

# Lipids and Cardiovascular Disease: Putting It All Together

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Since the early studies of Anichkov, the accumulation of cholesterol within the subendothelial space of the arterial wall and within lesions on the lumen-facing surface of arteries have been hallmarks of cholesterol and atherosclerotic cardiovascular disease (ASCVD). This issue of the *Methodist DeBakey Cardiovascular Journal* focuses on cholesterol in the context of atherosclerosis and cardiovascular disease and contains a series of articles from experts who discuss the role of cholesterol diagnostics in patient management, treatment options, reverse cholesterol transport, diabetes-linked ASCVD risk, and preclinical gene therapy. To set the stage for the main articles, we first review the illustrious history of cholesterol, the most famous and arguably most important lipid. In addition to its role in membrane and plasma lipoprotein structure and function, cholesterol is also important in regulatory aspects of metabolism because it is a precursor to numerous life-essential steroid hormones. This is likely the reason it has been so widely studied and the object of numerous Nobel Prizes in chemistry and physiology or medicine. In the context of this issue of the *Methodist DeBakey Cardiovascular Journal*, however, cholesterol takes on the more sinister role of the culprit in the etiology of ASCVD, making it both a diagnostic and therapeutic target.

The opening overview, “Cholesterol: Can’t Live With It, Can’t Live Without It,” discusses the evolution of cholesterol research, the recent changes of opinion about the role of high-density lipoprotein (HDL) in atheroprotection, the development of traditional and novel lipid-lowering therapies, and the role of reverse cholesterol transport in HDL cardioprotection. Following this overview, the first article describes a physician-patient interaction in the context of current lipid treatment guidelines, which must be integrated with physician judgment and recently emerging knowledge to bring about the best outcome (i.e., reducing risk for ASCVD). This is followed by two reviews on statin and nonstatin lipid therapeutics and, later in the series, another forward-looking article that describes preclinical gene delivery.

One mechanism for the suppression and reversal of ASCVD is the transfer of cholesterol from arteries to the liver for ultimate fecal disposal. This process is known as reverse cholesterol transport (RCT), which is covered in detail in the next two articles. In the current model, RCT comprises three steps: (1) free cholesterol (FC) efflux from macrophages in the subendothelial space of the arterial wall to form nascent (n)

HDL, (2) nHDL-FC esterification by plasma lecithin:cholesterol acyltransferase, and (3) hepatic FC extraction and transfer to bile and feces for disposal. This model, which has been used and cited by many researchers over the past 40 years, has been refined in response to the discovery of relevant transporters and receptors. Foremost among these are transporters ATP binding cassette A1 (ABCA1), ABCG1, and ABCG4, which mediate FC efflux, and the HDL scavenger receptor class B type 1 (SR-B1), which initiates HDL-cholesterol disposal by selective uptake. The final three articles summarize the work of three laboratories that study HDL, structure, speciation, and properties as well as HDL biogenesis and catabolism.

## MANAGING DYSLIPIDEMIA—CLINICAL JUDGMENT IN THE CONTEXT OF GUIDELINES

In the first review, Dr. Carl Orringer offers a practical guide to the real-world management of ASCVD lipid risk factors, promoting the use of published guidelines in the context of clinical judgment and the appearance of new data that have not yet been integrated into new guidelines. As new evidence emerges, guidelines are modified accordingly, yet there are usually gaps between updates. This finding reinforces the importance of clinical judgment in the process of shared decision making between physician and patient. Management of patients with severe hypercholesterolemia has been described by the 2013 American College of Cardiology/American Heart Association Blood Cholesterol Guideline and was recently updated in the 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APH/AASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol, which was published after the submission deadline for this issue. The guideline is based on the most recent high-quality evidence and identifies existing treatment gaps that should be considered in the care of such patients. Guideline documents are evidence-based to provide the best rationalized treatment plan. While all guidelines must be updated in accordance with newly published high-quality studies, treatment gaps must be addressed using clinical judgment. Using the example of a patient with severe hypercholesterolemia, Dr. Orringer walks us through these principles by collecting the patient history, lipid and nonlipid metabolic determinants, and current medications, which include a high-intensity statin therapy that resulted in only modest low-density lipoprotein cholesterol (LDL-C) reduction for the patient. He then goes through various decision trees: Should additional blood testing or biomarker testing be done? Is there

an evidence-based LDL-C goal? Should statin intensity be increased or a different statin prescribed? Should ezetimibe or a bile acid sequestrant be prescribed? Is the patient a candidate for a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor? These questions exemplify the importance of incorporating information published between guideline updates into patient management decisions.

