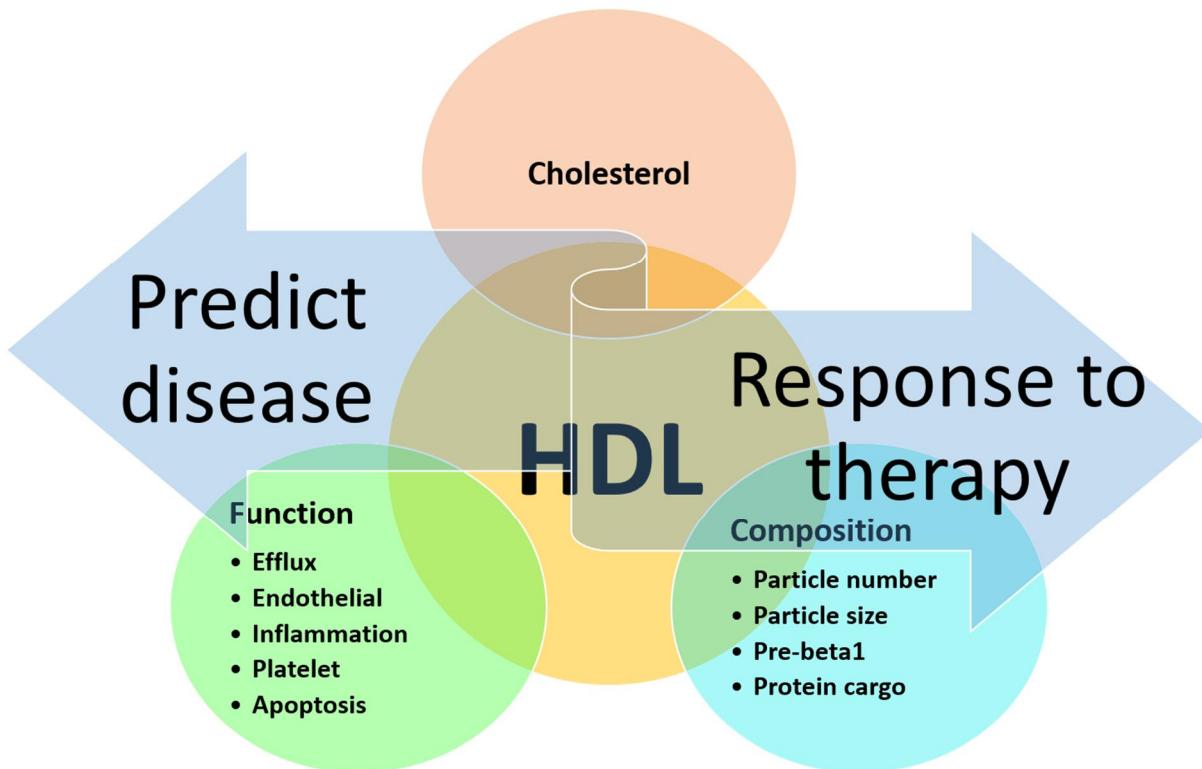


HDL Function: Redefining the HDL Hypothesis for the 21st Century



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Division of Cardiology

Internal Medicine Grand Rounds

UT Southwestern Medical Center

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Dr. Rohatgi has a research grant from Merck and serves as a consultant to Vascular Strategies and CSL Limited. He also serves on the Advisory Board of Cleveland HeartLab. He serves as the local site PI for the ACCELERATE trial (Evacetrapib).

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Purpose and Overview:

The purpose of this presentation is to review the emerging evidence that HDL cholesterol levels do not adequately predict atherosclerotic risk and response to therapy in part because they do not adequately reflect HDL function. The presentation illustrates how measurement of HDL function, in particular cholesterol efflux, is a better marker of atherosclerotic risk and perhaps a better therapeutic target.

Objectives:

- 1) Increase awareness that HDL cholesterol levels do not adequately predict disease and response to HDL-modifying therapies.
- 2) Describe the concept of HDL's central role in reverse cholesterol transport and its relevance to atherosclerosis.
- 3) Familiarize the audience with the measurement of cholesterol efflux and its association with atherosclerotic disease in humans.
- 4) Highlight the effects of previous and ongoing therapeutics on cholesterol efflux.

Introduction

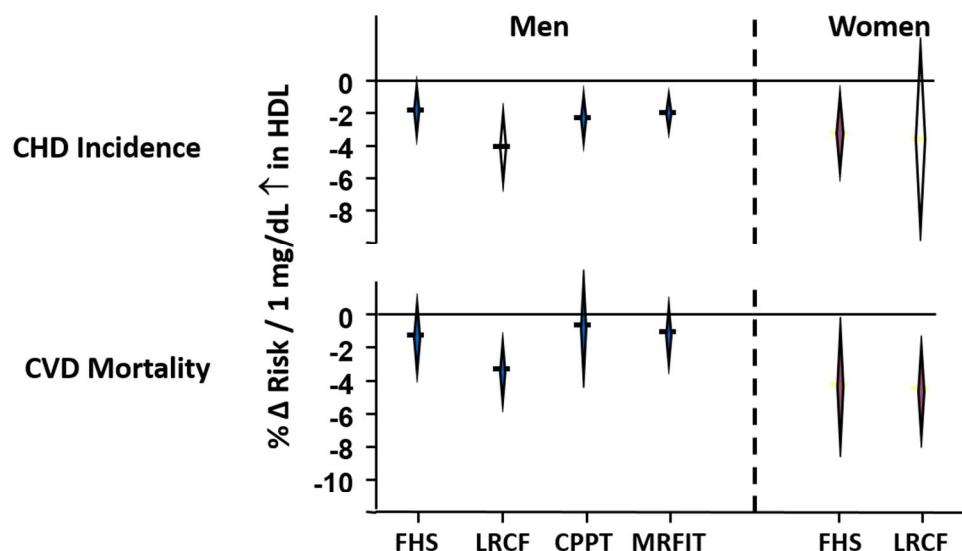
Heart disease is the number one cause of death in the U.S., with coronary heart disease (CHD) and stroke accounting for the majority of these deaths. As of 2011, about 735,000 people in the U.S. have heart attacks each year and an estimated 375,000 people die from heart disease per year.¹ The pathologic hallmark of coronary heart disease and atherosclerotic stroke is the lipid-laden arterial plaque. The majority of efforts to halt atherosclerosis have been focused on limiting cholesterol *influx* into resident macrophages within the arterial wall by reducing circulating levels of atherogenic lipoproteins, collectively reflected by non-High density cholesterol (non-HDL-C) levels. This strategy has been remarkably effective in reducing the incidence of atherosclerotic cardiovascular disease (ASCVD) in both primary and secondary prevention populations, largely through lifestyle modification and use of statin pharmacotherapies.²

