Therapeutic Lipidology

Contemporary Cardiology

Christopher P. Cannon, MD

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To the memory of my father, Dr. David M. Davidson, who died at age 47 of a myocardial infarction.

Michael H. Davidson

To RSM (especially MMQ, MMMc, MBA, and MYML) for teaching me the fundamentals of living my life with faith, fortitude, and ferocity.

Peter P. Toth

To the PCRC team, whose commitment to "doing well while doing good" contributes every day to our mission of helping people acheive greater quality of life by preventing diseases that erode good health and function.

Kevin C. Maki

Preface

Lipids constitute a diverse array of molecules with an astounding range of functions in biological systems in both health and disease. Many of the greatest scientific minds of the last century committed their energies to the identification of lipids and sterols and to characterizing how these molecules are synthesized and metabolized by a large number of cell types. This led to the identification of many species of lipids, including phospholipids, sphingolipids, cerebrosides, triglycerides, fatty acids and their metabolites (eicosanoids, leukotrienes), and cholesterol/cholesterol esters and other sterols. Lipids are involved in cell membrane and organelle formation, intracellular and intercellular signaling, cell surface receptor function, inflammation, and immunity. Cholesterol is a modulator of cell membrane fluidity, is a precursor to steroid hormones and bile salts, and, along with many lipid species, is a key modulator of risk for developing atherosclerotic disease. Cholesterol can also be a source of fatty acid in its esterified form. Fifteen Nobel Prizes have been awarded to investigators for work related to the biosynthesis and metabolism of cholesterol, and more are sure to follow. The identification of the enzymes and intermediates of the cholesterol biosynthetic pathway will forever be a milestone in human creativity and investigative ingenuity. The regulatory circuitry of lipid and cholesterol metabolism is understandably of enormous complexity, and many areas of uncertainty and gaps in knowledge remain.

In the clinical arena, the last 30 years have seen tremendous advances in the fields of lipidology and cardiovascular medicine. The Framingham Study and other epidemiologic investigations conducted around the world unequivocally demonstrated strong relationships between lipoproteins and risk for cardiovascular events. Seminal work by Fredrickson and colleagues classified dyslipidemias according to derangements in specific classes of lipoproteins. Goldstein and Brown ushered in the modern era of "therapeutic lipidology" by discovering the low-density lipoprotein (LDL) receptor and subsequently showing that (1) mutations in this receptor were etiologic for familial hypercholesterolemia and (2) inhibitors of the β-hydroxymethylglutaryl coenzyme A reductase upregulated this receptor and facilitated the clearance of LDL from the circulation. Gotto and coworkers subsequently sequenced the amino acid structure of apoprotein B100. Other apoproteins were identified and sequenced and their three-dimensional conformations and cell surface receptors elucidated. Mutations in these apoproteins were correlated with both gain and loss of function, yielding many identifiable and characteristic changes in lipoprotein distributions. More recently, the role of membrane cassette transport proteins in lipid trafficking between the gastrointestinal tract, liver, and blood vessel walls has been characterized. Mutations in these membrane cassette transport proteins have provided mechanistic insights into such lipid disorders as Tangier's disease and β-sitosterolemia. The role of lipolytic enzymes (lipoprotein lipase, hepatic lipase, endothelial lipase, phospholipase A2, and hormone-sensitive lipase) and their various mutant forms in lipid metabolism have also been elucidated and remain foci of intensive investigation. Polymorphisms from around the world continue to be identified and catalogued. The number of nuclear transcription factors

(e.g., peroxisomal proliferator-activated receptors, liver X receptor, and farnesoid X receptor) involved in lipid metabolism continues to expand. In the next few years, the areas of lipidomics, isoprenoid metabolism, and vascular biology will continue to provide us with more expansive vistas from which to view the relationship between lipid dynamics and atherogenesis.

Until the mid-1980s, serum LDL cholesterol (LDL-C) was recognized as a critical risk factor for atherosclerotic disease. However, prospective, placebo-controlled data showing that therapeutically reducing the burden of this lipoprotein in serum decreases risk for cardiovascular morbidity and mortality were lacking. The first proof of benefit from this approach came from the Lipid Research Clinics Coronary Primary Prevention Trial. In this trial, the bile acid binding resin cholestyramine was used to treat men at high risk for coronary artery disease. Patients experienced a 19% reduction in major coronary events with a 9% reduction in LDL-C. In its Adult Treatment Panel I, the National Cholesterol Education Program (NCEP) made its initial attempt to provide evidence-based guidelines for dyslipidemia management. The first statin, lovastatin, was approved in 1987. Over the course of the last 20 years, we have observed a remarkable evolution in our understanding of lipid metabolism and have greatly expanded the therapeutic armamentarium with which to decrease the burden of atherogenic lipoproteins. We have a large number of prospective clinical trials which demonstrate that lipid lowering with statins, fibrates, and niacin beneficially impacts risk for cardiovascular morbidity and mortality. Millions of patients have benefited from these therapies.

Despite these great successes, much work remains to be done. Even in the face of very aggressive lipid lowering with statins, the majority of acute cardiovascular events are not prevented. Clinicians must keep pace with rapidly changing guidelines, which call for ever greater reductions in both serum LDL-C and non-high-density lipoprotein cholesterol levels. The percentage of the population that develops the metabolic syndrome and diabetes is increasing, a greater percentage of the population is elderly with increased risk, and more patients are surviving ischemic strokes and acute coronary syndromes such as myocardial infarction and unstable angina. All of these patients warrant aggressive management according to updated NCEP guidelines. Many new drug classes are being developed to help address these challenges. Therapeutic approaches to more reliably increase serum levels of high-density lipoproteins, drugs to reduce appetite and weight gain, and deeper insights into specific dietary and lifestyle approaches to dyslipidemia are actively being developed and evaluated. The atherogenicity of novel pathways are burgeoning areas of exploration. Many new markers of risk have also been introduced into medicine in recent years. These include C-reactive protein, myeloperoxidase, lipoprotein-associated phospholipase A2, and soluble CD36. Evaluation of lipoprotein particle size and number offer valuable insights into lipoprotein metabolism and risk for cardiovascular events. Considerable skill is required to integrate information gleaned from these various modes of patient testing and to tailor and optimize therapeutic approaches. It is these approaches and skills that *Therapeutic Lipidology* addresses.

This book is intended to provide practicing clinicians with a focused and intensive, but useable, source of information on the identification and management of dyslipidemias. The need for a volume such as this has been longstanding. Since the creation of the American Board of Clinical Lipidology, many clinicians have asked us to create an up-to-date, comprehensive reference on lipidology. Subsequent editions are certain

to expand in size and scope as more species of atherogenic enzymes, lipids, and inflammatory mediators are identified and novel therapeutic interventions are introduced. The pace of scientific and clinical advances in lipidology is astounding. It is our sincerest wish that this reference will serve as a lifelong stimulus to the reader to continue to learn about the ever-changing and fascinating field of therapeutic lipidology. We also hope that this book will empower our readers to improve and extend the lives of the patients they serve.

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It is with much gratitude that we also acknolwedge colleagues who provided the images for the cover of *Therapeutic Lipidology*. The upper left panel was provided by Zahi Fayad, MD, of the Mount Sinai School of Medicine, New York. This image demonstrates inflamed carotid artery plaque within the red bars by measuring the uptake of 18-fluorine-fluorodeoxyglucose using fused positron emission tomography and computed tomography. Tony DeFranco, MD of the CVCTA Education Center in San Francisco provided the central panel, a color-enhanced 64-slice computed tomography image of the frontal view of a human heart. We also thank Dr. Eva Istvan of Washington University in Saint Louis for the bottom right panel, demonstrating the interaction of simvastatin with the active site of HMG CoA reductase (reproduced from Istvan and Diesenhofer [2001]; Science 292: 1160-4 with permssion).

