

# Cholesterol: Can't Live With It, Can't Live Without It

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**ABSTRACT:** Given its role in many biochemical processes essential to life, cholesterol remains a topic of intense research. Of all the plasma lipids, cholesterol is distinctive because it is a precursor to steroidogenic molecules, some of which regulate metabolism, and its blood concentration in the form of low- and high-density lipoprotein cholesterol (HDL-C) are positive and negative factors for atherosclerotic cardiovascular disease (ASCVD). New research, however, has challenged the widely held belief that high HDL-C levels are atheroprotective and is showing that both low and high plasma HDL-C levels confer an increased risk of ASCVD. Furthermore, it is disputing the widely cited mechanism involved in reverse cholesterol transport. This review explores the evolution of cholesterol research starting with the Gofman and Framingham studies, the development of traditional and emerging lipid-lowering therapies, and the role of reverse cholesterol transport in HDL cardioprotection.

## CHOLESTEROL IN LIFE

Cell membranes contain numerous lipids that are essential to multiple aspects of human metabolism and its regulation. Various lipids are also found in plasma lipoproteins, namely, the high-, low-, and very low-density lipoproteins (HDL, LDL, and VLDL, respectively); HDL is distributed into subpopulations of large (HDL<sub>2</sub>) and small (HDL<sub>3</sub>) particles. The major lipid membranes and lipoproteins are glycerophosphoryl lipids, sphingolipids, phospholipids, triglycerides, cholesterol, and cholesteryl esters. Of these, cholesterol is special because it is a precursor to bile salts and other steroidogenic molecules, some of which are metabolic regulators. Moreover, plasma concentrations of LDL- and HDL-cholesterol are positive and negative risk factors for atherosclerotic cardiovascular disease (ASCVD).

## A BRIEF HISTORY OF CHOLESTEROL

Given its role in many biochemical processes essential to life, cholesterol remains a topic of intense research. In fact, a PubMed search for cholesterol citations revealed more than 250,000 articles. Cholesterol is one of the most highly celebrated small molecules in biology. Thirteen Nobel Prizes have been awarded to scientists who devoted major parts of their careers to cholesterol (Table 1). In the first half of the 20th century, five Nobel Prizes in Chemistry were awarded for various refinements to the structure of cholesterol. In 1964, the Nobel Prize in Medicine or Physiology was awarded to Konrad Bloch for uncovering the mechanism for cholesterol biosynthesis from acetate, signaling the growing appreciation of cholesterol's importance in physiology. One year later, Robert B. Woodward received the Nobel Prize in Chemistry for the total synthesis of cholesterol from hydroquinone, and in 1975, John W. Cornforth received the Prize in Chemistry

for his structural studies of cholesterol. The 1985 Nobel Prize in Medicine or Physiology awarded to Michael S. Brown and Joseph L. Goldstein recognized their work on the regulation of cholesterol metabolism by structural and genetic factors. They and others thereafter identified different defects in the low-density lipoprotein (LDL) receptor gene that controlled various steps in LDL metabolism. These defects were associated with familial hypercholesterolemia, which is mechanistically linked to ASCVD and increases the risk of heart attack and stroke. These and other findings motivated scientists to search for ways to reduce plasma LDL-cholesterol (LDL-C) in a cardioprotective way.

## CHOLESTEROL: DIAGNOSTICS

Plasma lipoprotein cholesterol levels have been quantified by several methods, including electrophoresis,<sup>1</sup> analytical ultracentrifugation,<sup>2</sup> selective precipitation with polyanions and divalent cations,<sup>3</sup> size-exclusion chromatography,<sup>4</sup> gradient and rate zonal ultracentrifugation,<sup>5-7</sup> and sequential flotation at various densities.<sup>8</sup> Only the chromatographic and ultracentrifugal methods are used preparatively. While some of these methods are still used in a research setting, only selective precipitation is used for diagnostic purposes. For this method, plasma lipoprotein-cholesterol levels are determined by first assessing plasma total cholesterol (TC) and triglyceride (TG) concentrations. After this, the apolipoprotein B-containing lipoproteins are precipitated, and the TC of the supernatant, which contains HDL, is determined. According to the widely used Friedewald formula,<sup>9</sup>  $LDL-C = TC - HDL-C - TG/5$ . This formula is based on the approximate 5:1 ratio of TG to TC in VLDL and is not valid for the evaluation of hypertriglyceridemic patients. A newer formula that contains adjustments for hypertriglyceridemia has been reported but is not widely used.<sup>10</sup>