However, **reverse cholesterol transport**, the movement of cholesterol from tissues to the liver and out the body, is also an important mechanism for maintaining cellular homeostasis. Cholesterol *efflux* from macrophages is the first critical step of reverse cholesterol transport in the arterial wall. Preclinical and limited human clinical studies support the concept that improving cholesterol efflux promotes plaque regression, shifting macrophages from pro-inflammatory to anti-inflammatory states, promotes egress of lipid-laden macrophages out of the arterial wall, and reduces atherosclerotic lesion size.³ High-density lipoprotein (HDL) is the key mediator of cholesterol efflux and reverse cholesterol transport. Unfortunately, it has been incorrectly assumed that the cholesterol load carried by HDL, reflected by HDL-cholesterol levels (HDL-C), also represents the dynamic efflux function of HDL particles. However, recent studies in humans have revealed that cholesterol efflux capacity is a much more robust risk marker for incident ASCVD than HDL-C and cannot be predicted by HDL-C levels. This may explain the failure of therapies that raised HDL cholesterol quantity without improving HDL function.

HDL Cholesterol (HDL-C) Level as Risk Marker

Several large epidemiologic studies established that high-density lipoprotein cholesterol, HDL-C, is inversely associated with CHD.⁴ Four large American studies and one British study had been completed by the 1980s, with varying levels of risk factors but surprisingly similar levels of HDL-C among men and women separately. Taken together, a 1 mg/dL increase in HDL-C was associated with 1.9-2.3% decrease in CHD risk in men and 3.2% decreased risk in women. These risks were attenuated for CHD mortality in men except for one study but were magnified for CHD mortality in women. Adjustment for non-HDL-C (total – HDL-C) attenuated all associations between HDL and CHD risk but remained significant in 3 of the 5 studies (Figure 1).

Figure 1. HDL-C and Risk of Incident Coronary Heart Disease



FHS = Framingham Heart Study

LRCF = Lipid Research Clinics Prevalence Mortality Follow-up Study

CPPT = Coronary Primary Prevention Trial

MRFIT = Multiple Risk Factor Intervention Trial

Gordon DJ, et al. Circ 1989; 79:8

These consistent observations have led to the inclusion of low HDL-C (<40mg/dL in men; <50mg/dL in women) as a major risk factor for ASCVD in most risk prediction algorithms, including the recent 2013 Pooled Cohort Equation for estimation of 10-year ASCVD risk.² Unfortunately, whether HDL is causal in the development of or protection from CHD has not been proven in human studies.

Interestingly, more recent epidemiologic observations suggest that HDL-C is not, in fact, consistently inversely associated with incident ASCVD. Studies in the Multi-Ethnic Study of Atherosclerosis (MESA) and in our own Dallas Heart Study revealed that the inverse association between HDL-C and incident ASCVD is attenuated when accounting for HDL particle number (NMR) and may only be significant among non-Blacks.⁵⁻⁷ Furthermore, the inverse relationship may be only be present at lower HDL-C levels, below 40 mg/dL, with a relatively flat relationship at normal to higher levels. Lastly, several observation from randomized controlled trials have revealed that therapy with statins in the current era has blunted the inverse relationship between HDL-C and ASCVD (TNT, Dalcetrapib, AIM-HIGH, and JUPITER).

The inverse relationship between HDL-C and CHD risk in the general population has not been consistently observed in individuals and families with rare monogenic disorders of HDL-C. Part of the reason for this discordance is the remarkable phenotypic variability in HDL-C, atherosclerosis, and CHD among these individuals. Rare mutations in ABCA1, apoA-I, and LCAT lead to markedly low HDL-C

levels, whereas mutations in endothelial lipase (LIPG) and CETP lead to increased HDL-C.

Lastly, therapies leading to increases in HDL-C have not yielded consistent clinical benefits (Table 1). The Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes (AIM-HIGH) trial assessed if the addition of extended release niacin to intensive statin therapy would reduce the risk of events in patients with established ASCVD and atherogenic dyslipidemia with well controlled LDL-C.⁸ Most patients were taking a statin at entry (94%), with a median LDL-C of 71 mg/dl, median HDL-C of 35 mg/dl, and median triglycerides of 161 mg/dl. The AIM-HIGH trial was halted early after a planned interim analysis suggested futility. Those assigned to the niacin group achieved significant improvement in multiple lipid parameters, including decrease in LDL-C (13.6% compared to 7.6% in the placebo group), decrease in median triglycerides (30.8% compared to 9.9% in the placebo group), and increase in HDL-C (25% compared to 11.8% in the placebo group). The primary end point occurred in 16.4% of patients in the niacin group and 16.2% of patients in the placebo group (hazard ratio 1.02, 95% CI 0.87-1.21, p = 0.80).

The Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) study assigned over 25,000 patients with known vascular disease on statin therapy to receive extended release niacin with laropiprant (used to reduce facial flushing) versus placebo (Table 1).⁹ Patients in the niacin group had an average decrease in LDL-C of 10 mg/dl, average increase in HDL-C of 6 mg/dl, and average decrease in triglycerides of 33 mg/dl compared to placebo. Similar to AIM-HIGH, the study showed that despite favorable changes in HDL-C and other lipid parameters, there was no significant reduction in major vascular events with niacin (13.2% in niacin arm vs. 13.7% in the placebo group; HR 0.96, 95% CI 0.90-1.03, p = 0.29). However, assignment to the niacin arm was associated with significantly increased adverse effects, including gastrointestinal, musculoskeletal, infectious, and serious bleeding complications, worsened glycemic control amongst diabetics and non-diabetics (55% proportional increase in disturbances of diabetes control and 32% proportional increase in diabetes diagnosis in the study group), and even a trend towards increased risk of death (6.2% in study group vs. 5.7% in placebo group, HR 1.09, CI 0.99-1.21, p = 0.08). Given the adverse side effects noted, this trial was also stopped early, with median follow-up time of 3.9 years.

