Is raising HDL a futile strategy for atheroprotection?

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Abstract | The dramatic failure of clinical trials evaluating the cholesterol ester transfer protein inhibitor torcetrapib has led to considerable doubt about the value of raising high-density lipoprotein cholesterol (HDL-C) as a treatment for cardiovascular disease. These results have underscored the intricacy of HDL metabolism, with functional quality perhaps being a more important consideration than the circulating quantity of HDL. As a result, HDL-based therapeutics that maintain or enhance HDL functionality warrant closer investigation. In this article, we review the complexity of HDL metabolism, discuss clinical-trial data for HDL-raising agents, including possible reasons for the failure of torcetrapib, and consider the potential for future HDL-based therapies.

High-density lipoprotein (HDL). A class of cholesterol-rich lipoprotein particles that drive the return of cholesterol from the periphery back to the liver; cholesterol carried by these particles is colloquially referred to as 'good cholesterol'.

Atherosclerosis
A complex, multifactorial disease process that results in the development of arterial wall plaques, which can eventually occlude the arterial lumen and compromise blood flow, resulting in a heart attack or stroke depending on the affected arterial bed. Plasma lipids — especially cholesterol —in circulating lipoprotein particles have a key role at several stages of atherosclerosis.

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The inverse relationship between high-density lipoprotein cholesterol (HDL-C) levels and coronary heart disease (CHD) has stimulated interest in pharmacological agents that elevate plasma HDL. However, the recent unexpected association of torcetrapib — an agent that increases plasma HDL-C — with increased cardiovascular mortality has led to the discontinuation of further trials involving this drug[1]. Furthermore, imaging trials using surrogate endpoints of atherosclerosis did not demonstrate any benefits attributable to torcetrapib[2–4]. As a result of these findings, questions regarding the efficacy and safety of HDL elevation as a strategy for CHD prevention have been raised. For a systematic review of all trials evaluating HDL-C levels and atherosclerotic outcomes we refer the reader to a recently published article by Ansell and colleagues[5]. Here, we will discuss the importance of HDL in atherosclerosis, potential reasons for the failure of torcetrapib and future HDL-based strategies that might reduce or delay cardiovascular endpoints.

The HDL particle: structure and function

HDL structure. HDL particles are heterogeneous in shape, density, size and anti-atherogenic properties. Their shape can range from discoidal to spherical with densities ranging between 1.063 and 1.21 g/mL. In addition to a high protein content — approximately 50% by weight — HDL particles are composed of approximately 30% phospholipids, 25% cholesterol (of which about 70% is esterified) and 5% triglyceride. The larger spherical HDL particles contain a hydrophobic core of cholesteryl ester (CE) and triglyceride, whereas small discoidal HDL particles contain primarily apolipoprotein A-1 (APOA1) in a lipid monolayer that is composed of phospholipids and free cholesterol[6].

APOA1 and APOA2 are the major structural apolipoproteins of HDL. APOA1 is present on most HDL particles and accounts for almost 70% of their protein content. Another 20% of HDL protein is contributed by APOA2, which is present on two-thirds of HDL particles[7]. Minor HDL protein components include other apolipoproteins (APOA4, APOA5, APOC1, APOC2, APOC3, APOD and APOE), enzymes involved in lipid metabolism or with possible antioxidant activities — such as lecithin–cholesterol acyltransferase (LCAT), lipoprotein-associated phospholipase A_2 (also called platelet-activating factor-acetyl hydrolase, PAF-AH), paraoxonase 1 (PON1) and glutathione selenoperoxidase (GPX) — as well as other proteins, such as serum amyloid A, α-1-antitrypsin and amyloid-β[6].

HDL subclassifications and relationship to function. Plasma HDL-C level is currently the most accessible laboratory measurement of HDL. However, HDL-C simply quantifies the amount of cholesterol contained within the HDL lipoprotein fraction and does not necessarily correlate with the number of particles or with their net anti-atherogenic properties. In research laboratories, HDL particles can be subclassified according to their size or density using two-dimensional gel electrophoresis or density gradient ultracentrifugation, respectively, or by their apolipoprotein composition using immunological methods. Some evidence suggests that these subcategories define specific functional properties of HDL.

Apolipoprotein A-1
(APOA1). The major structural apolipoprotein on the vast majority of high-density lipoprotein particles, accounting for approximately 70% of the protein content of these particles.

Niacin
A member of the vitamin B family and effective cholesterol-lowering agent at high doses. It reduces low-density lipoprotein, triglyceride and total plasma cholesterol levels while raising high-density lipoprotein levels. It is the only known compound to effectively reduce the levels of lipoprotein A.

Low-density lipoprotein
(LDL). A class of cholesterol-rich lipoprotein particles that deliver cholesterol from the liver to peripheral cells, including cells within the evolving atherosclerotic plaque; cholesterol carried by these particles is colloquially referred to as 'bad cholesterol'.

Reverse cholesterol transport
(RCT). The general pathway through which cholesterol is transported from peripheral cells, such as cholesterol-laden macrophages, to the liver for excretion in bile. High-density lipoprotein (HDL) has a major role in RCT and this is thought to be the main mechanism by which HDL exerts its anti-atherogenic effects.

Cholesteryl ester transfer protein
(CETP). A hydrophobic glycoprotein that is primarily found bound to high-density lipoproteins (HDL). The major function of CETP is to facilitate the transfer of cholesteryl ester from HDL to low-density lipoproteins, intermediate density lipoproteins and very low-density lipoproteins in exchange for triglyceride from those particles during the latter stages of reverse cholesterol transport.

Fibrinolysis
The body's main mechanism of dissolving fibrin-formed clots and thus preventing adverse clot-initiated events such as heart attacks, deep vein thromboses or pulmonary emboli.

The predominant α subfraction of HDL was named because of its α mobility on gel electrophoresis. The α-HDL subfraction can be further subdivided into four categories, from α-1 (large, spherical) to α-4 (small, discoidal) HDL. APOA1 and APOA2 content differs across the α subfractions, with both α-1 and α-4 HDL containing APOA1 without APOA2, and with both α-2 and α-3 containing APOA1 and APOA2. Three other HDL subfractions, that are defined by electrophoretic mobility are pre-α, pre-β-1 (small, discoidal HDL) and pre-β-2 (large HDL), which all contain APOA1 but not APOA2 (REF. 8).

Both α-2 and pre-β-1 HDL correlate with free cholesterol efflux through the ATP-binding cassette subfamily A, member 1 (ABCA1) pathway, whereas α-1, α-2, α-3 and pre-β-1 HDL correlate with scavenger receptor class B member 1 (SCRB1)-mediated cholesterol efflux from liver cells (see below)[9]. Compared with controls, patients with CHD have lower α-1 and pre-α-1 HDL, but higher α-3 HDL subfractions[10]. In both the Framingham Offspring Study and the Veterans Affairs HDL Intervention Trial, low levels of α-1 and α-2 HDL were superior to overall HDL-C levels in predicting CHD[11,12]. Also, treatment with niacin plus simvastatin increased the α-1 HDL subfraction, which appeared to be correlated with reduced coronary atherosclerosis[13].