Foreword

As an investigator of the field of lipid disorders for the last four decades, I have been a first-hand witness to the many successes, controversies, and breakthroughs that have radically advanced the field of atherosclerotic cardiovascular disease prevention. Early skepticism through the mid-1980s that dyslipidemia and other risk factors could account for only about half of all coronary events has given way to a sophisticated understanding of the intricate interplay of cardiovascular risks and the realization that risk factor modification is critical to stemming the tide of cardiovascular disease.

I began in the field in 1967 at the National Institutes of Health (NIH), in the laboratory of Donald Fredrickson and Robert Levy, but the history of lipid research extends much farther back. Cholesterol, cholesteryl ester, and phospholipid circulate in blood in macromolecular complexes called lipoproteins. Michel Macheboeuf, working at the Pasteur Institute in 1929, first described the plasma lipoproteins by using ammonium sulfate fractionation of horse serum to isolate alpha-lipoproteins, what today almost certainly would be recognized as high-density lipoprotein (HDL). In the years surrounding World War II, Edwin J. Cohn and J.L. Oncley utilized fractionation procedures that included precipitation under different acid and salt conditions, as well as electrophoresis, to purify the constituents of blood plasma and serum.

In 1949, John Gofman and his colleagues at the University of California at Berkeley used the newly developed ultracentrifuge to observe that the lipoprotein fraction that corresponds with LDL was associated with increased risk for cardiovascular disease. Around the same time, at the Rockefeller University, E.H. "Pete" Ahrens studied the connection of diet with lipid metabolism and cholesterol homeostasis. At Cornell University Medical College (now Weill Cornell Medical College), Russ, Eder, and Barr used Cohn and Oncley's techniques to argue that higher concentrations of alpha-lipoprotein contributed to the lower cardiovascular disease event rate seen in pre-menopausal women.

At the NIH, Donald Fredrickson took advantage of the preparative ultracentrifuge and paper electrophoresis to characterize the lipoproteins further. In a series of landmark articles in the *New England Journal of Medicine*, Fredrickson, Robert Levy, and Robert Lees described a classification system based on which groups of lipoproteins were elevated. Fredrickson phenotyping has proved to be a popular and enduring principle for describing the dyslipidemias, although it does not distinguish between dyslipidemias with a primary or secondary etiology.

In those early days, two large observational trials were central to establishing the cardiac dangers of excess cholesterol levels. Ancel Keys' Seven Countries study, during the 1950s and 1960s, established the association between dietary fat consumption, dyslipidemia, and coronary risk on an international scale. In those populations that consumed a higher proportion of saturated fat as the total dietary caloric intake, there were higher levels of cholesterol in the blood and a higher incidence of CHD mortality. The other study began in 1948, under the auspices of the National Heart Institute (the precursor to the National Heart, Lung, and Blood Institute). Conducted in a working

class community in Massachussetts, the Framingham Heart Study began to collect longitudinal data on 1980 men and 2421 women. In 1961, the Framingham investigators showed that high blood pressure, smoking, and high cholesterol levels were major preventable factors in heart disease, a report that cemented the concept of modifiable and nonmodifiable "risk factors."

The crux of the lipid hypothesis was that reducing cholesterol would reduce coronary events. The epidemiology had made a clear connection between cholesterol and coronary risk, but compelling evidence that treating cholesterol would make a difference was not available until the publication of the Lipid Research Clinic Coronary Primary Prevention trial (LRC-CPPT) in 1984. The trial recruited 3806 middle-aged men with primary hypercholesterolemia, no evidence of coronary disease, and tolerance to the then-available preparation of cholestyramine, which participants compared to sand in terms of texture and palatability. Although the drug was supposed to be administered as 24 g/day, patients could manage an average dosage of around 12 g/day versus placebo. There was a 12% reduction in LDL-C and 9% in total cholesterol, corresponding to a reduction in CHD events of 19% after 7.4 years of follow-up.

While some investigators criticized the trial's design and statistical analysis, many others felt that LRC-CPPT confirmed the fundamental idea that lipid modification could reduce coronary disease. The study raised public awareness of the issue of cholesterol and heart disease and inspired the formation of an NIH committee to put together a National Cholesterol Consensus that was chaired by Dan Steinberg. The National Heart, Lung, and Blood Institute launched a program that turned into the National Cholesterol Education Program. The announcement of the positive results of the primary-prevention Helsinki Heart Study of gemfibrozil shortly thereafter provided additional affirmation of the lipid approach, although the paucity of evidence that treatment could improve survival hindered wider acceptance.

In September 1987, lovastatin became the first statin to be introduced into the market. The launch of the statins was a watershed moment for the field. These drugs lowered LDL-C more effectively than other pharmacotherapies that were available at the time, and early angiographic trials of these agents made a positive case for their use in slowing the progression of atherosclerotic disease. Beginning in 1994 and through the present day, large-scale clinical trials have overwhelmingly demonstrated that statins reduce the risks for clinical cardiovascular events across a broad range of patients. Indeed, three of the landmark statin trials have achieved the "Holy Grail" of a reduction in total mortality with active treatment: the Scandinavian Simvastatin Survival Study (4S); the Long-term Intervention with Pravastatin in Ischaemic Disease (LIPID); and the Heart Protection Study.

More recently, trials have investigated how aggressively we should treat LDL-C. On the whole, the findings suggest that achieving lower levels of LDL-C is associated with greater cardiovascular risk reduction, and guidelines now reflect that even lower LDL-C targets may be desirable in certain higher-risk groups. While no threshold has yet been convincingly identified, it is possible that we may eventually discover a lower bound of LDL-C below which no further coronary benefit may be achieved.

Although targeting LDL-C has taken primacy in the management of the dyslipidemias, other lipid fractions may also contribute to cardiovascular risk, such as HDL cholesterol (HDL-C) and triglyceride, and have garnered increasing attention. The Framingham Heart Study observed that the higher the HDL-C, the lower the risk for an event. Trials with gemfibrozil, such as the Helsinki Heart Study and the Veterans Affairs HDL-C

Intervention Trial (VA-HIT), showed primary HDL-cholesterol raising and triglyceride-lowering effects and reported fewer clinical events with treatment compared with placebo. Improved understanding of the cardioprotective effects of HDL has nurtured interest in potential therapeutic applications, although such efforts were dealt a blow in 2006 by the withdrawal of torcetrapib, a member of a new class of HDL-C-raising compounds, from further study because of cardiovascular toxicity. Nevertheless, the hypothesis that raising HDL-C may prevent cardiovascular disease remains a viable one, and other avenues are being explored.

In summary, atherosclerosis was once thought to be an irreversible, inevitable consequence of aging. The recognition of dyslipidemia as a major modifiable risk factor introduced the possibilities of both treatment and prevention. As time has passed and the body of evidence has grown, investigators and clinicians have increasingly appreciated the multifactorial nature of cardiovascular risk. There is now an emphasis on treating overall global risk for near-term cardiovascular disease, rather than treating individual risk factors per se. At the same time, basic science has further elucidated the complex pathologies that underlie atherosclerosis and has suggested novel therapeutic concepts.

All of this leads to the book that is now in your hands. This excellent text, edited by Michael H. Davidson, Peter P. Toth, and Kevin C. Maki, is an important synthesis of current information on the management of lipid disorders and cardiovascular risk. The authors and editors have produced a book that not only presents a thoughtful, complete, and authoritative guide to contemporary clinical challenges, but also often takes the long view of the field, putting information in the context of a rich five decades of research. Physicians who treat patients at risk for cardiovascular disease will find it an invaluable resource in their daily practice.

Although the advances of the last 50 years have been truly impressive, there is much about the origins and prevention of atherosclerosis that remains undiscovered. Continued exploration is imperative in order to maximize our efforts to reduce the burden of cardiovascular disease in our patients and around the world.

