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High-Density Lipoproteins in the Prevention of Cardiovascular Disease: Changing the Paradigm

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High-density-lipoprotein cholesterol (HDL-C) has been identified in population studies as an independent inverse predictor of cardiovascular events. Although the causal nature of this association has been questioned, HDL and its major protein, apolipoprotein (apo)A1, have been shown to prevent and reverse atherosclerosis in animal models. In addition, HDL and apoA1 have several putatively atheroprotective functions, such as the ability to promote efflux of cholesterol from macrophages in the artery wall, inhibit vascular inflammation, and enhance endothelial function. Therefore, HDL-C and apoA1 have been investigated as therapeutic targets for coronary heart disease. However, recent clinical trials with drugs that raise HDL-C, such as niacin and inhibitors of cholesteryl ester transfer protein, have been disappointing. Here, we review the current state of the science regarding HDL as a therapeutic target.

Advances in the treatment of hypercholesterolemia, primarily with statins, are responsible for the decline in mortality due to coronary heart disease (CHD); however, CHD is still the cause of death for one in six Americans.[1] Low-density-lipoprotein cholesterol (LDL-C) is a well-known causal factor for CHD and has been a primary target of therapy for >2 decades.[2] Meta-analyses of statin efficacy in the prevention of CHD indicate a 31% reduction in coronary events and a 29% reduction in CHD mortality.[3] However, there remains a high level of residual risk in susceptible individuals treated with statins.[2,4] Therefore, additional therapies beyond statins are needed to further reduce the risk of coronary events.

In many observational epidemiological studies, high-density-lipoprotein cholesterol (HDL-C) levels are a strong, independent, inverse predictor for cardiovascular events.[5–7] In subsequent analyses of large population studies, a 1-mg/dl increase in HDL-C was associated with a significant CHD risk reduction of 2% in men and 3% in women.[8] More recent data from the Emerging Risk Factors Collaboration, which included analyses of >300,000 individuals, upholds the strong inverse association of HDL-C with CHD risk, finding an adjusted hazard ratio of 0.78 (95% confidence interval (CI): 0.74–0.82) for CHD risk.[6] In addition, HDL-C and apolipoprotein (apo)A1 levels are associated with reduced risk of cardiovascular events in patients receiving statin therapy, even in those patients achieving on-statin LDL-C levels <50 mg/dl.[9] These data led to the concept of the "HDL-C hypothesis," which posits that interventions that increase HDL-C will prevent the occurrence of CHD.[10] The Veterans Affairs HDL Intervention Trial was one of the first prospective studies to demonstrate that a therapy that increased levels of HDL-C, gemfibrozil, reduced major cardiovascular events in CHD patients with low HDL-C.[11]

However, recent attempts at raising HDL-C through pharmacological intervention with niacin[12,13] and cholesteryl ester transfer protein (CETP) inhibitors[14,15] have raised serious doubts regarding whether simply raising HDL-C is atheroprotective. More irrefutable data against the HDL-C hypothesis come from human genetics; a loss-of-function variant in the *LIPG* gene encoding the enzyme endothelial lipase was associated with significantly elevated HDL-C levels but was not protective against the risk of myocardial infarction (MI).[16] Indeed, most common genetic variants associated solely with higher HDL-C levels are not associated with a lower CHD risk.[16] Although raising HDL-C concentrations may not equate to a CHD risk reduction, this does not eliminate the possibility that enhancing HDL function would be atheroprotective.

HDL-C has several well-established functions that could potentially protect against atherosclerotic vascular disease, including its most well-known function, reverse cholesterol transport (RCT). In addition, HDL has been shown to inhibit vascular inflammation, enhance endothelial function, and reverse atherosclerosis in animal models.[17] Although initially seen as a relatively homogeneous lipoprotein fraction, HDL particles are now known to be highly heterogeneous, with a function

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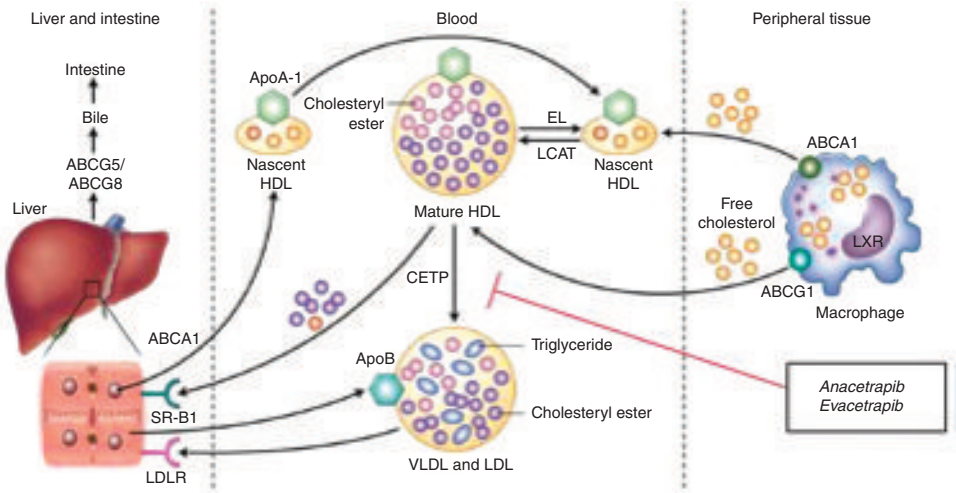