Ultracentrifugation subdivides HDL into HDL_2 and HDL_3 subclasses according to density. HDL_2 consists of larger, less dense CE-enriched particles compared with the smaller, dense HDL_3 particles. HDL_2 seems to be more atheroprotective than HDL_3 — an inverse correlation exists between HDL_2 and atherosclerosis, hypertriglyceridaemia and obesity[14–16]. HDL_3 protects low-density lipoprotein (LDL) from oxidative stress[17] and is more influenced by dietary and ethanol intake[18] than HDL_2. Although this HDL subclassification might define HDL function better than total HDL and predict cardiovascular endpoints, methodologies for such testing are not generally available at present.

HDL function — reverse cholesterol transport (RCT). Some of the key beneficial mechanisms of HDL are incompletely understood. Cholesterol-laden macrophages (or foam cells) have a key role in atherogenesis. The net flux of cholesterol from foam cells to the liver and ultimately, biliary excretion (a process referred to as reverse cholesterol transport (RCT)), is considered to be the primary mechanism by which HDL protects against atherogenesis[7]. A current working model of RCT invokes eight critical steps (FIG. 1) and involves the interplay of four organs/tissues (liver, intestine, arteries and kidney), six enzymes (LCAT, lipoprotein lipase (LPL), cholesteryl ester transfer protein (CETP), hepatic lipase (HL), endothelial lipase (EL) and secretory phospholipase A_2 (sPLA2)), three receptors (ABCA1, ATP-binding cassette subfamily G member 1 (ABCG1) and SCRB1) together with surface apolipoproteins, lipids and phospholipids.

Any of these molecules involved in RCT could potentially be targeted when aiming to increase HDL-C levels. However, the optimum point for intervention is unknown. If one considers steps one to four (building early HDL particles through the mobilization of cholesterol from cells) to be the key component of RCT, the appropriate targets to increase HDL would be APOA1, ABCA1, LCAT and LPL. But, if steps four to six (reshaping HDL particles) are considered to be most important in RCT, then key targets would be CETP, HL, EL or sPLA2. Finally, if steps seven and eight (disposing of HDL lipid content) are critical, then SCRB1 activity should be targeted. Modulating one part of the RCT pathway when another is more beneficial physiologically, might not be useful. Unfortunately, the most critical steps in RCT that will maximally affect clinical endpoints remain unclear.

Non-RCT functions of HDL. HDL possesses antioxidant, anti-inflammatory, antithrombotic and endovascular properties. By inhibiting LDL oxidation, HDL interferes with a process that initiates atherogenesis. Antioxidant properties are mediated through apolipoproteins (APOA1, APOE, APOJ, APOA2 and APOA4) and enzymes (PON1, LCAT, PAF-AH and GPX)[6,19,20]. HDL also stimulates nitric oxide and prostacyclin production, inhibits monocyte adhesion to endothelial cells and reduces platelet aggregation[21–24]. Hydrolysis of oxidized lipids by HDL contributes to an overall anti-inflammatory effect as oxidized LDL molecules are pro-inflammatory[25]. Furthermore, HDL is antithrombogenic because it reverses LDL-induced inhibition of fibrinolysis, inhibits tissue factors (factors Va, VIIIa and X) and increases the activity of protein S and activated protein C[26–30]. However, the ultimate clinical relevance of HDL's wide range of biological activities is uncertain.

Further complications. Plasma HDL-C levels vary widely, with the prevalence of low HDL-C ranging from 20% to 60% in patients with established cardiovascular disease[11,12,16,31,32]. Effective HDL function can be further complicated by a range of factors. For instance, in the context of inflammation, HDL can be transformed into a pro-atherogenic particle, which can either oxidize or glycosylate APOA1 amino-acid residues or alter the core lipid content[6]. HDL oxidation can further inhibit endothelial nitric oxide synthase and decrease vasodilation[33]. Oxidation of HDL_3 promotes expression of plasminogen activation inhibitor-1, which suppresses fibrinolysis[34]. Dysfunction or loss of such HDL-associated enzymes as PON1, LCAT and PAF-AH occurs with inflammation, type 2 diabetes and the metabolic syndrome[6]. Low PON1 activity correlates with increased CHD events among high risk men[35]. So, while HDL-C levels correlate indirectly with cardiovascular risk in large epidemiological studies, they are less informative of the risk for the individual patient, apart from a non-specific relationship between elevated HDL-C and a general increase in cardioprotective HDL subfractions.

CETP inhibition as a strategy to raise HDL

There has been intense interest in the development and application of pharmaceutical agents designed to raise HDL-C[36–41]. One target for HDL-C elevation has been CETP, a hydrophobic glycoprotein secreted primarily from the liver that circulates mainly bound to HDL.