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1 Lipoprotein Metabolism and Vascular Biology

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Contents

INTRODUCTION

Cholesterol is vitally important for the maintenance of cellular membranes, production of steroid hormones and bile acids, and a supply of energy for metabolic needs. However, an excess of cholesterol may lead to the development of atherosclerosis and coronary artery disease; imbalances in lipoprotein metabolism are responsible for arterial lipid deposition. As lipids are intricately entwined with the atherothrombotic disease process, therapeutic interventions aimed at favorably altering lipoprotein metabolism have shown success in limiting atherogenesis and its subsequent complications. To understand how these different therapeutic strategies work, one must possess a basic understanding of the metabolic pathways governing lipidology.

Lipoproteins are complex particles that are generally composed of a hydrophobic core of mainly nonpolar cholesterol esters (CEs) and triglycerides (TGs) surrounded by an amphipathic phospholipid monolayer that includes unesterified free cholesterol (FC) and proteins known as apolipoproteins or aproteins. Apolipoproteins play four critical roles: (1) they provide a framework for lipoprotein assembly; (2) they add structural stability to the lipoprotein complex; and (3) they determine the metabolic fate of the lipoprotein by activating or inhibiting key enzymes; and (4) they act as ligands

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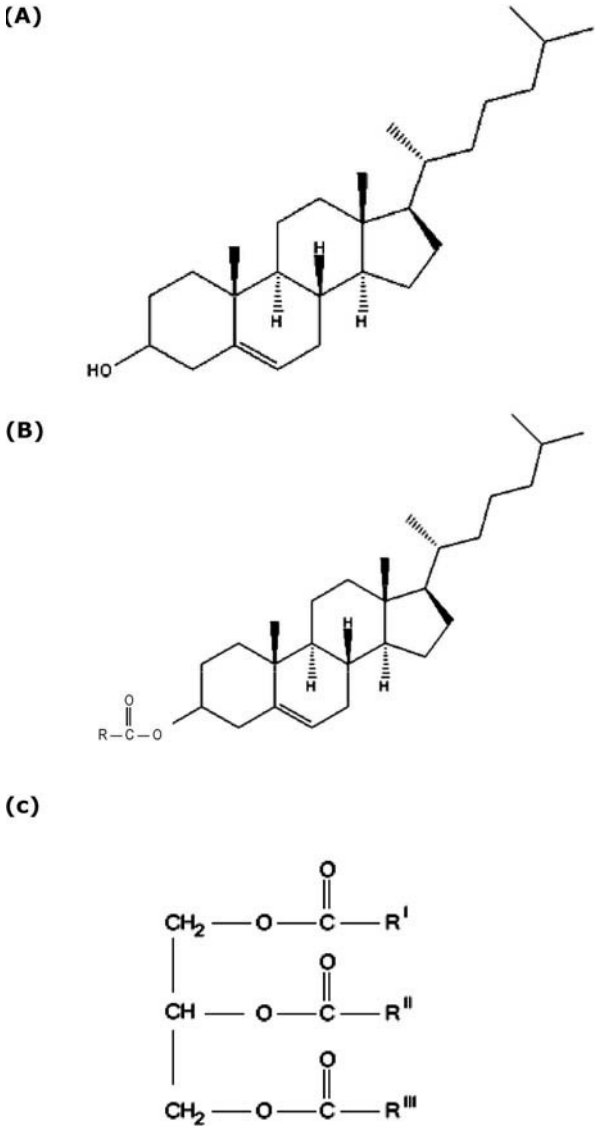

Fig. 1. **(A)** Free cholesterol (FC), **(B)** cholesterol ester (CE), and **(C)** triglyceride (TG). These nonpolar molecules are not water soluble and, therefore, require transport via lipoproteins.

for receptor molecules. Figure 1 details the chemical structures of these components; as these molecules are hydrophobic, they require a carrier to circulate in the aqueous bloodstream. Phospholipids have a polar head containing a glycerol group attached to two fatty acid chains. The hydrophilic outer shell of the glycerol backbones of the phospholipids allows lipoproteins to be water-soluble carriers of the hydrophobic inner contents through the circulating blood (Fig. 2).

LIPOPROTEIN CLASSIFICATION

There are five major classes of lipoproteins, defined by their respective density and electrophoretic mobility (Table 1). Most proteins have densities in the range of 1.3–1.4 g/mL, and lipid aggregate density is approximately 0.8 g/mL; hence,

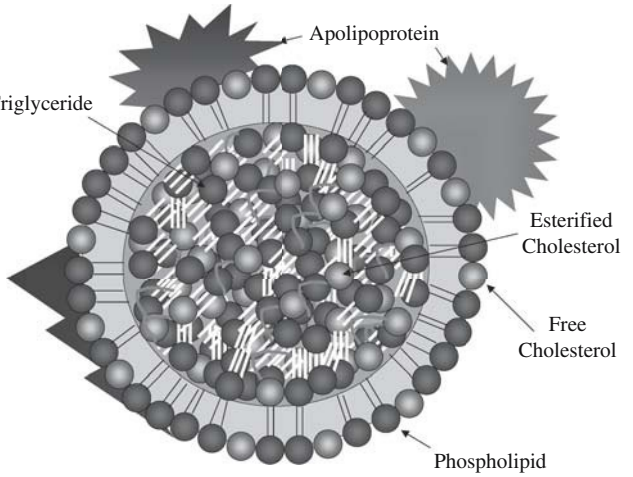

Fig. 2. Lipoprotein structure. A hydrophilic outer surface surrounds an inner hydrophobic core, allowing the transport of triglycerides and cholesterol in circulating blood. The apolipoproteins on the outer surface present to receptors on cell membranes and determine the ultimate destination for the lipoprotein molecule.

Table 1
Lipoprotein Classification and Properties *(90,91,94)*

Class	*Density (g/mL)*	*Electrophoretic mobility*	*Diameter (nm)*	*Molecular weight (Da)*
Chylomicrons	0.93	Remain at origin	75–1200	$(50–1000) \times 10^6$
Very low density lipoproteins	0.93–1.006	Pre-β	30–80	$(10–80) \times 10^6$
Intermediate-density lipoproteins	1.006–1.019	Slow pre-β	25–35	$(5–10) \times 10^6$
Low-density lipoproteins	1.019–1.063	β	18–25	$(2–3) \times 10^6$
High-density lipoproteins	1.063–1.21	α	5–12	$(65–386) \times 10^3$

lipoproteins with a higher protein:lipid ratio are denser than those with a lower protein:lipid ratio (Fig. 3). The least dense and largest lipoproteins are the TG-rich chylomicrons, and the densest and smallest lipoproteins are the high-density lipoproteins (HDLs). Between the chylomicrons and HDL are the very low density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), and low-density lipoproteins (LDLs), listed in order of increasing density.

APOLIPOPROTEIN CLASSIFICATION

Although lipoproteins are classified based on their density that is determined by their combination of components, their functional uniqueness is dependent on the apolipoproteins that they carry. The apolipoproteins play a critical role in the recognition of lipoproteins as they serve as receptor ligands and enzyme cofactors. The

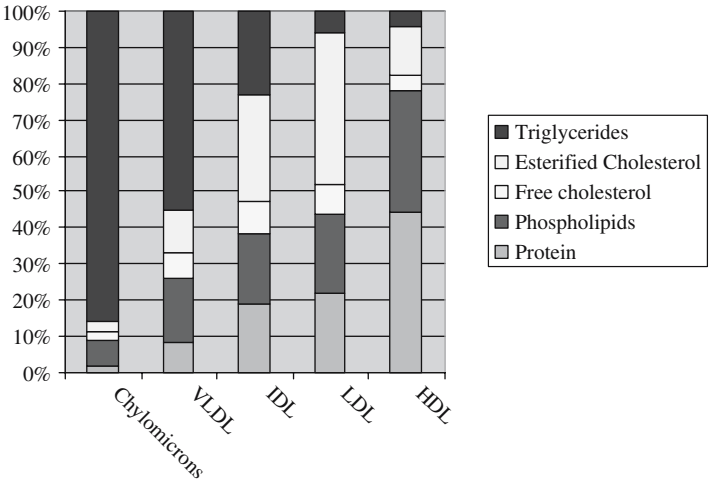

Fig. 3. Lipoprotein composition as percent of mass *(90,91)*.

apolipoproteins are typically classified by an alphabetical designation (A through E) with a Roman numeral suffix defined by the order in which the apolipoprotein emerges from a chromatographic column (Table 2). Apolipoproteins have four major functions: provide a framework for lipoprotein assembly, add structural stability to the lipoprotein complex, activate or inhibit processing enzymes, and signal to receptor molecules for uptake.