Cholesteryl-ester transfer protein (CETP) inhibitors are a potentially exciting addition to HDL-C raising therapies and initially held great promise to be the most potent HDL modulating class available. CETP promotes the exchange of cholesteryl esters from HDL to Apolipoprotein B-containing lipids (LDL and VLDL) while concurrently exchanging triglycerides from VLDL and LDL back to HDL, leading to smaller, lipid-poor HDL particles. Inhibition of CETP thus markedly increases HDL-C

concentration. The Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events (ILLUMINATE) study, the first randomized controlled trial of CETP inhibitors, compared patients on high intensity statin alone to those on high intensity statin plus torcetrapib. The study showed that while torcetrapib raised HDL-C by over 60% and reduced LDL-C by 20%, it led to an increase in adverse cardiovascular events and all-cause mortality.¹⁰ Furthermore, in the Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation (ILLUSTRATE) follow-up study, torcetrapib had no benefit on coronary atherosclerosis progression as measured by intravascular ultrasound.¹¹ The lack of efficacy of torcetrapib was confounded by its aldosterone-like blood pressure raising effect (an off-target toxicity not associated with other CETP inhibitors), which may partially explain the results of the ILLUMINATE trial. However, the dal-OUTCOMES trial, which studied dalcetrapib versus placebo, was also prematurely halted for lack of efficacy.¹²

Table 1. Randomized controlled trials of Niaspan and CETP Inhibitors

Drug Class	N	Trial	HDL-C change	Outcome
NIACIN	3,414	AIM-HIGH	25%	No benefit
	25,673	HPS2-THRIVE	15%	No benefit
CETPI	15,067	Torcetrapib	72%	Increased harm
	15,871	Dalcetrapib	35%	No benefit

AIM-HIGH: Boden WE, et al. NEJM 2011;365:2255-67

HPS2-THRIVE. NEJM 2014;371:203-12

Torcetrapib: Barter PJ, et al. NEJM 2007;357:2109-22

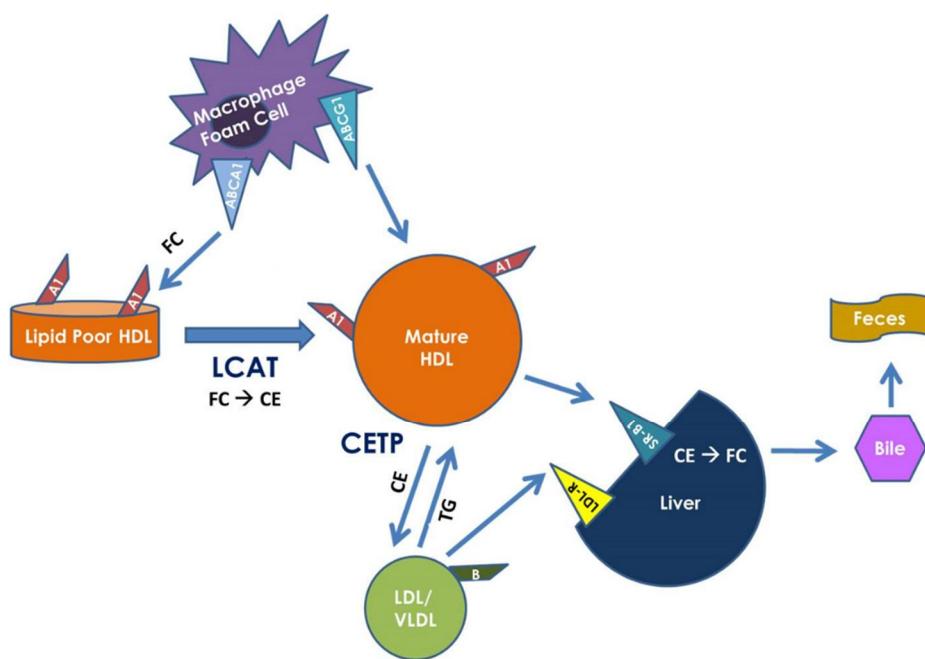
Dalcetrapib: Schwartz GG, et al. NEJM 2012;367:2089-99

Clearly, change in HDL-C levels do not accurately predict clinical benefit. It is reasonable to consider that HDL-C levels do not adequately reflect the remarkable heterogeneity of HDL composition, varying in shape, size, and protein and lipid composition. The protein make-up of HDL particles is also quite heterogeneous. Most of the apolipoprotein on the surface of HDL is apolipoprotein A-I (apoA-I). Various other apolipoproteins make up a smaller fraction of the remaining apolipoprotein component and have diverse biological activities as well. The recent advent of protein analysis by mass spectroscopy, or proteomics, has allowed remarkable discrimination in detecting proteins on the surface of HDL. In contrast to LDL which has 22 distinct proteins identified to date, the HDL proteome appears to have as many as 95 different proteins, many of which are not standard apolipoproteins known to be associated with HDL

(<http://homepages.uc.edu/~davidswm/HDLproteome.html>).^{13,14} These types of analyses suggest that the heterogeneous protein makeup of HDL reflects heterogeneous HDL functionality, including lipid metabolism, protease action, and immunity. Investigations into these diverse HDL functions are yielding new insights into how HDL may exert its anti-atherogenic effects and how therapeutic modulation can alter these functions in relation to HDL-C levels.

HDL Function

Figure 2



LCAT:Lechitin:Cholesterol Acyltransferase, CETP: Cholesteryl Ester Transfer Protein, FC: Free Cholesterol, CE: Cholesterol Ester, TG: Triglycerides, LDL-R LDL Receptor. Mani P, Rohatgi A. Curr Athero Reports 2015;17:521.

HDL exerts several key anti-atherosclerotic functions related to cholesterol transport, endothelial and vascular function, and inflammation. Reverse cholesterol transport (RCT), the ability of HDL to accept cholesterol from the periphery and deliver it to the liver for excretion, is the principal method for HDL biogenesis from nascent lipid-poor particles to mature cholesteryl-ester laden spherical particles.¹⁵ RCT is also considered the principal HDL function that impacts macrophage foam cell formation and

other functions such as endothelial activation of eNOS, monocyte adhesion, and platelet aggregation.¹⁶ Therefore, RCT is the overriding action of HDL on multiple cell types.

