Drugs
https://doi.org/10.1007/s40265-020-01265-4

CURRENT OPINION

The Rise and Fall "ing" of the HDL Hypothesis

Julien J. Feghaly[1] · **Arshag D. Mooradian**[2]

Abstract
Earlier epidemiological studies have shown an inverse correlation between high-density lipoprotein cholesterol (HDLc) and coronary heart disease (CHD). This observation along with the finding that reverse cholesterol transport is mediated by HDL, supported the hypothesis that the HDL molecule has a cardioprotective role. More recently, epidemiological data suggest a U-shaped curve correlating HDLc and CHD. In addition, randomized clinical trials of drugs that significantly increase plasma HDLc levels, such as nicotinic acid and cholesterol ester transfer protein (CETP) inhibitors failed to show a reduction in major adverse cardiovascular events. These observations challenge the hypothesis that HDL has a cardioprotective role. It is possible that HDL quality and function is optimal only when de novo synthesis of apo A-I occurs. Inhibition of turnover of HDL with currently available agents yields HDL molecules that are ineffective in reverse cholesterol transport. To test this hypothesis, newer therapeutic drugs that increase de novo production of HDL and apo A-I should be tested in clinical trials.

Key Points

The hypothesis that the high-density lipoprotein (HDL) and its main apolipoprotein apo A-I prevent coronary atherosclerosis is supported by earlier epidemiological studies and by experimental data in laboratory animals.

However, randomized clinical trials of drugs that significantly increase plasma HDL levels failed to show a reduction in major adverse cardiovascular events.

It is possible that HDL quality and function is optimal only when de novo synthesis of apo A-I occurs. Inhibition of turnover yields dysfunctional HDL that is ineffective in reverse cholesterol transport.

1 Introduction

Cardiovascular mortality remains the leading cause of death in industrialized countries despite recent advancements and greater insight into the prevention of cardiovascular disease (CVD). Statin-based therapies have contributed an important role in the evolution of the management of CVD, reducing the risk of major coronary events by 31% and all-cause mortality by 21% [1]. Yet, these favorable outcomes remain short of expectations as substantial residual risk of cardiovascular deaths persists. This has led to the pursuit of alternative pathways and therapeutic targets for further reduction of CVD. One such therapeutic target is high-density lipoprotein (HDL). This lipoprotein along with its main apoprotein, the apolipoprotein A-I (apo A-I), have many coronary antiatherosclerotic properties, most notably its role in mediating the reverse cholesterol transport pathway that facilitates the clearing of cholesterol deposits in coronary arteries [2]. Additional cardioprotective properties of HDL and apo A-I include antioxidant [3] and anti-inflammatory effects [4], inhibition of adhesion molecule expression [5], prostacyclin stabilization [6], promotion of nitric oxide production [7], and decreasing platelet activation and coronary thrombus formation [8]. Hence, the hypothesis that increasing the HDL levels or improving its functionality would have favorable effects on CVD has strong biological plausibility. This manuscript aims to review the empirical evidence supporting or opposing a role of HDL and apo A-I in the

✉ Arshag D. Mooradian
arshag.mooradian@jax.ufl.edu

[1] Department of Medicine, School of Medicine, Saint Louis University, Saint Louis, MO, USA

[2] Department of Medicine, University of Florida College of Medicine, 653-1 West 8th Street, 4th Floor-LRC, Jacksonville, FL 32209, USA

Published online: 04 February 2020

pathogenesis of cardiovascular disease and review current and future directions in studying the role of HDL.

2 Evidence for Cardioprotective Effects of HDL/apo A-I

The first association of HDL and CVD dates back to 1977 when the Framingham study found an inverse relationship between the HDL cholesterol (HDLc) level and coronary heart disease (CHD), and identified HDLc as an independent negative predictor of CHD even in people with low plasma levels of low-density lipoprotein cholesterol (LDLc) [9, 10]. In addition, HDLc remains inversely associated with cardiovascular risk in patients on statin therapies with low LDLc levels [11]. However, several classical CVD risk factors such as obesity [12–14], insulin resistance [15–19], diabetes [15], chronic inflammatory disorders [20–25], and smoking [26] have been associated with low plasma levels of HDLc. These observations lead to the question whether HDL is truly cardioprotective or is simply a marker of CHD [27].

Subsequent to these early observations, some studies reported an association of higher HDLc levels with longevity [28, 29]. The Long Life Family Study (LLFS) revealed probands and offspring who had higher HDLc levels and were healthier in terms of cardiovascular risk [28]. Similarly, higher HDLc levels were associated with survival to 85 years of age in a prospective cohort of aging male veterans [29]. Conversely, the Cardiovascular Health Study found that HDLc along with other cardiovascular lipid risk factors had minimal effects on CVD risk in the elderly [30], while CVD risk was associated with glomerular filtration rate, diabetes, C-reactive protein, and N-terminal pro-B-type natriuretic peptide levels [30].

The major protein component of HDL, apo A-I, is likewise inversely associated with cardiovascular risk [31]. Genetically determined variation in plasma HDLc levels is attributed to specific changes in hepatic lipase and *AI/CIII/AIV* gene loci [32], with certain mutations accounting for lower total cholesterol and HDLc levels and higher frequency of angina and myocardial infarction [33]. Amongst 15 loci related to plasma HDLc, six loci (*LPL, TRIB1, APOA1-APOC3-APOA4-APOA5 cluster, CETP, ANGPTL4, GALNT2*) have had allele associations with an elevated plasma HDLc level, reduced risk of myocardial infarction, and reduced LDLc and triglyceride levels [34].

