Contemporary Cardiology
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Michael H. Davidson
Peter P. Toth
Kevin C. Maki *Editors*

Therapeutic Lipidology

Second Edition

Humana Press

Contemporary Cardiology

Series Editor

Peter P. Toth
Ciccarone Center for the Prevention of Cardiovascular Disease
Johns Hopkins University School of Medicine
Baltimore, MD
USA

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Editors
Michael H. Davidson
The University of Chicago Pritzker School of Medicine
Chicago, IL
USA

Kevin C. Maki
Department of Applied Health Science
Indiana University School of Public Health
Bloomington, IN
USA

Peter P. Toth
Cicarrone Center for the Prevention of Cardiovascular Disease
Johns Hopkins Univerity School of Medicine
Baltimore, MD
USA

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Preface

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

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

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

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

Chicago, IL, USA — Michael H. Davidson, MD
Baltimore, MD, USA — Peter P. Toth, MD, PhD
Bloomington, IN, USA — Kevin C. Maki, PhD

Contents

List of Contributors

Judith A. Aberg, MD Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai/Mount Sinai Health System, New York, NY, USA

Shone Almeida, MD Division of Cardiology, Lundquist Institute, Torrance, CA, USA

Karen E. Aspry, MD, MS Lipid and Prevention Program, Lifespan Cardiovascular Institute, Providence, RI, USA

Department of Medicine (Clinical), Brown University, Alpert Medical School, Providence, RI, USA

Department of Cardiovascular Medicine, Rhode Island Hospital and Miriam Hospital, Providence, RI, USA

Maciej Banach, MD, PhD Department of Hypertension, Medical University of Lodz, Lodz, Poland

Polish Mother's Memorial Hospital Research Institute (PMMHRI), Lodz, Poland

Marcin A. Bartlomiejczyk, MSc Department of Hypertension, Medical University of Lodz, Lodz, Poland

Lane Benes, MD Department of Cardiology, Evergreen Hospital, Kirkland, WA, USA

Vera Bittner, MD, MSPH Department of Medicine, Division of Cardiovascular Disease, University of Alabama at Birmingham, Birmingham, AL, USA

Michael B. Boffa, PhD Department of Biochemistry, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada

Eric J. Brandt, MD Division of Cardiovascular Diseases, Department of Internal Medicine, University of Michigan, Ann Arbor, MI, USA

George A. Bray, MD, MACP, MACE Department of Clinical Obesity, Pennington Center Louisiana State University, Baton Rouge, LA, USA

Julie A. Brothers, MD Department of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA

Division of Cardiology, The Children's Hospital of Philadelphia, Philadelphia, PA, USA

Alan S. Brown, MD Division of Cardiology and Lipid Clinic, Advocate Lutheran General Hospital, Park Ridge, IL, USA

Matthew Budoff, MD Division of Cardiology, Department of Medicine, Lundquist Institute, Torrance, CA, USA

Adib Chaus, MD Division of Cardiology, Advocate Lutheran General Hospital, Park Ridge, IL, USA

Vadzim Chyzhyk, MD Prairie Cardiovascular Consultants, Southern Illinois Healthcare, Department of Cardiology, Clinical Associate Professor, Southern Illinois University, Carbondale, IL, USA

Ehab G. Dababneh, MD Division of Cardiology, Advocate Lutheran General Hospital, Park Ridge, IL, USA

Stephen R. Daniels, MD, PhD Department of Pediatrics, University of Colorado School of Medicine, Aurora, CO, USA

Department of Pediatrics, Children's Hospital Colorado, Aurora, CO, USA

Maryam Darabi, PhD ICANalytics Team, Institute of Cardiometabolism and Nutrition (ICAN), Hospital Pitie – Salpetriere, Paris, France

Michael H. Davidson, MD The University of Chicago Pritzker School of Medicine, Chicago, IL, USA

Thomas Dayspring, MD Attia Medical, PC, San Diego, CA, USA

Christopher W. DeVille, BS, CHCP American Board of Clinical Lipidology, Jacksonville, FL, USA

Mary R. Dicklin, PhD Midwest Biomedical Research: Center for Metabolic & Cardiovascular Health, Addison, IL, USA

Matthew Evans, MD Department of Internal Medicine, University of Maryland Medical Center, Baltimore, MD, USA

Steven Feinstein, MD Department of Medicine, Cardiology, Rush University Medical Center, Chicago, IL, USA