Figure 1 High-density-lipoprotein cholesterol (HDL-C) metabolism and reverse cholesterol transport. Apolipoprotein A1 (apoA1) is synthesized by both the liver and the intestine and is secreted in the lipid-free form. Lipidation of the particle occurs with the acquisition of phospholipids and free cholesterol via hepatocyte adenosine triphosphate (ATP)–binding cassette A1 (ABCA1) to form nascent HDL. Nascent HDL can acquire additional free cholesterol via ABCA1 from peripheral tissues. Mature HDL is generated by the actions of lecithin cholesterol acyltransferase (LCAT), which esterifies free cholesterol to cholesteryl esters. Mature HDL can serve as an acceptor of cholesterol efflux via the macrophage ABCG1 pathway and transport it back to the liver for uptake via the hepatic scavenger receptor class B type 1 (SRB1). Alternatively, cholesteryl ester transfer protein (CETP) can facilitate transfer of cholesteryl esters from HDL to apoB-containing lipoproteins and subsequent uptake in the liver. Selective inhibition of CETP by CETP inhibitors enhances cholesterol efflux. From ref. 22.

that is probably more complicated than once believed. As an example, small human studies infusing recombinant HDLs, mainly comprising apoA1, have suggested the potential for atherosclerosis regression.[18,19] However, a recent randomized phase IIb study of CER-001 (Cerenis Therapeutics, Toulouse, France), a synthetic HDL mimetic, failed to show regression in >500 patients post acute coronary syndrome (ACS).[20] Therefore, it is critical to understand the biology of HDL function and its potential contributions to atheroprotection.

Here, we review the state of the art of recent findings that have brought the HDL-C hypothesis into question, including genetic data and failed recent clinical trials targeting HDL-C. We then discuss what is known about HDL function and data that support the "HDL flux hypothesis" and reevaluate the role of HDL-C in the management of hypercholesterolemia.

HDL METABOLISM AND RCT

The metabolism of HDL is distinct from that of other major lipoprotein fractions in that most of its lipid and apolipoprotein components are assembled after secretion and because it is subject to active remodeling in the plasma compartment (**Figure 1**).[21,22] The biosynthesis of HDL begins with the secretion of lipid-poor apoA1 by the liver and intestine, followed quickly by the acquisition of free cholesterol and phospholipids from these tissues through efflux of cholesterol from cells by active transport via adenosine triphosphate (ATP)–binding cassette A1 (ABCA1) to form pre-β HDL. Then, lecithin cholesterol acyltransferase esterifies free cholesterol, producing cholesteryl ester, which is hydrophobic and forms the HDL particle's core. One fate of the cholesteryl ester in the mature HDL-C particle is to be selectively taken up by the liver via hepatic scavenger receptor class B type 1. Alternatively, HDL cholesteryl ester can be transferred to apoB-containing lipoproteins, very-low-density lipoprotein (VLDL), and LDL by a plasma glycoprotein, CETP, in exchange for triglycerides (TGs).[23] The action of CETP results in decreased HDL-C concentrations and cholesterol-enriched LDL-C. HDL particles can also be remodeled by the action of lipases such as hepatic lipase and endothelial lipase, resulting in smaller, lipid-depleted HDL particles.

One of the mechanisms by which HDL is believed to exert atheroprotective properties is through RCT.[21,22] This is the process by which excess cholesterol from peripheral, extrahepatic, and arterial tissues undergoes efflux to apoA1 or HDL and is returned to the liver for elimination in the bile. This process maintains cholesterol homeostasis by preventing toxic buildup of cholesterol in arterial foam cells, a key initiating step in the development of atherosclerotic plaques. Cholesterol efflux is facilitated by active transport via ABCA1 and ABCG1, which are expressed on the surface of macrophages. Each transporter displays unique acceptor specificities; ABCA1 promotes cholesterol efflux to lipid-poor apoA1, whereas ABCG1 is responsible for cholesterol efflux to mature HDL particles. Enhancement of cholesterol efflux through these pathways appears to be atheroprotective.[24]

In addition to promoting cholesterol efflux, HDL has several additional functions that would contribute to its ability to reduce cardiovascular risk (**Table 1**). This topic has recently been reviewed by Rye and Barter.[17] Notably, HDL has been shown to inhibit vascular inflammation, enhance endothelial function, and increase angiogenesis. Moreover, HDL possesses antioxidant and antithrombotic properties and is reported to have beneficial antidiabetic properties. The HDL subfractions that are responsible for these properties are unknown and will require further investigation. Recent work investigating the molecular mechanism of the anti-inflammatory effects of HDL identified a transcriptional regulator, *ATF3*, as an HDL-inducible gene

Table 1 Pleiotropic and anti-inflammatory effects of HDL

Reverse cholesterol transport
Antioxidant activity
Anti-inflammatory effect
Antithrombotic action
Enhancement of endothelial repair
Restoration of endothelial function
Promotion of angiogenesis
Inhibition of hematopoietic stem proliferation
Antidiabetic effects
Inhibition of apoptosis
Component of innate immunity

Adapted from refs. 17 and 26.

in macrophages that downregulates the expression of Toll-like receptor–mediated proinflammatory cytokines.[25] Increasing evidence points to the concept that HDL is an important part of the innate immune system and mediates functions related to antimicrobial activity.[26] Because HDL is expressed at high levels in plasma in lower vertebrates, it is suggested that HDL might have evolved as a component of the primitive innate immune system.