It is noteworthy that most epidemiological studies have used HDLc as a surrogate measure of HDL. In general, the levels of HDLc correlate well with HDL and apo A-I levels as the turnover rate of HDL and apo A-I is modulated by the cholesterol content of HDL [35]. Thus, plasma clearance of HDL and apo A-I is increased when cholesterol content of the molecule is decreased while cholesterol-rich HDL has a prolonged plasma half-life [35]. However, plasma HDLc measurements do not always reflect the biologic activity of HDL as a key protein in the reverse cholesterol transport process [35]. The amount of HDL and apo A-I molecules, HDL function or composition are important determinants of HDL cardioprotective potential.

Experiments in animal models of atherosclerosis have also supported an antiatherosclerotic role of HDL and apo A-I. In apo E-deficient mice, vector-mediated expression of human apo A-I resulted in a reduction of the development of atherosclerosis [36]. Similarly, infusions of apo A-I in the lipid-free form or as a constituent of discoidal reconstituted high-density lipoproteins, (A-I)rHDL, inhibit acute vascular inflammation in normocholesterolemic New Zealand White (NZW) rabbits [37]. This effect was partly attributed to increased arterial 3β-hydroxysteroid-Δ24 reductase expression [37].

Animal experiments were followed by a promising human pilot study, which used a recombinant apo A-I Milano/phospholipid complex infusion to demonstrate significant coronary atherosclerosis regression, as measured with an intravascular ultrasound (IVUS) [38]. HDL therapy using apo A-I Milano/phospholipid complex infusion at a rate of 1.2 g weekly for 5 weeks decreased the total atheroma volume by 4.2% [38]. This remarkable outcome was deemed highly significant when compared to the 0.4% atheroma volume reduction observed after 2–3 years of 40- to 80-mg atorvastatin therapy [39].

Overall, these older studies support the hypothesis that HDL/apo A-I has a protective role in CVD. However, contemporary studies so far do not support this hypothesis.

3 Evidence Against Cardioprotective Effects of HDL

In recent years, accumulating evidence suggests that subgroups of people with high plasma HDLc levels are at increased risk of CHD. Experiments in scavenger receptor BI (SR-BI) knockout mice and population-based studies have found that elevated plasma HDLc levels were paradoxically correlated with an increased risk of atherosclerosis and CHD (odds ratio = 1.79; $p = 0.018$) [40]. In humans, SR-BI is the major hepatocellular receptor for HDLc uptake. Therefore, it is expected that any loss of function of SR-BI may interrupt hepatic cholesterol uptake and the HDLc level would increase. However, it is noteworthy that the significance of SR-BI in human physiology is questionable as cholesterol ester transfer protein (CETP)-mediated exchange to apo B-containing lipoproteins is the major mechanism by which HDLc is transported to the liver in humans [41].

Unlike older epidemiological studies, a recent observational study of 5291 adults with established CVD determined

that low (< 41 mg/dL) and very high (> 60 mg/dL) HDLc levels were associated with increased cardiovascular risk; as high as twofold for very high HDLc levels [42]. This observation suggests that there is a U-shaped curve correlating HDLc and CVD. The U-shaped association is in line with many risk factors associated with disease states where the optimal outcome occurs when the variable is not at its extreme ends of the spectrum.

Mendelian randomization analyses, using single nucleotide polymorphisms (SNPs) in the endothelial lipase gene associated with HDLc, failed to elicit an association with increased plasma HDLc levels and lower myocardial infarction risk [34]. A subsequent genetic analysis, as part of the Copenhagen City Heart Study, determined that the risk of myocardial infarction was higher in individuals with lower plasma HDLc levels. However, this was not the case in individuals with low HDLc levels attributed to the lecithin-cholesterol acyltransferase (LCAT) gene, suggesting that genetically low plasma HDLc levels do not always increase the risk of myocardial infarction [43]. These observations do not support the hypothesis that raising plasma HDLc will necessarily translate to decreased cardiovascular events.

A more direct approach for testing the HDL hypothesis in humans requires interventions aimed at increasing plasma HDL and apo A-I levels and measuring the subsequent incidence of major adverse cardiovascular events.

4 Outcome of Studies Targeting HDL/apo A-I

The clinical outcomes of studies using reconstituted HDL (rHDL) infusions containing human apo A-I and a phospholipid have been inconsistent (Table 1). In one study rHDL infusions enhanced angiogenesis and wound healing by restoring hypoxia-inducible factor-1a stability and vascular endothelial growth factor signaling in patients with diabetes [44]. These favorable effects were attributed to rHDL initiating a signaling cascade via an apo A-I scavenger receptor class B type 1 interaction [44]. Additional favorable outcomes were observed when rHDL containing apo A-I Milano infusion resulted in reduced atheroma volume [38] and preparations with CSL-112 (human apo A-I and phosphotidylcholine complex) increased in the capacity of serum to efflux cholesterol from macrophages [45, 46]. In contrast, studies with another rHDL preparation labelled CSL-111 did not result in any significant change in atheroma volume [47], while CER-001, an alternative rHDL preparation, has demonstrated mixed results, both improving efflux of cholesterol and decreasing carotid wall area [48, 49], without altering the atheroma volume [50]. In the MILANO-PILOT trial, a randomized control trial using the rHDL MDCO-216 infusion, regression of coronary atherosclerosis in statin-treated patients with an acute coronary syndrome was observed [51]. Similarly, there was no regression of atherosclerosis with CER-001 infusions in the CER-001 Atherosclerosis Regression Acute Coronary Syndrome Trial (CARAT) [52]. Another ongoing Phase 3 trial (AEGIS-II, NCT03473223) in ~ 17,400 patients with acute coronary syndrome is evaluating the effect of CSL112 on clinical endpoints [53]. Overall, it appears that the data supporting the effectiveness of rHDL infusions in atherosclerosis regression is limited. In addition, infusions of rHDL preparations are not a practical solution to treating a chronic disease such as CVD. Hence, the importance of identifying easily administered HDL/apo A-I boosting agents.