AWARDEE	YEAR	NOBEL ACHIEVEMENT
Heinrich O. Wieland	1928	Prizes in Chemistry for delineating the structure of cholesterol
Adolf O.R. Windaus	1928	
Leopold Ruzicka	1939	
Robert Robinson	1947	
Otto P.H. Diels	1950	
Konrad Bloch and Feodor Lynen	1964	Prize in Medicine or Physiology for uncovering the mechanisms for cholesterol biosynthesis from acetate
Robert B. Woodward	1965	Prize in Chemistry for the synthesis of cholesterol from hydroquinone in nearly 40 steps
Derek H.R. Barton and Odd Hassel	1969	Prize in Chemistry for establishing the conformation of cholesterol
John W. Cornforth	1975	Prize in Chemistry for identifying the orientation of hydrogen atoms in cholesterol
Michael S. Brown and Joseph L. Goldstein	1985	Prize in Medicine or Physiology for discoveries about the regulation of cholesterol metabolism

*Table 1.*

Nobel Prizes associated with cholesterol.

## PLASMA LDL-C AND HDL-C CONCENTRATIONS AS ASCVD RISK FACTORS

Whereas plasma total cholesterol levels are a traditional risk factor for ASCVD, risk in recent years is assessed by the amount of cholesterol occurring in specific plasma lipoprotein fractions and subfractions. In the early 1950s, University of California researcher John Gofman and associates discovered that men who developed atherosclerosis commonly had elevated plasma levels of LDL and low levels of HDL. They later showed in a prospective 10-year study of ~2,000 Livermore Lab employees that there were more heart disease cases in men with low plasma HDL<sub>2</sub> and HDL<sub>3</sub> levels.<sup>2</sup> A 29-year follow-up confirmed earlier findings showing that increased HDL<sub>2</sub> was more atheroprotective than HDL<sub>3</sub>. Many other studies, most notably the extant Framingham Heart Study that began in 1948, confirmed these findings and implicated other factors in ASCVD. Though important, these studies did not provide a mechanistic link between LDL- and HDL-C and atherogenesis.

## LDL-C THERAPEUTICS

The discovery and subsequent validation of the Gofman and Framingham studies prompted searches for ways to reduce

plasma LDL-C concentrations and raise those of HDL. One early landmark trial, The Lipid Research Clinic Coronary Primary Prevention Trial,<sup>11</sup> randomized hypercholesterolemic (> 265 mg/dL) asymptomatic men between ages 35 and 59 years to either control or treatment with the bile acid sequestrant cholestyramine. Both groups were advised to follow a low-cholesterol and low-fat diet and were followed for a mean time of 7.4 years. During this time, the cholestyramine group experienced a 13% greater reduction in plasma LDL-C than the placebo group as well as a 24% reduction in CVD death and a 19% reduction in nonfatal myocardial infarction. The incidence rates for related morbidities were also reduced in the cholestyramine group. These findings correlated reduced LDL-C levels with reduced incidence of CVD morbidity and mortality in men who started with higher-than-normal plasma LDL-C levels and provided convincing evidence for a causal role of LDL-C in CVD pathogenesis; however, cholestyramine was associated with side effects and did not lower LDL-C enough in many patients.

Thus, there arose a compelling rationale for developing and testing more tolerable and effective LDL-C lowering drugs that worked by inhibiting HMG-CoA reductase, the rate-limiting step