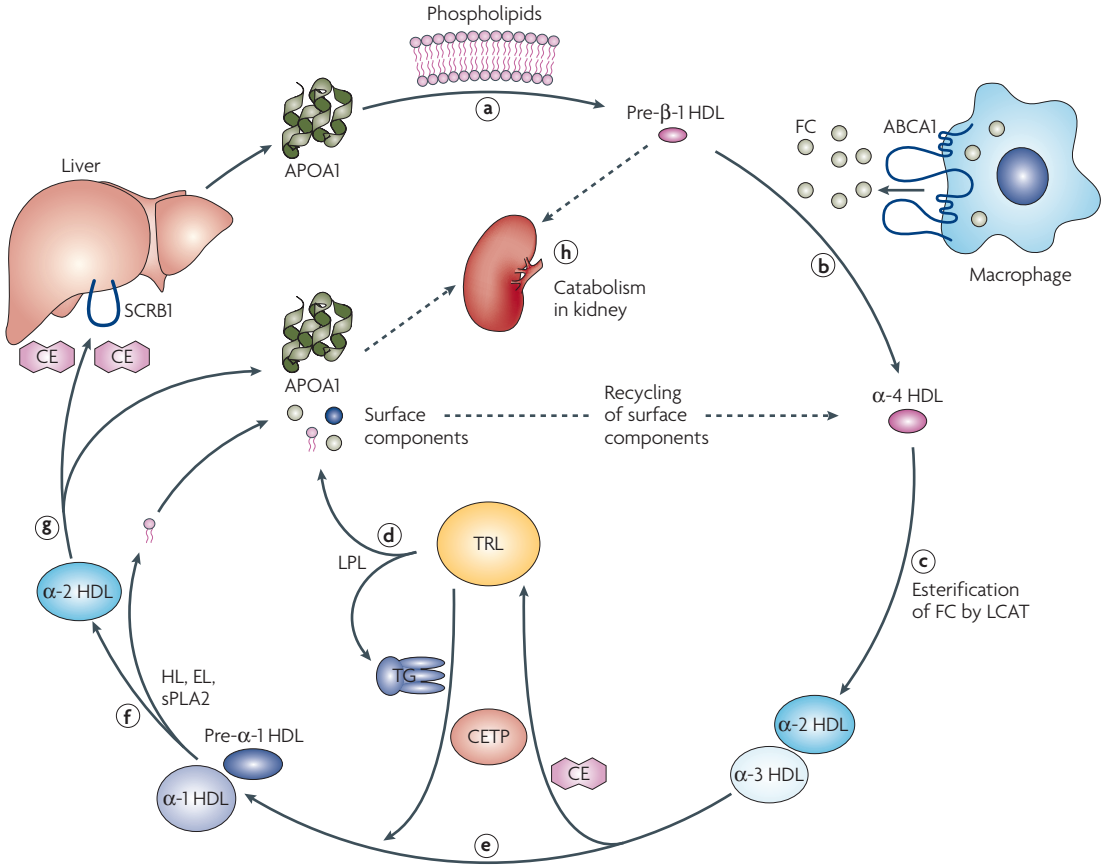

Figure 1 | **Reverse cholesterol transport (RCT).** Apolipoprotein A-1 (APOA1) secreted by the liver and intestine combines with phospholipids to form small, discoidal pre-β-1 high-density lipoproteins (HDL) that can bind cholesterol (**a**). Pre-β-1 HDL is converted into small, discoidal α-4 HDL by the efflux of cellular free cholesterol (FC) (from macrophages in the arterial wall, for example) by the ABCA1 and ABCG1 (not shown) transporters (**b**). This cholesterol is then esterified by lecithin–cholesterol acyl transferase (LCAT) (**c**). The movement of cholesteryl ester (CE) to the core of the HDL particle converts the α-4 HDL into larger α-2 and α-3 HDL. Lipoprotein lipase (LPL) hydrolyses the triglyceride (TG) carried on TG-rich lipoproteins (TRL) to provide surface components (phospholipids, FC and apolipoproteins) to the HDL particle (**d**). TG from TRL is exchanged for CE on HDL through cholesterol ester transfer protein (CETP), resulting in the formation of pre-α and pre-α-1 HDL (**e**). The plasma enzymes hepatic lipase (HL), endothelial lipase (EL) and secretory phospholipase A2 (sPLA2) hydrolyse and remove PL from the newly formed HDL, converting α-1 HDL into α-2 HDL with recycling of surface APOA1 protein (**f**). The liver then takes up CE from HDL, by scavenger receptor-B1 (SCRB1) (**g**) and recycling of the surface components of HDL. The final step of RCT is the catabolism of free APOA1 and pre-β-1 HDL by the kidney with excretion in the urine (**h**) [8,31].

The small fraction of CETP that does not circulate could have a role in intracellular lipid metabolism[42,43]. In plasma, CETP primarily facilitates the movement of CE from HDL to LDL, intermediate-density lipoproteins (IDL), and very low-density lipoproteins (VLDL) in exchange for triglyceride during the latter stages of RCT. The disparate predicted effects of 'normal' CETP are summarized in BOX 1.

CETP was first recognized as a potential target when rodents lacking plasma CETP activity were found to have elevated HDL and were resistant to diet-induced atherosclerosis[44]. Subsequently, it was discovered that some patients with CETP mutations seemed to have elevated HDL levels and decreased CHD[45,46]. As a result, both Japan Tobacco and Pfizer began development of CETP inhibitors, namely JTT-705 and torcetrapib, respectively. In rabbits with diet-induced atherosclerosis, JTT-705 and torcetrapib increased plasma HDL-C levels by two- and threefold, respectively, and reduced atherosclerotic plaque area by 70% and 60%, respectively[47,48]. The total cholesterol to HDL-C ratio was directly correlated with atherosclerosis in torcetrapib-treated rabbits[47]. However, another study showed that high-doses of JTT-705 increased triglyceride and HDL-C levels by approximately 200%, with no change in atherosclerosis[49]. Initial human trials of both JTT-705 and torcetrapib were promising. A Phase II trial of JTT-705 demonstrated a 34% increase in HDL-C levels and a 7% decrease in LDL-C levels[50]. Torcetrapib increased HDL-C up to 91% and decreased LDL-C up to 42% at high doses, with no apparent significant adverse effects[51].

However, in retrospect, the evidence supporting the potential of CETP inhibition for atherosclerosis prevention is not straightforward. First, although some

Box 1 | **Predicted effects of 'normal' physiological CETP activity**

Pro-atherogenic

- Transfer of cholesteryl ester (CE) by cholesterol ester transfer protein (CETP) increases CE content in apolipoprotein B (APOB) containing lipoproteins (low-, very low- and intermediate-density lipoproteins).
- Increased CE in low-density lipoprotein particles as a result of CETP-mediated transfer can be taken up by macrophages in the vessel wall for atheroma formation.

Anti-atherogenic

- Decrease in CE (high-density lipoprotein 'remodelling') facilitates the use of lipid-poor apolipoprotein A-1 (APOA1) for ATP-binding cassette subfamily A, member 1 (ABCA1)-mediated cholesterol efflux.
- CE transferred to APOB-containing lipoproteins (low-, very low- and intermediate-density lipoproteins) for possible excretion by the liver through scavenger receptor class B, member 1.
- Total pool of APOA1 and high-density lipoprotein cholesterol levels are increased.

patients with CETP mutations were initially found to have elevated HDL-C levels and decreased CHD, other *CETP* gene variants — namely *I405V*, *D442G*, *c.629C→A*, and *TaqIB B2* — were associated with increased CHD risk[45,46,52–55]. Second, animal studies were also inconsistent. In rabbits, CETP inhibition reduced atherosclerosis[47,48,56–58], but studies in mice were confounding. Mice, in contrast to both humans and rabbits, normally lack CETP. Both APOE- and LDL-receptor knockout mice developed extensive atherosclerosis when cross-bred with CETP transgenic mice[59]. Atherosclerosis was increased in LDL-receptor knockout mice that had been transplanted with bone marrow from CETP transgenic mice[60]. Conversely, CETP expression in both APOC3 transgenic mice and SCRB1-deficient mice was associated with decreased atherosclerosis[61,62]. In LCAT transgenic mice, CETP expression decreased atherosclerosis and improved HDL dysfunction. Cross-breeding of CETP and LCAT transgenic mice normalized plasma HDL clearance and hepatic CE accumulation[63]. Conflicting results from transgenic CETP expression on different mouse backgrounds could have been the result of epigenetic effects (that is, the interaction between allelic variants), position effects (due to the random insertion of the transgene at different genomic sites) or the presence of passenger genes (that is, variable movement of genes within the region flanking the selected locus across generations, or chance persistence of unlinked donor chromosomal regions)[64,65]. Evidence for the pro- and anti-atherogenic consequences of CETP inhibition is summarized in TABLE 1.