Few medications can increase plasma HDL/apo A-I levels [54, 55]. Examples of such drugs are the fibrates, nicotinic acid, and the experimental class of cholesterol ester transfer protein (CETP) inhibitors. Various studies have examined the utility of fibrates in patients with low plasma HDLc and high triglycerides. In the Veterans Administration HDL intervention trial (VA-HIT), gemfibrozil led to a 6% increase in the mean plasma HDLc levels with a statistically significant 22% reduction in cardiovascular events; however, this did not affect all-cause mortality and the external validity was limited as the study only included male veterans [56]. Although the major effect of fibrates is lowering of triglycerides, in the VA-HIT the reduction in cardiovascular events was attributed to the modest rise in HDLc rather than the reduction in triglyceride levels [56]. In the Helsinki Heart Study (HHS), randomization to gemfibrozil treatment was associated with a 14% increase in plasma HDLc and a 34% reduction in CHD without significant effect on all-cause mortality [57]. Further trials examining bezafibrate and fenofibrate failed to achieve statistical significance with the primary endpoint of reduced cardiovascular events [58–60]. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study group failed to show a benefit of adding fenofibrate to simvastatin in reducing cardiovascular mortality [60]. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study did not significantly reduce the risk of the primary outcome of coronary events while improvements in secondary outcomes, such as a reduction in non-fatal myocardial infarctions and rate of revascularizations, were observed [59]. Post hoc analysis of data from clinical trials suggest that fibrates may have some cardioprotective effects in those with high plasma triglycerides and low HDLc levels [54]. It is possible that the neutral outcome of the FIELD trial may have been the consequence of a large "drop in" statin therapy [59]. In ACCORD, one possible reason for failure to demonstrate benefit of add-on fenofibrate therapy was that the treatment duration of 4.7 years was not long enough to detect a treatment effect [61]. However, the extension of the latter study for an additional 5 years did not show evidence of any legacy effect of fenofibrate therapy [61].

Table 1 Clinical outcomes of studies using reconstituted HDL (rHDL) infusions

rHDL preparation	References	Participants/study design	Dosing	Outcome
ETC-216: Apo A-I Milano and phospholipid complex	Nissen SE et al. 2003 [16]	123 patients post-ACS RCT	15 or 45 mg/kg, weekly for 5 weeks	Decrease in atheroma volume (IVUS)
CSL-111: human apo A-I and phosphotidylcholine complex	Tardif J-C, et al. 2007 [25]	183 patients post-ACS RCT	40 or 80 mg/kg, weekly for 4 weeks	No change in atheroma volume (IVUS). Improvement in coronary score (QCA)
CSL-112: human apo A-I and phosphotidylcholine complex	Gille A, et al. 2014 [23]	58 healthy subjects Open label	5–135 mg/kg, single dose	Increase in apo A-I and cholesterol efflux
	Tricoci P, et al. 2015 [24]	45 patients with stable CHD Open label	1.7–6.8 mg/kg, single dose	Increase in apo A-I and cholesterol efflux
	Duffy D, et al. 2019 [31]	Estimated enrollment: 17,400 patients post-ACS RCT	Not stated	Ongoing study, estimated completion June 2022
CER-001: recombinant human apo A-I and two phospholipid complex	Kootte RS, et al. 2015 [26]	7 patients with FHA Open label	8 mg/kg, 20 infusions over 6 months	Increase in apo A-I, HDLc, and cholesterol efflux. Decrease in carotid wall area (MRI and PET/CT)
	Hovingh GK, et al. 2015 [27]	23 patients with homozygous FH Open label	8 mg/kg, 12 infusions over 24 weeks	Decrease in carotid vessel wall area (MRI)
	Tardif J-C, et al. 2014 [28]	507 patients post-ACS RCT	3–12 mg/kg, weekly for 6 weeks	No reduction in atheroma volume (IVUS or QCA)
	Nicholls SJ, et al. 2018 [29]	272 statin-treated patients post-ACS RCT	3 mg/kg, weekly for 10 weeks	No regression in atheroma volume (IVUS)
MDCO-216: Apo A-I Milano and phospholipid complex	Nicholls SJ, et al. 2018 [30]	122 statin-treated patients post-ACS RCT	20 mg/kg, weekly for 5 weeks	No regression in atheroma volume (IVUS)

ACS acute coronary syndrome, *CHD* coronary heart disease, *FH* familial hypercholesterolemia, *FHA* familial hypoalphalipoproteinemia, *HDLc* high-density lipoprotein cholesterol, *IVUS* intravascular ultrasound, *MRI* magnetic resonance imaging, *PET/CT* positron emission tomography/computed tomography, *QCA* quantitative coronary angiography, *RCT* randomized controlled trial

The addition of niacin to the treatment of CVD in patients on statin therapy did not result in any clinical benefit, despite an improvement in plasma HDLc and triglyceride levels [62]. Similarly, the addition of a niacin–laropiprant combination in statin-treated patients not only failed to reduce the incidence of major vascular events but led to an increased incidence of serious adverse events [63].

Fatty acids are known to alter apo A-I expression and the use of pharmacologic doses of n-3 fatty acids can modestly increase plasma HDLc level [64–66]. The role of n-3 fatty acids in the management of hypertriglyceridemia is well established [67–69] and a recent trial with 4 g/day of an ethyl ester form of eicosapentanoic acid (EPA) found an approximately 25% relative risk reduction in major adverse cardiovascular events ($p < 0.001$) [70]. However, other preparations of n-3 fatty acids prescribed at a dose of 1 g/day did not reduce the risk of cardiovascular events in three large randomized studies [71–73]. Preliminary results of the Phase 3 STRENGTH trial (NCT02104817; $n = 13{,}086$) [74] indicate that omega-3 carboxylic acids are unlikely to demonstrate clinical benefit in patients with mixed dyslipidemia who are at high risk for CV disease and receiving optimal statin therapy [75].