- *ApoA-I*: principal structural protein of HDL but also found in chylomicrons and activates lecithin:cholesterol acyltransferase (LCAT).
- *ApoA-II*: another structural protein of HDL also found in chylomicrons and activates hepatic lipase (HL).
- *ApoA-IV*: predominantly found in HDL and activates LCAT and lipoprotein lipase (LPL).
- *ApoB-48*: exclusively found in chylomicrons, derived from the apoB-100 gene, and reduced to 48% of the N-terminal component of B-100 by RNA editing, with no LDL receptor (LDLr) binding domain.
- *ApoB-100*: principal structural protein of LDL, alsofound in VLDL, IDL, and Lp(a); ligand for LDLr.
- *ApoC-I*: primarily found in HDL and chylomicrons and also in IDL and VLDL and activates LCAT.
- *ApoC-II*: protein primarily of VLDL and chylomicrons and also found in HDL and IDL and activates LPL.
- *ApoC-III*: found broadly in HDL, IDL, LDL, VLDL, and chylomicrons but more consistently in VLDL and chylomicrons and inhibits LPL *(1)*.
- *ApoD*: exclusively found in HDL and possibly activates CE transfer protein (CETP) *(2)*.
- *ApoE*: also found broadly in HDL, IDL, LDL, VLDL, and chylomicrons and binds to LDLr with varying affinity dependent on the inherited apoE allele. Three different apoE isoforms exist in humans: apoE2 with lower affinity to LDLr, apoE3 with intermediate binding affinity, and apoE4 with higher affinity *(3)*.
- *Apo(a)*: distinguishing structural protein of Lp(a) that is covalently bound to apoB-100 and inhibits plasminogen activation *(4,5)*.

Table 2
Apolipoprotein Classification *(95)*

	Predominant lipoprotein	*Minor lipoproteins*	*Plasma concentration (mg/dL)*	*Role*	*Molecular weight (kDa)*	*Chromosome*
ApoA-I	HDL	Chylomicrons	90–160	LCAT activation	28.3	11q23
ApoA-II	HDL	Chylomicrons	25–45	HL activation	17	1q21–23
ApoA-IV	HDL		10–20	LCAT and LPL activation	45	11q23
ApoB-48	Chylomicrons		0–100	Structural	241	2q23–24
ApoB-100	LDL, VLDL	IDL, Lp(a)	50–150	LDLr binding	512	2q23–24
ApoC-I	Chylomicrons, HDL	IDL, VLDL	5–6	LCAT activation	6.63	19q13.2
ApoC-II	Chylomicrons, VLDL	HDL, IDL	3–5	LPL activation	8.84	19q13.2
ApoC-III	Chylomicrons, VLDL	HDL, IDL, LDL	10–14	LPL activation	8.76	11q23
ApoD	HDL		4–7	CETP activation	33	3q26.2
ApoE	Chylomicron remnants, IDL	HDL, LDL, VLDL	2–8	LDLr binding	34	19q13.2
Apo(a)	Lp(a)		0–200	Plasminogen activation inhibition	250–800	6q27

CETP, cholesterol ester transfer protein; HDL, high-density lipoprotein; HL, hepatic lipase; IDL, intermediate-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low-density lipoprotein; LDLr, LDL receptor; LPL, lipoprotein lipase; VLDL, very low density lipoprotein.

LIPID METABOLISM

Lipoprotein metabolism serves the two major functions of providing TGs to adipose and muscle for storage or energy substrate and oftransporting cholesterol for cellular membrane, steroid hormone, and bile acid syntheses. The majority of carbon and hydrogen that is used for cellular fuel passes through lipid intermediaries prior to ultimate use. Lipid metabolism has two pathways to maintain the movement of lipids from diet to blood to cells: (1) the exogenous pathway (Fig. 4) that starts with intestinal absorption of dietary fat and cholesterol and (2) the endogenous pathway (Fig. 5) that starts with VLDL production from the liver.

Exogenous Pathway

Consumed dietary fat is emulsified in bile salts within the intestinal lumen and then converted to monoglycerides or diglycerides and free fatty acids (FFAs) by pancreatic

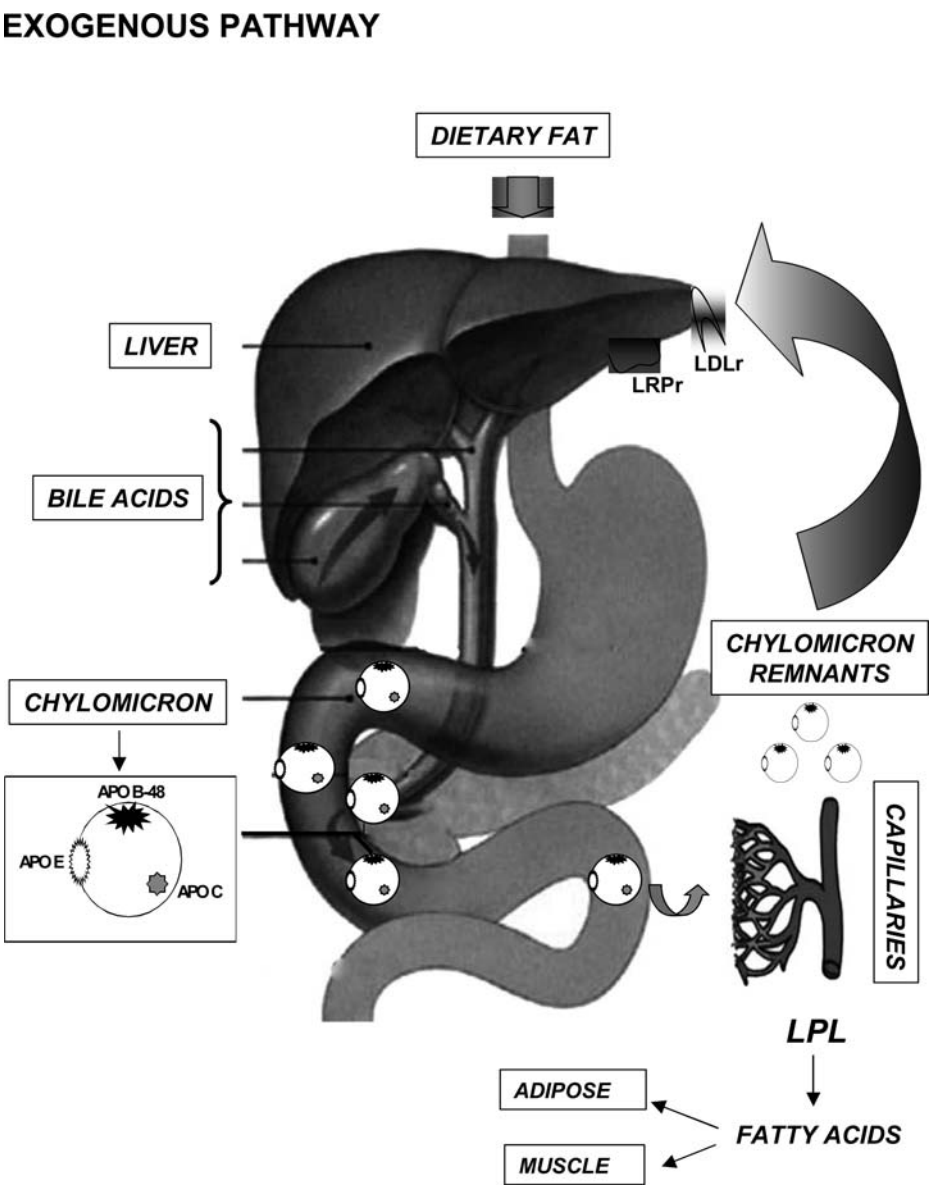

Fig. 4. Lipoprotein metabolism: exogenous pathway. The exogenous pathway starts with dietary consumption of fat that is broken down by pancreatic enzymes and then absorbed by intestinal cells. Here, they are packaged into chylomicrons for export into circulation. Triglycerides carried by the chylomicrons are hydrolyzed into fatty acids by lipoprotein lipase (LPL) for local metabolic needs or for storage in adipose; the remnants return to the liver for removal from circulation.