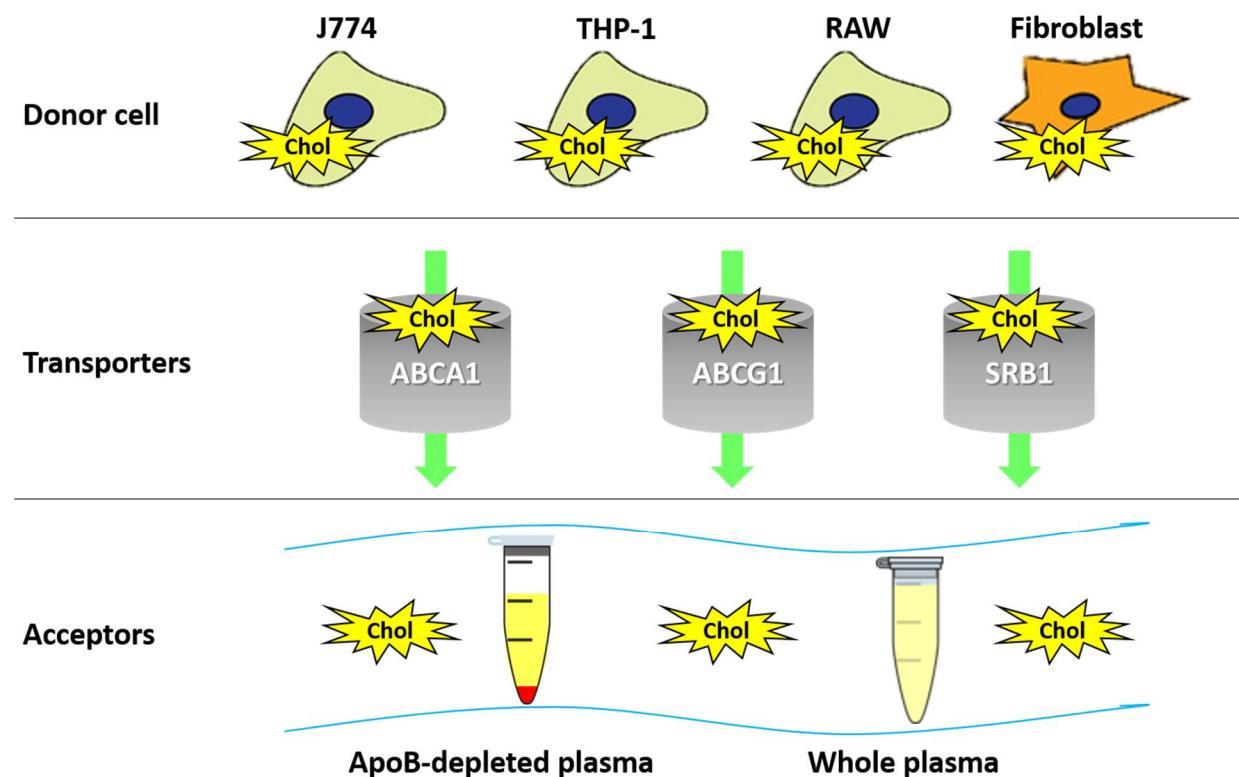
Macrophage-specific cholesterol efflux is the key initial step in RCT and has been shown in genetic and pharmacologic animal studies to be more closely associated with atherosclerosis than circulating levels of HDL-C.¹⁵ Whether this function is operative in humans remains to be seen, but recent studies assessing cholesterol efflux in humans suggest that the cholesterol efflux capacity (CEC) of human plasma or serum is a potent marker of ASCVD risk.

Measuring Cholesterol Efflux

There is no standardized method for measuring CEC in humans and protocols vary considerably; however, they all measure the movement of labeled cholesterol from cells to an extracellular acceptor (Figure 3).¹⁷ In general, most studies in humans have only tested the cholesterol acceptor aspect of efflux, specifically, the differential capacity of human serum/plasma to accept cholesterol from cells in a unidirectional manner. This approach does not take into account the ability of a patient's own macrophages to efflux cholesterol and does not assess cholesterol influx, or net efflux.

Macrophages are the most relevant cell type for studies of atherosclerosis given the central role of macrophage "foam" cells in disorders of lipid accumulation. Macrophages efflux cholesterol via several transporters, including ATP-binding cassette transporters ABCA1 and ABCG1, scavenger receptor SRB1, as well as via aqueous diffusion. CEC assays can reflect all of these pathways in aggregate or can be modified to interrogate a specific transporter. Choice of cholesterol acceptor can have significant impact on assessment of CEC and is the largest source of variation across studies. Cholesterol acceptor mediums can range in specificity for HDL from isolated pure HDL to apolipoprotein B-depleted (apoB-depleted) plasma/serum to whole plasma/serum. The use of ApoB-depleted plasma eliminates the role of LDL and VLDL in assessing cholesterol efflux, making it more specific for HDL-mediated CEC. When whole or apoB-depleted plasma/serum is used, other cholesterol acceptors and shuttles such as albumin can also play a role in CEC; however, studies have shown that apolipoprotein A-I (apoA-I), the main protein constituent of HDL particles, is responsible for ~75-80% of the CEC from macrophage cell lines with amplified ABCA1 transporter pathways.^{18,19} Ascertaining the specific methodology used to assess CEC is critical when evaluating the reported findings in human studies.

Figure 3. Cholesterol Efflux Assay



Cholesterol efflux capacity is assessed ex-vivo by incubating donor cells with labeled cholesterol and cholesterol acceptors such as plasma. The fraction of labeled cholesterol that effluxes from donor cells to extracellular acceptors is quantified as cholesterol efflux. Rohatgi A. Progress in CV Disease 2015;58:32-40.