Cholesterol ester transfer protein (CETP) inhibitors increase plasma levels of HDL and apo A-I through interfering with the transport of cholesterol from HDL to other lipoproteins [35]. Yet four trials with CETP inhibitors have failed to prove the clinical usefulness of this class of agents (Table 2) [76–79]. Torcetrapib, in the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial, increased plasma HDLc by 72.1% and decreased LDLc by 24.9%. However, the trial had to be discontinued early following an increase in all-cause mortality that was partly attributed to increased blood pressure [76]. Another CETP inhibitor, dalcetrapib, appeared to lack some of the undesirable neurohormonal and hemodynamic effects of torcetrapib. Yet in the dal-OUTCOMES trial, dalcetrapib failed to show a reduction in cardiovascular risk and the trial was stopped early due to futility [77]. Subanalysis of the dal-OUTCOMES trial did hint at potential benefit in selected patients, and this is currently being evaluated in the ongoing phase-3 dal-GenE trial (NCT02525939) [80]. The latter is a double-blind, randomized, placebo-controlled study to evaluate the effects of dalcetrapib on cardiovascular risk in a genetically defined population with a recent acute coronary syndrome.

Lack of clinical benefit with CETP inhibitors was also observed in the Assessment of Clinical Effects of Cholesteryl Ester Transfer Protein Inhibition with Evacetrapib in Patients at a High Risk for Vascular Outcomes (ACCELERATE) trial [78]. In the Randomized Evaluation of the Effects of Anacetrapib through Lipid Modification (REVEAL) trial, anacetrapib decreased the incidence of major coronary

Table 2 Clinical outcomes of randomized controlled trials of cholesterol ester transfer protein (CETP) inhibitors

Study	Participants/study design	Dosing	Outcome	Limitations/criticism
ILLUMINATE Barter PJ, et al. 2007 [51]	15,067 patients with high cardiovascular risk	Atorvastatin plus torcetrapib 60 mg orally once daily versus atorvastatin plus placebo. Median follow-up period 550 days	Increase in HDLc, systolic blood pressure, serum sodium, bicarbonate, and aldosterone. Decrease in LDLc and serum potassium	Stopped early due to increased all-cause mortality. Attributed to increased blood pressure
Dal-OUTCOMES Schwartz GG, et al. 2012 [52]	15,871 patients post-ACS	Dalcetrapib 600 mg orally once daily versus placebo. Median follow-up period 31 months	Increase in HDLc but no reduction in cardiovascular risk	Stopped early due to futility
ACCELERATE Lincoff AM, et al. 2017 [53]	12,092 patients post-ACS, CHD or vascular disease	Evacetrapib 130 mg orally once daily versus placebo. Median follow-up period 28 months	No reduction in cardiovascular risk	No clinical benefit
REVEAL The HPS3/TIMI55–REVEAL Collaborative Group 2017 [54]	30,449 patients with atherosclerotic vascular disease	Anacetrapib 100 mg orally once daily versus placebo. Median follow-up period of 4.1 years	Decreased incidence of major coronary events. No effect on cardiovascular or all-cause mortality	Benefit attributed to LDLc reduction

ACS acute coronary syndrome, *CHD* coronary heart disease, *HDLc* high-density lipoprotein cholesterol, *LDLc* low-density lipoprotein cholesterol

events in patients with established CVD who were on intensive statin therapy but did not affect cardiovascular or all-cause mortality [79]. The apparent reduction in the incidence of major coronary events was attributed to the concomitant reduction in plasma LDLc levels especially in patients with baseline higher LDLc levels [79].

The failure of CETP inhibitors and niacin in reducing cardiovascular events despite the increase in plasma HDLc level could have been secondary to off-target effects of these agents or related to accumulation of dysfunctional HDL when HDL turnover is reduced along with reduced reverse cholesterol transferring function [35].

5 Future Directions

It is apparent that the available clinical trials using interventions to increase plasma HDL/apo A-I levels have failed to establish the clinical utility of increasing the HDL/apo A-I. However, most of these trials have either used rHDL made of recombinant apo A-I along with a phospholipid moiety or used agents that interfere with the plasma half-life of HDL/apo A-I. It is possible that these interventions have interfered with the functional quality of the HDL/apo A-I particles. Prior to abandoning the HDL hypothesis, it is important to test the clinical effectiveness of increasing endogenous de novo production of HDL/apo A-I, which tends to have more efficient reverse cholesterol transport capacity. In pursuing agents that can increase apo A-I transcription and production, structure function analysis of the apo A-I gene promoter has yielded invaluable clues for potential therapeutic agents to increase HDL/apo A-I levels [81]. The insulin-responsive core element (IRCE) modulates the apo A-I gene promoter activity via insulin, growth factors, protein kinase A, and protein kinase C [81]. A second site, site A, is regulated via thyroid hormone, retinoids, and peroxisome proliferator activated receptor-α and -γ (PPARα/γ) agonists, tumor necrosis factor-α, vitamins C, D, and E, and endocannabinoids [81]. Site B is regulated by estradiol, glucocorticoids, and bile acids, while additional sites are regulated via liver receptor-homologue-1 (LRH-1) and a thyroid hormone-responsive element that has inhibitory (negative) effects on promoter activity (nTRE) [81]. Through these promoter sites, various drugs regulate apo A-I synthesis [82], such as histamine (H_1)-receptor antagonists including azelastine, fexofenadine, cetirizine, and diphenhydramine [83]. This outcome is attributed to antagonizing the inhibitory action of histamine on apo A-I gene expression that occurs