GENETICS OF HDL

As described, the metabolism of the HDL particle is a multistep process involving several apolipoproteins, enzymes, and transporters; therefore, genetic variation in genes regulating each of these steps will greatly affect HDL-C concentrations.[27] Mendelian disorders of high and low HDL-C levels have provided clues about the biology of HDL.[28] Candidate gene studies have identified Mendelian causes of low HDL-C levels, including mutations in *ABCA1*, *APOA1*, and *LCAT*. Conversely, mutations in *CETP* and endothelial lipase (*LIPG*) result in high HDL-C levels. Cardiovascular gene–centric genomic association studies and genome-wide association studies have confirmed the association of candidate HDL-C genes and have also identified novel loci previously unknown to regulate HDL metabolism.[27,29,30] Unbiased approaches to date have identified 60 validated independent loci associated primarily with plasma HDL-C levels, explaining 13.7% of the variance in plasma HDL-C levels in the Framingham Heart Study.[29,30]

Endothelial lipase (*LIPG*), a member of the TG lipase family, is one of several HDL-C candidate genes; endothelial lipase specifically hydrolyzes phospholipids on HDL particles.[31] Overexpression of endothelial lipase in mice decreases HDL-C levels, whereas deletion of endothelial lipase in mice increases HDL-C levels. Sequencing the *LIPG* gene in a cohort comprising subjects with extremely high and low HDL-C levels (mean: 103 and 34 mg/dl, respectively) led to the discovery of the low-frequency Asn396Ser loss-of-function variant that is significantly associated with elevated HDL-C levels.[31] To determine whether this variant was also protective against MI risk, Voight *et al.*[16] conducted a Mendelian randomization study. On the basis of the principle of random assortment of alleles at the time of gamete formation, this analytical approach can determine whether genetic variation influencing plasma levels of a biomarker such as HDL-C is directly involved with disease risk because inherited variation affecting plasma HDL-C should affect risk of MI in the direction and magnitude predicted by the HDL-C plasma concentrations.[32] Carriers of the *LIPG* 396Ser allele had significantly higher HDL-C levels (5.4 mg/dl higher, $P = 8 \times 10^{-13}$), which would be expected to confer a 13% decreased risk of MI. However, this variant, tested in 20,913 MI cases and 95,407 controls, was not associated with a reduced risk of MI (odds ratio (OR): 0.99, 95% CI: 0.88–1.11, $P = 0.85$). In addition, a genetic score consisting of 14 single-nucleotide polymorphisms exclusively associated with HDL-C levels was not associated with the risk of MI, whereas a genetic score exclusively comprising LDL-C-associated single-nucleotide polymorphisms was associated with MI risk.

For the genetic risk score, the authors excluded variants with the largest effects on HDL-C because of known associations with other lipid fractions; as a result, the 14 variants explain a small amount of variation of plasma HDL-C, minimizing their influence even when combined. For example, variants in *CETP* are associated with elevated HDL-C levels and a lower risk of CHD, but these also influence LDL-C[33]; therefore, their protective effect cannot solely be attributed to HDL-C.

In a recent analysis of >180,000 participants examining 185 independent loci associated with LDL-C, HDL-C, or TGs, investigators applied multivariable analysis to establish that LDL-C and TGs were causally and independently associated with CHD, whereas HDL-C failed to show a similar association.[34] Several HDL-C loci have also been associated with lipid subfractions and sphingolipids.[30] For example, *LIPC* variants were strongly associated with plasmalogen levels, and *ABCA1* variants were associated with sphingolipid levels. Understanding these subphenotypes may provide clues that can reconcile the disconnect between the genetic studies showing lack of association between HDL-C and CHD and the epidemiological studies that show strong evidence of association of HDL-C with CHD risk.

CLINICAL TRIALS TARGETING HDL-C

Niacin

Niacin has long been used to beneficially modulate lipids.[35] Niacin is the most effective medication for raising low HDL-C levels, but it also decreases LDL-C and TG levels. In addition, niacin has been shown to lower lipoprotein a (Lp(a)), an independent risk factor for CHD.[35,36] However, the mechanism by which niacin raises HDL-C is not well understood. Human kinetic studies show that niacin decreases the fractional catabolic rate of HDL–apoA1 without affecting apoA1 synthesis.[37] *In vitro* studies in human HepG2 cells and *CETP* transgenic mice corroborate niacin's ability to reduce apoA1 catabolism.[37] In addition, niacin was found to inhibit CETP expression and activity in mice, which resulted in increased cholesteryl ester content of HDL.[38] *In vitro*, niacin decreases expression of the hepatocyte β-chain of ATP synthase, an HDL holoparticle receptor, thereby inhibiting uptake of HDL from plasma (**Figure 2**).[37,39]

