Treating low high-density lipoprotein cholesterol: what is the evidence?

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***Abstract*:** Epidemiological studies have shown an inverse association between high-density lipoprotein cholesterol (HDL-C) and cardiovascular disease (CVD) risk. However, genetic and interventional studies have failed to consistently support this relationship. There is an increasing body of evidence that the function of HDL, including its antiatherogenic properties and its reverse cholesterol transport activity, has a greater impact on CVD risk compared with levels of HDL alone. Targeting HDL has become a growing interest. Nevertheless, raising HDL pharmacologically has failed to show a considerable, if any, impact on cardiovascular outcome. Efforts should focus on improving HDL quality in addition to raising HDL levels when developing new therapies. Ongoing and future research will help determine the most safe and effective approach to improve cardiovascular outcome and establish the safety, efficacy and impact on atherosclerosis of the emerging HDL-raising therapies.

Keywords: cardiovascular risk, cholesterol ester transfer protein inhibitors, dysfunctional high-density lipoprotein cholesterol, high-density lipoprotein cholesterol

Introduction

Despite reaching optimal low-density lipoprotein cholesterol (LDL-C) levels in patients treated with statins, the residual risk of cardiovascular events in some remains elevated. Targeting high-density lipoprotein cholesterol (HDL-C) to reduce this residual risk has been the focus of great interest and a major challenge [Brewer, 2011]. Indeed, epidemiological data support an inverse association between serum HDL-C and cardiovascular disease (CVD) risk [Gotto and Brinton, 2004; Toth, 2004]. However, association does not necessarily equal causation, and indeed, genetic and interventional CVD trials failed to consistently support this relationship.

In this review, we consider the evidence for and against a causative role for HDL in CVD from epidemiological, interventional and genetic studies. We then examine the described functions of HDL in atherosclerosis. Finally, we review the cholesteryl ester transfer protein (CETP) inhibitors as HDL-raising therapies and their impact on cardiovascular outcome.

Literature search

We conducted a PubMed search until January 2013 through the English literature using the search terms high-density lipoprotein cholesterol, cardiovascular events, cardiovascular deaths, statins, fibrates and cholesterol ester transfer protein inhibitors. We also included references from the articles identified and publications available in the authors' libraries.

Overview of HDL metabolism

Apolipoprotein A-I (apoA-I), a major protein component of HDL, is produced by the liver and the intestine and released in small amounts from very low density lipoprotein (VLDL) and chylomicrons [Lewis and Rader, 2005; Rader and deGoma, 2012]. ATP-binding cassette transporter A1 (ABCA1) strongly expressed by the liver and intestines releases unesterified cholesterol and phospholipids to apoA-I forming a nascent pre-beta HDL. The free cholesterol in HDL is esterified by the enzyme lecithin-cholesterol acyltransferase (LCAT), resulting in the formation of a larger mature HDL particle: alpha HDL.

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CETP transfers cholesteryl esters (CEs) from HDL to VLDL in exchange for triglycerides (TGs) [Tall, 1993]. The resulting TG-rich HDL can be hydrolyzed by hepatic lipase to form smaller HDL3 particles that are rapidly catabolized. Endothelial lipase releases lipid-poor apoA-I from HDL phospholipids that is filtered by renal glomeruli and degraded by proximal tubular cell receptors. HDL-C can also be removed from the circulation via selective hepatic uptake mediated by scavenger receptor class B type I (SR-BI) or a holoparticle uptake mechanism that is less clear [Trigatti *et al.* 2003].