## STATINS—THEN AND NOW

Next, Dr. Peter Toth discusses the history of the statin class of plasma cholesterol-lowering drugs and its emergence as one of the safest and most widely prescribed drug classes. The accumulation of cholesterol has long been one of the traditional hallmarks of ASCVD and was first described by Nikolay Anichkov, who observed lesions in rabbits after feeding them pure cholesterol. Studies including those by Gofman, the Framingham Heart Study, and Seven Countries Study associated serum cholesterol levels, especially LDL-C, with myocardial infarction. Brown and Goldstein published multiple papers showing that some patients with familial hypercholesterolemia had defects in the LDL-receptor pathway. The Lipid Research Clinics Coronary Primary Prevention Trial showed that cholestyramine, a bile-acid-binding resin sequestrant, reduced total cholesterol and LDL-C while reducing cardiovascular events and nonfatal myocardial infarction. This demonstrated the cardioprotective value of reducing serum LDL-C and provided a rationale for more potent and better tolerated LDL-C-lowering agents. Although the first successful LDL-C-lowering drug was lovastatin, statin efficacy was firmly established by the Scandinavian Simvastatin Survival Study, which showed that simvastatin reduced cardiovascular events, cardiovascular mortality, and total mortality in patients with coronary disease; thereafter came many statin imitators. After multiple prospective randomized trials, statins became accepted as a safe, well-tolerated, and effective means for reducing the atherogenic lipoprotein burden. Outcomes studies showed that statins reduced the risk for nonfatal myocardial infarction and ischemic stroke, the need for revascularization, and cardiovascular and all-cause mortality. In addition, statins stabilize and regress established atherosclerotic plaque. Although statin dosing was initially determined by risk-stratified LDL-C goals, the recent trend is away from LDL-C goal attainment and more toward cardiovascular risk and percent LDL-C reduction. Reports of statin-associated cognitive impairment have been anecdotal and unsubstantiated. Although some trials revealed increased risk for diabetes mellitus with statin therapy, moderate- to high-dose statin therapy will add one person per 500 to the diabetic population. In context, patients who develop diabetes on statin therapy are already prediabetic, and diabetic patients derive as much benefit from statin therapy as do patients without diabetes. One currently

open question is “How low is too low for serum LDL-C levels?” Dr. Toth ends by citing the disconcerting fact that long-term statin adherence rates are low and, even when used, statins tend to be under-dosed; this is unfortunate for an inexpensive drug with such an impressive record of safety and efficacy.

## NONSTATIN MANAGEMENT OF DYSLIPIDEMIA

Drs. Xiaoming Jia, Patrick Lorenz, and Christie Ballantyne go on to affirm that while statins remain the first-line therapy for patients with elevated LDL-C and at high risk for ASCVD, other options should be considered for patients who are statin unresponsive or intolerant. The most adverse side effect of statins is rhabdomyolysis, which is fortunately rare. There are other options that are better tolerated, including ezetimibe, which inhibits intestinal cholesterol absorption, and PCSK9 inhibitors, which increase LDLR levels on plasma membranes of cells, thereby lowering plasma LDL-C levels. As pointed out by Li et al., trials of ezetimibe and PCSK9 inhibitors revealed that lowering LDL-C to the lowest level possible, even below the current recommendation of 70 mg/dL, confers additional ASCVD outcomes benefit, and adding select nonstatin agents to background statin therapy confers additional benefit. As new nonstatin therapeutics are validated, they should be considered if maximally tolerated statin monotherapy does not bring LDL-C to target levels. Noninvasive imaging techniques—calcium scoring, protein biomarkers, and genetic assays—will guide practitioners to prescribe the optimal medications for the reduction of ASCVD risk. Advances in risk assessment and diagnostic approaches will set the stage for better managing dyslipidemia and ASCVD risk in the emerging age of precision medicine.

## HDL FUNCTION, RCT, AND BIOMARKERS

Drs. Kayla Riggs and Anand Rohatgi then shift the focus to RCT and HDL function, summarizing the current status of HDL research at a time when traditional thought about the salutary properties of HDL is segueing from quantity to quality. Whereas conventional wisdom has been that a high plasma HDL-C level is protective, more recent studies show the relationship to be more complex and confounded by other factors.

Niacin, apoA1/phospholipid infusions, and cholesteryl ester transfer protein inhibitors profoundly increase plasma HDL levels, but their failure to reduce cardiovascular events in recent trials suggests that plasma HDL-C levels are not causally linked to ASCVD. Other reports, including a Mendelian randomization study of an HDL-raising gene, support this conclusion. Thus, HDL may simply be a biomarker for other salutary metabolic factors but may not be related to ASCVD risk reduction. Studies revealing that the ASCVD hazard ratio and total mortality exhibit

a U-shaped curve with respect to plasma HDL levels suggest that different factors may contribute to ASCVD at high versus low plasma HDL levels. For example, further analysis shows that a low plasma HDL-C level is likely a marker of insulin resistance and associates with ASCVD but not always with mortality. On the other hand, the mechanism by which a high plasma HDL-C increases ASCVD risk is unknown and deserves additional study. Collectively, the JUPITER trial and several other studies reveal that HDL particle number is a stronger predictor of incident cardiovascular events than HDL-C or apoA1 levels. Given its predictive strength, it will be important to have more accurate and simple methods to determine HDL particle number for diagnosis and the evaluation of new ASCVD therapeutics.