The mechanisms of niacin's effects on TG synthesis and apoB-containing particles such as VLDL and LDL-C were long thought to be a result of its antilipolytic actions.[35] Stimulation of the G-protein-coupled receptor 109A (GPR109A or $HCAR_2$) on adipocytes by niacin results in the inhibition of lipolysis and a reduction in plasma free fatty acids, a key pharmacodynamic readout of niacin action.[40] A reduction in the supply of free fatty acids returning to the liver for TG synthesis in turn reduces formation of TGs, VLDL and LDL (**Figure 2**).[41] However, a recent study showed that genetic deletion of GPR109A in mice prevented the acute antilipolytic effects of niacin, but the mice demonstrated reductions in TG and LDL-C.[42] In addition, two GPR109A agonists (MK-1903 and SCH900271) failed in phase II trials because, although they markedly lowered free fatty acids, neither had the desired effects on serum lipids.[42] *In vitro* studies suggest that niacin directly inhibits TG synthesis by inhibition of diacylglycerol acyltransferase 2 in the liver, a key enzyme catalyzing the final step of TG synthesis.[37] A reduction in liver TG pools would prevent assembly of VLDL and result in posttranslational degradation of apoB and subsequent decrease in secretion of VLDL and LDL into the plasma.[37]

Benefits of niacin were first shown in the Coronary Drug Project, in which 8,341 men who survived an MI were randomized to estrogen, thyroxine, clofibrate, niacin, or placebo.[43] Niacin was the only therapy that significantly reduced the risk of MI and stroke at the end of 6 years. A long-term follow-up of the study participants showed that niacin imparted a mortality benefit at 12 years postrandomization.[44] At the time of study, statins were not available on the market and therefore were not included in the trial design. Since then, niacin has demonstrated beneficial effects on surrogate outcomes such as carotid intima-media thickness (CIMT), a standardized method for assessing plaque progression. In the Arterial Biology for the Investigation of Treatment of Reducing Cholesterol (ARBITER) 2 study, 1-year treatment with extended-release (ER) niacin + statin showed plaque stabilization with no change in CIMT, as opposed to significant plaque progression in the control group receiving statin alone.[45] An additional 12-month follow-up of this study demonstrated that niacin treatment for a total of 24 months decreased CIMT, indicating regression of atherosclerotic plaque.[46] As a follow-up to these studies, in the ARBITER 6 study, 363 statin-treated patients with CHD were randomized to ezetimibe or ER niacin to compare the effects of additional LDL-C lowering with ezetimibe or HDL-C raising by niacin on atherosclerosis as measured by CIMT. The trial was stopped early at 14 months due to the superior outcome demonstrated by niacin on CIMT at both the 8-month and 14-month time points, as compared with ezetimibe, which showed no change in CIMT.[47]

However, the most recent studies aimed at hard clinical event reduction have met with disappointing results. The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes (AIM-HIGH) trial was a randomized, placebo-controlled study to evaluate the effect of ER niacin in patients with cardiovascular disease with well-controlled LDL-C (40–80 mg/dl) levels and undergoing simvastatin ± ezetimibe therapy.[12] The primary end point was the composite of death from coronary disease, nonfatal MI, ischemic stroke, hospitalization for an ACS, or symptom-driven coronary or cerebral revascularization over 5 years (**Table 2**). A total of 3,414 men and women with mean age of 64 years were randomized in the trial. The trial was stopped early at 3 years for futility, due to no evidence of reduced cardiovascular events in the ER niacin group as compared with the placebo group. The primary end point occurred in 16.4% of niacin-treated patients and 16.2% in the placebo group (hazard ratio: 1.02, 95% CI: 0.87–1.21, $P = 0.80$). HDL-C levels had significantly increased by 25% to 42 mg/dl in the ER niacin group, compared with an increase of 9.8% to 38 mg/dl in the placebo group at the end of 2 years ($P < 0.001$). In addition, TGs decreased by 28.6% in the niacin group and by 8.1% in the placebo group.

