that had the most patients' LDLC level less than 70 mg/dl at 52 weeks [26].

Adverse effects occurred in 74.8% of the evolocumab group compared to 74.2% in the placebo group [35]. The most common adverse events were nasopharyngitis, upper respiratory tract infection, influenza and back pain. A small percentage, 2.2% or 13 patients of the evolocumab group was discontinued from the study drug compared to 1% (3 patients) from the placebo group.

Injection-site reactions (pruritus, erythema, hematoma, or pain) were reported in 5.7% of evolocumab treated patients and 5.0% in the placebo group.

Another method of blocking the effect of PCSK9 was reported by Ding et al. Genome editing with the CRSPR-Cas9 system, using an adenovirus, was found to disrupt the PCSK9 gene in vivo permanently with high efficiency, and also permanently reduced blood cholesterol levels in mice. There are plans to study genome editing in humans [27].

Microsomal Triglyceride Transfer Protein (MTP) inhibition.

MTP inhibition is not dependent upon an interaction with the LDL receptor, compared to PCSK9 inhibitors, and therefore has potentially great promise for hoFH and heFH patients.

MTP resides in the endoplasmic reticulum, where it transfers neutral lipids including triglycerides on to newly synthesized apolipoprotein B (apoB) to assemble chylomicrons in the intestinal enterocyte and very low-density lipoprotein (VLDL) in the hepatocyte. VLDL is the precursor to LDLC. Loss-of-function mutations of the gene encoding MTP (MTTP) cause the rare genetic condition abetalipoproteinemia. In this condition there is no lipidation of apoB, resulting in the absence of chylomicrons and VLDL production, and therefore absence of LDL in the plasma. Consequently there is failure to absorb dietary fat and fat-soluble vitamins and vitamin E transport from the liver to the periphery is reduced. Patients have impaired oral fat tolerance upon eating a high-fat meal, leading to nausea, flatulence, and diarrhea as well as progressive retinal and spinocerebellar degeneration caused by vitamin E deficiency.

Lomitapide (BMS-201038/Juxtapid) was one of the first to be developed. Lomitapide is a small molecule that inhibits lipid transfer by directly binding to MTP in the liver and intestines [28].

A phase 3 study of lomitapide was published in 2013 [29]. Twenty-nine patients were enrolled in a single-arm, open-label trial in which lomitapide was titrated from 5 mg–60 mg on top of standard care including apheresis. Twenty-three patients who

completed the 26-week efficacy phase showed a mean LDLC decrease of 50%. The same 23 patients completed the 78-week safety phase. Apheresis was discontinued in three and reduced in frequency in three subjects. Sixteen achieved an LDLC of less than 100 mg/dL. At up to four and a half years of the ongoing long-term extension study there was a stable reduction in LDLC and apoB. There was a transient reduction in HDLC.

Gastrointestinal side effects were minimized with adherence to a low-fat diet, dosing in the fasting state, and a gradual dose-escalation regimen [29]. Three of 29 patient subjects were discontinued within the first 12 weeks because of gastrointestinal symptoms but the frequency and intensity decreased substantially after 12 weeks with no additional discontinuances through 78 weeks. Lomitipide had no significant effects on plasma levels of vitamins A and D and vitamin K assessed by international normalized ratio. Vitamin E supplement of 400 IU/d was provided with the ratio of Vitamin E to total lipids remaining in the normal range. Increased serum transaminase levels were transient and reversible. There was no comcomitant increase in bilirubin or alkaline phosphatase. In subjects with alanine aminotransferase (ALT) or aspartate aminotransferase (AST) elevations greater than five times the upper limits of normal (ULN), dose reduction or interruption led to a rapid decrease in the transaminase levels. In subjects with elevations that were less than five times the ULN, continuation of lomitapide at the same dose was generally associated with a decrease in the transaminase levels to baseline [29]. The greatest elevations in transaminases were observed in patients with the high alcohol consumption.

Hepatic fat increased from a baseline average of 1% measured by magnetic resonance imaging (MRI) to an average of 8.6% at six months at which time the mean dose of lomitapide was 40 mg/d. ALT and AST were correlated with maximum hepatic fat. In a phase 2-hoFH study follow-up MRI was obtained after a 4-week discontinuance of lomitapide, and hepatic fat was found to be rapidly reversible [30].

Fasting blood sugar, insulin levels and hemoglobin A1c were unchanged with lomitapide treatment [31].

Mipomersen (KYNAMRO), unlike lomitapide, blocks the translation of apoB mRNA reducing the synthesis of apoB and subsequent production of VLDL and LDLC. The discovery of about 60 mutations of apoB that inhibited its own synthesis supported the strategy of finding a molecule that would result in a similar reduction [32].

A phase 3 trial of 51 hoFH patients not on apheresis were randomized in a 2:1 ratio with 34 allocated to mipomersen 200 mg weekly SC [33]. Mipomersen produced a mean 25% reduction in LDLC from baseline. There were concordant reductions in serum apoB levels by 27% and lipoprotein (a) by 32%. Three pediatric hoFH patients < 18 years of age were included and responded similarly to adults. HDLC increased 15%.

