Lipids and cardiovascular disease 2

HDL and cardiovascular disease

Daniel J Rader, G Kees Hovingh

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This is the second in a **Series** of three papers about lipids and cardiovascular disease

Department of Medicine and Department of Genetics, Institute for Translational Medicine and Therapeutics, and Cardiovascular Institute, Perelman School of Medicine of the University of Pennsylvania, Philadelphia, PA, USA (Prof D J Rader MD); **and Department of Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands** (G K Hovingh MD)

Correspondence to: Prof Daniel J Rader, 11–125 Smilow Center for Translational Research, 3400 Civic Center Blvd, Philadelphia, PA 19104, USA **rader@mail.med.upenn.edu**

The cholesterol contained within HDL is inversely associated with risk of coronary heart disease and is a key component of predicting cardiovascular risk. However, despite its properties consistent with atheroprotection, the causal relation between HDL and atherosclerosis is uncertain. Human genetics and failed clinical trials have created scepticism about the HDL hypothesis. Nevertheless, drugs that raise HDL-C concentrations, cholesteryl ester transfer protein inhibitors, are in late-stage clinical development, and other approaches that promote HDL function, including reverse cholesterol transport, are in early-stage clinical development. The final chapters regarding the effect of HDL-targeted therapeutic interventions on coronary heart disease events remain to be written.

Genesis of the HDL hypothesis

HDLs were first described in the 1960s after isolation by ultracentrifugation. Advances in precipitation of apoB-containing lipoproteins made it possible to measure the cholesterol content of HDL (HDL-C) in many individuals and enabled large-scale epidemiological studies of the relation between HDL-C concentrations and coronary heart disease. The first compelling reports of the strong inverse association between HDL-C and coronary heart disease were from the Framingham Heart Study.[1] These observational data formed the basis for the widely acknowledged concept of HDL as the good cholesterol and led to the idea that HDL might have properties that protect against coronary heart disease, and therefore that intervention to raise HDL-C would reduce risk of coronary heart disease (the HDL hypothesis). Indeed, Glomset and colleagues[2] originated the concept of reverse cholesterol transport and speculated that HDL, by promoting this process, protected against coronary heart disease.

The hypothesis that HDL is protective against atherosclerosis was supported by a series of animal studies in the 1980s and 1990s. Badimon and colleagues[3] infused HDL into rabbits and reported inhibition of atherosclerosis. Rubin and colleagues[4] showed that mice overexpressing the major HDL protein apolipoprotein A-I (apoA-I) are protected from atherosclerosis. Viral overexpression of apoA-I in mice with pre-existing atherosclerosis resulted in regression of pre-existing atherosclerotic disease.[5] These preclinical data matched the epidemiological data and strongly reinforced the HDL hypothesis, making HDL a major target for novel therapeutic approaches to decrease atherosclerosis.

Search strategy and selection criteria

We searched PubMed using the terms "high density lipoprotein cholesterol", "cholesterol efflux", and "reverse cholesterol transport" from 2004 to 2014. Relevant articles were chosen and their references were secondarily searched for additional relevant articles with no limit on the original date of the article.

In parallel, major advances were made in the understanding of the molecular and physiological regulation of plasma HDL-C concentrations. The comprehensive, but probably incomplete, model of HDL metabolism, based on work from many laboratories worldwide[6] is shown in the figure. Biogenesis of HDL occurs in the liver and intestine, which both synthesise and secrete apoA-I. Shortly after secretion as a lipid-poor protein, apoA-I interacts with the cholesterol–phospholipid transporter ABCA1 (ATP Binding Cassette A1) expressed by hepatocytes and enterocytes to acquire lipids, thereby generating a nascent HDL particle.[7] HDL acquires additional lipids and apolipoproteins derived from the hydrolysis of triglyceride-rich lipoproteins, and this process partly accounts for the strong inverse relation between triglycerides and HDL-C. The enzyme lecithin cholesteryl acyl transferase (LCAT) acts on cholesterol in nascent HDL particles to generate cholesteryl ester, which forms the core of the mature HDL particle.[8]