CEC and Atherosclerotic Cardiovascular Disease (ASCVD)

Studies assessing the association between CEC and ASCVD are summarized in Table 2. Perhaps the first reported study of CEC and CAD in humans, a small case-control study in the mid 1990's showed that CEC was lower in patients with prevalent CAD and was the lowest in those with both CAD and diabetes.²⁰ Among low-risk groups, the first cohort study evaluated CEC in 204 healthy white males undergoing carotid intima media thickness (CIMT) evaluation.²¹ CEC was found to be inversely proportional to CIMT, a surrogate marker that is associated with increased atherosclerotic disease risk,^{22,23} independent of HDL-C and ApoA-I levels. Interestingly, HDL-C levels were significantly correlated with CEC ($r=0.58$, $p < 0.0001$), but failed to correlate with CIMT. These findings further the notion that the relationship between CEC and atherosclerosis is not completely explained by HDL-C levels. Another study explored the relationship between CEC and coronary atherosclerosis via CT angiography in patients with psoriasis without known coronary artery disease (CAD).²⁴ Low CEC was associated with a higher coronary plaque burden — specifically non-calcified plaque — independent of cardiovascular risk factors, including hyperlipidemia.

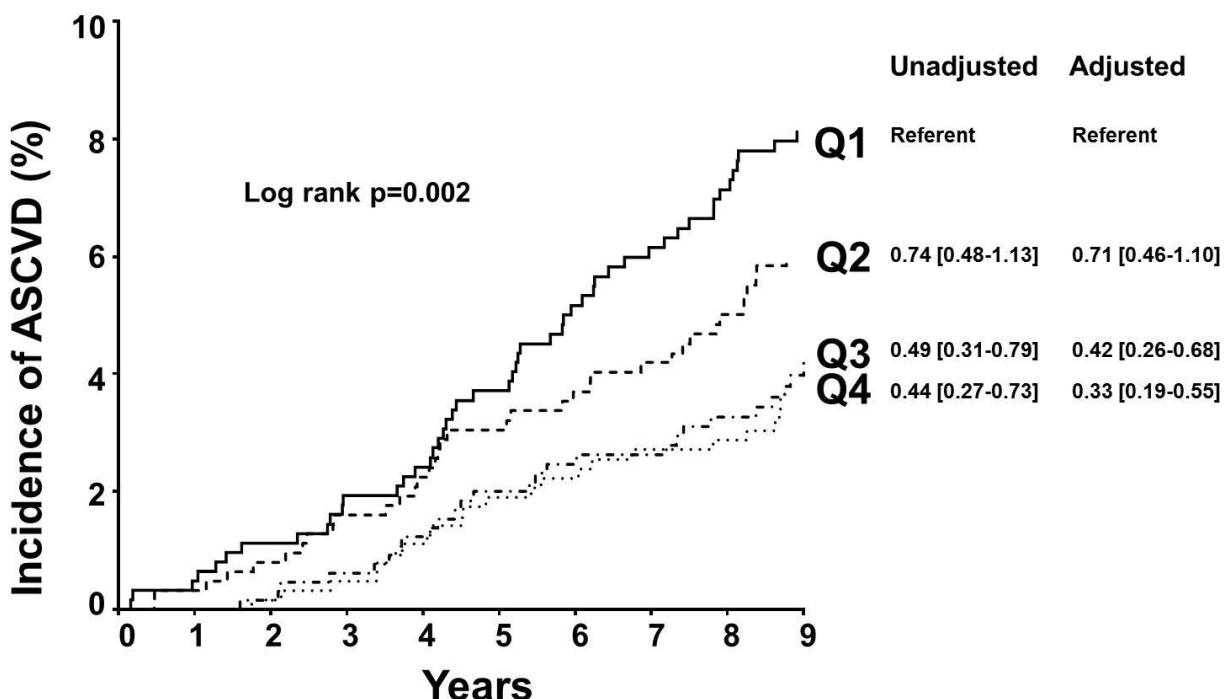
The relationship between CEC and coronary atherosclerosis has also been assessed in higher risk cohorts undergoing coronary angiography. The first large study of CEC in humans utilized a sample of 793 individuals without acute coronary syndrome presenting for coronary angiogram in a case-control design.²⁵ In this study sample, CEC was positively correlated with HDL-C ($r=0.51$, $p<0.0001$). Increasing CEC was associated with decreased prevalence of angiographic CAD, even when adjusted for traditional risk factors and HDL-C. CEC was also inversely associated with severity of angiographic CAD. This study established the relevance of measuring CEC in humans with regard to ASCVD, but its cross-sectional design limited the ability to determine whether impaired efflux occurred prior to the onset of CAD.

Our group studied CEC and incident ASCVD events in 2924 American individuals without known CAD from the Dallas Heart Study.⁷ The average age of this low-risk population cohort was 42, with almost 50% Blacks, and a median LDL-C of 104 mg/dL. ASCVD was defined as myocardial infarction, stroke, coronary revascularization, or cardiovascular death. Baseline HDL-C was not associated with ASCVD after adjustment for risk factors and total HDL particle concentration (HDL-P) by NMR (HR 1.08, 95% CI 0.59-1.99). CEC was associated with increasing lipid levels but very few other traditional risk factors. The correlation between CEC and HDL-C was weak ($r=0.07$, $p<0.05$) and modest with HDL-P ($r=0.15$, $p<0.05$). CEC was inversely associated with ASCVD with no attenuation of the point estimate after adjustment for risk factors, HDL-C, or HDL-P (HR for quartile 4 vs. 1: 0.33, 95% CI 0.19-0.55) (Figure 4). This inverse association was graded across increasing quartiles and was similar for the hard end point of nonfatal and fatal myocardial infarction and stroke. The hazard for 1 standard deviation increase in continuous CEC was 0.68 (95% CI 0.55-0.84). As the first multiethnic study of HDL function, the Dallas Heart Study showed no interaction by race/ethnicity, an important observation as therapies targeting HDL and HDL function are developed.