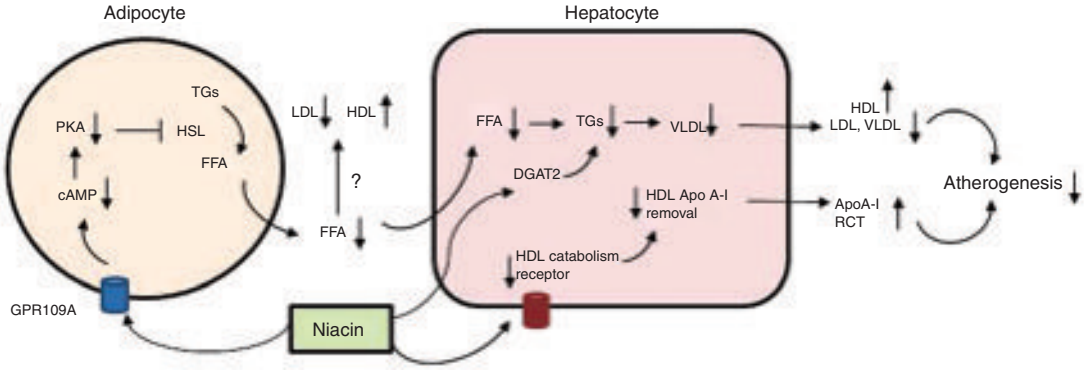

Figure 2 Mechanism of action of niacin. Niacin acts on its receptor, GPR109A, on adipocytes to suppress lipolysis. Stimulation of GPR109A lowers cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) levels, preventing the phosphorylation of hormone-sensitive lipase (HSL) and triglyceride (TG) hydrolysis. The reduced supply of free fatty acids (FFAs) returning to the liver inhibits the hepatic synthesis of TGs, very-low-density lipoprotein (VLDL), and low-density lipoprotein (LDL). Although the suppression of FFAs is receptor dependent, the lipid effect is not; therefore, this effect of niacin is unlikely to be the mechanism by which niacin modulates lipids. Niacin also directly inhibits diacylglycerol acyltransferase 2 (DGAT2), which reduces hepatic TG and VLDL synthesis. Lower levels of VLDL and LDL reduce the exchange of cholesteryl esters and TGs between LDL and high-density lipoprotein (HDL), thereby increasing HDL-cholesterol (HDL-C). Niacin has also been shown to decrease the apolipoprotein A1 fractional catabolic rate and to inhibit the HDL particle receptor (β-chain of adenosine triphosphate (ATP) synthase), thereby inhibiting HDL uptake from plasma. From ref. 41.

Table 2 Results of contemporary outcomes studies for drugs that target HDL-C

Trial	Intervention	Primary end point	Change in lipoproteins		Cardiovascular outcomes[b]
			Statin + active drug	Statin + placebo	
AIM-HIGH[12]	Niacin + simvastatin vs. simvastatin[a]	Death from cardiovascular causes, nonfatal MI, nonfatal stroke, acute coronary syndrome, or revascularization	HDL-C: +25%; LDL-C: −12%; TG: −29%	HDL-C: +10%; LDL-C: −6%; TG: −8%	Hazard ratio: 1.02 (95% CI: 0.87–1.21, $P = 0.80$)
HPS2-THRIVE[13]	Niacin/laropiprant + simvastatin vs. simvastatin ± ezetimibe	Death from cardiovascular causes, nonfatal MI, nonfatal stroke, or revascularization	HDL-C: +6 mg/dl; LDL-C: −10 mg/dl; TG: −33 mg/dl	NA	Relative risk: 0.96 (95% CI: 0.90–1.03, $P = 0.29$)
ILLUMINATE[14]	Torcetrapib + atorvastatin vs. atorvastatin	Death from cardiovascular causes, nonfatal MI, nonfatal stroke, or acute coronary syndrome	HDL-C: +72%; LDL-C: −25%; TG: −9%	HDL-C: +2%; LDL-C: +3%; TG: +1%	Hazard ratio: 1.25 (95% CI: 1.14–2.19, $P = 0.0006$)
Dal-OUTCOMES[15]	Dalcetrapib + statin vs. statin	Death from cardiovascular causes, nonfatal MI, nonfatal stroke, unstable angina, or cardiac arrest	HDL-C: +40%; TG: +10%	HDL-C: +11%; TG: +17%	Hazard ratio: 1.04 (95% CI: 0.93–1.16, $P = 0.52$)

AIM-HIGH, Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides Impact on Global Health Outcomes; CI, confidence interval; HPS2-THRIVE, Heart Protection Study 2: Treatment of HDL to Reduce the Incidence of Vascular Events; HDL-C, high-density-lipoprotein cholesterol; ILLUMINATE, Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events; LDL-C, low-density-lipoprotein cholesterol; MI, myocardial infarction, NA, not available.

[a]Ezetimibe could be used as needed in either arm to achieve a target LDL-C level of 40–80 mg/dl. [b]The hazard ratio or relative risk is for the comparison between interventional drug + statin and statin alone.

A second, larger trial, similar in design to the AIM-HIGH trial, was conducted in Europe and Asia. The Second Heart Protection study (HPS2-THRIVE) randomized 25,673 patients with vascular disease to treatment with simvastatin + ER niacin combined with laropiprant (LRPT), a prostanoid receptor (DP1) antagonist, to minimize the flushing effects of niacin, as compared with treatment with simvastatin + placebo.[13] Lipid values at enrollment were well controlled with mean total cholesterol of 128 mg/dl, LDL-C of 63 mg/dl, HDL-C of 44 mg/dl, and TGs of 125 mg/dl. Over the average 4-year follow-up, the niacin/LRPT treatment resulted in an additional reduction of 10 mg/dl in LDL-C relative to the placebo group, a further 6-mg/dl increase in HDL-C, and a 33-mg/dl decrease in TGs. There were no differences in the occurrence of the primary composite end point by treatment group (**Table 2**). However, excess serious adverse events occurring in the niacin/LRPT group led to the early termination of the trial. Higher occurrences of diabetic complications (HR: 1.55, 95% CI: 1.34–1.78), serious bleeding (HR: 1.38, 95% CI: 1.17–1.62), and serious infection (HR: 1.22, 95% CI: 1.12–1.34) were seen in the niacin/LRPT group.[13] Overall, there was a higher incidence of definite myopathy seen in the niacin/LRPT–treated patients, with a risk ratio (RR) of 4.4 (95% CI: 2.6–7.5, $P < 0.001$), with a significantly higher rate occurring in Chinese participants treated with niacin/LRPT (RR: 5.2, 95% CI: 3.4–7.8) as compared with European participants (RR: 1.5, 95% CI: 0.7–3.3), with a significant interaction between treatment and region ($P = 0.008$).