lipases. Micelles of monoglycerides, FFAs, phospholipids, and bile salts diffuse into intestinal mucosal cells where FFAs are recombined with glycerols to produce TGs, and absorbed dietary cholesterol is esterified by acyl:cholesterol acyltransferase (ACAT) to form CEs *(6)*. Micelle absorption may occur via a Niemann-Pick C1-like 1 protein *(7)*. The TGs and CEs are packaged within chylomicrons intracellularly and then extruded into either the portal circulation or the lymphatic circulation and travel via the thoracic

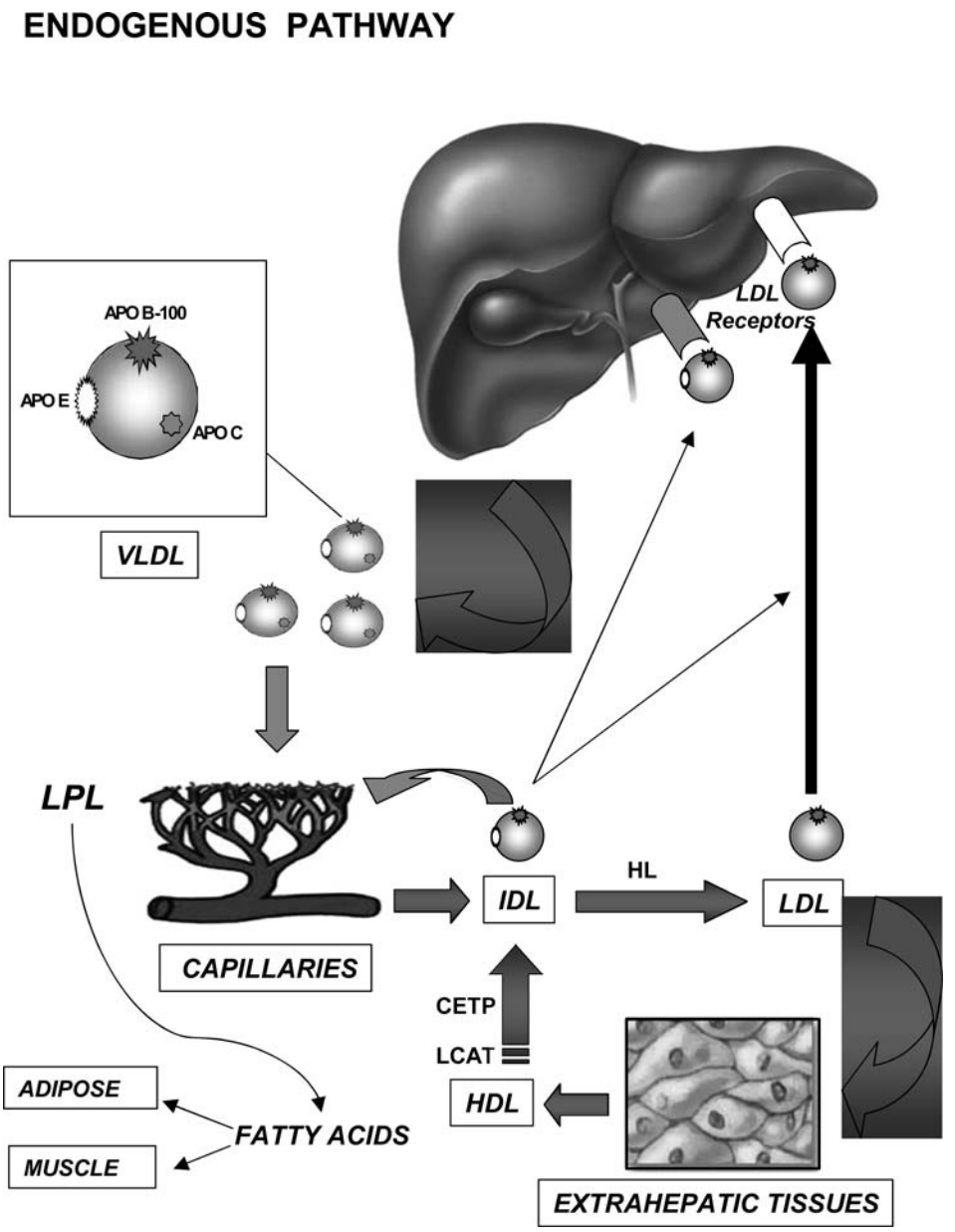

Fig. 5. Lipoprotein metabolism: endogenous pathway. The endogenous pathway starts with very low density lipoprotein (VLDL) production from the liver. The triglycerides carried by the VLDL are hydrolyzed by LPL into fatty acids for local metabolic needs or for storage in adipose; the remaining VLDL remnants, intermediate-density lipoproteins (IDLs), may be absorbed by the liver for removal from circulation or further hydrolyzed by hepatic lipase to form low-density lipoprotein (LDL). LDLs are either returned to the liver or absorbed by nonhepatic tissues to provide cholesterol esters for metabolic needs such as hormone production or cell membrane synthesis.

duct before entering systemic circulation. The chylomicrons initially contain mostly the apolipoproteins apoA-I, apoA-II, and apoB-48 but later acquire increased concentration of apoC-I, apoC-II, apoC-III, and apoE via transfer from HDL and lose their apoA-I with passage to the lymph. By binding to the C-terminal portion of LPL, the apoC-II activates LPL located on luminal side of capillary endothelial cells, which hydrolyzes TG into glycerol (a 3-carbon alcohol) and FFA *(8)*; FFA may then be used for local metabolic needs or stored in adipose. TG hydrolysis is modulated by apoC-III that acts as an LPL inhibitor *(9)*. The remaining glycerol is returned to the liver and intestines via the systemic circulation. ApoE on the remaining chylomicron remnants binds to hepatic LDLr, facilitating the removal of circulating chylomicrons into the liver. Hepatic uptake may also occur via LDL-related protein receptor (LRPr) *(10)*. Thus, the major fate of dietary fat via the exogenous pathway, unless used for immediate metabolic needs, is storage in adipocytes or transfer to the liver.

Endogenous Pathway

The endogenous pathway starts with the production of TG-rich VLDLs in the liver. In situations where dietary fat consumption is insufficient to meet metabolic needs for FFAs (i.e., starvation), the liver can synthesize VLDLs for exportation to other organ systems. Alternatively, cells may generate cholesterol de novo using acetate as a substrate in a process regulated by HMG-CoA reductase and HMG-CoA synthase, but this process is much more metabolically intensive.