Table 3 HDL/apo A-I augmenting effects of contemporary and experimental drugs

Drug class	Effect on HDLc	Mechanism of HDL increase
Contemporary drugs		
Nicotinic acid (niacin)	↑ 15–35%	Mostly reduced HDL turnover and uptake by the liver; Minor effect of increased apo A-I gene promoter activity
Fibrates (fibric acid derivatives)	↑ 10–35%	Increased apo A-I gene promoter activity
PPAR gamma agonists	↑ 5–28%	Increased apo A-I gene promoter activity
HMG-CoA reductase inhibitors (statins)	↑ 5–15%	Increased apo A-I gene promoter activity
PCSK9 inhibitors	↑ 5%	Unknown
Experimental drugs		
rHDL infusions		
ETC-216	Not evaluated	Increasing plasma levels of rHDL
CSL-111	Not evaluated	Increasing plasma levels of rHDL
CSL-112	↑ 81–300%	Increasing plasma levels of rHDL
CER-001	↑ 2–17%	Increasing plasma levels of rHDL
MDC0-216	↓ 8%	Unknown
CETP inhibitors		
Torcetrapib	↑ 72%	Reduced HDL clearance
Dalcetrapib	↑ 31–40%	Reduced HDL clearance
Evacetrapib	↑ 133%	Reduced HDL clearance
Anacetrapib	↑ 112%	Reduced HDL clearance
BET inhibitors		
Apabetalone	↑ 11%	Increased apo A-I gene promoter activity, changing the epigenetics

Data from Mooradian et al. [35] and The HPS3/TIMI55–REVEAL Collaborative Group [79]

↑ indicates an increase, ↓ indicates a decrease, *BET* bromodomain and extra-terminal domain, *CETP* cholesterol ester transfer protein, *HMG-CoA* β-hydroxy β-methylglutaryl-CoA, *PCSK9* proprotein convertase subtilisin/kexin type 9, *PPAR* peroxisome proliferator-activated receptors, *rHDL* reconstituted high-density lipoprotein

through H1-receptor-mediated increase in NF-κB expression, a known suppressor of apo A-I promoter activity [84].

Another relevant therapeutic target is the bromodomain and extra-terminal domain (BET) inhibitors. The leading agent in this category is the small molecule apabetalone (RVX-208), a selective BET inhibitor [85]. This agent has been shown to activate apo A-I gene transcription and increase HDLc in cell cultures and in vivo [85]. The Study of Quantitative Serial Trends in Lipids with Apolipoprotein A-I Stimulation (SUSTAIN) trial confirmed the safety of RVX-208 in patients and observed an increase in plasma apo A-I and HDLc levels [86]. This was in concurrence with the results of the Apo A-I Synthesis Stimulation and Intravascular Ultrasound for Coronary Atheroma Regression Evaluation (ASSURE) trial in high-risk CVD patients [87]. The latter trial demonstrated a significant increase in plasma apo A-I and HDLc levels alongside a statistically insignificant (0.6%) regression in coronary artery plaque as measured by IVUS [87]. The extension of the ASSURE trial did not show incremental change in plasma HDLc, LDLc, or plaque regression [87, 88]. However, the BET inhibitors have favorable biochemical effects that extend beyond their effects on apo A-I production [89]. Nevertheless, apabetalone did not meet its primary endpoint of reduction in major adverse cardiovascular events (MACE) in the randomized, double-blind, placebo-controlled phase-3 BEToneMACE trial (NTC02586155) [90]. Table 3 summarizes the HDL/apo A-I increasing effects of some of the cotemporary and experimental drugs. An ongoing Phase 3 dal-GenE trial is evaluating dalcetrapib in a genetically defined population with a recent acute coronary syndrome [80]. The results of this study are expected to be available in the first quarter of 2021.

6 Conclusions

Although epidemiological and biochemical studies provide strong support for the plausibility of the HDL hypothesis, interventional clinical trials have shown that currently available HDL-targeting drugs are not effective in cardioprotection and at times are unsafe. It is tempting to speculate that the failure of current therapeutic agents that increase HDL levels are ineffective in cardioprotection because of poor quality and functionality of HDL produced. It is possible that HDL quality and function is optimal only when de novo synthesis of apo A-I occurs. Inhibition of turnover of apo A-I or HDL with currently available agents yields HDL molecules that are ineffective in reverse cholesterol transport [35, 81]. Newer therapeutic drugs that increase de novo production of HDL and apo A-I should be tested in clinical trials to establish their clinical safety and efficacy.

Compliance with Ethical Standards

Funding No external funding was used in the preparation of this manuscript.

Conflict of interest Julien J. Feghaly and Arshag D. Mooradian declare that they have no potential conflicts of interest that might be relevant to the contents of this article.

References

1. LaRosa JC, He J, Vupputuri S. Effect of statins on risk of coronary disease: a meta-analysis of randomized controlled trials. JAMA. 1999;282:2340–6.
2. Rosenson RS, Brewer HB, Davidson WS, et al. Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. Circulation. 2012;125:1905–19.
3. Bielicki JK, Oda MN. Apolipoprotein A-I(Milano) and apolipoprotein A-I(Paris) exhibit an antioxidant activity distinct from that of wild-type apolipoprotein A-I. Biochemistry. 2002;41:2089–96.
4. Cameron SJ, Morrell CN, Bao C, Swaim AF, Rodriguez A, Lowenstein CJ. A novel anti-inflammatory effect for high density lipoprotein. PLoS One. 2015;10(12):e0144372.
5. Ashby DT, Rye KA, Clay MA, Vadas MA, Gamble JR, Barter PJ. Factors influencing the ability of HDL to inhibit expression of vascular cell adhesion molecule-1 in endothelial cells. Arterioscler Thromb Vasc Biol. 1998;18:1450–5.
6. Yui Y, Aoyama T, Morishita H, Takahashi M, Takatsu Y, Kawai C. Serum prostacyclin stabilizing factor is identical to apolipoprotein A-I (Apo A-I). A novel function of Apo A-I. J Clin Investig. 1988;82:803–7.
7. Vaisar T, Couzens E, Hwang A, Russell M, Barlow CE, DeFina LF, Hoofnagle AN, Kim F. Type 2 diabetes is associated with loss of HDL endothelium protective functions. PLoS One. 2018;13(3):e0192616.
8. Chung DW, Chen J, Ling M, Fu X, Blevins T, Parsons S, Le J, Harris J, Martin TR, Konkle BA, Zheng Y, López JA. High-density lipoprotein modulates thrombosis by preventing von Willebrand factor self-association and subsequent platelet adhesion. Blood. 2016;127:637–45.
9. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease: the Framingham study. Am J Med. 1977;62:707–14.
10. Miller M, Stone NJ, Ballantyne C, et al. Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association. Circulation. 2011;123:2292–333.
11. Boekholdt SM, Arsenault BJ, Hovingh GK, et al. Levels and changes of HDL cholesterol and apolipoprotein A-I in relation to risk of cardiovascular events among statin-treated patients: a meta-analysis. Circulation. 2013;128:1504–12.
12. Vergès B, Adiels M, Boren J, Barrett PH, Watts GF, Chan D, et al. Interrelationships between the kinetics of VLDL subspecies and HDL catabolism in abdominal obesity: a multicenter tracer kinetic study. J Clin Endocrinol Metab. 2014;99:4281–90.
13. Haas MJ, Mazza AD, Wong NC, Mooradian AD. Inhibition of apolipoprotein A-I gene expression by obesity-associated endocannabinoids. Obesity (Silver Spring). 2012;20:721–9.
14. Mooradian AD, Haas MJ, Wehmeier KR, Wong NC. Obesity-related changes in high-density lipoprotein metabolism. Obesity (Silver Spring). 2008;16:1152–60.