A similar study was conducted in the EPIC-Norfolk cohort, using a prospective nested case-control sample of European Caucasians.²⁶ There were 1745 cases, defined as those who were hospitalized or died from unstable angina, stable angina, or myocardial infarction. A total of 1749 participants were in the control group, free of coronary disease at mean follow up. The 1745 incident cases at baseline were older and more overweight, had worse lipid profiles, and more likely to have diabetes, hypertension or a history of smoking. Among the controls, CEC was associated with female sex, total cholesterol, and alcohol intake, and inversely with measures of adiposity and diabetes. CEC was moderately correlated with HDL-C ($r=0.40$, $p<0.05$), in contrast with a minimal correlation in the Dallas Heart Study, and modestly with ApoA-I ($r=0.22$, $p<0.05$) among controls. Despite this differing correlation between CEC and HDL-C, a similar inverse dose-response relationship with events was found: increasing tertiles of CEC were associated with reduced incidence of coronary heart

disease, without attenuation from risk factor adjustment, including HDL-C (OR tertile 3 vs. 1: 0.64, 95% CI 0.51-0.80). Similar findings were seen with adjustment for Apo A-I levels. The odds ratio for 1 standard deviation increase in continuous CEC was also similar to that seen in the Dallas Heart Study (OR per 1 SD: 0.80, 95% CI 0.70-0.90).

Figure 4. Cholesterol Efflux Capacity and Incident ASCVD in the Dallas Heart Study



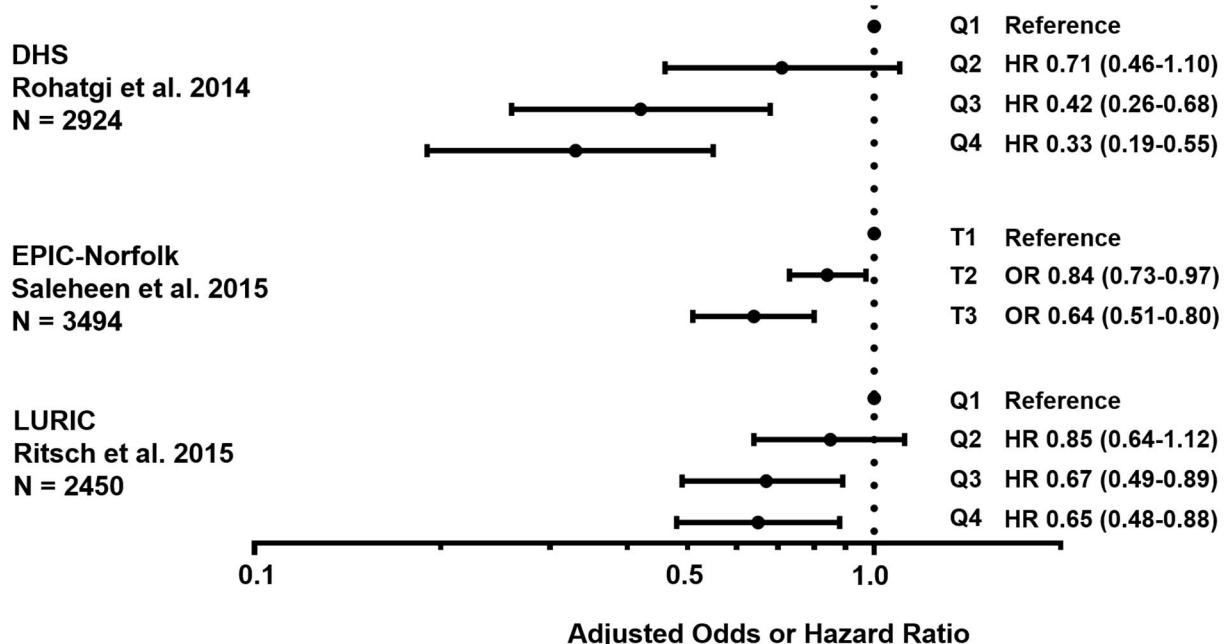
Quartiles of Cholesterol Efflux Capacity and Incident ASCVD: nonfatal MI or stroke, coronary revascularization, or CV death (N=132). HR adjusted for age, sex, ethnicity, hypertension, diabetes, smoking, total cholesterol, statins, BMI, HDL-C, and HDL-P. Rohatgi et al., NEJM 2014;371:2383-93.

The Dallas Heart Study and the EPIC-Norfolk study both were low risk populations free of heart disease at baseline. Despite some differences, including CEC assay methodology, levels of risk factors and ethnicity make-up, both studies revealed similar inverse associations with incident ASCVD. The end points in the Dallas Heart Study included stroke and all CV death, whereas those in the EPIC-Norfolk study were specific to coronary ischemia or myocardial infarction. Taken together, these large longitudinal studies extend prior cross-sectional associations with prevalent coronary disease by showing that low baseline CEC predicts incident ASCVD.

Lastly, in a letter publication, the LURIC study assessed efflux in 2450 European Caucasians presenting for coronary angiogram. There was a strong, graded, inverse association between efflux at the time of angiogram and incident CV death. The LURIC study has a large number of end points but is not in fully published format.

Overall, these studies have established that CEC can be measured in high-throughput fashion in thousands of human subjects and exhibits a validated inverse association with incident coronary disease (Figure 5). Several intriguing observations from these studies deserve comment. HDL-C levels appear to explain only about 20-25% of the variation in efflux capacity, suggesting that the cholesterol load of HDL particles does not fully reflect the efflux functionality of HDL particles. In addition, efflux in these studies quantified *total macrophage efflux* through the various cholesterol transporters, of which 30-40% is ATP-binding cassette transporter A1 (ABCA1)-specific.²⁵ The ABCA1 transporter is essential to the maturation of HDL from small dense particles containing mostly protein (ApoA-I) to larger spherical lipoproteins containing more cholesteryl ester. In contrast, non-ABCA1 transporters play a significant role in efflux to larger mature HDL particles, resulting in high correlations with circulating HDL-C levels. Efflux via ABCA1 is promoted by lipid-poor apoA-I, often termed “prebeta-1” particles and correlates poorly with HDL-C levels.²⁷ Intriguingly, impaired ABCA1-specific efflux in animal studies consistently leads to increased atherosclerosis whereas impaired non-ABCA1-efflux pathways on their own do not.¹⁵ In support of these observations, our recent population-based study utilized an assay more specific for ABCA1-efflux than previously used methods and found almost no correlation between efflux and HDL-C levels yet striking inverse associations with incident ASCVD.⁷

Figure 5. Cholesterol Efflux Capacity (CEC) and Risk of Incident Cardiovascular Events.



The relationship between increasing cholesterol efflux capacity and incident cardiovascular events is shown for 3 cohorts. The depicted hazards ratios (HR) and odds ratios (OR) with 95% CI are adjusted for traditional risk factors. Q=quartile; T=tertile.