Jennifer Fleming, PhD, MS, RDN Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, USA

Abhimanyu Garg, MD Division of Nutrition and Metabolic Diseases, Department of Internal Medicine, Center for Human Nutrition, UT Southwestern Medical Center, Dallas, TX, USA

Antonio Gotto Jr, MD, DPhil Weill Cornell Medicine, New York, NY, USA

Christian R. Hamilton-Craig, MBBS, PhD, FRACP, FCSANZ, FSCCT The Prince Charles Hospital, Griffith University School of Medicine, Chermside, Brisbane, QLD, Australia

Ian R. Hamilton-Craig, MBBS, PhD, FRACP, FCSANZ, FLS Flinders Rural Health South Australia, Flinders University School of Medicine, Victor Harbor, SA, Australia

Kristin M. Hirahatake, PhD, RD AbbVie, Inc., Irvine, CA, USA

Emily A. Johnston, MPH, RDN, CDE Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, USA

Kenneth Kellick, PharmD, CLS Department of Pharmacy, VA Western New York Healthcare System, Buffalo, NY, USA

Sarah M. King, PhD Department of Pediatrics, University of California, San Francisco, San Francisco, CA, USA

Anatol Kontush, PhD Hospital Pitie – Salpetriere, Sorbonne University, INSERM Unit 1166, Paris, France

Marlys L. Koschinsky, PhD Department of Physiology and Pharmacology, Schulich School of Medicine and Dentistry, The University of Western Ontario, Robarts Research Institute, London, ON, Canada

Karam Kostner, MD, PhD Department of Cardiology, Mater Hospital Brisbane, Brisbane, QLD, Australia

Ronald M. Krauss, MD Departments of Pediatrics and Medicine, University of California, San Francisco, San Francisco, CA, USA

Penny M. Kris-Etherton, PhD, RD Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, USA

Kevin C. Maki, PhD Midwest Biomedical Research: Center for Metabolic & Cardiovascular Health, Addison, IL, USA

Department of Applied Health Science, Indiana University School of Public Health, Bloomington, IN, USA

Carlos D. Malvestutto, MD, MPH Department of Medicine/Infectious Diseases, Ohio State University Wexner Medical Center, Columbus, OH, USA

Victor Marinescu Department of Cardiology, Advocate Medical Group at Advocate Aurora Health, Naperville, IL, USA

Peter A. McCullough, MD, MPH Department of Internal Medicine, Division of Cardiology, Baylor University Medical Center, Baylor Heart and Vascular Hospital, Baylor Heart and Vascular Institute, Dallas, TX, USA

Jeffrey W. Meeusen, PhD Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

Michael Miller, MD Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA

Pamela B. Morris, MD Department of Medicine, The Medical University of South Carolina, Mount Pleasant, SC, USA

Dragana Nikolic, BSc, MSc, PhD Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, Palermo, Italy

Børge G. Nordestgaard, MD, DMSc Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark

Carl E. Orringer, MD Department of Cardiovascular Medicine, University of Miami Miller School of Medicine, Miami, FL, USA

Johnathon Seth Parham, MD Department of Cardiology, NYU Langone Hospital, New York, NY, USA

Peter E. Penson, MPharm, Ph.D. School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK

Kristina Petersen, PhD, BNutDiet (Hons) Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, USA

Gregory S. Pokrywka, MD, FACP, FNLA, FASPC, NCMP Assistant Professor of General Internal Medicine, Johns Hopkins University School of Medicine, Director: Baltimore Lipid Center, Baltimore, MD, USA

Nataliya Pyslar, MD Division of Cardiology, Cook County Health, Chicago, IL, USA

Department of Medicine, RUSH Medical College, Chicago, IL, USA

Anupama K. Rao, MD Department of Medicine, Cardiology, Rush University Medical Center, Chicago, IL, USA

Ronak Rengarajan, MD Department of Internal Medicine, Division of Cardiology, Baylor University Medical Center, Baylor Heart and Vascular Hospital, Dallas, TX, USA

Manfredi Rizzo, MD, PhD Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo, Palermo, Italy

Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of South Carolina, Palermo, Italy

Joyce L. Ross, MSN, CRNP, CS Accreditation Council for Clinical Lipidology, School of Graduate Nursing, West Chester, PA, USA

Raul D. Santos, MD, PhD, MSc Department of Cardiology and Lipid Clinic, Heart Institute (InCor) University of Sao Paulo Medical School Hospital, Sao Paulo, Brazil