Two metabolic pathways of clearance of cholesteryl ester in HDL have been described: direct uptake by the liver or steroidogenic tissues via the HDL receptor scavenger receptor B1 (SR-BI), or transfer to apoB-containing lipoproteins (usually in exchange for triglyceride) by the plasma protein cholesteryl ester transfer protein (CETP). Uptake via SR-BI is selective and after removal of cholesteryl ester the smaller apoA-I containing HDL particle dissociates and recycles.[9] The effect of CETP is not only depletion of cholesteryl ester from the HDL particle, but also triglyceride enrichment of the particle. This triglyceride-enriched HDL is susceptible to lipolytic modification by hepatic lipase and endothelial lipase. Upon modification by these two enzymes, a smaller HDL particle is formed, which is susceptible to faster catabolism. At least some apoA-I is catabolised in the kidneys after the lipid-poor form is filtered and then taken up via a process involving the proteins cubilin and megalin, and degraded by proximal tubular cells.[10]

Thus, many apolipoproteins, enzymes, lipid transfer proteins, cellular lipid transporters, and cell surface receptors act together to regulate HDL metabolism in a way that ultimately determines the plasma HDL-C

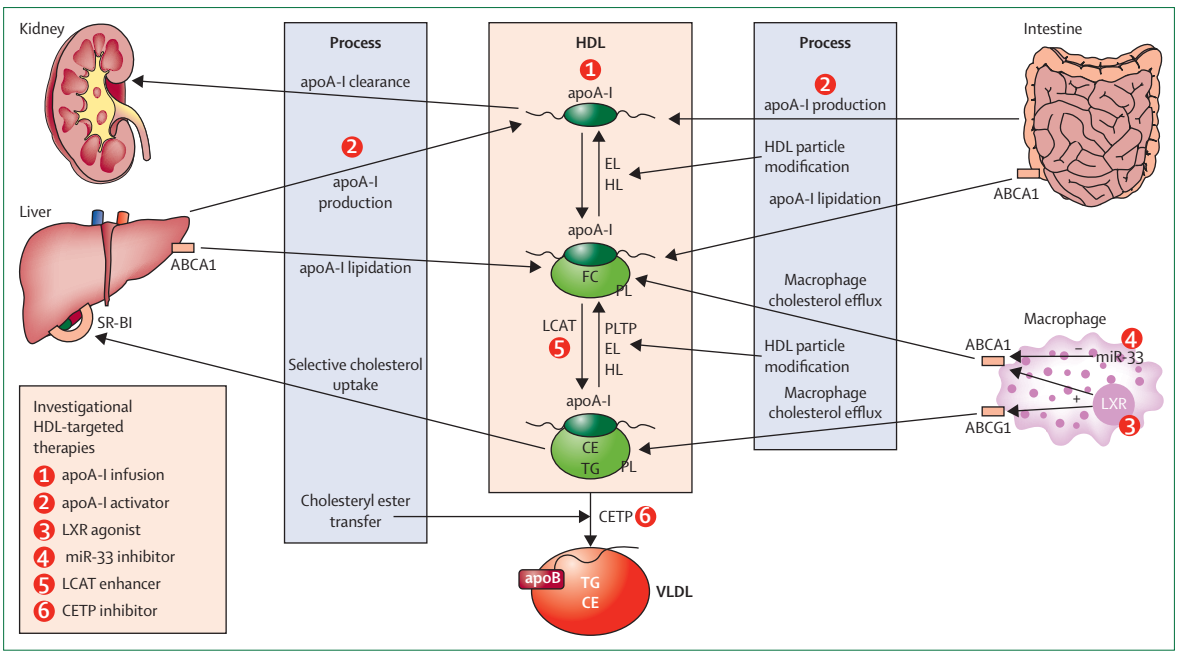

Figure: **HDL metabolism and the action of HDL-targeted therapies**
apoA-1=apolipoprotein A-I. LXR=liver X receptor. LCAT=lecithin cholesteryl acyl transferase. CETP=cholesteryl ester transfer protein. CE=cholesteryl ester. TG=triglyceride. PL=phospholipid. EL=endothelial lipase. HL=hepatic lipase. FC=free cholesterol. PLTP=phospholipid transfer protein. VLDL=very low-density lipoprotein.