Clinical effects of torcetrapib

Torcetrapib monotherapy (60 mg/day) caused a non-significant decrease in LDL-C of 8% with significant increases in HDL-C, HDL_2 and HDL_3 by 45%, 67%, and 31%, respectively[66]. When combined with 20 mg atorvastatin, LDL-C decreased significantly (by 16%), whereas HDL-C, HDL_2 and HDL_3 increased by 33%, 74% and 26%, respectively[67]. These favourable biochemical outcomes were predicted to translate into beneficial clinical outcomes. However, in late 2006 all torcetrapib trials were halted abruptly due to the interim findings of the ILLUMINATE (Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events) trial. This study randomized 15,067 individuals at high risk for cardiovascular disease to treatment with atorvastatin (10 to 80 mg/day) plus placebo, or atorvastatin plus torcetrapib (60 mg/day). After 12 months, those in the torcetrapib arm demonstrated an increased risk of cardiovascular events (hazard ratio, 1.25; 95% confidence interval, 1.09 to 2.19, p=0.006) despite a 72.1% increase in HDL-C and 24.9% decrease in LDL-C levels. Both cardiovascular and non-cardiovascular deaths were higher among those in the torcetrapib arm compared with placebo[1].

Three concurrent trials evaluating the efficacy of torcetrapib on either coronary or carotid atherosclerosis were also disappointing. The ILLUSTRATE (Investigation of Lipid Level Management Using Coronary Atherosclerosis by CETP Inhibition and HDL Elevation), RADIANCE-1 (Rating Atherosclerosis Disease Change with a New CETP Inhibitor) and RADIANCE-2 trials compared the effect of torcetrapib plus atorvastatin with atorvastatin alone in decreasing coronary atherosclerosis in patients with CHD, and in decreasing carotid atherosclerosis in patients with heterozygous familial hypercholesterolaemia and with mixed dyslipidaemia, respectively. Despite an approximate 60% increase in HDL-C, there was no difference in progression or regression of atherosclerosis in the torcetrapib arm of these studies[2–4]. In the ILLUSTRATE trial, the secondary endpoint of change in total atheroma volume improved in the torcetrapib plus atorvastatin arm compared with atorvastatin alone[2]. However, the marginal treatment-associated reduction of total atheroma volume over 24 months was much smaller than the regression seen in similar imaging studies performed with statins[68,69]. Meanwhile, patients in the torcetrapib arm of the RADIANCE-1 trial had a significant increase in the secondary endpoint of annualized change in intima-media thickness (IMT) in the common carotid artery[3]. Taken together, these studies indicated that increased HDL-C levels in torcetrapib-treated patients were not associated with improved clinical surrogate markers or hard clinical endpoints.

Torcetrapib actually came under earlier scrutiny when Phase II studies revealed mean increases of 1.3–2.2 mmHg in systolic blood pressure (SBP) and 0.9–1.1 mmHg in diastolic blood pressure at doses of 60 or 90 mg/day[66,67]. Approximately 4% of individuals in the Phase II trials experienced an increase in blood pressure of greater than 15 mmHg[70]. As a result, the torcetrapib dose was restricted to 60 mg/day in subsequent Phase III trials. Despite this dose restriction, the relative mean SBP increases in RADIANCE-1, RADIANCE-2 and ILLUSTRATE were 2.8, 5.4 and 4.6 mmHg, respectively[2,3]. In fact, 5% of individuals in RADIANCE-2 and 9% of individuals in ILLUSTRATE demonstrated an increase in blood pressure of more than 15 mmHg[2–4]. The recently reported results of the ILLUMINATE trial also revealed that treatment with torcetrapib was associated with a relative increase in mean SBP of 4.5 mmHg[1].

Familial hypercholesterolaemia (FH). A genetically inherited lipoprotein disorder characterized by cutaneous manifestations of hypercholesterolaemia, very high levels of low-density lipoprotein and total cholesterol, as well as early, often fatal, cardiovascular disease. FH is most often caused by defects in the low-density lipoprotein receptor (*LDLR*) gene.

Table 1 | **Selective evidence for beneficial and detrimental effects of CETP inhibition**

Study Details	Study Outcome	Refs
Beneficial		
Human CETP transgene expression in APOE knockout mice or LDL-receptor knockout mice	Expression of CETP in APOE knockout mice resulted in:	59
	Decreased HDL-C levels by 34%	
	Increased atherosclerotic lesion area by approximately 2-fold after 2–4 months, and 1.4- to 1.6-fold after 7 months when compared with APOE knockout mice without CETP	
	Expression of CETP in LDL-receptor knockout mice resulted in:	
	Increased mean lesion area by 1.8-fold	
Simian CETP expression in cholesterol-fed C57BL/6 mice	Significant increase in aortic lesion area when compared with non-CETP transgenic mice	152
CETP inhibitor (torcetrapib) given to cholesterol-fed white New Zealand rabbits	3-fold increase in HDL-C levels	47
	60% reduction in aortic atherosclerosis after 16 weeks of therapy	
	Lesion area in the torcetrapib-treated group was strongly correlated with the ratio of total plasma cholesterol to HDL-C	
CETP inhibitor (JTT-705) or simvastatin given to cholesterol-fed white Japanese rabbits	90% increase in HDL-C levels by JTT-705 versus 28% by simvastatin	48
	JTT-705 caused a 70% reduction in atherosclerotic lesion size, an effect similar to the one observed with simvastatin	
Honolulu Heart Study examined the prevalence of CHD among men of Japanese ancestry in relation to the presence of CETP mutations	High prevalence of two different *CETP* gene mutations (*D442G*, 5.1%; intron 14 G>A, 0.5%) was found in the 3,469 men	52
	These mutations were associated with decreased CETP (–35%) and increased HDL-C levels (+10% for *D442G*)	
	No increased risk of CHD in those with decreased CETP levels compared with those without, when HDL-C levels were greater than 1.54 mmol/L	
Examined cholesterol efflux capacity from HDL-2 particles from CETP-deficient versus control individuals	CETP-deficient HDL-2 resulted in a 2- to 3-fold stimulation of net cholesterol efflux compared with control HDL-2 through an ABCG1-dependent pathway	74
Investigated the effects of 60 mg/day or 120 mg/day of torcetrapib on cholesterol efflux by human HDL particles	60 mg/day torcetrapib increased HDL-mediated net cholesterol efflux, primarily by increasing HDL concentrations, compared with baseline	75
	120 mg/day torcetrapib increased cholesterol efflux both by increasing HDL concentration and by causing increased efflux at matched HDL concentrations compared with baseline	
Detrimental		
Human CETP expression in APOC3 hypertriglyceridaemic mice	Lower average number of aortic lesions per mouse among CETP/APOC3 mice compared with either APOC3 or control mice	61
	Decrease in mean lesion area per mouse by 55% compared with APOC3 mice	
CETP expression among LCAT transgenic mice (which normally have 'dysfunctional HDL' —that is, APOE and CE-rich HDL with impaired RCT and increased atherosclerosis despite elevated HDL)	Mean aortic lesion area was reduced by 41% in CETP–LCAT transgenic mice compared with LCAT only transgenics	63, 153
	Normalized plasma clearance of cholesteryl esters from HDL	
Honolulu Heart Study examined the prevalence of CHD among men of Japanese ancestry in relation to the presence of CETP mutations	Increased risk of CHD in those with decreased CETP levels compared with those without, when HDL-C levels were less than 1.54 mmol/L	52
Examined the frequency of heterozygotes for the CETP intron 14 splicing mutation among Japanese individuals in the Omagari region	The frequency of the CETP intron 14 splicing mutation was higher in patients with CHD than in healthy control subjects	154
	Frequency of heterozygotes for the CETP intron 14 splicing defect was much lower among elderly individuals (> 80 years) compared with those who were younger (< 80 years), thereby implying possibly no beneficial effects of CETP deficiency to longevity	