Epidemiological and interventional trials

Epidemiological studies have supported an inverse relationship between HDL-C level and cardiovascular risk. Early data from the Framingham Heart Study demonstrated a significant increase in coronary heart disease (CHD) events in individuals with low HDL less than 34 mg/dl [Gordon *et al.* 1977]. Furthermore, Genest and colleagues identified low HDL as a strong negative marker for cardiovascular risk second only to cigarette smoking in a population of men with premature coronary artery disease (CAD) [Genest *et al.* 1991]. In the Emerging Risk Factors Collaboration Trial which included data from 302,430 subjects without CHD from 68 population-based studies, HDL-C was strongly associated with CHD risk (hazard ratio 0.78 [95% CI 0.74–0.82]) and an increase in HDL cholesterol by 15 mg/dl was associated with a 22% reduction in CHD risk [Di Angelantonio *et al.* 2009]. A meta-analysis of four large prospective studies concluded that every 1 mg/dl decrease in HDL-C is associated with a 2–3% increase in CVD risk independent of other risk factors [Gordon *et al.* 1989]. This inverse relationship between HDL and CHD holds true even in patients treated with statins, as evidenced by the Treating to New Targets clinical trial in which HDL remained an important risk factor despite a reduction in LDL to below 70 mg/dl [Barter *et al.* 2007b]. Similarly, statins did not alter the relationship between HDL-C and cardiovascular risk in a recent meta-analysis of 20 large randomized controlled trials of statins independent of the type of statin, age, hypertension, diabetes mellitus and tobacco use [Jafri *et al.* 2010]. However, does association imply causation?

Data from randomized controlled clinical trials involving HDL-raising therapies are inconsistent. Early trials involving fibrates showed a 22% and 34% reduction in CHD with gemfibrozil in the Helsinki Heart Study and Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial trials respectively [Rubins *et al.* 1999; Tenkanen *et al.* 2006]. In contrast to these results, the Bezafibrate Infarction Prevention, and Fenofibrate Intervention and Event Lowering in Diabetes studies failed to find a significant benefit of bezafibrate and fenofibrate *versus* placebo on cardiovascular outcomes [Bezafibrate Infarction Prevention Study, 2000; Keech *et al.* 2005]. Furthermore, in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) lipid study, fenofibrate added on to statin therapy did not further reduce cardiovascular events compared with statin alone in patients with type 2 diabetes [ACCORD Study Group *et al.* 2010]. However, *post hoc* analyses of several of these fibrate trials suggest that the subgroup of patients with metabolic features including overweight subjects with low HDL and high TG levels benefit the most from fibrates in terms of cardiovascular event reductions [Barter and Rye, 2008]. Recently, results from the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes (AIM-HIGH) and Heart Protection Study 2 Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) trials were a disappointment to the cardiovascular field [AIM-HIGH Investigators *et al.* 2011]. The rationale behind the AIM-HIGH trial was to evaluate whether the addition of niacin which raises HDL-C to optimal LDL lowering therapy provides any additional clinical benefit. This trial included 3414 high-risk subjects with history of vascular disease, low HDL-C and high TG, all treated with simvastatin and randomized to either high-dose extended release niacin (n = 1718) or placebo (n = 1696). Treatment with niacin did not confer any additional benefit on cardiovascular outcome despite an increase in HDL levels by 25% and a reduction in TG levels by 30%. Therefore, the trial was halted prematurely after 3 years [AIM-HIGH Investigators *et al.* 2011]. In retrospect, the study had some methodological flaws, including a relatively small sample size, use of low-dose immediate release niacin in the placebo group that resulted in a difference in HDL-C of only 4 mg/dl between the two groups and the early cessation of the study. It is worth noting that in the Coronary Drug Project, a late benefit of niacin on cardiovascular events and mortality was seen in its 9-year post-trial follow-up data [Canner

et al. 1986]. Similarly, the HPS2-THRIVE trial failed to show a significant reduction in the risk of cardiovascular events with the combination of extended-release niacin and laropiprant added to statin therapy with or without ezetemibe compared with LDL-lowering therapy alone in high-risk patients after a median follow up of 3.9 years. There was also a significant increase in unspecified nonfatal serious adverse events in the niacin plus laropiprant group [Merck, 2012].