concentration. This complex metabolism results in various HDL particles of different density, size, and composition. The plasma HDL-C concentration is a reflection of the net state of production, modification and catabolism of HDL particles and, as a result, should not be interpreted as a measure of reverse cholesterol transport-mediated flux.

Several medical and environmental factors influence HDL metabolism, including those that tend to reduce HDL-C concentrations (eg, obesity, type 2 diabetes, inflammation, and smoking) and those that increase HDL-C concentrations (eg, oestrogen, thyroid hormone, exercise, and alcohol use).[11] HDL-C concentration is therefore a reflection of various metabolic and inflammatory processes, and this partly explains its strong inverse association with coronary heart disease. Nevertheless, improved understanding of HDL metabolism gave rise to therapeutic targets with potential to increase HDL-C concentrations and, according to the HDL hypothesis, reduce risk of coronary heart disease.

HDL-C still has value as a predictor of cardiovascular risk

Although the HDL hypothesis has been challenged, it is important to emphasise that its value as a predictor of cardiovascular risk remains largely unchallenged. Many prospective studies from different racial and ethnic groups worldwide have confirmed that HDL-C is a strong, consistent, and independent predictor of incident cardiovascular events (myocardial infarction, ischaemic stroke).[11,12] Strong data also exist regarding HDL-C as a predictor of incident cardiovascular events in the setting of secondary prevention in individuals who have already been diagnosed with cardiovascular disease. In the statin era, the relation of HDL-C to cardiovascular events in patients treated with statins is less clear. In some large clinical trials with statins, on-treatment HDL-C was predictive of incident cardiovascular events,[13] whereas in others it was not.[14] In the JUPITER trial, the ability of HDL-C to predict incident events in patients treated with high-dose statin was weak.[15] However, in a large meta-analysis of eight statin trials, among statin-treated patients baseline HDL-C concentrations were strongly predictive of subsequent cardiovascular events.[16]

The usefulness of HDL-C as an independent risk predictor is shown by its continued central use in cardiovascular risk equations. For example, in the recent American Heart Association and American College of Cardiology risk calculator HDL-C is a critical component in risk prediction,[12] in agreement with the European guidelines issued a few years before.[17] Thus, regardless of the nature of the relation of HDL-C with cardiovascular disease, its use as a predictor of risk remains unchallenged.

There is substantial interest in other measures of HDL-related analytes and whether they could provide predictive power that exceeds that of HDL-C itself. For example, many prospective studies have assessed plasma apoA-I concentrations and compared them with HDL-C in predicting incident cardiovascular events. Overall, the conclusion is that apoA-I might be slightly better at predicting risk than HDL-C,[18] although not all large

studies support this conclusion.[19] Although there has been historical interest in the HDL subfractions HDL_2 and HDL_3, neither is significantly better at predicting risk than HDL-C itself.[20] More recently, there has been interest in the measurement of HDL particle number (HDL-P), which is done through nuclear magnetic resonance lipoprotein analysis. Studies that have compared HDL-P with HDL-C,[21] including those in the setting of high-dose statin therapy,[22] have concluded that HDL-P could be better than HDL-C in predicting incident events. However, a change to measurement of HDL-P instead of HDL-C in clinical practice will probably need more head-to-head comparative data.