Philip A. Sapp, MS Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA, USA

Joseph J. Saseen, PharmD, CLS Department of Clinical Pharmacy and Family Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, USA

Alexandros G. Sachinidis, MD, MSc, PhDc 2nd Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, Hippokration General Hospital of Thessaloniki, Thessaloniki, Greece

Kashif Shaikh, MD Division of Cardiology, Lundquist Institute, Torrance, CA, USA

Vinaya Simha, MD Internal Medicine, Division of Endocrinology, Mayo Clinic, Rochester, MN, USA

Allan D. Sniderman, MD Department of Cardiology, McGill University, Montreal, QC, Canada

Anca Pantea Stoian, MD, PhD Department of Diabetes, Nutrition and Metabolic Diseases, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

Neil J. Stone, MD Feinberg School of Medicine/Northwestern University, Department of Medicine (Cardiology), Northwestern Memorial Hospital, Chicago, IL, USA

Kristen M. Tecson, PhD Baylor Heart and Vascular Institute, Baylor Scott and White Research Institute, Dallas, TX, USA

Peter P. Toth, MD, PhD Cicarrone Center for the Prevention of Cardiovascular Disease, Johns Hopkins University School of Medicine, Baltimore, MD, USA

James A. Underberg, MS, MD Department of Medicine, NYU Center for Prevention of Cardiovascular Disease, NYU Langone Medical Center & NYU Grossman School of Medicine, New York, NY, USA

Anette Varbo, MD, PhD Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark

Natalie C. Ward, BSc (Hons), PhD Medical School, University of Western Australia, Perth, WA, Australia

School of Public Health, Curtin University, Perth, WA, Australia

Karol E. Watson, MD, PhD Department of Cardiology, University of California, Los Angeles, Los Angeles, CA, USA

Gerald F. Watts, DSc, MD, PhD Medical School, University of Western Australia, Perth, WA, Australia

Lipid Disorders Clinic, Royal Perth Hospital, Perth, WA, Australia

Robert A. Wild, MD, MPH, PhD Department of Obstetrics/Gynecology, Oklahoma University Health Sciences Center, Oklahoma City, OK, USA

Michael J. Wilkinson, MD Division of Cardiovascular Medicine, Department of Medicine, University of California San Diego, La Jolla, CA, USA

Emile Zakiev, MSc Department of Medicine, Hospital Pitie – Salpetriere, Sorbonne University, Paris, France

History of Lipidology

1

Antonio Gotto Jr. and Michael H. Davidson

Discovery of Cholesterol and the Link to Atherosclerosis

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

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

Elucidation of the Correct Structure of Cholesterol in 1932

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

A. Gotto Jr.
Weill Cornell Medicine, New York, NY, USA
e-mail: amg2004@med.cornell.edu

M. H. Davidson (✉)
The University of Chicago Pritzker School of Medicine, Chicago, IL, USA
e-mail: mdavidso@bsd.uchicago.edu

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

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

Elucidation of the Cholesterol Synthetic Pathway

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

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

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

Discovery of LDL and the Birth of Lipidology

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

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

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

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

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

Developing Drugs to Lower Cholesterol

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

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

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

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

The Statin Era Begins

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Appendix

List of Important Discoveries

Anitschkow: Discovery of cholesterol inducing atherosclerosis in rabbits

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

Michel Macheboeuf: Discovery of plasma lipoproteins

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

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

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

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

Fredrickson and Levy: Classification of plasma lipoprotein disorders

Brown and Goldstein: Discovery of LDL receptors

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

Endo: Discovery of statins

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

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2 Atherogenesis and Vascular Biology

Peter P. Toth

Atherogenesis

Introduction

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

P. P. Toth (✉)
Cicarrone Center for the Prevention of Cardiovascular Disease, Johns Hopkins University School of Medicine, Baltimore, MD, USA
e-mail: Peter.toth@cghmc.com