ABCG1, ATP-binding cassette, subfamily G, member 1; APOC3, apolipoprotein C3; APOE, apolipoprotein E; CE, cholesteryl ester; CETP, cholesterol ester transfer protein; CHD, coronary heart disease; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; LCAT, lecithin–cholesterol acyltransferase; LDL, low-density lipoprotein; RCT, reverse cholesterol transport.

Given that every 10 mmHg increase in SBP is associated with a 25% increase in cardiovascular disease, stroke and vascular death, this effect of torcetrapib might have negated the benefit of HDL-C elevation[71,72]. The increase in SBP might have been due to some unknown generalized vascular off-target drug effect, such as calcium-mediated vasospasm or hyperaldosteronism. Post-hoc analysis of the results of ILLUMINATE revealed an increase in aldosterone levels in the torcetrapib group, although a direct comparison of the mean values was not possible due to the fact that 53–57% of the samples had aldosterone levels below the lower limit of quantification and that only 87–88% of the samples were analysed[1]. The mechanism underlying the hypertension observed in patients receiving torcetrapib remains to be elucidated.

Alternative explanations for the failure of torcetrapib are less straightforward. HDL function might have been impaired either by CETP inhibition or by torcetrapib itself. Indirect human studies have produced conflicting findings: HDL from CETP-deficient subjects has been shown both to impair[73] and promote[74] cholesterol efflux from cultured macrophages, although the heparin-affinity column used in the former study probably removed APOE-rich HDL[73]. More recently, torcetrapib given to mildly hyperlipidaemic patients at a dose of 60 mg/day increased HDL-C levels by 50% and also increased cholesterol efflux efficiency[75]. A 4-week study in 19 subjects treated with 120 mg of torcetrapib once or twice daily showed no effect on RCT as assayed by faecal sterol clearance[76], and the increased HDL-C levels were associated with decreased APOA1 catabolism[76]. It thus seemed unlikely that this aspect of HDL function would be compromised by torcetrapib.

An alternative proposal for torcetrapib-associated HDL dysfunction is the formation of non-productive complexes. Torcetrapib binds CETP in a 1:1 ratio, forming a larger complex with CETP and HDL, and shifting the percentage of total plasma CETP found in HDL from 25% to 75%. Recovery of CETP in the 1.13 to 1.21 g/mL density range did not clarify whether the complex involved HDL_2 or HDL_3 (REF. 77). As plasma CETP concentration is low compared with HDL, the interaction between CETP and HDL as a result of torcetrapib would probably not affect HDL function, although this presumes 1:1 binding of HDL to the torcetrapib–CETP complex, which has not been shown[51]. In addition, because these studies were limited and short, the effect of a long duration of torcetrapib on parameters such as cholesterol efflux, RCT or non-productive complex formation remains to be determined. It would be important to understand whether the adverse events in ILLUMINATE were the result of the torcetrapib molecule rather than the CETP inhibition mechanism; this will require careful analysis of the effects of torcetrapib on lipid metabolism in the patients from that study.

In contrast, another CETP inhibitor, JTT-705, did not affect blood pressure, suggesting that some of the effects of torcetrapib were not class effects of CETP inhibition[78]. In addition, patients with CETP deficiency do not have hypertension. The results of Phase III clinical trials with JTT-705 will address the efficacy and safety of CETP inhibition with other agents, perhaps extending the viability of this approach. Unfortunately, torcetrapib's failure has caused many to question whether HDL-raising itself is a truly effective general strategy for preventing cardiovascular disease.

Heparin-affinity column
A chromatographic method that is used to separate and purify proteins based on their differential affinity to heparin.

Is raising HDL still a valid strategy?

Several pharmacological interventions associated with increased HDL-C appear to reduce atherosclerosis. Most trials have involved the administration of niacin, although other strategies include fibrates, $APOA1_{Milano}$ and reconstituted HDL[36–41,79–81]. Positive results from studies using these agents for secondary prevention or atherosclerosis regression are summarized in TABLE 2 and underscore the need for ongoing research in HDL-C elevation for the prevention of cardiovascular morbidity, and possibly mortality.

Niacin and increased HDL-C. Clinical trials with niacin have provided substantial evidence that increased HDL-C level (albeit in combination with other favourable biochemical effects) is associated with improved outcomes, as seen in the Coronary Drug Project (CDP), and with plaque regression, as seen in the non-randomized Familial Atherosclerosis Treatment Study (FATS), HDL Atherosclerosis Treatment Intervention Study (HATS), University of California, San Francisco, Specialized Center of Research (UCSF-SCOR) trial, Cholesterol Lowering Atherosclerosis Study (CLAS) and the randomized Arterial Biology for the Investigation of the Treatment Effects of Reducing cholesterol trials (ARBITER-2 and ARBITER-3)[36,38,39,82–85].

CDP was the first major clinical trial to demonstrate an association between reduced vascular events and an improved lipoprotein profile, including increased HDL-C levels. Among 8,341 men aged 30–64 years with a history of myocardial infarction (MI), niacin therapy decreased MI risk by 26%, coronary revascularization by 67% and stroke by 24% after a mean follow-up of 6 years[36]. At the 15-year follow-up, all-cause mortality decreased by 11% among those randomized to niacin compared with placebo[37].

The recent ARBITER-2 and ARBITER-3 studies evaluated carotid IMT after adding extended-release (ER)-niacin to statin therapy in CHD patients with low HDL-C. In ARBITER-3, ER-niacin was associated with regression at 24 months, and this was in turn independently associated with HDL-C after correcting for LDL-C and triglycerides[82,83].