15. Duvillard L, Pont F, Florentin E, Gambert P, Vergès B. Inefficiency of insulin therapy to correct apolipoprotein A-I metabolic abnormalities in non-insulin-dependent diabetes mellitus. Atherosclerosis. 2000;152:229–37.
16. Mooradian AD, Haas MJ, Wong NC. Transcriptional control of apolipoprotein A-I gene expression in diabetes. Diabetes. 2004;53:513–20.
17. Murao K, Wada Y, Nakamura T, Taylor AH, Mooradian AD, Wong NC. Effects of glucose and insulin on rat apolipoprotein A-I gene expression. J Biol Chem. 1998;273:18959–65.
18. Lam JK, Matsubara S, Mihara K, Zheng XL, Mooradian AD, Wong NC. Insulin induction of apolipoprotein AI, role of Sp1. Biochemistry. 2003;42:2680–90.
19. Mooradian AD, Albert SG, Haas MJ. Low serum high-density lipoprotein cholesterol in obese subjects with normal serum triglycerides: the role of insulin resistance and inflammatory cytokines. Diabetes Obes Metab. 2007;9:441–3.
20. Haas MJ, Mooradian AD. Inflammation, high-density lipoprotein and cardiovascular dysfunction. Curr Opin Infect Dis. 2011;24:265–72.
21. Haas MJ, Mooradian AD. Regulation of high-density lipoprotein by inflammatory cytokines: establishing links between immune dysfunction and cardiovascular disease. Diabetes Metab Res Rev. 2010;26:90–9.
22. Beers A, Haas MJ, Wong NC, Mooradian AD. Inhibition of apolipoprotein AI gene expression by tumor necrosis factor alpha: roles for MEK/ERK and JNK signaling. Biochemistry. 2006;45:2408–13.
23. Parseghian S, Onstead-Haas LM, Wong NC, Mooradian AD, Haas MJ. Inhibition of apolipoprotein A-I expression by TNF-alpha in HepG2 cells: requirement for c-jun. J Cell Biochem. 2014;115:253–60.
24. Haas MJ, Horani M, Mreyoud A, Plummer B, Wong NC, Mooradian AD. Suppression of apolipoprotein AI gene expression in HepG2 cells by TNF alpha and IL-1beta. Biochim Biophys Acta. 2003;1623(2–3):120–8.
25. Palacio C, Alexandraki I, Bertholf RL, Mooradian AD. Transient dyslipidemia mimicking the plasma lipid profile of Tangier disease in a diabetic patient with gram negative sepsis. Ann Clin Lab Sci. 2011;41:150–3.
26. Naem E, Alcalde R, Gladysz M, Mesliniene S, Jaimungal S, Sheikh-Ali M, Haas MJ, Wong NC, Mooradian AD. Inhibition of apolipoprotein A-I gene by the aryl hydrocarbon receptor: a potential mechanism for smoking-associated hypoalphalipoproteinemia. Life Sci. 2012;91:64–9.
27. Mooradian AD. Is high-density lipoprotein cardioprotective or simply a marker of cardiovascular disease? Am J Ther. 2014;21:438–9.
28. Newman AB, Glynn NW, Taylor CA, et al. Health and function of participants in the Long Life Family Study: a comparison with other cohorts. Aging (Albany, NY). 2011;3:63–76.
29. Rahilly-Tierney CR, Spiro A, Vokonas P, Gaziano JM. Relation between high-density lipoprotein cholesterol and survival to age 85 years in men (from the VA Normative Aging Study). Am J Cardiol. 2011;107:1173–7.
30. Odden MC, Shlipak MG, Whitson HE, et al. Risk factors for cardiovascular disease across the spectrum of older age: the Cardiovascular Health Study. Atherosclerosis. 2014;237:336–42.
31. Stampfer MJ, Sacks FM, Salvini S, Willett WC, Hennekens CH. A prospective study of cholesterol, apolipoproteins, and the risk of myocardial infarction. N Engl J Med. 1991;325:373–81.
32. Cohen JC, Wang Z, Grundy SM, Stoesz MR, Guerra R. Variation at the hepatic lipase and apolipoprotein AI/CIII/AIV loci is a major cause of genetically determined variation in plasma HDL cholesterol levels. J Clin Investig. 1994;94:2377–84.
33. Reguero JR, Cubero GI, Batalla A, et al. Apolipoprotein A1 gene polymorphisms and risk of early coronary disease. Cardiology. 1998;90:231–5.
34. Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet. 2012;380(9841):572–80.
35. Mooradian AD, Haas MJ. Targeting high-density lipoproteins: increasing de novo production versus decreasing clearance. Drugs. 2015;75:713–22.
36. Pastore L, Belalcazar LM, Oka K, et al. Helper-dependent adenoviral vector-mediated long-term expression of human apolipoprotein A-I reduces atherosclerosis in apo E-deficient mice. Gene. 2004;327:153–60.
37. Patel S, Di Bartolo BA, Nakhla S, et al. Anti-inflammatory effects of apolipoprotein A-I in the rabbit. Atherosclerosis. 2010;212:392–7.
38. Nissen SE, Tsunoda T, Tuzcu EM, et al. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes. JAMA. 2003;290:2292.
39. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. JAMA. 2004;291:1071–80.
40. Zanoni P, Khetarpal SA, Larach DB, et al. Rare variant in scavenger receptor BI raises HDL cholesterol and increases risk of coronary heart disease. Science. 2016;351:1166–71.
41. Schwartz CC, VandenBroek JM, Cooper PS. Lipoprotein cholesteryl ester production, transfer, and output in vivo in humans. J Lipid Res. 2004;45:1594–607.
42. Allard-Ratick M. Association between very high levels of HDL-c and increased risk of CV events. In: Atlanta: ESC; 2018. https://ipccs.org/2018/09/04/association-between-very-high-levels-of-hdl-c-and-increased-risk-of-cv-events/. Accessed Aug 6, 2019.
43. Haase CL, Tybjærg-Hansen A, Ali Qayyum A, Schou J, Nordestgaard BG, Frikke-Schmidt R. LCAT, HDL cholesterol and ischemic cardiovascular disease: a mendelian randomization study of HDL cholesterol in 54,500 individuals. J Clin Endocrinol Metab. 2012;97:E248–56.
44. Tan JTM, Prosser HCG, Dunn LL, et al. High-density lipoproteins rescue diabetes-impaired angiogenesis via scavenger receptor class B type I. Diabetes. 2016;65:3091–103.
45. Gille A, Easton R, D'Andrea D, Wright SD, Shear CL. CSL112 enhances biomarkers of reverse cholesterol transport after single and multiple infusions in healthy subjects. Arterioscler Thromb Vasc Biol. 2014;34:2106–14.
46. Tricoci P, D'Andrea DM, Gurbel PA, et al. Infusion of reconstituted high-density lipoprotein, CSL112, in patients with atherosclerosis: safety and pharmacokinetic results from a phase 2a randomized clinical trial. J Am Heart Assoc. 2015;4(8):e002171.
47. Tardif J-C, Grégoire J, L'Allier PL, et al. Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: a randomized controlled trial. JAMA. 2007;297(15):1675–82.
48. Kootte RS, Smits LP, van der Valk FM, et al. Effect of open-label infusion of an apoA-I-containing particle (CER-001) on RCT and artery wall thickness in patients with FHA. J Lipid Res. 2015;56:703–12.
49. Hovingh GK, Smits LP, Stefanutti C, et al. The effect of an apolipoprotein A-I–containing high-density lipoprotein–mimetic particle (CER-001) on carotid artery wall thickness in patients with homozygous familial hypercholesterolemia: The Modifying Orphan Disease Evaluation (MODE) study. Am Heart J. 2015;169:736–42.
50. Tardif J-C, Ballantyne CM, Barter P, et al. Effects of the high-density lipoprotein mimetic agent CER-001 on coronary atherosclerosis in patients with acute coronary syndromes: a randomized trial. Eur Heart J. 2014;35:3277–86.