Nascent TG-rich VLDLs prior to leaving the liver express apoE and apoB-100 around a core of mostly TGs and some CEs; upon export, nascent VLDLs interact with HDLs, and apoC-I, apoC-II, and apoC-III are added to form mature VLDLs. The apoC-II serves as a cofactor for LPL so that while VLDL is in circulation the TG core of VLDL is hydrolyzed by LPL on capillary endothelial cells forming relatively TG-depleted VLDL remnants, or IDL. The half-life of circulating VLDL is about 30–60 min *(11)*. The hydrolysis of TG by LPL is again moderated by apoC-III that inhibits LPL. The FFA liberated by LPL can again be used for local metabolic needs or stored in adipose as in the endogenous pathway.Because the IDL contains apoB-100 and apoE, these VLDL remnants can be resorbed by the liver via LDLr. Alternatively, approximately half of IDLs may be further hydrolyzed by HL to form LDLs.

Whereas VLDLs are mostly TGs with some CEs, LDLs are mostly CEs with less than 10% TGs. In a TG-rich milieu, LDLs can absorb more TGs, resulting in smaller, denser LDL subfractions that are thought to be more atherogenic. The primary structural apolipoprotein of LDL is apoB-100 that allows LDL to return to the liver for resorption via the LDLr. In the liver, the LDL may be transformed to bile acids and excreted via the biliary tree into the intestinal lumen. From the intestinal lumen, the bile acids may either be removed with the feces (approximately 1 g/day) or reabsorbed via the exogenous pathway in the distal ileum, thus completing the enterohepatic circulatory process.

The physiologic half-life of LDLs in humans is 2–3 days. Approximately half of LDLs, however, are absorbed by nonhepatic tissue. The primary physiologic purpose of LDL is to deliver CEs for critical cellular metabolic needs such as hormone production and cell membrane synthesis. Rapidly dividing cells, for example, because of their need for cellular membrane synthesis exuberantly express LDLr, and the highest level of expression, as expected, occurs in malignant cells. Peripheral cells also express LDLr, which recognize apoE and, to a lesser extent, apoB-100.

HDL METABOLISM

HDLs play a critical role in preventing the deposition of lipids in the periphery. HDL metabolism starts with the synthesis of pre-β lipid-poor apoA-I in the liver and gut, approximately half from each. The discoid-shaped apoA-I is the primary structural protein of HDL. The apoA-I interacts with hepatic and peripheral ATP-binding cassette transporter A1 (ABCA1), which mediates efflux of phospholipids and FC from intracellular storage pools to the structural scaffold provided by apoA-I, thereby forming lipid-laden nascent pre-β-HDL *(12,13)*. LCAT, also activated by apoA-I, esterifies the FC to form CE by transferring a fatty acid chain from lecithin to cholesterol, thereby transforming the nascent pre-β-HDL to the more spherical α-HDL. α-HDL may further accept FC via SRB1 receptors and by passive diffusion *(14)*. SRB1 receptors on hepatic cells may facilitate the selective uptake of α-HDL to the liver *(15)*. The subfractions of HDLs that contain apoE may also undergo hepatic uptake via the LDLr. Thus, HDL, particularly in its HDL_2 subfraction form, serves as the primary vehicle for the return of FC from the periphery back to the liver, a process termed reverse cholesterol transport (Fig. 6). Several epidemiologic studies have postulated an antiatherogenic effect associated with high HDL levels, but the first experimental evidence of the

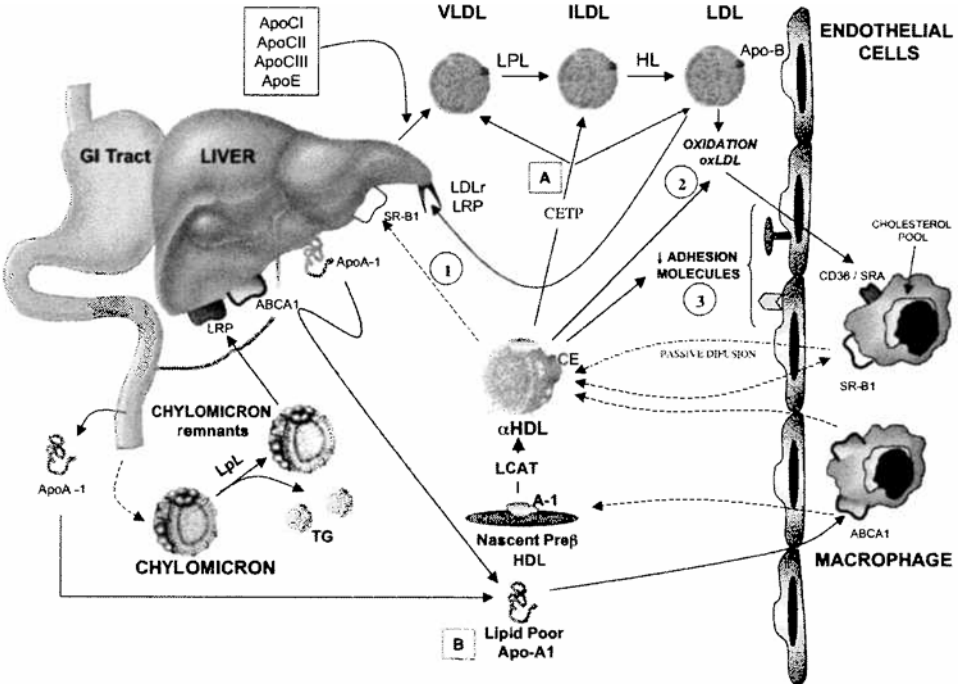

Fig. 6. The role of high-density lipoprotein (HDL) in lipoprotein metabolism. Modified from Brewer *(92)*. The accepted mechanisms behind the potential benefit of HDL are (1) reverse cholesterol transport (RCT) from lipid-laden macrophages in the vessel wall to the liver, (2) shunting of low-density lipoprotein (LDL) from being oxidized, thereby preventing accumulation of lipids within foam cells, and (3) blocking monocyte entry into the vessel wall via downregulation of endothelial cell adhesion molecules (ECAMs). Two sites of action for pharmacologic intervention are identified: (A) the inhibition of cholesterol ester transfer protein (CETP) and (B) augmentation of RCT by increase in lipid-poor apoA-I (i.e., apoA-I Milano infusion).

HDL-mediated reverse cholesterol transport comes from an experimental model by the decrease in lipid deposition in rabbits treated with weekly HDL infusion *(16,17)*. ApoD on α-HDL may also activate CETP, which transfers CEs from α-HDLs to VLDLs, IDLs, and LDLs. HDLs also serve as a reservoir of apoC and apoE for transfer to VLDLs and chylomicrons.

LIPOPROTEIN METABOLISM REGULATION

In the nonpathologic state, consumed dietary fat provides necessary cholesterol for metabolic needs via the exogenous pathway, and in times of fasting, the endogenous pathway supplies cholesterol. The balance between cholesterol deficit and excess is maintained by key regulatory processes.

In the fed state, LPL in adipose is upregulated so that as chylomicrons enter the circulation from the gut, they are hydrolyzed for storage into adipose. This process appears to be insulin mediated. Decreased adipose LPL activity during fasting allows chylomicrons to be used for metabolic demands of other tissues, but also in the fasting state, LPL activity is upregulated in more metabolically active cardiac and skeletal cells to steer the FFA energy substrate toward the organ systems with a greater requirement. Local demands for cholesterol are also regulated by LDLr expression. If cells are in positive cholesterol balance, LDLr is downregulated so that cellular intake of CEs is reduced. Excess cellular cholesterol in the form of 27-hydroxycholesterol also binds to the liver X receptor (LXR) that dimerizes with retinoid X receptor (RXR); this LXR/RXR complex binds to the LXR response element (LXRE) promoter to increase the expression of ABCA1 and SRB1 *(18–20)*. This upregulation of ABCA1 and SRB1 promotes the efflux of FC from the lipid-overloaded cells to HDL for disposal in the liver.