Niacin is the most effective currently available drug to increase HDL-C (typically by 15–30%). It also reduces total cholesterol, triglyceride, LDL and lipoprotein levels (by 20–40%)[86], which makes it difficult to directly implicate the changes in HDL-C as the primary beneficial effect of niacin. The key pharmacological receptors for niacin are HM74A (GPR109A) and PUMA-G (mouse homologue) and activation leads to decreased lipolysis through inhibition of adenyl cyclase in adipocytes. As a result, free fatty-acid levels decrease, leading to decreased triglyceride levels[87–89]. However, the widespread acceptance of niacin has

Table 2 | **Secondary prevention and atherosclerosis imaging studies of HDL-based therapies: positive results**

Study	Population	Duration (years)	Treatment	Results	Refs
Niacin					
Coronary Drug Project	8,341 males, post-MI	6	Niacin or clofibrate versus placebo	↓ risk of stroke by 24%, MI by 26% and coronary revascularization by 67% in niacin group	36,37
		15	Niacin or clofibrate versus placebo	↓ mortality in niacin group only versus placebo by 11%	
Stockholm Ischaemic Heart Disease Study	555 post-MI patients	5	Niacin plus clofibrate versus no treatment	↓ mortality by 26% ↓ ischaemic heart disease mortality by 36%	155
CLAS I and II	162 post-CABG patients	2 and 4	Niacin plus colestipol versus placebo	Significant lesion regression at 2 and 4 years At 4 years, lesion non-progression in 52% versus 15%, and regression in 18% versus 6% of patients (active versus placebo)	84,156
FATS	146 patients with CHD	2.5	Colestipol plus niacin or lovastatin versus placebo	Atherosclerotic regression in 39% versus 11% (active niacin versus placebo) ↓ clinical events (death, MI or revascularization) by 78% in niacin group	38,157
FATS Extended follow-up	176 patients	10	Niacin plus lovastatin plus colestipol versus standard care	Significant reduction in mortality by 96% and coronary events by 72% in the triple therapy group	
UCSF-SCOR	72 patients with FH and CHD	2	Niacin plus colestipol ± lovastatin (+ diet) versus diet alone	Significant coronary lesion regression in active therapy (p=0.039)	85
HATS	160 patients with CHD, low HDL-C and normal LDL	3	Niacin (IR or ER) plus simvastatin versus antioxidants versus niacin plus simvastatin plus antioxidants versus placebo	Regression of coronary lesions in only the niacin plus simvastatin group (p<0.0001) ↓ clinical events (death, MI, stroke or revascularization) by 88% in only the niacin plus simvastatin group (p=0.04)	39
ARBITER-2	167 patients with CHD and low HDL-C levels	1	ER-niacin versus placebo (added onto stable statin therapy)	↓ rate of carotid IMT progression among those in ER-niacin group without insulin resistance No difference in the overall difference in IMT progression between the niacin and placebo groups.	83
ARBITER-3	130 patients from ARBITER-2	2	ER-niacin versus placebo (added onto stable statin therapy)	Net regression of carotid IMT among those in ER-niacin group at 1 year with even more significant regression at 2 years	82
Fibrates					
Helsinki Heart Study	4,081 men with non-HDL ≥ 5.2 mmol/L	5	Gemfibrozil versus placebo	↓ incidence of CHD by 34%	40
BECAIT	92 men ≤ 45 years old, post-MI	5	Bezafibrate versus placebo	↓ coronary event rate (p=0.02) ↓ coronary atherosclerosis progression (p<0.05) with bezafibrate	98
Frick *et al.*	395 men post-CABG	3	Gemfibrozil versus placebo	↓ progression of native and vein-graft atherosclerosis	158
DAIS	731 type 2 diabetic patients	3	Micronised fenofibrate versus placebo	↓ coronary atherosclerosis progression with fenofibrate	99
VA-HIT	2,531 men with CHD	5	Gemfibrozil versus placebo	↓ rate of death from CHD or non-fatal MI by 22% with gemfibrozil	41
HDL therapy					
ERASE	145 patients with ACS	1 month	Reconstituted HDL versus placebo	↓ total atheroma volume in HDL infusion arm by 3.4% (post-infusion versus pre-infusion) ↓ plaque characterization index and coronary score	81
Nissen *et al.*	47 patients with ACS	5 weeks	$APOA1_{Milano}$ versus placebo	Significant regression of coronary atherosclerosis	80

ACS, acute coronary syndrome; APOA1, apolipoprotein A1; ARBITER, Arterial Biology for the Investigation of the Treatment Effects of Reducing cholesterol trials; BECAIT, Bezafibrate Coronary Atherosclerosis Intervention Trial; CABG, coronary artery bypass graft; CHD, coronary artery disease; CLAS, Cholesterol Lowering Atherosclerosis Study; DAIS, Diabetes Atherosclerosis Intervention Study; ER, extended-release; FATS, Familial Atherosclerosis Treatment Study; FH, Familiar Hypercholesterolemia; ERASE, Effect of rHDL on Atherosclerosis Safety and Efficacy trial; HATS, HDL Atherosclerosis Treatment Intervention Study; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; IR, immediate-release; LDL, low-density lipoprotein; MI, myocardial infarction; USCF-SCOR, University of California, San Francisco, Specialized Center Of Research; VA-HIT, Veterans Affairs HDL Intervention Trial.

been thwarted by its main side effect: skin flushing, and related vasodilatory symptoms. The combination of ER-niacin and a selective prostaglandin D_2 receptor 1 antagonist, laropiprant (MK-0524), has been shown to reduce niacin-related flushing by about 50%[90]. This MK-0524A combination (MK-0524 and niacin) and a MK-0524B combination (MK-0524A and simvastatin) are currently the focus of two separate clinical outcome trials[91]. Other future clinical trials will formally evaluate the effect of adding niacin to existing statin therapies on atherosclerotic outcomes[92].

Despite evidence that niacin affects lipolysis, the precise mechanisms of action are incompletely defined[93]. Niacin treatment of wild-type mice does not raise HDL-C levels, whereas treatment of CETP transgenic mice does increase HDL-C levels, suggesting that niacin might affect CETP[94]. Niacin also prevents endothelial-cell-mediated oxidation of LDL[93]. Furthermore, niacin decreases APOA1 catabolism, causing a rise in APOA1-containing HDL particles, which are more efficient in RCT[95]. Niacin inhibits fatty acid synthesis and triglyceride synthesis, thus increasing APOB degradation, decreasing APOB secretion and reducing triglyceride levels[93]. As HDL-C and triglyceride levels are inversely correlated, lowering triglycerides attenuates CETP-mediated depletion of CE in HDL, and possibly prolongs APOA1 half-life. Niacin has also been associated with increased large HDL, no change in small HDL and decreased small, dense LDL particles[96]. Niacin might also promote cholesterol efflux through a peroxisome proliferator-activated receptor (PPAR)-dependent pathway[93]. Furthermore, niacin reduces C-reactive protein and fibrinogen[86]. Thus, niacin exerts multiple cardioprotective mechanisms, and its observed favourable efficacy suggests that its continued evaluation might be very worthwhile.