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

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

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

Arterial Structure

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

Endothelial Cell Function and Dysfunction

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

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

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

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

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

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

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

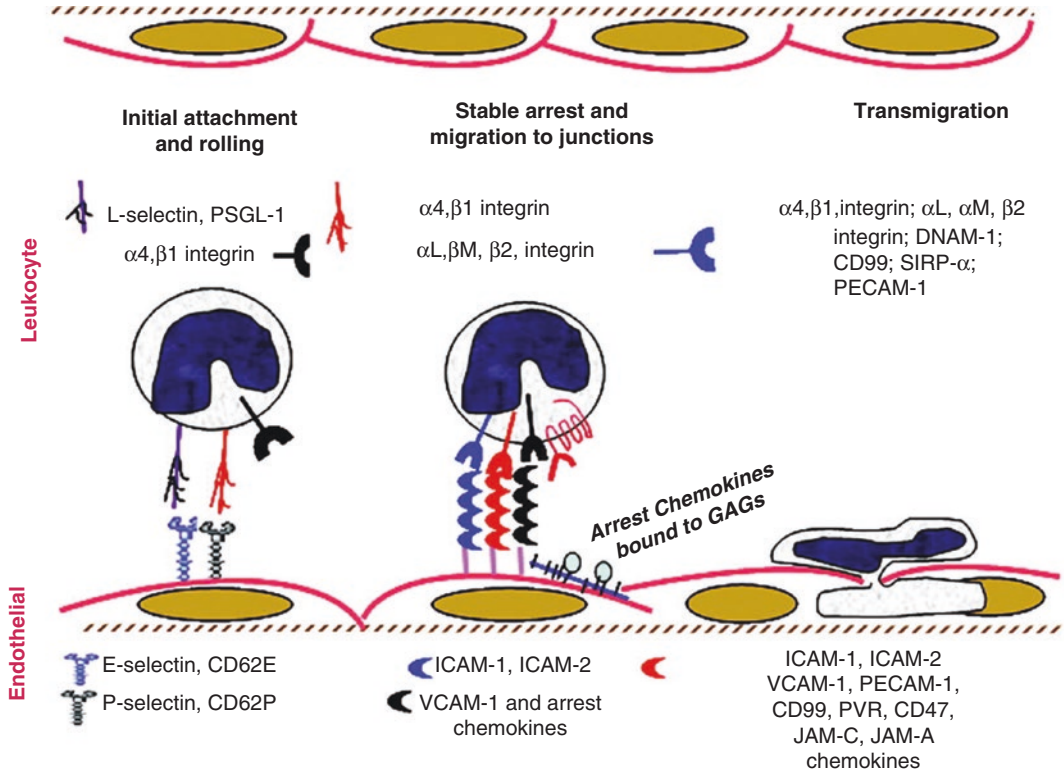

Fig. 2.1 Leukocyte recruitment from blood into the subendothelial space. Complex orchestration of cell attachment and diapedesis with sequential expression of different integrins, selectins, and adhesion molecules. Initial attachment and rolling, arrest, and migration to cell-cell borders and transmigration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