Challenges to the HDL hypothesis and the causality of HDL in cardiovascular disease risk

Challenges to the HDL hypothesis are driven by data derived from human genetics studies and randomised controlled trials. Much has been learned from the study of Mendelian disorders of low HDL-C. The three Mendelian disorders causing primary extreme low HDL-C include mutations in apoA-I, ABCA1, and LCAT.[23] Despite HDL-C concentrations that are less than the 5th percentile, none of these disorders is unequivocally associated with premature coronary heart disease.[23–26] Heterozygosity for structural mutations in apoA-I (eg, $apoA\text{-}I_{Milano}$[27]) has been associated with no increased risk of atherosclerosis despite low concentrations of HDL-C (there are a few exceptions such as the L178P mutation in apoA-I[28]). Similar conflicting data are from patients with deleterious ABCA1 mutations, which when they occur in both alleles cause Tangier disease. Despite the almost undetectable plasma HDL-C and apoA-I concentrations, these patients do not routinely develop premature coronary heart disease,[29,30] but the rarity of the disease makes it difficult to define the risk for coronary heart disease. Whether heterozygotes for loss-of-function mutations in ABCA1, who have about half the normal concentrations of HDL-C, are at increased risk of coronary heart disease is still debated. Although an imaging study suggested increased atherosclerosis in Tangier heterozygotes,[31] larger population-based studies have suggested that there is no increased risk of cardiovascular events.[32] Homozygous deficiency of LCAT causes very low concentrations of HDL-C and early chronic kidney disease but premature coronary heart disease is not a common clinical observation in these individuals.[33,34] Heterozygosity for loss-of-function mutations in LCAT slightly reduces HDL-C concentrations, and whether this translates into increased risk of coronary heart disease is debated. Whereas some imaging studies have shown increased atherosclerosis in LCAT heterozygotes,[35,36] others have not.[37] Overall, the cardiovascular risk associated with genetically reduced LCAT activity and lower HDL-C concentrations is not increased.[38] Conversely, CETP deficiency is a Mendelian cause of markedly raised HDL-C but despite being described more than 20 years ago, it is not clear whether CETP deficiency protects against coronary heart disease.[39]

This confusing and seemingly paradoxical picture regarding the rare Mendelian disorders is further compounded by recent data on low-frequency and common genetic variants associated with variation in HDL-C. For example, loss-of-function variants in endothelial lipase (gene name *LIPG*) are associated with raised HDL-C concentrations.[40] A *LIPG* variant N396S present in 1–2% of individuals of European ancestry raises HDL-C and apoA-I concentrations[40] but was found not to be associated with protection from coronary heart disease.[41] By contrast with LDL-C, in which the most common variants associated with LDL-C are also associated with coronary heart disease, most common variants associated with HDL-C have no association with coronary heart disease.[41,42]

Many variants not only affect HDL-C concentrations, but also have an effect on other lipoproteins. As a result, the question as to whether HDL-C concentrations are directly related with cardiovascular disease risk cannot be answered by studies of these variants. Johannsen and colleagues[43] found a highly significant association between genetic variation in the CETP gene that caused higher HDL-C concentrations and reduced cardiovascular disease risk. However, LDL-C, non-HDL-C, and triglyceride concentrations were significantly lower in carriers of the HDL-increasing CETP alleles, which makes it hard to understand the direct effect of the HDL-C phenotype on the risk of cardiovascular disease. Variants associated with triglycerides are often associated with coronary heart disease even after adjusting for their effects on HDL-C, whereas variants associated with HDL-C are rarely associated with coronary heart disease after adjusting for their effects on triglyceride concentrations.[42] Thus, the aggregate lesson from human genetics is that genetically altered HDL-C concentrations do not necessarily translate to an altered risk of coronary heart disease.