Fibric acid derivatives (fibrates) and increased HDL-C. Fibrates are weak PPAR-α agonists that raise HDL-C levels from 5 to 50% while lowering triglycerides and both total and LDL cholesterol. Downregulation of APOC3 increases VLDL clearance whereas upregulation of APOA1, SCRB1 and ABCA1 transporter genes increases APOA1-incorporation into HDL and enhances cholesterol efflux through RCT. Fibrates also decrease levels of fibrinogen and C-reactive protein. The commonly used fibrates, namely gemfibrozil, fenofibrate, bezafibrate and ciprofibrate, have inter-individual differences in both molecular and biochemical effects[97].

Some fibrate trials have shown reduced cardiovascular morbidity, for instance in the Helsinki Heart Study (HHS) and Veterans Affairs HDL Intervention Trial (VA-HIT), or reduced progression of angiographically-defined coronary atherosclerosis, for instance in the Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) and Diabetes Atherosclerosis Intervention Study (DAIS)[40,41,98,99]. The HHS reported that gemfibrozil increased HDL-C levels by 11% and reduced CHD by 34%[40]. The VA-HIT reported that gemfibrozil increased HDL-C by 6% and reduced CHD death or non-fatal MI by 22%[41]. Each 0.13 mmol/L increase in HDL-C was associated with an 11% reduction in cardiovascular events[79].

However, two large fibrate trials have given negative results. The Bezafibrate Infarction Prevention (BIP) study evaluated the effects of bezafibrate versus placebo in 3,090 individuals with prior MI or stable angina. The study showed no difference in the primary endpoint of fatal or non-fatal MI or sudden death, despite an 18% increase in HDL-C and a 21% decrease in triglyceride levels in those on bezafibrate[100]. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial demonstrated no difference in CHD events or mortality among diabetic patients randomized to either fenofibrate or placebo[101]. The lack of benefit in BIP and FIELD could have been due to the high numbers of patients in the placebo groups who received additional 'drop-in' lipid-lowering treatment, mainly statin drugs[100,101]. Also, fenofibrate therapy in FIELD increased HDL-C by only 3%, an effect that is perhaps too small to be expected to produce any benefit[101].

Future HDL-based therapies

Phospholipids. Phospholipid liposomes mediate RCT and promote cholesterol efflux[102]. Liposomes are too large to cross the endothelium and instead accept cholesterol that has been shuttled across the endothelium by HDL. The interaction of the liposome with HDL creates a cholesterol-poor, phospholipid-rich particle that is thought to have enhanced cholesterol efflux capability[103]. In animal models, phospholipids increase transcription of APOA1, enhance the inhibition of monocyte migration, increase HDL-C levels and decrease atheroma formation[104,105]. Anionically charged phosphatidylinositol (PI), which is a small component of the phospholipid layer, inhibited LCAT activity, reduced synthesis and storage of CE, increased cholesterol efflux and decreased cholesterol synthesis and esterification[106,107]. In patients, PI administration increased HDL-C and APOA1 levels by up to 18% and 6%, respectively (without apparent side effects) after two weeks[108]. As PI is derived from soy lecithin, potential adverse effects include an allergic response. Further investigation in humans, particularly examining primary and surrogate atherosclerotic outcomes, will help to determine the utility of this class of agents.

Reconstituted HDL. Reconstituted HDL not only induces effective cholesterol efflux but also inhibits pro-inflammatory changes and platelet aggregation[103,109,110]. Infusion of reconstituted HDL in cholesterol-fed rabbits significantly reduced atheroma size compared with oral atorvastatin. Interestingly, particle composition seemed to be an important determinant, with certain phospholipid combinations apparently being more effective than others[111]. Mean HDL-C levels were increased by 69% and 225% among patients with hypercholesterolaemia and Tangier disease, respectively, and intravenous infusion of reconstituted HDL normalized endothelium-mediated vasodilation through increased nitric oxide bioavailability[112,113]. Reconstituted HDL *in vitro* directly induced endothelial progenitor-cell differentiation and increased angiogenesis within ischaemic muscle[114]. Theoretically, these activities make reconstituted HDL an ideal agent for

Tangier disease
A rare autosomal recessive lipoprotein disorder characterized by low to absent high-density cholesterol levels with deposition of cholesterol esters in reticuloendothelial cells, leading to tonsillar enlargement, hepatomegaly, splenomegaly and lymphadenopathy. The disease is caused by mutations in the ATP-binding cassette subfamily A, member 1 (*ABCA1*) gene.

patients with acute coronary syndromes. Indeed, reconstituted HDL infusion in patients with acute coronary syndrome was associated with reduced atheroma volume when compared with baseline (pre-infusion) values, and with an improvement of plaque quality compared with placebo[81]. Even though reconstituted HDL infusion increases HDL-C, the torcetrapib experience indicates that this may not lead to positive clinical outcomes.

APOA1 mimetic peptides. APOA1 is found on virtually all HDL particles and accounts for about 70% of the total protein content of the HDL particle[6]. APOA1 is composed of 243 amino acids arranged in 10 amphipathic helices. In animal models, peptide analogues as short as 18 amino acids mimicking the lipid-binding domain of APOA1 have shown beneficial effects, including reduced atherosclerotic lesions (by up to 79%), reduced lipid peroxidation, improved endothelial dysfunction, decreased pro-inflammatory HDL and depressed monocyte recruitment, without increased HDL-C levels[115–118]. In animal models, the orally stable, APOA1 mimetic peptide D4F decreased plaque size by 43% and lipid content by 70% in evolving lesions within vein bypass grafts, but had no significant effect on established lesions. However, another study showed that D4F decreased established atherosclerotic lesions[116]. This discrepancy might relate to the timing of D4F administration, with earlier initiation leading to greater benefit[116,119]. Oral D4F inhibits lipid hydroperoxides, increases PON1 activity, increases the formation of pre-β HDL and improves the ability of HDL to efflux cholesterol[120]. So, although D4F has potential benefits, consistent reductions in atheroma need to be demonstrated for this therapeutic class before it can be taken forward.