Table 2. Association of Cholesterol Efflux and Heart Disease in Human Studies

Study	Population	Endpoint	Cross-sectional/Incident	Efflux Result
Linsel-Nitschke et al., 2009 ²⁸	<ul style="list-style-type: none"> • Cross-sectional case-control • Presenting for angiogram • N=90 cases • N=52 controls 	<ul style="list-style-type: none"> • >50% angiographic coronary stenosis 	Cross-sectional	<u>Lower in CAD:</u> <ul style="list-style-type: none"> • 46% in CAD vs. 51% in controls, P=0.028
Khera et al., 2011 ²⁵	<ul style="list-style-type: none"> • Cross-sectional case-control • Presenting for angiogram • N=442 cases • N= 351 controls 	<ul style="list-style-type: none"> • >50% angiographic coronary stenosis 	Cross-sectional	<u>Lower in CAD:</u> <ul style="list-style-type: none"> • Q4 vs. Q1: OR 0.48 [0.30-0.78] • 1 SD: OR 0.75 [0.63-0.90]
De Vries et al., 2011 ²⁹	<ul style="list-style-type: none"> • Nested case-control • Presenting for angiogram • N=113 cases • N=111 controls 	<ul style="list-style-type: none"> • Incident CVD: CV death, hospitalization for MI, PCI/CABG 	Incident	<u>No difference:</u> <ul style="list-style-type: none"> • HR 1.06 [0.88-1.29], p=0.52
Li et al., 2013 ¹⁸	<ul style="list-style-type: none"> • Cross-sectional case-control and longitudinal • Outpatient: N=146 cases, N=431 controls • Angiographic: N=871 cases, N=279 controls 	<ul style="list-style-type: none"> • CAD vs. no CAD for outpatient and angiographic cohort • Incident CVD: MI, stroke, death at 3 years for angiographic cohort 	Cross-sectional Incident	<u>Lower in outpatient CAD:</u> <ul style="list-style-type: none"> • T3 vs. T1: OR 0.2, p<0.05 <u>No difference in angiographic CAD:</u> <ul style="list-style-type: none"> • T3 vs T1: OR 1.2, p=ns <u>Higher in incident CVD:</u> <ul style="list-style-type: none"> • T3 vs. T1: HR 1.9 [1.1-3.1]
Rohatgi et al., 2014 ⁷	<ul style="list-style-type: none"> • Longitudinal • Population-based sample free of CVD • N=2924 	<ul style="list-style-type: none"> • Incident CVD: MI, stroke, coronary revascularization, CV death 	Incident	<u>Inverse with CVD:</u> <ul style="list-style-type: none"> • Q4 vs. Q1: HR 0.33 [0.19-0.55] • 1 SD: HR 0.68 [0.55-0.84]
Ritsch et al., 2015 ³⁰	<ul style="list-style-type: none"> • Longitudinal • Angiographic cohort • N=2450 	<ul style="list-style-type: none"> • Incident CV death 	Incident	<u>Inverse with CV Death</u> <ul style="list-style-type: none"> • Q4 vs. Q1: HR 0.65 (0.48-0.88)
Saleheen et al., 2015 ³¹	<ul style="list-style-type: none"> • Longitudinal • Population-based sample free of CVD • Cases: 1749 • Controls: 1745 	<ul style="list-style-type: none"> • Incident CHD: hospitalization or death from angina or MI 	Incident	<u>Inverse with CVD:</u> <ul style="list-style-type: none"> • Q3 vs. Q1: HR 0.64 [0.51-0.80] • 1 SD: HR 0.80 [0.70-0.90]

Modified from Rohatgi A. Progress in CV Disease 2015;58:32-40.

Effect of Therapies Targeting HDL Metabolism on Cholesterol Efflux

(Reviewed in Bhatt A, Rohatgi A. *Curr Athero Reports* 2015; *in press*)

Niacin

The combination of niacin and statin therapy, when compared to statin therapy alone, failed to decrease the risk of recurrent cardiovascular events in the AIM-HIGH and HPS2-THRIVE clinical trials ^{9,32}, despite an increase in HDL-C levels. Though niacin does have a modest effect on ABCG1-specific CEC ³³, the overall effect on CEC was likely due to increases in HDL-C concentration. Niacin has not been shown to have a significant effect on ABCA1-specific efflux, especially in patients already on statin therapy ^{34,35}. In addition, the combination of niacin and statin therapy in the AIM-HIGH trial trivially increased ApoA-I levels compared to statin therapy alone. Overall, niacin does not decrease the risk of future cardiovascular events in addition to statin therapy, perhaps partially explained by the lack of significant modulation of CEC—specifically ABCA1-specific efflux. An intriguing question would be to clarify whether there would be any clinical benefits of niacin in those with low total efflux and HDL-C levels, allowing a more targeted approach.

CETP Inhibitors

CETP inhibitors have not reduced cardiovascular outcomes in large, randomized controlled trials ^{12,36}. In the Dal-ACUTE trial ³⁷, 300 patients were randomized to 600mg/day of dalcetrapib or placebo, within one week of an acute coronary syndrome. While HDL-C was increased by almost 34% at 4 weeks in the dalcetrapib arm, CEC was only increased by 9.5%. Importantly, this increase in CEC was mostly via non-ABCA1-specific efflux.

Yvan-Charvet et al. compared torcetrapib 60mg/day vs. 120mg/day dosing in a small sample of patients without cardiovascular disease (n=8 in each arm) for 8 weeks ³⁸. At the 60mg daily dosing, no significant difference in CEC was noted at 8 weeks; however, the 120mg daily dosing did significantly raise CEC. The ILLUMINATE trial used 60mg daily dosing to evaluate the efficacy of torcetrapib, but was stopped early due a high rate of adverse outcomes ³⁶. As noted with dalcetrapib, a significant proportion (40-50%) of torcetrapib's effect on cholesterol efflux was non-ABCA1-specific.