In addition to the human genetics, reports from several randomised clinical trials of HDL-raising drugs have failed to show a reduction in cardiovascular events. Although each of these trials have their caveats, taken together they contribute to the perception that raising HDL-C concentrations per se does not necessarily confer a cardiovascular benefit or result in a beneficial effect on coronary heart disease outcome measures. Nicotinic acid (niacin) has been used for more than 50 years as a cholesterol-lowering and HDL-raising drug. Historically, one major clinical outcome trial with niacin (using immediate-release niacin), the Coronary Drug Project, was done in the pre-statin era. The trial investigated lowering of cholesterol in men with hypercholesterolaemia and showed the benefit of niacin treatment compared with placebo in reducing cardiovascular events.[44] Administration of niacin resulted in significant

cholesterol reduction in this trial, which probably contributed to, or even accounted for, its benefit. However, two recent trials of niacin (using extended-release niacin; AIM-HIGH[45] and HPS2-THRIVE[46]) were done on the background of statin therapy and were primarily designed to show the benefit of the HDL-raising effects of niacin. Neither trial met its primary endpoint and niacin failed to reduce cardiovascular events in both trials. Based on this, extended-release niacin added to a statin in patients with reasonably controlled LDL-C concentrations does not confer a cardiovascular benefit despite a slight increase in HDL-C concentrations. As a result, niacin should not be considered a therapeutic option for raising HDL-C concentrations.

Experience with CETP inhibitors has been most problematic for the HDL hypothesis. Torcetrapib was the first CETP inhibitor to enter a phase 3 cardiovascular outcome trial (ILLUMINATE). This trial was stopped prematurely due to increased coronary heart disease events and total mortality in patients who were being randomised to torcetrapib plus standard statin treatment.[47] Torcetrapib was subsequently described to have off-target effects on blood pressure and adrenal hormone production, which could have contributed to the adverse outcomes, despite the more than 70% increase in HDL-C concentrations in torcetrapib-treated individuals.[48] More troublesome for the HDL hypothesis was the experience with dalcetrapib; its phase 3 trial dal-OUTCOMES in patients who had acute coronary syndrome was ended prematurely for futility; there was no trend for benefit despite a more than 25% increase in HDL-C.[49] Although the increase in HDL-C was modest, the epidemiology of HDL-C and coronary heart disease risk would have predicted a benefit of such an increase if the relation were causal.

No association was shown between HDL-C concentrations at baseline and cardiovascular outcome in either group of the dal-OUTCOMES study, which is different from the findings of some secondary prevention studies. HDL particles in patients with acute coronary syndrome could have lost their protective capacity, which is supported by data in patients with stable and unstable coronary heart disease.[50] In the dal-ACUTE study, the effect of dalcetrapib on HDL efflux capacity was disproportionately lower than on HDL-C concentrations, which suggests that improvements in HDL function and HDL-C concentrations are dissociated.[51] Dalcetrapib had no effect on lowering LDL-C concentrations, compared with the CETP inhibitors in clinical development (eg, anacetrapib,[52] evacetrapib,[53] and TA8995[54]). Thus, if these CETP inhibitors significantly reduce cardiovascular events, this could be due to reduction in LDL-C and will not serve as a formal test of the HDL hypothesis.[55]

In a phase 2 trial using coronary atheroma volume by intravascular ultrasound as an endpoint, a novel apoA-I transcriptional upregulator (RVX-208) was found to have no significant effect on plaque size.[56] All trials with HDL-directed therapies have been carefully examined,[57] and certainly have their caveats, but taken together with the human genetics data create an unmistakable impression that the HDL cholesterol hypothesis is not proven and, in its simplest form, is unlikely to be correct.