Three other phase 3 trials have been reported. All were designed with randomization ratio of 2:1 active to placebo and 26 weeks of mipomersen SC weekly. Apheresis was excluded and all were on a low-fat diet tolerating maximum lipid-lowering therapy. Across these phase 3 studies average baseline reduction for

LDLC ranged from 28–36%, for apoB 25–34%, and for lipoprotein (a) 21–33%. Triglyceride levels fell significantly, possibly related to the drop in apolipoprotein C-III [34]. LDL particle size (sLDLC) was also reduced with mipomersen therapy [35, 36, 37].

Two of these phase 3 trials were in non-FH patients not on apheresis with severe hypercholesterolemia on maximally tolerated lipid-lowering therapy as well as a low-fat diet. Both were double-blind placebo-controlled studies enrolling at total of 216 patients. Mipomersen reduced LDLC by 36% and 36.9%, respectively [38, 39].

After subcutaneous injection mipomersen is readily absorbed and distributed to tissues with the highest concentrations in the liver and kidney, and the parent drug and metabolites are predominantly excreted in the urine. Plasma half-life ranges from 1–2 months which allows weekly dosing. Mild to moderate erythema or pain at the injection site was the most frequently reported adverse event in 84% with mipomersen, and 33% in placebo requiring discontinuance of treatment in 5%. Injection-site reactions decreased incrementally at 6-month intervals. Reaction events were generally self-limited and resolved spontaneously within 2–5 days. Thirty percent of patients on mipomersen experienced flu-like symptoms (fatigue, chills, aches) compared to 16% on placebo and held constant at 6-month intervals with continued treatment. No significant changes were found in hsCRP, erythrocyte sedimentation rate or IgG levels over time.

Serum liver transaminase elevated equal to or greater than three times ULN on two consecutive measurements, 7 days apart, occurred in 8% of mipomersen treatment patients. Most were reversible on continued treatment. There was no accompanying change in total bilirubin, alkaline phosphatase, prothrombin, coagulation factors and albumin.

A median 10% increase in liver fat was found in treated patients. This was reversible as liver fat returned to normal in the post-treatment follow-up period. A limited number had liver biopsies, all confirming hepatic steatosis with minimal signs of inflammation and minimal to no liver fibrosis.

Summary

Three promising new therapies for lowering LDL cholesterol are available. Two (lomitipide and mipomersen) may be most helpful in patients with FH. PCSK9 inhibition has the promise of low side effects, and if similar to those that occur in nature as loss-of-function alleles, may provide a therapy tolerable by many. Long-term studies are needed to assess adverse reactions and reduction in cardiovascular morbidity and mortality (Table 15.1).

Table 15.1 A summary of four new lipid therapies with their mechanisms of actions, indicated uses and reported adverse effects. HoFH: homozygous familial hyperlipidemia

	Alirocumab	Evolocumab	Lomitapide/ JUXTAPID	Mipomersen/ KYNAMRO
	Regeneron/ Sanofi	Amgen	Aegerion	Genzyme
Mechanism of action	PCSK9 antibody blocking interaction with LDL receptor	PCSK9 antibody blocking interaction with LDL receptor	Blocks lipid transfer by binding MTP	Blocks translation of apoB in RNA
Indicated uses	Not at LDL target with statin	Not at LDL target with statin	HoFH	HoFH
Common adverse side effects	Hepatic steatosis	Hepatic steatosis	The most common adverse reactions were gastrointestinal, reported by 27 (93%) of 29 patients. Adverse reactions reported by 8 (28%) patients in the HoFH clinical trial included diarrhea, nausea, vomiting, dyspepsia, and abdominal pain. Other common adverse reactions, reported by 5–7 (17–24%) patients, included weight loss, abdominal discomfort, abdominal distension, constipation, flatulence, increased ALT, chest pain, influenza, nasopharyngitis, and fatigue. The safety and effectiveness of lomitapide have not been established in patients with hypercholesterolemia who do not have HoFH. The effect of lomitapid on cardiovascular morbidity and mortality has not been determined	Eighteen percent of patients on drug and 2% of patients on placebo discontinued treatment due to adverse reactions. The five most common adverse reactions in patients treated with drug that led to treatment discontinuation and occurred at a rate greater than placebo were injection site reactions (5.0%), alanine aminotransferase increased (3.4%), flu-like symptoms (2.7%), aspartate aminotransferase increased (2.3%), and liver function test abnormal (1.5%).: The safety and effectiveness of mipomersen have not been established in patients with hypercholesterolemia who do not have HoFH. The effect of mipomersen on cardiovascular morbidity and mortality has not been determined. The use of Mipomersen as an adjunct to LDL apheresis is not recommended

Table 15.1 (continued)

	Alirocumab	Evolocumab	Lomitapide/ JUXTAPID	Mipomersen/ KYNAMRO
	Regeneron/ Sanofi	Amgen	Aegerion	Genzyme
Trials completed with results [40]	None	None	7	6
Trials completed [40]	11	13	3	7
Trials pending [40]	15	8	5	3
FDA approval	No	No	Yes	Yes

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