APOA1$_{Milano}$. Individuals with the APOA1$_{Milano}$ mutation have low HDL-C levels without increased CHD risk[121]. Recombinant APOA1$_{Milano}$ administration to mice halted atherosclerosis progression, induced a favourable change in plaque composition by decreasing lipid and macrophages, and increased cholesterol efflux capacity[122,123]. Yet the precise atheroprotective mechanisms for APOA1$_{Milano}$ remain unclear; cholesterol efflux with APOA1$_{Milano}$ did not differ from wild-type APOA1[124]. In addition, vector-based gene transfer of APOA1$_{Milano}$ or wild-type APOA1 into LDL-receptor deficient mice showed similar delays in atherosclerosis progression[125]. Conversely, serum from APOA1$_{Milano}$-carriers was more efficient in cholesterol efflux through the ABCA1 pathway compared with control serum[126]. In addition, a randomized clinical trial of an intravenous infusion of APOA1$_{Milano}$ complexed with phospholipid in individuals with acute coronary syndrome demonstrated significant regression of coronary atherosclerosis compared with baseline values after just 6 weeks[80]. As with reconstituted HDL, the need for intravenous infusion limits the long-term application of this potential treatment, and efficacy might depend on optimizing the set of phospholipid complexes for administration of APOA1$_{Milano}$. Further studies are needed to elucidate pathways affected by APOA1$_{Milano}$ and to define the ideal APOA1$_{Milano}$-infusion features.

APOA1$_{Milano}$ mutation
A cysteine to arginine substitution at position 173 in the apolipoprotein A-1 (*APOA1*) gene. Individuals possessing this mutation have low levels of high-density lipoprotein cholesterol and increased levels of triglycerides without an associated increased risk for cardiovascular disease.

Other potential therapies. Therapies to increase HDL-C can focus on either increasing HDL/APOA1 production or on decreasing catabolism. PPAR-α agonism increases APOA1 and APOA2 production while stimulating VLDL catabolism, whereas PPAR-γ agonism enhances adipocyte differentiation, improves insulin sensitivity and induces lipid efflux from macrophages[127]. Thus, dual PPAR-α/γ agonists might not only raise HDL-C levels, but also improve metabolic and cardiovascular outcomes. The first such agent, muraglitazar, raised HDL-C levels by up to 19%[128,129]. However, in 2005, muraglitazar failed to receive FDA approval due to associated excess deaths, major cardiovascular events and congestive heart failure[130]. In 2006, AstraZeneca suspended further trials of another dual PPAR agonist, tesaglitizar, due to an inadequate benefit-to-risk ratio and an unacceptable increase in serum creatinine[131]. The concerns surrounding adverse events have curbed enthusiasm for dual PPAR agonists.

Future potential therapies to reduce HDL catabolism include inhibition of HL, EL or SCRB1. Both HL and EL hydrolyse phospholipids from HDL during RCT and thereby promote HDL catabolism. Although HL has been shown to positively correlate with APOA1 catabolism, the role of HL is complex as it also appears to clear atherogenic APOB-containing particles[7]. Thus, inhibition of HL might increase not only APOA1 and HDL-C levels but also APOB-containing particles; also patients with genetic HL deficiency develop early atherosclerosis despite very high HDL-C levels (R.A.H., unpublished data).

Interfering with EL has been proposed as another therapeutic possibility to increase HDL-C. EL can be inactivated by hepatic pro-protein convertases (PCs); inhibiting PCs leads to increased EL function with subsequent decreases in HDL-C and decreases in RCT[132]. In humans, plasma EL levels have been associated with coronary atherosclerosis[133]. Thus, strategies to either inhibit EL or enhance PC activity could help increase HDL-C levels and perhaps decrease cardiovascular disease risk, although such strategies are less well-developed than others already mentioned.

SCRB1 is a hepatic receptor involved in the uptake of cholesterol esters from HDL, leading to recycling of APOA1 particles during the terminal phase of RCT. Although SCRB1 knockout mice have decreased hepatic HDL uptake, and thus increased HDL-C levels, SCRB1-deficiency increases atherosclerosis in murine models[134–137]. Strategies to increase SCRB1 activity might reduce atherosclerosis, despite the associated decrease in HDL-C levels, and could be a promising area for research.

ABCA1 and ABCG1 are important transporters in early RCT that efflux cholesterol from peripheral cells to HDL particles. They are primarily regulated by liver X-receptors (LXR), which are nuclear transcription factor receptors that are also involved in intestinal cholesterol absorption, lipogenesis and bile-acid synthesis [138]. Early studies of pharmacological LXR activation showed increases in HDL-C and triglyceride levels due to increased sterol regulatory element-binding protein

expression[139]. Recently, treatment with selective LXR agonists increased HDL-C levels by up to 48%, without increasing triglyceride levels, and improved atherosclerosis in animal studies[140–142]. Additional research, including evaluation of surrogate markers, is needed to determine the potential use of these agents.

The endocannabinoid system has recently been shown to be important in insulin resistance and dyslipidaemia. The selective cannabinoid-1 receptor antagonist rimonabant increased HDL-C levels by up to 8% and decreased triglycerides by up to 13% after one year in both the Rimonabant in Obesity (RIO)-North America and RIO-lipids trials[143,144]. The RIO-diabetes trial, involving overweight and obese patients, revealed similar improvements in HDL-C and triglyceride levels. Importantly, 57% of the total increase in HDL-C could not be attributed to the observed weight loss[145]. The precise mechanism by which rimonabant increased HDL-C levels is unknown. Unfortunately, the side effects of rimonabant (nausea and central nervous system effects such as dizziness, anxiety, insomnia, depression and possible increased suicidality) have delayed FDA approval[143–146].

Diet and exercise

The combination of a low saturated fat diet and increased exercise raises HDL-C levels by 5–14% and lowers triglyceride, LDL-C and total cholesterol levels by 4–18%, 7–15% and 7-18%, respectively[147]. Thus, simple lifestyle measures including a prudent diet and increased activity represent a cost-effective and low-risk intervention that is associated with a range of other additive health benefits including improvements in dysglycaemia, blood pressure and CHD risk[148–150].

Conclusions

The primary focus of pharmaceutical lipid modulation has been LDL-C reduction; this strategy has reduced cardiovascular morbidity and mortality by up to 25%. Now, raising HDL-C has emerged as a potential strategy to tackle the residual risk that remains even after lowering LDL-C. Because of the biological complexity discussed above, simple elevation of HDL-C levels alone might not translate into clinical benefit. It is possible that future validated measurements of either HDL subclasses or HDL function might be more useful in determining the potential use of future HDL-based therapies. Current animal models have key differences in HDL metabolism and transgenic experiments require careful interpretation. Discrepant effects of drugs on lipid metabolism in animals compared with humans are not uncommon[151]. However, even with newer methods and models to understand HDL, the torcetrapib experience indicates that any new HDL-based therapy will require demonstration of a beneficial effect on the atherosclerotic plaque early in development, through validated surrogate imaging endpoints or benefit on clinical outcomes. This will require closer collaboration between industry and academic researchers. Despite the disappointments of torcetrapib and fibrate trials, HDL-based therapies such as D4F, reconstituted HDL, phospholipids and $APOA1_{Milano}$ have shown some promising results. Moreover, trials involving niacin seem to consistently indicate favourable clinical and surrogate outcomes. At present, no evidence suggests that all HDL-based therapies will be ineffective in atherosclerosis. Thus, the development of alternative agents and strategies focusing on HDL should, in our opinion, continue to be an important goal of future cardiovascular research.

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