Primary forms of low HDL, some of which have been associated with premature CAD, have helped in the understanding of the complex metabolism of the HDL particle. In monogenic disorders, data on the association of very low HDL with increased cardiovascular risk remains unclear. An absence of cardiovascular risk has been associated with some monogenic low HDL disorders, including apoA-I Milano, some ABCA1 variants and LCAT deficiency. When comparing the intima media thickness of a number of individuals with mutations in apoA-I, ABCA1, LCAT or CETP, carriers of apoA-I mutation displayed the most accelerated progression of atherosclerosis and that is the greatest elevation in cardiovascular risk compared with carriers of ABCA1 or LCAT mutations. However, elevated HDL secondary to a loss of CETP function did not significantly affect atherosclerosis progression compared with family controls [Hovingh *et al.* 2005]. Indeed, mutations in apoA-I are associated with a marked reduction in HDL and with premature cardiovascular disease [Ordovas *et al.* 1989; Schaefer *et al.* 1982]. However, carriers of a variant of apoA-I that is apoA-I Milano (apoA-IArg-173Cys) had very low HDL but a reduced risk of CAD. Although some variants of ABCA1 contribute to low levels of HDL and risk of ischemic heart disease, this association appears to be independent of plasma HDL levels [Frikke-Schmidt, 2010]. Subjects with LCAT deficiency have marked corneal opacification and an increased risk of renal failure but are not apparently at a higher risk of cardiovascular disease [Calabresi *et al.* 2012]. It has been suggested that subjects with classic LCAT deficiency and fish eye disease are protected from CAD due to a preferential catabolism of LpA-I:A-II particles that are less antiatherogenic [Rader *et al.* 1994]. However, patients with a deficiency of CETP have markedly elevated HDL levels but not necessarily a decreased risk of CHD, and an increased risk was even reported by some investigators [Hirano *et al.* 1995, 1997]. In addition, mutations in the hepatic lipase gene lead to higher plasma HDL levels yet a possible increased risk of CAD [Dugi *et al.* 2001]. Furthermore, recent Mendelian randomization studies failed to demonstrate causal associations between decreased or increased HDL-C and the risk of myocardial infarction [Frikke-Schmidt *et al.* 2008; Haase *et al.* 2012; Johannsen *et al.* 2009; Voight *et al.* 2012]. For instance, Haase and colleagues reported that low HDL due to LCAT genetic variations were not associated with increased risk of ischemic heart disease, although low plasma HDL levels were robustly associated with an increased risk of myocardial infarction. Similarly, Voight and colleagues demonstrated that single nucleotide polymorphisms in genes selectively affecting HDL levels did not associate with cardiovascular events [Voight *et al.* 2012]. Therefore, genetic studies do not appear to support a causative link between plasma HDL levels and cardiovascular link.

How can HDL reverse atherosclerosis?

One of the main mechanisms by which HDL is antiatherogenic is through reverse cholesterol transport. This pathway involves transfer of excess cholesterol from peripheral tissues, including arterial wall macrophages, to the liver for excretion [Rader, 2006; Tall, 2008]. In addition, HDL displays anti-inflammatory, antioxidative antithrombotic and vasodilatory properties [Calabresi *et al.* 2003; Mineo *et al.* 2006]. HDL protects LDL from oxidative damage through a number of apolipoproteins (apoA-I, apoE, apoJ, apoA-II and apoA-IV) and enzymes such as paraoxonase 1 (PON1), platelet-activating factor-acetyl hydrolase (PAF-AH), glutathione selenoperoxidase (GSPx) and LCAT [Kontush *et al.* 2003; Kontush and Chapman, 2006; Tabet and Rye, 2009]. Furthermore, HDL inhibits expression of adhesion molecules, prevents monocyte chemotaxis, stimulates nitric oxide synthase activity and reduces endothelial dysfunction [Chen and Mehta, 1994; Cockerill *et al.* 1995; Nicholls *et al.* 2005]. The antithrombotic effects of HDL have been further linked to an inhibition of coagulation factors (Va and VIIa) and a stimulation of protein C and S activity [Griffin *et al.* 1999, 2001].

However, there is an increasing body of evidence that HDL can lose its protective effects and thus become 'dysfunctional' in the setting of systemic inflammatory states, such as infection, diabetes, uremia and coronary atherosclerosis [Ansell,