in cholesterol biosynthesis. The first of these were mevastatin and compactin, which were isolated by two different groups from *Penicillium citrinum* and *Penicillium brevicompactum*, respectively.<sup>12,13</sup> Neither was marketed because of adverse effects. In 1987, a team at Merck started marketing lovastatin under the trade name Mevacor, the first statin-class drug. As awareness grew about plasma LDL-C levels being a risk factor for ASCVD, so did the number of patients who started receiving lovastatin to reduce ASCVD risk. The 5,608 men and 997 women with elevated plasma LDL-C levels in the Air Force/Texas Coronary Atherosclerosis Prevention Study received lovastatin 20 to 40 mg daily or a placebo and a diet low in saturated fat and cholesterol.<sup>14</sup> The intervention group had a 25% reduction in LDL-C as well as less fatal or nonfatal MI, unstable angina, or sudden cardiac death, thereby confirming the benefit of plasma LDL-C reduction. Given the efficacy and safety of lovastatin, other pharmaceutical companies followed suit, with at least seven other statin-class drugs now on the market. Trials of some of these drugs showed that patients without a history of myocardial infarction<sup>15</sup> or with diabetes also benefitted.<sup>16</sup> For patients who are statin intolerant or insensitive, other options include ezetimibe, which blocks cholesterol absorption by gastrointestinal tract epithelial cells,<sup>17</sup> niacin and fibrates, which are also triglyceride-lowering drugs, and lomitapide, which reduces hepatic VLDL secretion.<sup>18-20</sup> Although statins are judged to be safe and effective, a meta-analysis has linked statin therapy with a 9% increase in diabetes, an effect that is more prevalent in older patients.<sup>21</sup> However, the benefit in ASCVD risk reduction outweighs the risk of developing diabetes.

## HDL-C THERAPEUTICS

The association between high plasma HDL-C concentrations and reduced ASCVD risk is widely accepted. Moreover, the Helsinki Heart Study and the Veterans Administration HDL Intervention Trial (VA-HIT) showed that increased plasma HDL-C levels in patients receiving gemfibrozil versus placebo are associated with fewer coronary heart disease (CHD) events; however, this benefit was more strongly correlated with insulin resistance than HDL-C in VA-HIT. Moreover, in the Helsinki Heart Study, those with low plasma total cholesterol and HDL-C and hypertriglyceridemia benefitted most.<sup>22-24</sup>

Moderate exercise and regular moderate alcohol consumption increase plasma HDL-C concentration nearly equally, but their effects are not additive.<sup>25</sup> Moreover, regular moderate alcohol consumption is associated with reduced CHD incidence and mortality, an effect initially assigned to attendant increased HDL-C.<sup>26-28</sup> The hypothesis that raising HDL levels is salutary has begun to show cracks due to numerous confounders, failed trials, genetic studies, and new alternative mechanisms.

For example, although fibrates increase plasma HDL-C levels, they also lower plasma TG, another ASCVD risk factor. Alcohol also has multiple metabolic effects via its terminal metabolite acetate, which inhibits adipocyte lipolysis,<sup>29</sup> reduces plasma fatty acid concentrations in man,<sup>30</sup> and inhibits the chylomicron-TG lipolysis.<sup>31</sup> These effects may be mediated by the adipocyte-fatty acid receptor 2, for which acetate, the terminal metabolite of alcohol, is a ligand.<sup>32</sup>

## THE FAILED TRIALS

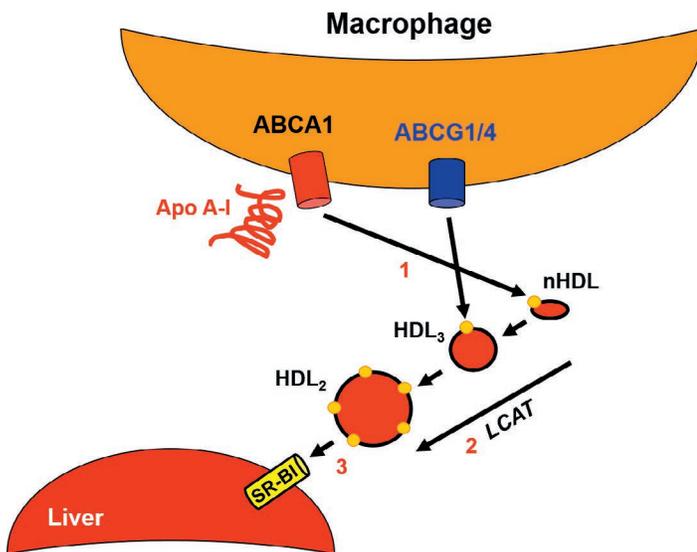
The very high plasma HDL-C concentrations found in patients with cholesteryl ester transfer protein (CETP) deficiency do not commensurately reduce CVD incidence.<sup>33</sup> Likewise, CETP inhibitors, which profoundly increase plasma HDL-C levels, do not reduce CVD events.<sup>34</sup> Although niacin reduces cardiovascular events and cardiovascular and all-cause mortality,<sup>35</sup> these benefits are not uniformly observed. Given the residual risk in patients who achieve their LDL-C targets, it was important to test whether the addition of niacin, which raises HDL-C, to statin therapy reduces residual risk. However, clinical trials have shown that niacin does not reduce CVD events. Although the AIM-HIGH trial showed that niacin increased HDL-C concentration and lowered triglyceride and LDL-C concentrations, the trial was stopped for futility at the end of 3 years.<sup>36</sup> In the larger HPS2-THRIVE trial, addition of niacin and the flushing inhibitor laropiprant to statin therapy produced modest HDL-C changes, no clinical benefit, and toxicity.<sup>37</sup> A subgroup analyses of ACCORD and other fibrate trials suggested possible benefits to patients with low HDL-C and hypertriglyceridemia.<sup>38</sup> Pemafibrate, a new fibrate that is not approved in the United States, has been shown to improve lipid profiles among patients with type 2 diabetes. Two recent trials of HDL mimetics have failed to reduce ASCVD.<sup>39,40</sup>