51. Nicholls SJ, Puri R, Ballantyne CM, et al. Effect of infusion of high-density lipoprotein mimetic containing recombinant apolipoprotein A-I Milano on coronary disease in patients with an acute coronary syndrome in the MILANO-PILOT Trial: a randomized clinical trial. JAMA Cardiol. 2018;3:806–14.
52. Nicholls SJ, Andrews J, Kastelein JJP, et al. Effect of serial infusions of CER-001, a pre-β high-density lipoprotein mimetic, on coronary atherosclerosis in patients following acute coronary syndromes in the CER-001 Atherosclerosis Regression Acute Coronary Syndrome Trial: a randomized clinical trial. JAMA Cardiol. 2018;3:815–22.
53. Duffy D. Study to investigate CSL112 in subjects with acute coronary syndrome (AEGIS-II)—NCT03473223. https://clinicaltrials.gov/ct2/show/NCT03473223. Accessed Aug 8, 2019.
54. Chehade JM, Gladysz M, Mooradian AD. Dyslipidemia in type 2 diabetes: prevalence, pathophysiology and management. Drugs. 2013;73:327–39.
55. Mooradian AD. Diabetes and atherogenic dyslipidemia. In: Rodriguez-Saldana SJ, editor. The diabetes textbook. Clinical principles, patient management and public health issues. Basel: Springer Nature; 2019. p. 587–96.
56. Rubins HB, Robins SJ, Collins D, et al. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. N Engl J Med. 1999;341:410–8.
57. Frick MH, Elo O, Haapa K, et al. Helsinki Heart Study: Primary-Prevention Trial with gemfibrozil in middle-aged men with dyslipidemia. N Engl J Med. 1987;317:1237–45.
58. The BIP Study Group. Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease. Circulation. 2000;102:21–7.
59. FIELD Study Investigators. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet. 2005;366:1849–61.
60. The ACCORD Study Group. Effects of combination lipid therapy in type 2 diabetes mellitus. N Engl J Med. 2010;362:1563–74.
61. Elam MB, Ginsberg HN, Lovato LC, Corson M, Largay J, Leiter LA, et al. Association of fenofibrate therapy with long-term cardiovascular risk in statin-treated patients with type 2 diabetes. JAMA Cardiol. 2017;2:370–80.
62. The AIM-HIGH Investigators. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med. 2011;365:2255–67.
63. The HPS2-THRIVE Collaborative Group. Effects of extended-release niacin with laropiprant in high-risk patients. N Engl J Med. 2014;371:203–12.
64. Mooradian AD, Haas MJ, Wong NCW. The effect of select nutrients on serum high density lipoprotein cholesterol and apolipoprotein A-I levels. Endocr Rev. 2006;27:2–16.
65. Mooradian AD. The effect of nutrients on apolipoprotein AI gene expression. In: Fielding CJ, editor. High density lipoproteins. From basic biology to clinical aspects. Weinheim: Wiley-VCH; 2007. p. 399–423.
66. Haas MJ, Horani MH, Wong NCW, Mooradian AD. Induction of apolipoprotein AI promoter by Sp1 is repressed by saturated fatty acids. Metabolism. 2004;53:1342–8.
67. Mooradian AD. Evidence based cardiovascular risk management in diabetes. Am J Cardiovasc Drugs. 2019;19:439–48.
68. Mooradian AD. Dyslipidemia of type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab. 2009;5:150–9.
69. Bays HE, Maki KC, McKenney J, et al. Long-term up to 24-month efficacy and safety of concomitant prescription omega-3-acid ethyl esters and simvastatin in hyper-triglyceridemic patients. Curr Med Res Opin. 2010;26:907–15.
70. Bhatt D, Steg PG, Michael Miller M, et al. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. N Engl J Med. 2019;380:11–22.
71. ORIGIN Trial Investigators, Bosch J, Gerstein HC, et al. n-3 fatty acids and cardiovascular outcomes in patients with dysglycemia. N Engl J Med. 2012;367:309–18.