The inability of niacin to significantly improve cardiovascular outcomes in both AIM-HIGH and HPS2-THRIVE calls into question the HDL cholesterol hypothesis and the role of niacin as an HDL-C-raising drug to reduce risk of CHD. The study populations in both trials were very well controlled in terms of LDL-C levels, with a baseline mean level of 71 mg/dl in the AIM-HIGH study and 63 mg/dl in HPS2-THRIVE. It may be that HDL raising with niacin in the face of aggressively controlled LDL-C may not allow for a perceivable clinical benefit. The design of both these trials does not reflect lipid management in a clinical setting because very few patients would be prescribed niacin when LDL-C concentrations are already < 70 mg/dl. Consequently, in stable patients on statin therapy with very well-controlled LDL-C levels, adding niacin therapy may not further reduce residual cardiovascular risk. However, because these trials did not enroll patients with acute cardiac events (e.g., following MI or ACS), it is unknown what benefits, if any, may be seen with the addition of niacin to statin treatment in high-risk patient populations with conditions such as ACS. Because niacin is effective in lowering LDL-C, it may still play a role in patients who are statin intolerant and unable to reach LDL-C goals or in subpopulations (e.g., patients with high TG and low HDL-C levels) who will still derive benefit from niacin.

CETP INHIBITORS

Torcetrapib

Several CETP inhibitors have been developed to determine whether raising HDL-C by inhibition of this pathway would result in improved cardiovascular outcomes and reduced CHD risk. Torcetrapib, a reversible, noncompetitive inhibitor of the HDL–CETP complex was the first of this drug class to be tested in humans.[48,49] Early short-term studies demonstrated a dose-dependent, 33–106% increase in plasma HDL-C values.[50,51] In the phase III outcomes study, Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events, 15,067 subjects on atorvastatin (dose ranging from 10 to 80 mg/day) were randomized to torcetrapib (60 mg/day) or placebo.[14] Although torcetrapib treatment significantly raised HDL-C levels by 72% and lowered LDL-C by 25% at 1 year, the trial was terminated early due to high mortality in patients receiving torcetrapib. It was discovered that torcetrapib-treated subjects exhibited higher systolic blood pressure and hypokalemia due to unexpected modulation of the renin–angiotensin–aldosterone system. *Post hoc* measurements indicated that torcetrapib significantly increased plasma aldosterone concentrations

at 3 months. *In vitro* studies and animal studies confirmed increased circulating aldosterone levels and upregulation of *CYP11B2* (aldosterone synthase) by torcetrapib.[52,53]

Further analysis revealed that the excess mortality due to torcetrapib was confined to the subjects who received the 10-mg atorvastatin dose.[54] The torcetrapib plus 10-mg atorvastatin group, as compared with the placebo plus 10-mg atorvastatin dose group, experienced an increased risk of death due to cardiovascular causes (30 vs. 13, $P < 0.008$) and noncardiovascular causes (19 vs. 6, $P < 0.008$) for a HR of 2.68 (95% CI: 1.58–4.54; $P < 0.001$). Excess mortality was not experienced in any other atorvastatin dose group. Although the mechanism of the cause of death in this dose group alone is unclear, it appears to be independent of torcetrapib-induced changes in blood pressure, serum aldosterone levels, electrolytes, or plasma lipoproteins. Mechanistic studies suggest that torcetrapib may cause endothelial dysfunction, independent of CETP inhibition, as assessed by ultrasound imaging of acetylcholine-induced changes in a rabbit model.[55] Torcetrapib, when administered in therapeutic doses, resulted in elevated arterial pressures and significantly reduced central ear artery diameter in the rabbit, which was not seen with another structurally distinct CETP inhibitor despite similar degrees of CETP inhibition and elevation in HDL-C.[55] Additional work revealed that torcetrapib decreased release of nitric oxide, a potent vasodilator, while increasing production of endothelin-1, a potent endothelial vasoconstrictor.[56] These findings together raise the possibility that higher doses of statin may have counteracted the effects of torcetrapib-induced endothelial dysfunction. A randomized study to evaluate the effect of torcetrapib on atherosclerosis progression as measured by intravascular ultrasonography revealed that the percentage of atheroma was not different in the torcetrapib–atorvastatin group vs. the atorvastatin-only group.[57] However, significant regression in percentage of atheroma volume was observed in patients who had the greatest change in HDL-C. Moreover, changes in HDL-C independently predicted the effect on atherosclerosis progression.[58] Future CETP inhibitors in development that do not share the off-target effects of torcetrapib may be beneficial in slowing the progression of atherosclerosis.

Dalcetrapib

The next agent in this class to enter phase III trials, dalcetrapib, is an irreversible inhibitor of CETP activity with much lower potency (half-maximal inhibitory concentration >1,000 nmol/l) than torcetrapib.[49] Phase II studies demonstrated a more modest effect on lipids, with a 28–34% increase in HDL-C and 5–7% decrease in LDL-C.[59,60] Dalcetrapib did not appear to share the off-target effects on blood pressure and aldosterone seen with torcetrapib.[53] Dalcetrapib exhibited minimal benefits on surrogate end points such as atherosclerosis progression as assessed by imaging[61] and neutral effects on endothelial function.[62] Dal-OUTCOMES, the pivotal phase III outcomes trial with dalcetrapib in 15,871 patients with a recent acute coronary event, was stopped due to futility. The addition of 600 mg/day dalcetrapib did not reduce the risk of recurrent cardiovascular events compared with placebo (cumulative event rate 8.0 and 8.3% with dalcetrapib and placebo, respectively, $P = 0.52$) at the 31-month follow-up period.[15] At randomization, the mean HDL-C and LDL-C levels were 42 and 76 mg/dl, respectively. Although dalcetrapib treatment significantly raised HDL-C by 31–40%, compared with 4–11% for placebo, relative to baseline, it did not affect LDL-C concentrations. As naturally occurring *CETP* variants that have been shown to have atheroprotective influence on levels of both HDL-C and LDL-C,[33] the success of CETP inhibitors currently in development may hinge on their ability to modulate both lipoproteins. In addition, HDL-C levels at baseline before randomization did not correlate with CHD risk, as shown in previous epidemiological studies, raising the questions of whether plasma HDL-C is a good marker of cardiac future events in the setting of a recent coronary event and whether the cardioprotective function of HDL is defective after ACS.