### RCT IN THE CONTEXT OF HDL FREE CHOLESTEROL BIOAVAILABILITY

In keeping with the topic of RCT, Drs. Corina Rosales, Henry Pownall, and colleagues review nHDL transport *in vitro* and *in vivo*. Previous studies in mice and humans respectively showed that HDL-FC was hepatically cleared on a time scale of ~2 and ~10 min, times during which little HDL-FC esterification had occurred. Studies using nHDL labeled with [<sup>3</sup>H]FC, [<sup>14</sup>C]PL, and [<sup>125</sup>I] to follow the distinct metabolic pathways of each HDL analyte showed that the plasma clearance times of each analyte was different; PL and FC were hepatically cleared within a few minutes, whereas apoA1 was cleared with a half time of ~7 hours. As with HDL in mice and humans, the clearance of nHDL-FC involved little esterification (~2%), so that there was only a minimal role for LCAT in RCT. Moreover, at longer times, FC appeared in all major organs albeit at different concentrations, with the highest being in erythrocytes. These findings support the concept of high FC bioavailability, whereby it diffuses to many tissue sites by a spontaneous transfer mechanism that likely includes transintestinal FC transport in addition to the better-known transhepatic RCT route. The design of future therapeutic strategies to improve RCT will have to be formulated in the context of these dual RCT mechanisms and the role of FC bioavailability.

### THE TRANSINTESTINAL CHOLESTEROL EXCRETION PATHWAY

Next, Drs. Aldo Grefhorst, Henkjan Verkade, and Albert Groen continue this theme by reviewing recent studies on an alternative pathway that is responsible for about 25% of whole-body RCT. Unlike the transhepatic RCT pathway, cholesterol in the transintestinal cholesterol excretion (TICE) pathway is taken up from very low-density lipoprotein particles at the basolateral side of the enterocyte, after which the sterol translocates to the apical side of the enterocyte. On the apical side, the ABCG5/ABCG8 heterodimer transports cholesterol into the intestinal lumen. Current evidence suggests that much of the

secreted cholesterol is reabsorbed via the cholesterol-influx transporter Niemann-Pick C1-Like 1. Many of the pathways and proteins involved in intracellular cholesterol trafficking in the enterocyte have not been identified. A major TICE pathway is likely spontaneous cholesterol transfer that is independent of protein carriers. TICE may be part of a larger process by which FC moves throughout the body according to the total whole-body cholesterol burden. Both reuptake and efflux can be influenced by pharmacologic agents, making the TICE pathway an attractive target for the reduction of whole-body cholesterol, including arterial wall macrophages, an important cell type in atherogenesis.

### HDL SUBSPECIES FUNCTION AMONG ADOLESCENTS

From here, Drs. Sean Davidson and Amy Shah discuss the evolving role of HDL subspecies, which can be distinguished on the basis of charge, size, composition, and function, thus connecting functionality of HDL subspecies with type 2 diabetes (T2D). Patients with T2D have lower plasma levels of cholesterol-rich, larger HDL<sub>2</sub> species but higher levels of cholesterol-poorer HDL<sub>3</sub>; moreover, women with T2D carry lower plasma levels of large HDL particles and higher levels of the lipid-poor HDL. In their review, the authors consider the impact of obesity, insulin resistance, and hyperglycemia on HDL subspeciation and cite the importance of HDL quality versus HDL quantity. They suggest that the subtle shift in HDL particle size may be a diagnostic for incipient T2D. Notably, they also report that phospholipid content of large HDL decreases according to obesity-linked diabetes status, the same order as increased vascular stiffness, an ASCVD predictor. Surgically assisted weight loss among obese adolescents increases levels of large phospholipid-rich HDL subspecies to nearly that of lean, insulin-sensitive controls. Although dysfunctional HDL associates with other metabolic abnormalities, functional HDL may also play a role in atheroprotection.

### GENE DELIVERY IN LIPID RESEARCH AND POTENTIAL THERAPEUTIC APPLICATIONS

Delving more into basic science research, Drs. Marco De Giorgi and William Lagor close this issue with a discussion on new ways to edit genes in the context of lipoprotein metabolism. Several animal-tested technologies developed to somatically overexpress, silence, or disrupt genes have advanced our understanding of metabolism, especially with regard to the liver, and have the potential as gene therapies for lipid disorders. The authors address several gene transfer technologies used in lipid research—including adeno-associated viral vector overexpression, antisense oligonucleotides and small interfering RNAs, and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 genome editing

system—and discuss their potential therapeutic applications for lipid disorders.

## CONCLUSIONS

The field of human plasma cholesterol diagnostics and therapeutics is clearly changing. The statin class of LDL-C lowering drugs has matured, and their penetrance into the CVD market in some countries is nearing a plateau. Unfortunately, hypercholesterolemia is frequently underdiagnosed, and even after statin therapy has been prescribed, subsequent adherence is poor. Drugs that block cholesterol absorption are frequently prescribed for statin-insensitive and nonresponsive patients.

Identification of interventions that modify HDL quantity and quality in a cardioprotective way is still needed. Moreover, newer gene-based therapies such as ASOs and siRNAs are still in their infancy, and other therapies based on CRISPER/CAS9 and AAV are mostly in preclinical stages. In the future, some of these are likely to make their way into clinical practice for subgroups of patients at ASCVD risk for which current therapies are not adequate.

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