LIPIDS IN VASCULAR BIOLOGY AND ATHEROTHROMBOSIS

In 1852, Virchow first identified the importance of lipid accumulation in the formation of atherosclerotic plaques *(21)*. Perturbations in lipoprotein metabolism, whether by inherited disorders or by environmental factors, may promote atherogenesis. For example, if dietary fat and cholesterol consumption exceed metabolic needs and fecal excretion (i.e., Western diet), then this positive cholesterol balance may result in pathogenic consequences.

Atherothrombosis Phases

As the balance of lipid influx exceeds that of lipid efflux, atherosclerotic plaques progress through a series of five progressive phases (Fig. 7) as described by Fuster et al. *(21)*, based on a classification scheme previously described by the American Heart Association Committee on Vascular Lesions *(22)* and Stary *(23)*. Lipid influx/efflux plays a greater role in atherogenesis in Phases 1–3, whereas Phases 4 and 5 are more lipid independent.

PHASE 1

Early Phase 1 consists of type I lesions that have lipid-laden foam cells, but as lipid accumulates, smooth muscle cells migrate into the vessel intima, and extracellular lipid deposits are formed (type II). Type II lesions can be further divided into the progression-prone type IIa and the progression-resistant type IIb *(24)*. The anatomic

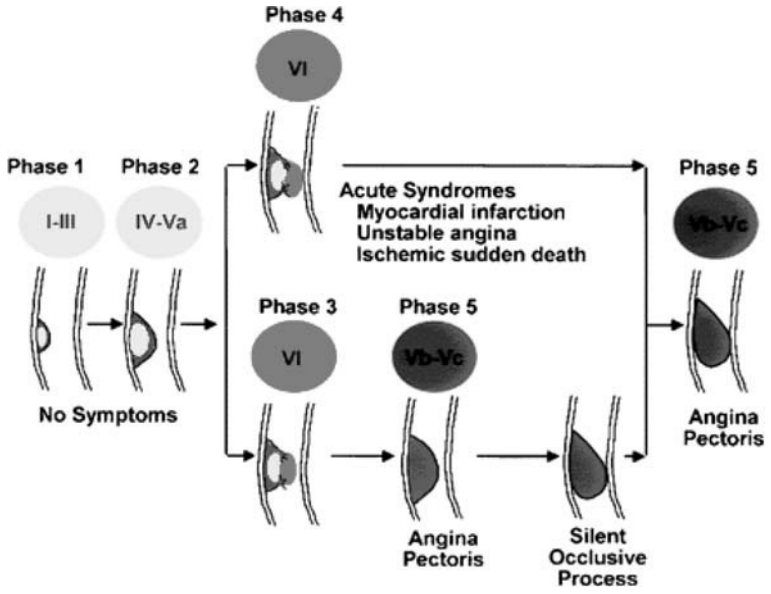

Fig. 7. Clinicopathologic correlation of asymptomatic atherosclerosis leading to symptomatic atherothrombosis. Modified from Corti and Fuster *(93)*.

location of the lesion may determine its fate as IIa or IIb. Departure of blood from laminar flow (e.g., at bifurcation points, curvatures, or stenoses) results in turbulence and areas of relative low shear stress; type IIa lesions are morelikely to be in areas of low shear stress, as LDL particles and monocytes have increased time to migrate into the vessel wall *(25)*. Type III lesions have more smooth muscle cells that have started to form extracellular matrix and have increased extracellular lipid deposition.

Phase 2

The more advanced Phase 2 lesions have a high lipid content and a thin fibrous cap over the lipid core, making them more prone to rupture, especially in an inflammatory state. Plaques that have high free:esterified cholesterol ratios at the plaque center but high esterified cholesterol level at the plaque edge, possibly reflecting high local macrophage activity, are especially prone to rupture *(26)*. These plaques are categorized morphologically as one of two variants: (1) type IV lesions consist of confluent cellular lesions with a great deal of extracellular lipid intermixed with normal intima, which may predominate as an outer layer or cap; and (2) type Va lesions possess an extracellular lipid core covered by an acquired fibrous cap. The American Heart Association classification falls short of identifying plaque erosion or the thin-cap fibroatheroma (TCFA); new classifications have been proposed including these two categories, as proposed by Virmani et al. *(27)*. Once rupture occurs, the lesions are characterized as Phase 3 (clinically silent) or Phase 4 (symptomatic).

Phase 3

Phase 3 lesions are the acute "uncomplicated" type VI lesions, originating from ruptured (type IV or Va) or eroded lesions, and leading to mural, non-obstructive thrombosis. This process is clinically silent but occasionally may lead to the onset of angina *(28)*. As LDL is associated with lipid accumulation and HDL with reverse

cholesterol transport, plaque rupture was more associated with increased total cholesterol:HDL ratio than with either smoking or hypertension *(29)*. HDL may exert an antiapoptotic effect on vascular endothelial cells, which suggests that a plaque may be less vulnerable to disruption and rupture *(30)*.

Phase 4

Phase 4 lesions are characterized by acute "complicated" type VI lesions, with fixed or repetitive, occlusive thrombosis. This process becomes clinically apparent in the form of acute coronary syndrome (ACS), although not infrequently it is silent *(31,32)*. In about two-thirds of ACS, occlusive thrombosis occurs on a nonstenotic plaque. In the remaining one-third, the thrombus occurs on the surface of a stenotic plaque *(33)*. In Phases 3 and 4, changes in the geometry of ruptured plaques, as well as organization of the occlusive or mural thrombus by connective tissue, can lead to occlusive or significantly stenotic and fibrotic plaques. Lipids do not play a direct role in the development of the Phase 4 lesion; however, increased lipid deposition within plaques can make Phase 4 more severe by increasing the thrombus size *(34)*. Lipid-rich atheromatous plaque core contains the most concentrated amount of tissue factor; thus, if disruption of the plaque results in exposure of the lipid core, a larger, more occlusive thrombus may develop *(35)*.

Phase 5

Phase 5 is characterized by type Vb (calcific) or Vc (fibrotic) lesions that may cause angina; however, if preceded by stenosis or occlusion with associated ischemia, the myocardium may be protected by collateral circulation and such lesions may then be silent or clinically inapparent *(36,37)*. Lipid deposition does not play a role in Phase 5 development; Phase 5 may be described as a "healed" lesion, but if it results in a tight stenosis of the vessel lumen without adequate collaterals, these lesions result in significant morbidity.

Pathogenesis of Phase 1: Endothelial Dysfunction

The endothelium is a cellular monolayer that lines the blood vessels, separating the blood from the vascular media, and endothelial dysfunction is the first pathogenic sign in atherogenesis (Fig. 8) and initiates Phase 1 development *(38)*. Endothelial cells perform both autocrine and paracrine functions, and by secreting nitric oxide (NO), they can signal for downregulation of cell adhesion molecules (CAMs), prevent vasoconstriction, shift the balance from thrombosis to fibrinolysis, prevent lipoprotein oxidation, and decrease vessel permeability to lipids *(39)*. Shear stress, caused by blood flow perturbations at bifurcation segments or bends in vessel course, disturbs endothelial function, making the artery at these points more vulnerable to lipid infiltration *(40,41)*. The endothelium responds to shear stress by activating CAMs; these CAMs attract monocytes to enter the vessel wall, which accelerates the atherogenic process. As the monocytes enter and transform into macrophages and become foam cells with the absorption of lipids, they begin to express cytokines that activate the formation of extracellular matrix, fibrosis, and migration of smooth muscle cells into the intima *(42)*.