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

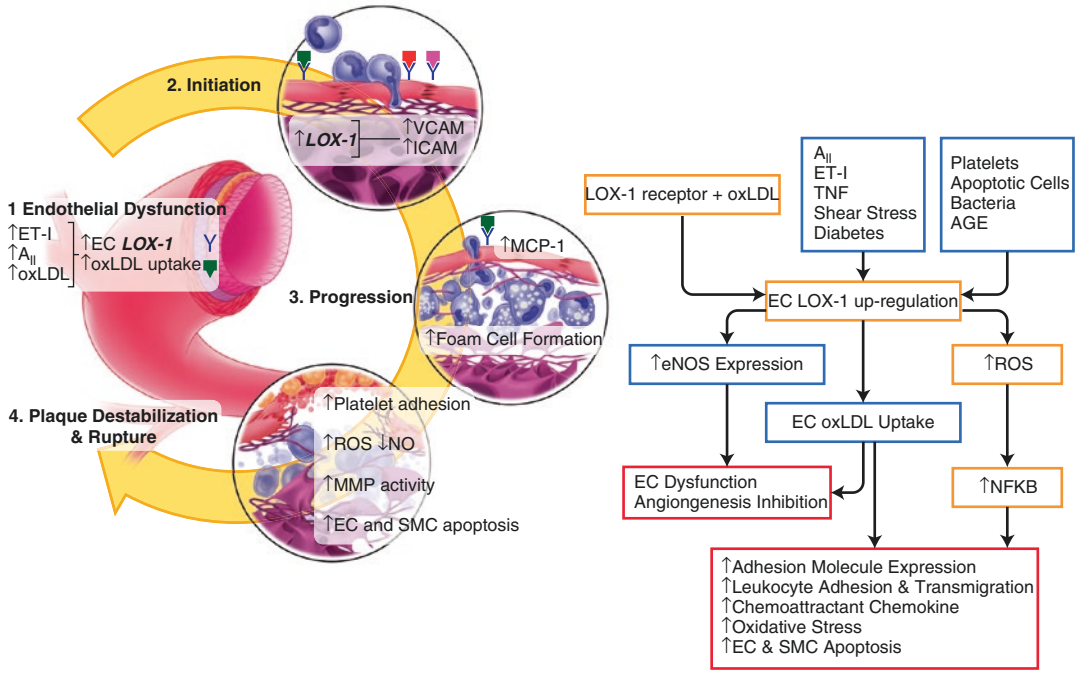

Fig. 2.2 LOX-1 and inflammation. LOX-1 plays a role in the initiation, progression, and destabilization of atherosclerotic plaques. The steps in atherogenesis it impacts are shown on the left and summarized in greater detail on the right. LOX-1 binding and signaling initiate a series of molecular and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

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

Receptors of Advanced Glycosylated End Products

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

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

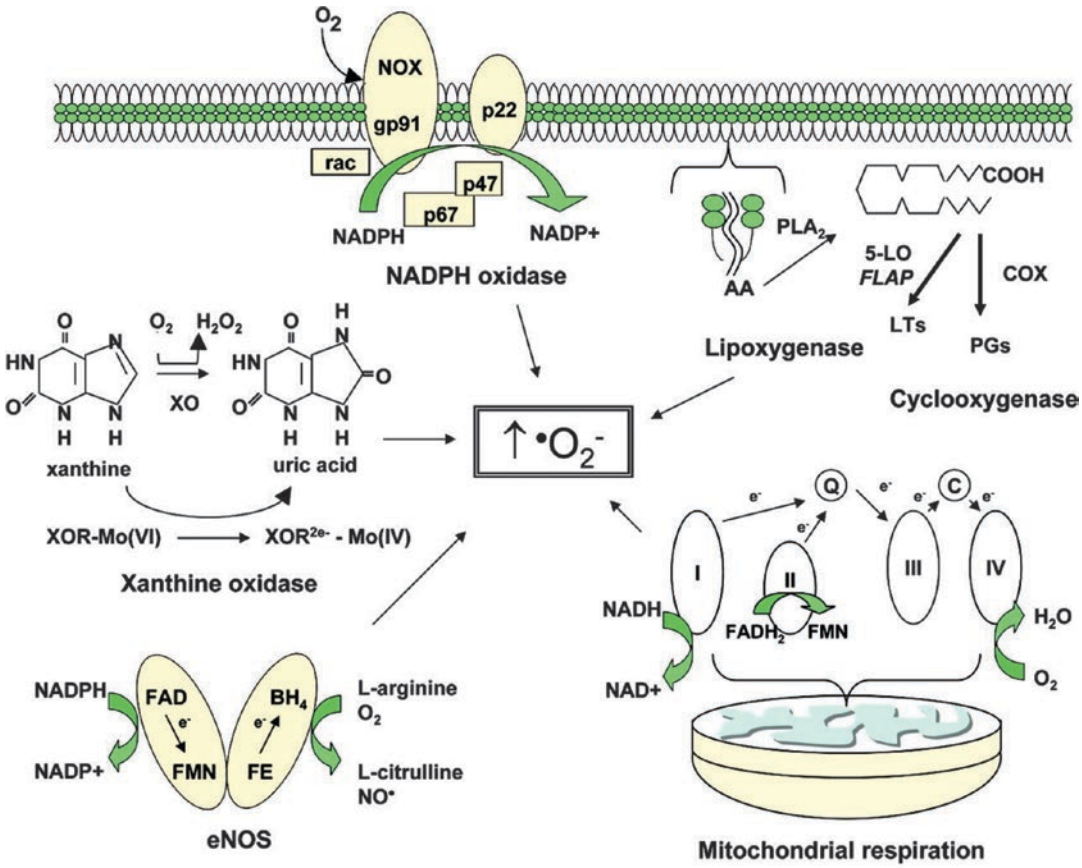

Fig. 2.3 Metabolic and enzymatic sources of superoxide anion in the vasculature. Superoxide anion (•O2⁻) is formed by several metabolic and enzymatic sources within the cell. NADPH oxidase is composed of multiple membrane-bound and cytoplasmic subunits. The enzyme is activated when the cytoplasmic subunits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

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