Anacetrapib

Anacetrapib is a potent inhibitor of CETP activity (half-maximal inhibitory concentration: 21 nmol/l). Early studies have not demonstrated adverse effects on blood pressure with anacetrapib.[63–65] Anacetrapib significantly raised HDL-C by 129–139% and decreased LDL-C by 38–40% with doses of 150–300 mg/day.[63,64] In addition, it was shown to favorably decrease Lp(a) concentrations by 40–50%.[64] To mitigate cardiovascular safety concerns, a randomized, double-blind placebo-controlled trial was conducted in 1,623 patients with LDL-C <100 mg/dl. In the Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib (DEFINE) study,[65,66] subjects were randomized to receive anacetrapib 100 mg/day or placebo in addition to statin therapy and were followed for 18 months. At 24 weeks, anacetrapib decreased LDL-C by 44.5% as compared with a decrease of 4.8% with placebo, representing a 39.8% reduction beyond that seen with placebo ($P < 0.001$). HDL-C also increased significantly with anacetrapib treatment (percentage change from baseline: 152.8% for anacetrapib vs. 14.7% for placebo; $P < 0.001$). Safety end points assessed at 76 weeks did not reveal any adverse effects on blood pressure, electrolyte values, or aldosterone levels. There were no differences in cardiovascular events between anacetrapib (2.0%) and placebo (2.6; $P = 0.40$), providing a 94% predictive probability that anacetrapib treatment would not result in a higher cardiovascular event rate as seen with torcetrapib. Interestingly, a recent report on the safety of anacetrapib during a 12-week reversal period following the 18 months of treatment in the DEFINE trial showed residual plasma lipid changes ~50–60% of those observed during the active treatment phase, with detectable anacetrapib drug concentrations 40% of on-treatment trough levels.[67] Although an increase in adverse events was not observed in this study, the clinical ramifications of the prolonged clearance of the drug due to its lipophilic nature are still uncertain. The phase III outcomes trial, Randomized Evaluation of the Effects of Anacetrapib Through Lipid-Modification (REVEAL), is currently ongoing and will enroll 30,000 patients with occlusive arterial disease to determine the efficacy and safety of CETP inhibition (ClinicalTrials.gov identifier NCT01252953).

Evacetrapib

Evacetrapib, another potent CETP inhibitor (half-maximal inhibitory concentration: 5 nmol/l), is currently in clinical development.[68] Evacetrapib did not adversely affect blood pressure in preclinical studies performed in rats. Moreover, *in vitro* studies did not demonstrate any effects of evacetrapib on aldosterone or cortisol synthesis.[68] A single study has been published evaluating the safety and efficacy of evacetrapib monotherapy or in combination with statins after 12 weeks of treatment in 398 patients.[69] Evacetrapib monotherapy raised HDL-C in a dose-dependent manner by 54–129% ($P < 0.001$) and decreased LDL-C by 14–36% ($P < 0.001$) compared with placebo. Compared with statin monotherapy, the addition of evacetrapib increased HDL-C by 79–89% ($P < 0.001$) and decreased LDL-C by 11–14% ($P < 0.001$). No significant increases in blood pressure were observed in the study, with the exception of the treatment arm that received evacetrapib (100 mg/day) in combination with simvastatin (40 mg/day) as compared with simvastatin monotherapy ($P = 0.02$). No significant changes were seen in aldosterone, cortisol, or electrolyte levels with evacetrapib administration. The much-anticipated phase III outcomes trial, A Study of Evacetrapib in High-Risk Vascular Disease (ACCELERATE) trial (ClinicalTrials.gov identifier NCT01687998), has completed enrollment and will shape the future of this class of novel therapeutics.

THE FUTURE: EVALUATING FUNCTIONALITY OF HDL-C

Evolving science around RCT

Because pharmacological attempts at raising HDL-C levels have not yet proven successful in reducing cardiovascular events, it might be concluded that raising HDL-C concentrations alone is not sufficient to reduce risk and that improved HDL function may be required for benefit. Plasma HDL-C concentration is a static snapshot of the cholesterol pool and does not provide information regarding the flux of cholesterol from peripheral cells returning to the liver or regarding other aspects of HDL function. Promotion of cholesterol efflux through the RCT pathway may be a key atheroprotective function of HDL, and understanding the biological underpinnings of this pathway will be pivotal in developing novel therapies targeting HDL.