2007; Navab *et al.* 2001; Smith, 2010]. This impaired proinflammatory HDL particle has a reduced reverse cholesterol transport pathway activity. ApoA-I, a mediator of cellular cholesterol efflux, is decreased secondary to reduced apoA-I synthesis in the liver, enhanced HDL catabolism and replacement of apoA-I by serum amyloid A protein, a pro-oxidant acute phase reactant [Cabana *et al.* 1989; Sammalkorpi *et al.* 1988]. Furthermore, apoA-I is oxidized by myeloperoxidase which severely reduces ABCA1-dependent cholesterol efflux from macrophages [Zheng *et al.* 2005]. Indeed, an elevated 'HDL inflammatory index' reflecting the presence of proinflammatory HDL particles was seen in patients with CHD or CHD equivalents, with yet increased HDL levels demonstrating that the inflammatory effects of HDL are independent of plasma HDL levels [Ansell *et al.* 2003]. Therefore, determining HDL functionality can be expected to improve cardiovascular risk assessment and help optimize targets for HDL-modifying therapies. However, HDL-C measurements do not take into account its functionality. Developing a new generation of robust HDL biomarkers and integrating structure–function relationships may provide a better means to identify patients who should receive therapy directed at improving HDL quality.

Effect of cholesteryl ester transfer protein inhibition on HDL-C

Targeting CETP to raise HDL is a growing interest. CETP is a protein complex that allows the transport of CEs from the antiatherogenic HDL to the atherogenic apoB-containing particles. As previously mentioned, deficiencies in CETP have been linked in epidemiological studies to a reduced CAD risk [Curb *et al.* 2004; Tall, 2009]; however, this has not been consistent across all studies [Hirano *et al.* 1995, 1997]. Four CETP inhibitors have been developed so far. Indeed, inhibiting CETP led to a substantial increase in HDL of 30 to 130% and this is expected to translate into reduced adverse cardiovascular events. However, this beneficial effect on cardiovascular outcome was not seen with the two CETP inhibitors torcetrapib and dalcetrapib. Torcetrapib, the first CETP inhibitor, raised HDL dose dependently by 28–91% [Barter *et al.* 2007a; Clark *et al.* 2004; Kuivenhoven *et al.* 2005]. However, the development of this drug was halted after the Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial revealed an increase in major cardiovascular events and mortality in the torcetrapib-treated group [Barter *et al.* 2007a]. It has been suggested that the failure of torcetrapib was due to nonclass related off-target effects, including an increase in blood pressure and lowering of serum potassium levels related to a rise in serum aldosterone [Hu *et al.* 2009]. Similarly, dalcetrapib which raised HDL by 31–40% failed to reduce the risk for recurrent cardiovascular events in patients with a recent acute coronary syndrome in the Dal-OUTCOMES trial [Schwartz *et al.* 2012]. Endpoint studies on anacetrapib and evacetrapib are currently underway. So far, CETP inhibition has been relatively unsuccessful. The reason for this remains unclear. The generation of nonfunctional HDL particles with CETP inhibition has been raised [Ng *et al.* 2013]

Other novel therapies aiming at raising HDL include apoA-I mimetics, reconstituted HDL, peroxisome proliferator activated receptor α/γ agonists, and liver X receptor agonists. These have shown some promising results and are summarized in Table 1 but will not be discussed any further in this review.

Conclusion

High HDL levels do not always confer protection. Low HDL has been correlated with increased cardiovascular risk in epidemiological studies; however, this correlation was not consistent in genetic and interventional CVD trials. Raising HDL pharmacologically failed to show a considerable, if any, impact on cardiovascular outcome. Improving HDL quality besides raising HDL levels should be considered when developing new therapies. In addition, measuring HDL levels may represent a crude marker of functionally active HDL and validated measurements of HDL function might be more helpful when evaluating the efficacy of HDL-raising therapies. In the interim, relying on non-pharmacological measures such as weight loss and exercise to increase HDL should not be discontinued. Ongoing and future research will help determine the safest and most effective approach to improve cardiovascular outcome and establish the safety, efficacy and impact on atherosclerosis of the emerging HDL-raising therapies.

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Table 1. Summary of HDL-raising therapies, mode of action, available clinical evidence on atherosclerosis and cardiovascular risk.