Hyperlipidemia, in and of itself, also induces endothelial dysfunction; other risk factors for endothelial dysfunction include hypertension (which increases mechanical shear forces), advanced glycation end products (found in diabetics and the elderly),

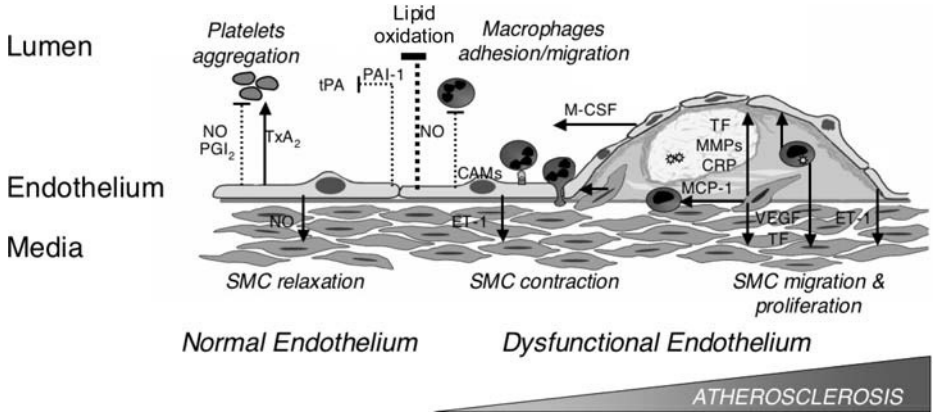

Fig. 8. Hyperlipidemia augments endothelial dysfunction. Dysfunctional endothelium facilitates atherosclerosis development via impaired endothelium-dependent vasodilation, upregulated platelet aggregation and thrombogenicity, increased macrophage adhesion and migration, and smooth muscle cell (SMC) proliferation *(47)*. CAM, cell adhesion molecule; CRP, C-reactive protein; ET, endothelin; MCP, monocyte chemotactic protein; M-CSF, monocyte colony-stimulating factor; MMP, matrix metalloproteinase; NO, nitric oxide; PAI, plasminogen activator inhibitor; PGI_2, prostacyclin; TF, tissue factor; tPA, tissue-type plasminogen activator; TxA2, thromboxane A2; VEGF, vascular endothelial growth factor.

tobacco smoke, vasoactive amines, and immune complexes *(43–46)*. With high lipid levels, NADPH oxidase is upregulated causing nitric oxide (NO) breakdown and superoxide formation *(47)*. Lower NO translates to poorer flow-mediated dilation and increased monocyte and platelet adhesion. Thus, as hyperlipidemia induces endothelial dysfunction, the endothelium loses its ability to prevent the inward migration of lipids and to prevent the oxidation of LDL, which also enhances lipid accumulation, causing a cycle of dysregulation. LDL apoB binds to the extracellular matrix proteins such as proteoglycans, collagen, and fibronectin, leading to the retention of LDL in the vessel wall *(48)*. Once in the wall, LDL, particularly in its oxidized form, may induce inflammation, further increasing plaque vulnerability to rupture *(48)*.

HDL, however, may enhance endothelial function through its anti-inflammatory and antioxidant properties *(49)*, and HDL mediates other mechanisms that may enhance endothelial function (Fig. 9). HDL decreases CAM expression by inhibiting the expression of IL-1α-induced E-selectin *(50,51)*. Also, HDL binds to endothelial SRB1 receptors, thereby activating endothelial NO synthase *(52)*; this mechanism may improve endothelial function by restoring NO bioavailability *(53,54)*. Therefore, by inhibiting endothelial dysfunction, HDL may play a critical role in limiting atherogenesis in addition to its role in reverse cholesterol transport.

Progression into Phase 2: Lipid Influx

If LDL plasma concentrations are high, excess circulating LDLs may be absorbed by macrophages and foam cells. As lipids accumulate, the atherosclerotic lesions progress to Phase 2. Macrophages and foam cells also express LDLr that facilitates the selective uptake of LDL, but these cells also express unregulated scavenger receptors, CD36 or SRA, that absorb oxidized LDL, making oxidized LDL a particularly potent chemoattractant molecule for these cells *(55–57)*. Oxidized LDL contains oxidized

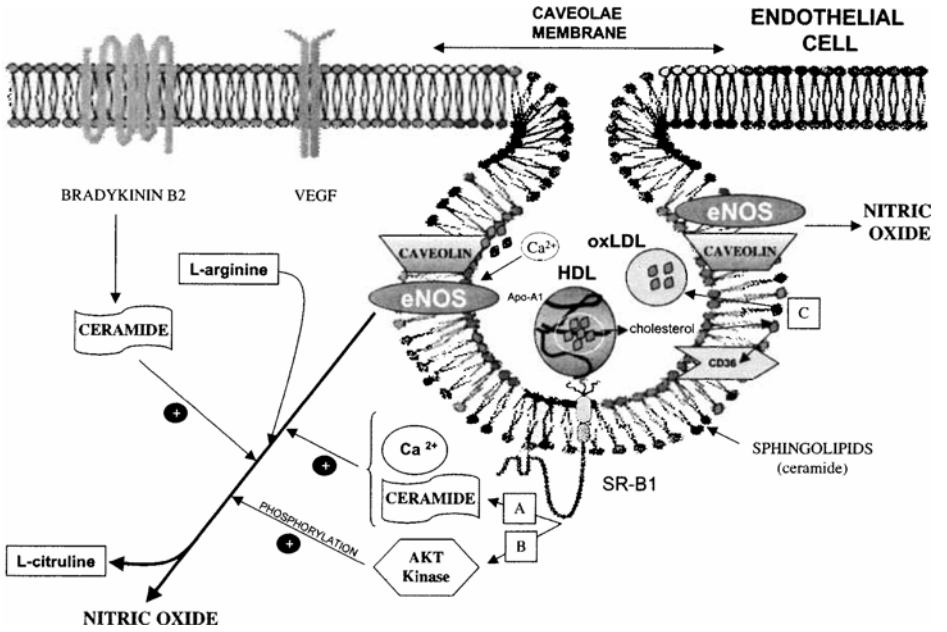

Fig. 9. Nitric oxide dysregulation results in increased lipid accumulation in the vessel wall which may be counterbalanced by high-density lipoprotein (HDL). (A) HDL binding to SRB1, a class B scavenger receptor, can stimulate eNOS by a ceramide-dependent pathway. (B) SRB1–HDL interaction also induces the activation of Akt kinase, which subsequently phosphorylates eNOS and stimulates the enzyme to synthesize nitric oxide. (C) Oxidized low-density lipoprotein (LDL)-initiated CD36-dependent depletion of caveola cholesterol is countered, which allows eNOS to remain associated with caveolae, thereby maintaining the ability of nitric oxide production.

phospholipids that are generated under situations of oxidative stress such as infection and other inflammatory states, and the oxidated state allows binding to these receptors, but native LDL does not *(58)*. These responses to excess oxidized LDLs are adaptive in that they may prevent the LDLs from otherwise causing endothelial injury *(59)*, but as these macrophages continue to absorb LDLs and begin to foam, lipid accumulates within the vessel wall, thereby increasing atherosclerosis *(60)*. Endothelial cells may also selectively uptake oxidized LDLs via the Lox-1 receptor *(61)*. The uptake of oxidized LDLs triggers the activation of transcriptional nuclear factor-kappa-B (NFκB), which upregulates the expression of CAMs that encourage monocyte entry into the vessel wall *(62)*.

In addition to the inhibition of plasminogen, another role that Lp(a) plays is in lipid influx. Lp(a), a modified form of LDL that carries the apo(a), binds with especially high affinity to specific receptor on macrophages that result in rapid accumulation of LDL and foaming *(63)*. Oxidized LDLs may also augment the atherogenicity of Lp(a) *(64)*.

As the vessel thickens from lipid influx and remodeling of the vessel wall, the diffusion of oxygen from blood to the outer vessel wall is impaired, and without neovascularization, oxygen demand may exceed supply at the cellular level, resulting in either anaerobic metabolism or necrosis. Macrophages, however, express cytokines that encourage neovessel formation in the wall to supply oxygenated blood to the thickened large vessels *(65,66)*. Vasa vasorum neovascularization surrounds the adventitia and