## THE GENETIC STUDIES

In the Copenhagen City Heart Study, patients with genetically elevated plasma levels of HDL-C did not have a reduced ASCVD risk.<sup>41</sup> According to a Mendelian randomization study, an HDL-C-raising endothelial lipase variant was not associated with reduced myocardial infarction.<sup>42</sup> In the Cohort of Norway Population, controlling for HDL-C did not affect the magnitude of the negative relationship between alcohol intake and death from ASCVD, indicating that HDL should not be implicated in the atheroprotective effects of alcohol ingestion.<sup>43</sup> Thus, many interventions that increase HDL-C are not atheroprotective.

## THE NEW MECHANISMS

According to one model (Figure 1),<sup>44</sup> HDL elicits its cardioprotective effect by supporting reverse cholesterol



**Figure 1.**

Schematic representation of the Glomset/Ross model of reverse cholesterol transport comprises three steps. (1) Formation of nHDL via the interaction of apolipoprotein AI with the macrophage ABCA1 transporter; direct transfer of cholesterol to HDL via ABCG4 is also shown. (2) FC esterification by LCAT to give a mature spherical form of HDL. (3) Transfer of HDL lipids to liver hepatocytes via SR-B1. nHDL: nascent high-density lipoprotein; ABCA1: ATP-binding cassette transporter; ABCG1/4: ATP-binding cassette subfamily G member 1 and 4; FC: free cholesterol; LCAT: lecithin:cholesterol acyltransferase; SR-B1: scavenger receptor class B type 1

transport. This begins with the efflux of macrophage-free cholesterol (FC) in the arterial subendothelium to apolipoprotein (apo) AI and HDL,<sup>45</sup> processes that are respectively mediated by macrophage ATP-binding cassette and ATP-binding cassette subfamily G member 4 transporters. This produces early forms of HDL, or nascent HDL. Nascent HDL (nHDL) diffuses into the plasma, where FC is esterified by lecithin:cholesterol acyltransferase (LCAT), producing a mature HDL. In the final step, HDL-lipids are hepatically removed by selective nibbling mechanism<sup>46</sup> while the HDL-apos are extruded into the plasma. Given the mixed effects of interventions that raise plasma HDL-C concentrations in ASCVD, studies segued from plasma HDL concentrations to HDL quality and function, especially FC efflux.

Two large studies revealed that FC efflux from macrophages to plasma is higher in controls than in patients with ASCVD.<sup>47,48</sup> This finding is relevant because macrophages are an important cell type in all stages of atherogenesis. Free cholesterol efflux can be enhanced by several mechanisms, including reassembled (r) HDL. Some rHDL formulations (CSL-111, CSL-112, and ETC-216) mobilize cellular cholesterol and regress atherosclerotic plaques in animal models, even after only a few

treatments.<sup>49</sup> Synthetic peptide analogs of apos also mediate cholesterol efflux.<sup>50</sup> Although they remove cholesterol from cells, an intrinsic problem with all of these infused agents (be they peptides, rHDL, delipidated HDL, or lipid vesicles) is that they lack directionality and would likely remove cholesterol from all tissue sites, including the liver, the very target of therapeutic disposal. Therefore, agents that selectively target macrophage FC efflux to the liver are needed.