As a way to measure cholesterol efflux capacity of an individual's HDL, macrophage acceptor cells are labeled with radiolabeled cholesterol and then incubated with apoB-depleted serum from patients; then, the amount of cholesterol efflux is calculated based on radioactive cholesterol counts in the medium.[70] The assay quantifies total efflux by macrophages involving ABCA1, ABCG1, scavenger receptor class B type 1, and passive diffusion pathways. To test whether the ability of HDL to accept cholesterol from macrophages is associated with atherosclerotic disease, this assay was applied to sera from 203 healthy volunteers with CIMT measurements, 442 patients with angiographically confirmed coronary artery disease, and 351 controls without disease.[71] A significant inverse correlation was observed between cholesterol efflux capacity and CIMT, whereas no relationship was observed between CIMT and HDL-C levels in healthy control subjects. Patients with coronary disease had significantly lower levels of cholesterol efflux compared with control subjects. Although HDL-C was the strongest predictor of efflux capacity, it accounted for only 26% of the variation. An increased efflux capacity was associated with a decreased risk of coronary artery disease (OR per increase of 1 SD in efflux capacity: 0.70, 95% CI: 0.59–0.83, $P < 0.001$), even after adjustment for traditional risk factors.

Cholesterol efflux capacity was also measured in additional cohorts following pharmacological interventions.[71] Treatment with pioglitazone significantly increased cholesterol efflux as compared with administration of placebo,[71] which could be related to enhanced transcription of apoA1 by pioglitazone.[72] However, no change in cholesterol efflux was noted after 16 weeks of statin treatment, which implies that the mechanisms of lipid modulation by statins are independent from promotion of cholesterol efflux.[71]

To assess HDL function following niacin treatment, 39 patients with known carotid atherosclerosis were randomized to 6 months of treatment with simvastatin + placebo or simvastatin + ER niacin.[73] Niacin increased HDL-C by 29% as compared with a 2% increase in the placebo group. However, no differences were observed in cholesterol efflux capacity or measures of antioxidant properties of HDL. On the basis of this study, it appears that niacin does not improve HDL function; this finding may help explain why recent clinical trials did not show CHD risk reduction with niacin.

One question regarding CETP inhibition has been whether the elevated numbers of large HDL-C particles generated by inhibiting this pathway are truly functional. HDL-C was isolated from subjects receiving 60 and 120 mg of torcetrapib to assess its ability to promote cholesterol efflux from macrophage foam cells. At matched HDL concentrations, the 120-mg dose demonstrated increased cholesterol efflux, accompanied by increased content of apoE and lecithin cholesterol acyltransferase.[74] In a similar study, the same authors showed a similar result with higher doses of anacetrapib.[75] These studies indicate that there was no adverse effect of torcetrapib or anacetrapib on HDL-mediated cholesterol efflux. Quantification of dynamic cholesterol flux through the RCT pathway will be crucial in assessing the impact of future therapeutics aimed at enhancing RCT. Novel methods to assess RCT *in vivo* have been investigated and will provide a complete picture of RCT in humans.[76]

Modulating ApoA1 to improve HDL functionality

ApoA1, which comprises 70% of HDL protein, has been shown to be antiatherogenic. In animal models, apoA1 overexpression increases plasma HDL levels, promotes RCT, and decreases atherosclerosis.[77,78] RVX-208 (Resverlogix, Calgary, Canada) is a small molecule that promotes hepatic apoA1 expression.[22] Early data suggested that treatment with RVX-208 increased efflux capacity with only a slight increase in HDL-C levels. Another approach has been to directly infuse apoA1 combined with phospholipids in the form of reconstituted HDL into the circulation.[22] Preclinical studies in humans have found transient increases in plasma apoA1 levels, cholesterol efflux capacity, and fecal sterol excretion.[79,80] In a randomized clinical trial of 183 patients, treatment

with reconstituted HDL resulted in significant reduction in atheroma volume, measured by intravascular ultrasonography, as compared with baseline; however, the trial was underpowered to demonstrated difference compared with placebo.[18] Another reconstituted HDL product consists of apoA1 engineered to harbor the *apoA1 Milano* variant,[19] a naturally occurring variant discovered in Italy for which carriers have very low levels of HDL-C (10–30 mg/dl) and increased longevity.[81] Weekly infusions of apoA1 Milano in patients with ACS induced significant reduction of atheroma volume, compared with placebo, at the end of 5 weeks.[19] This general approach appears to have promise; however, much larger studies with primary clinical end points will be required to assess its efficacy in reducing cardiovascular risk.

CONCLUSIONS

Recent studies have brought into question the validity of the HDL cholesterol hypothesis—whether increasing HDL-C will reduce the risk of CHD.[10] The results of long-standing epidemiological studies and animal models demonstrating the atheroprotective effects of HDL are undeniable. The next developments in the story of HDL will be focused on understanding the function and biology underlying its major atheroprotective functions, namely, promotion of cholesterol efflux and RCT. The expanding population of patients with diabetes, obesity, and metabolic syndrome will only increase the number of people with low or dysfunctional HDL levels. Future studies aimed at understanding HDL biology and function will be required to expand our therapeutic armamentarium and to address the residual risk of CV events in statin-treated patients.

CONFLICT OF INTEREST

D.J.R. is a consultant for Merck & Co and Eli Lilly; end cofounder for Vascular Strategies. S.T. declared no conflict of interest.

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