Drugs	Mode of action	Effect on HDL	Available clinical evidence on atherosclerosis and cardiovascular risk
Niacin (nicotinic acid)	↑ Hepatic apoA-I production and ABCA1 transcription ↓ Hepatic clearance of apoA-I and HDL ↓ CETP activity ↓ Lipolysis of triacylglyerol	↑ HDL-C 15–40%	AIM-HIGH and HPS2-THRIVE: lack of benefit on cardiovascular outcomes [AIM-HIGH Investigators *et al.* 2011; Merck, 2012]
Fibrates	↑ ApoA-I transcription and ABCA1 production	↑HDL-C 2–20%	VA-HIT and HHS: ↓ 22–34% in CHD with gemfibrozil [Frick *et al.* 1987; Rubins *et al.* 1999]. BIP and FIELD: no benefit of bezafibrate and fenofibrate in reducing CHD events [Bezafibrate Infarction Prevention Study, 2000; Keech *et al.* 2005]. ACCORD-lipid: no further reduction in CV events with fenofibrate plus simvastatin compared with simvastatin alone [ACCORD Study Group *et al.* 2010] *Post hoc* analyses: subgroup of patients with low HDL and high triglycerides benefited from fibrates in terms of cardiovascular risk reduction
Inhibitors of CETP	Inhibit CETP	↑HDL-C 28–138%	Torcetrapib: increase in major cardiovascular events and mortality; increase in BP and aldosterone concentration [Barter *et al.* 2007a] Dalcetrapib: lack of benefit on cardiovascular outcomes [Schwartz *et al.* 2012] Ongoing trials with anacetrapib and evacetrapib
PPAR-α and -δ agonists	↑ApoA-I, ApoA-II, LPL activity and RCT	↑ HDL-C 5–15%	Toxicity of first-generation drugs: tesaglitazar and muriglitazar AleCardio phase III trial with aleglitazar halted due to safety signals and lack of efficacy
ApoA-I mimetics	Cause a shift from α-HDL particles to pre-β1-HDL particles ↑ Other aspects of RCT	No effect on HDL-C levels	Improved inflammatory index in patients with clinical cardiovascular disease [Bloedon *et al.* 2008]
Apo-AI synthesis stimulators (RVX-208)	Induce de novo hepatic synthesis of apoA-I	↑ HDL-C 3.2-8.3% ↑HDL particle 11 -21%	ASSURE: no incremental benefit on atherosclerotic plaque compared with placebo [Nicholls *et al.* 2013]
Recombinant HDL infusions	Mimic native HDL	↑HDL-C 64%	EFFECT: significant reduction in coronary atheroma volume compared to baseline but not to placebo [Tardif *et al.* 2007] Favorable impact on endothelial function [Spieker *et al.* 2002]
Delipidated HDL	↑ RCT	No effect on HDL-C Preβ HDL ↑28×	Nonsignificant decrease in mean atheroma volume [Waksman *et al.* 2010]
LXR agonists	↑ Mobilization of intracellular cholesterol ↑ Transcription of ABCA1 and ABCG1 ↑ RCT ↑ Intestinal HDL generation	↑ HDL-C up to 48%	Reduced atherosclerosis in animal studies
LCAT activators	Contributes to maturation of HDL particles ↑ RCT	↑ HDL-C 40–68%	Reduced atherosclerosis in animal studies
MicroRNA-33	Inhibits ABCA1 mRNA degradation	↑ HDL-C 50%	Inhibition of microRNA-33 promotes regression of atherosclerosis in LDLr–/– mice [Rotllan *et al.* 2013]

ABCA-1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette subfamily G member 1; ACCORD, Action to Control Cardiovascular Risk in Diabetes; AIM-HIGH, Atherothrombosis Intervention in Metabolic Syndrome with low HDL/High Triglycerides and Impact on Global Health Outcomes; apoA-I, apolipoprotein A-I; BIP, Bezafibrate Infarction Prevention; CETP, cholesterol ester transfer protein; FIELD, Fenofibrate Intervention and Event Lowering in Diabetes; HDL-C, high-density lipoprotein cholesterol; HHS, Helsinki Heart Study; HPS2-THRIVE, Heart Protection Study 2 Treatment of HDL to Reduce the Incidence of Vascular Events; LCAT, lecithin-cholesterol acyltransferase; LDLr, low-density lipoprotein receptor; LPL, lipoprotein lipase; LXR, liver X receptor; PTLP, phospholipid transfer protein; PPAR, peroxisome proliferator-activated receptor; RCT, reverse cholesterol transport; VA-HIT, Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial.

Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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