72. ASCEND Study Collaborative Group, Bowman L, Mafham M. Effects of n-3 fatty acid supplements in diabetes mellitus. N Engl J Med. 2018;379:1540–50.
73. Manson JE, Cook NR, Lee IM, et al. Marine n-3 fatty acids and prevention of cardiovascular disease and cancer. N Engl J Med. 2019;380:23–32.
74. Nicholls SJ, Lincoff AM, Bash D, Ballantyne CM, Barter PJ, Davidson MH, et al. Assessment of omega-3 carboxylic acids in statin-treated patients with high levels of triglycerides and low levels of high-density lipoprotein cholesterol: rationale and design of the STRENGTH trial. Clin Cardiol. 2018;41:1281–8.
75. Anon. Update on Phase III STRENGTH trial for Epanova in mixed dyslipidaemia (AstraZeneca press release dated January 13, 2020). https://www.astrazeneca.com/media-centre/press-releases/2020/update-on-phase-iii-strength-trial-for-epanova-in-mixed-dyslipidaemia-13012020.html. Accessed 22 Jan 2020.
76. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med. 2007;357:2109–22.
77. Schwartz GG, Olsson AG, Abt M, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. N Engl J Med. 2012;367:2089–99.
78. Lincoff AM, Nicholls SJ, Riesmeyer JS, et al. Evacetrapib and cardiovascular outcomes in high-risk vascular disease. N Engl J Med. 2017;376:1933–42.
79. The HPS3/TIMI55–REVEAL Collaborative Group. Effects of anacetrapib in patients with atherosclerotic vascular disease. N Engl J Med. 2017;377:1217–27.
80. A phase III, double-blind, randomized placebo-controlled study to evaluate the effects of dalcetrapib on cardiovascular (CV) risk in a genetically defined population with a recent acute coronary syndrome (ACS): the Dal-GenE Trial. https://clinicaltrials.gov/ct2/show/NCT02525939. Accessed Jan, 2020.
81. Haas MJ, Mooradian AD. Therapeutic interventions to enhance apolipoprotein A-I-mediated cardioprotection. Drugs. 2010;70:805–21.
82. Haas MJ, Onstead-Haas L, Kurban W, et al. High-throughput analysis identifying drugs that regulate apolipoprotein A-I synthesis. Assay Drug Dev Technol. 2017;15:362–71.
83. Haas MJ, Plazarte M, Chamseddin A, et al. Inhibition of hepatic apolipoprotein A-I gene expression by histamine. Eur J Pharmacol. 2018;823:49–57.
84. Haas MJ, Jurado-Flores M, Hammoud R, et al. Regulation of apolipoprotein A-I gene expression by the histamine H1 receptor: Requirement for NF-κB. Life Sci. 2018;208:102–10.
85. Bailey D, Jahagirdar R, Gordon A, et al. RVX-208. J Am Coll Cardiol. 2010;55:2580–9.
86. Nicholls SJ, Gordon A, Johannson J, et al. ApoA-I induction as a potential cardioprotective strategy: rationale for the SUSTAIN and ASSURE studies. Cardiovasc Drugs Ther. 2012;26:181–7.
87. Nicholls SJ, Puri R, Wolski K, et al. Effect of the BET protein inhibitor, RVX-208, on progression of coronary atherosclerosis: results of the phase 2b, randomized, double-blind, multicenter, ASSURE Trial. Am J Cardiovasc Drugs. 2016;16:55–65.
88. Shishikura D, Kataoka Y, Honda S, et al. The effect of bromodomain and extra-terminal inhibitor apabetalone on attenuated coronary atherosclerotic plaque: Insights from the ASSURE Trial. Am J Cardiovasc Drugs. 2019;19(1):49–57. https://doi.org/10.1007/s40256-018-0298-8.

89. Nicholls SJ, Ray KK, Johansson JO, et al. Selective BET protein inhibition with apabetalone and cardiovascular events: a pooled analysis of trials in patients with coronary artery disease. Am J Cardiovasc Drugs. 2018;18(2):109–15. https://doi.org/10.1007/s40256-017-0250-3.

90. Effect of BET protein inhibition with apabetalone on cardiovascular outcomes in patients with acute coronary syndrome and diabetes—BETonMACE. https://www.acc.org/latest-in-cardiology/clinical-trials/2019/11/15/17/25/betonmace. Accessed Jan 2020.