Two new agents in this class are currently undergoing evaluation. We await the results of the ongoing phase 3 trial, REVEAL, in which cardiovascular outcomes are being studied in response to therapy with anacetrapib 100mg daily in addition to statin therapy [clinicaltrials.gov, identifier: NCT01252953]. Anacetrapib has been shown to

increase HDL-C by up to 100%³³. Perhaps more importantly, this study of 20 patients given 300mg of anacetrapib showed that at 8 weeks, there was up to a 2.4 fold increase in CEC when compared to the control groups³³. Furthermore, the increased efflux potential seen with anacetrapib was partly dependent on ABCA-1 expression.

Another phase 3 trial in progress, ACCELERATE, is evaluating the effect of evacetrapib on cardiovascular risk [clinicaltrials.gov, identifier: NCT01687998]. Evacetrapib therapy alone increases HDL-C by 54-129% in a dose dependent fashion³⁹, and has been shown to increase total CEC by 28%, and ABCA1-specific efflux by 17% [Rader et al. Effects of the cholesteryl ester transfer protein inhibitor, evacetrapib, administered as monotherapy or in combination with statins on cholesterol efflux and HDL particles in patients with dyslipidemia. *Circulation* 2014; 130: A12252].

In contrast to torcetrapib and dalcetrapib, anacetrapib and evacetrapib seem to increase both global CEC and ABCA1-specific efflux. It remains to be seen whether these differential effects on the various efflux pathways will translate into clinical benefit. However, due to the variety of lipid effects of CETP inhibitors, including a reduction in LDL, it may be difficult to assess the specific clinical effects of modulating CEC via CETP inhibition.

ApoA-I

ApoA-I accepts cholesterol from the periphery and forms Prebeta-1 HDL, considered the primary acceptor of cholesterol via ABCA-1. ApoA-I levels have been inversely associated with incident cardiovascular events⁴⁰. ApoA-I mimetics have been studied in murine and human models, stimulate CEC^{41,42}, and have atheroprotective effects⁴³⁻⁴⁶.

The ERASE trial⁴⁷ in 2007 randomized 183 patients with recent acute coronary syndromes to placebo or different doses of CSL-111, a recombinant HDL particle consisting of human ApoA-I and phosphatidylcholine. Coronary angiography with IVUS was performed at time of incident event and at a mean of forty-four days later. Though there was a 3.4% improvement in the primary endpoint—atheroma volume, as measured by intravascular ultrasound—this was not significant compared to placebo. There was a significant improvement in plaque characterization index and coronary score in the CSL-111 treated arm.

AEGIS-1 is an ongoing phase 2B, randomized placebo-controlled study investigating recurrent cardiovascular event rates with a newer recombinant HDL particle, CSL-112, therapy in 1,200 patients with recent acute coronary syndrome [clinicaltrials.gov, identifier: NCT02108262]. CSL-112 has been associated with increases in HDL-C levels, Prebeta-1 HDL particles⁴⁴ and ABCA1-specific CEC^{41,42}.

RVX-208 is a novel agent that selectively upregulates ApoA-I synthesis, and has been shown in animal models to increase ApoA-I levels, increase ABCA1-specific and

non-ABCA1-specific cholesterol efflux, as well as promote a shift in HDL particle size distribution⁴⁸. This prompted a phase 1 study of patients receiving RVX-208 infusions for seven days, showing significant increases in ApoA-I levels and ABCA1-specific cholesterol efflux without significant increases in HDL-C levels⁴⁸. A phase II trial investigated 299 patients with stable CAD on statin therapy⁴⁹. After twelve weeks of RVX-208 infusion, both increases in ApoA-I and HDL-C levels were noted.

The ASSURE and SUSTAIN trials are completed phase 2B trials of RVX-208⁵⁰. In ASSURE, 310 patients with low HDL-C and angiographic evidence of CAD (at least one stenosis >20% in an epicardial coronary artery on a clinically indicated coronary angiogram) were randomized to RVX-208 or placebo for twenty-six weeks. The primary outcome was intravascular ultrasound guided assessment of atheroma volume. There was no significant difference in atheroma volume in the treated cohort versus placebo⁵¹. In SUSTAIN, 172 patients with low HDL-C already on statin therapy were randomized to RVX-208 or placebo, with change in HDL-C levels as the primary efficacy marker. Both of these trials analyzed the incidence of MACE as a secondary outcome. Preliminary results report a 55% relative risk reduction in MACE ($p = 0.02$) in the RVX-208 cohort, with a larger beneficial effect in those with diabetes (77% relative risk reduction; $p = 0.01$) [Wong et al. Abstract 338: Effects of RVX-208 a Selective Bromodomain Extra-Terminal Protein Inhibitor Beyond Raising ApoA-I/HDL. *Arterioscler Thromb Vasc Biol.* 2015;35:A338].

Therapies mimicking ApoA-I or targeting ApoA-I metabolism more directly modulate CEC without significant effects on other lipids and will potentially be able to answer the critical question of whether targeting efflux capacity can lead to clinical benefit.

Summary of Therapeutics Section

The clinical relevance of manipulating the reverse cholesterol transport pathway in humans remains unknown. The failure of niacin and two CETP inhibitors to improve cardiovascular outcomes in addition to statins, despite increases in HDL-C, certainly dispels *change in HDL-C* as a valid sole therapeutic target but leaves unanswered whether changes in efflux can affect outcomes. However, it will remain challenging to fully assess the therapeutic impact of targeting cholesterol efflux in studies testing therapies like niacin and CETP inhibitors that not only affect HDL but also lower non-HDL-C levels and other lipids. Questions that remain include what amount of change in efflux is necessary to confer clinical benefit, whether the mechanism by which a therapy changes total or ABCA1-specific efflux matters, and whether altering efflux will affect plaque stability and plaque regression similarly across the continuum of plaque morphology from nascent fatty streaks to calcified fibrotic plaques to lipid-laden inflammatory lesions.

Conclusion

In summary, measuring the dynamic functionality (efflux) versus simply the cholesterol load (HDL-C) of HDL particles imparts significantly more information about HDL metabolism. The key anti-atherosclerotic function of HDL is to promote cholesterol efflux and reverse cholesterol transport, and this function has been inversely associated with ASCVD in both low- and high-risk cohorts, independent of HDL-C levels. Future studies will need to ascertain the determinants of cholesterol efflux, validate associations with CV events in high-risk populations, and determine whether targeting cholesterol efflux will promote plaque regression and improve clinical outcomes, and if disease and treatment status modify these relationships.

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