#### NEW INSIGHTS INTO REVERSE CHOLESTEROL TRANSPORT

Although the Glomset/Ross model of reverse cholesterol transport (RCT) has served well for several decades, part of it is inconsistent with current data (Figure 1). Hepatic clearance of mouse and human HDL-FC occurs on a fast time scale—2 and 8 minutes, respectively—which is rapid compared to LCAT activity. Similarly, the clearance of nHDL, the particle formed during FC efflux to apoAI in the first RCT step, is also fast compared to its esterification. Within these halftimes, only about 2% of FC is esterified<sup>51–53</sup>; this means that > 95% of HDL-FC is cleared without esterification, and LCAT plays only a minor role in RCT. Studies in mice also show that most HDL-FC is cleared via hepatic scavenger receptor class B type 1 (SR-B1), so that in SR-B1<sup>-/-</sup> mice, which are atherosusceptible, the plasma HDL is FC-rich (60 vs 15 mol% in wild-type mice). This suggests the hypothesis that HDL derives its dysfunctionality from both a high FC content and high plasma HDL concentration. However, epidemiological studies in humans are consistent with this hypothesis; the correlation between plasma HDL-C levels and ASCVD hazard ratio is U-shaped, with the extremes of high and low HDL-C concentrations being associated with more all-cause<sup>54</sup> and, in some reports, ASCVD mortality.<sup>55–58</sup> The underlying cause of the high hazard ratio at low HDL-C concentrations was linked to factors that cluster with low plasma HDL-C levels—smoking, physical inactivity, elevated body mass index, high blood pressure—collectively simulating metabolic syndrome phenotype.<sup>54</sup> The underlying cause for the increased ASCVD hazard ratio in the highest quintile of HDL-C levels (i.e., “too much of a good thing”) is not known,<sup>54</sup> but it could be due to the combined effects of high plasma concentrations of HDL particle with high FC content that supports high FC bioavailability to extrahepatic tissues, including the arterial wall. Notably, rHDL with FC ≤ 10 mol% supports cellular FC efflux, whereas rHDL with FC ≥ 20 mol% supports cellular FC influx; the switch from efflux to influx occurs at ~15 mol% rHDL-FC. The magnitudes of efflux at low rHDL-FC content and influx at high rHDL-FC content are potentiated by increased plasma HDL levels.<sup>59</sup> Extrapolation of these data to human physiology suggests that high plasma concentrations of HDL that have a high FC content drives net FC transfer into cells, including arterial wall macrophages, in a way that supports atherogenesis. These data provide a rationale

for future human studies testing the hypothesis that HDL-FC bioavailability in patients with ASCVD is greater than that of matched controls without ASCVD.

## FUTURE DIRECTIONS

There is little doubt about the association of high and low plasma HDL-C levels with increased ASCVD. Given that many interventions that increase plasma HDL-C levels do not reduce ASCVD risk, there is a need to identify mechanisms that increase plasma HDL-C levels in an atheroprotective way. It seems likely that one approach would be to treat the comorbidities that associate with low plasma HDL-C—especially physical inactivity, insulin resistance, and a poor diet—to potentially reduce ASCVD events. The strategy for managing high plasma concentrations of dysfunctional HDL is a bit more elusive. Given the current knowledge, it would be worth comparing FC bioavailability in patients with ASCVD versus controls. If these tests support the hypothesis that higher-than-normal HDL-FC bioavailability is mechanistically linked to ASCVD, then new means for its control should be developed.

## KEY POINTS

- Recent studies support changes to the widely cited reverse cholesterol transport (RCT) mechanism. Foremost among these is the minimal involvement of lecithin:cholesterol acyltransferase (LCAT)-mediated free cholesterol esterification—interesting given that the discovery of LCAT provoked formulation of the traditional RCT mechanism.
- Mechanistically, free cholesterol (FC) transfer to the liver occurs via spontaneous transfer and scavenger receptor class B type 1-mediated uptake.
- Lastly, the FC content of high-density lipoprotein (HDL) and plasma HDL concentration are both determinants of FC bioavailability, which may be an important determinant of several pathological states, including atherosclerosis.

### Conflict of Interest Disclosure:

Dr. Gotto is a consultant for Esperion and KOWA Pharmaceuticals America, Inc., and is on the Data Safety Monitoring Board for Ionis Pharmaceuticals.

### Keywords:

lipid risk factors, reverse cholesterol transport, lipoproteins, atherogenesis, lipid therapeutics

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