Concept of HDL function and cardiovascular disease risk: the HDL function hypothesis

The above developments have led to a recasting of the HDL hypothesis to a different and more subtle concept, namely the HDL function hypothesis.[58] In this view, it is not HDL cholesterol itself that has a causal relation to atheroprotection, but rather HDL function, which cannot be reliably estimated through the simple measurement of HDL-C. In test tubes, model systems, and even in some human studies, HDL has been shown to have various properties that might reasonably be expected to confer cardiovascular protection.[59] The best known and best studied of these properties is the ability to promote cholesterol efflux from cells (such as macrophages) and the related complex physiological process of reverse cholesterol transport.[60] Studies have shown that lipid-poor apoA-I promotes efflux of cholesterol via the transporter ABCA1 and that mature HDL promotes cholesterol efflux via ABCG1, SR-BI, and probably other mechanisms. The ABC transporters also regulate myelopoesis and inflammatory responses and in model systems have major effects on atherosclerosis.[61] Modification of apoA-I by myeloperoxidase impairs its ability to promote cholesterol efflux, and myeloperoxidase-modified apoA-I is abundant in human atheroma.[62] Methods to test the capacity of HDL from an individual to promote cholesterol efflux from macrophages (HDL cholesterol efflux capacity) have been developed. Most studies have reported a slight correlation between HDL cholesterol efflux capacity and HDL-C concentration, suggesting that HDL-C concentration does not predict the ability of an individual's HDL to promote macrophage cholesterol efflux. Cross-sectional studies of prevalent coronary heart disease and measures of atherosclerosis have indicated that HDL cholesterol efflux capacity is a significant inverse predictor of coronary heart disease even after adjusting for HDL-C concentrations.[63,64] However, in patients recruited from a coronary catheterisation laboratory, HDL cholesterol efflux capacity was positively associated with incident cardiovascular events.[64] Data from large prospective observational studies will be needed to better refine the role of HDL efflux capacity in the risk of coronary heart disease.

Methods of tracing movement of cholesterol from macrophages to the liver and faeces (macrophage reverse cholesterol transport) in animal models have been developed.[60] Overall, the relation of macrophage reverse cholesterol transport to atherosclerosis in the same model system is much stronger (inverse) than of HDL-C to atherosclerosis, suggesting that macrophage reverse cholesterol transport might come closer to a causal pathway leading to atheroprotection. Studies of

macrophage reverse cholesterol transport in human beings are needed to validate this concept. A method of reverse cholesterol transport in human beings[65] was used to show a defect in tissue cholesterol efflux in patients with a loss-of-function mutation in apoA-I.[66] The application of this and a different macrophage-specific method of reverse cholesterol transport in human beings[67] to therapeutic interventions will be important.

HDL and apoA-I have been reported to have various other properties that could be atheroprotective.[59,68] These include anti-inflammatory and anti-oxidant effects, NO-promoting effects, and anti-apoptotic effects. Experiments in model systems have indicated that some of these properties, such as the anti-inflammatory effects, occur in vivo[69,70] and could contribute towards anti-atherosclerotic effects of HDL infusion. Some data in human beings have suggested that these effects, particularly related to enhancing NO production,[71] might be relevant to cardiovascular disease. However, more work will be needed to show the importance of these properties in human beings. A key issue is whether these measures are quantitatively and inversely related to incident coronary heart disease independent of HDL-C concentrations.

Considerations regarding the therapeutic target of HDL

What implications does the above information have for the development of HDL-targeted therapeutics? In our opinion, it cannot be assumed that an intervention that raises HDL-C concentrations will reduce cardiovascular risk (the converse is also true: interventions that reduce HDL-C concentrations cannot be assumed to confer increased cardiovascular risk). However, not all HDL-raising interventions are doomed to failure. It will depend on the mechanism by which the HDL-raising occurs, the effects on relevant HDL functions, and the other effects on lipoprotein fractions (ie, LDL-C, triglyceride-rich lipoproteins, and Lp(a)). An example is CETP inhibitors, of which there are still several in clinical development, including two (anacetrapib and evacetrapib) in phase 3 cardiovascular outcome trials (REVEAL, NCT01252953; ACCELERATE, NCT01687998). These two compounds are very effective in raising HDL-C (>100% at the doses being used in the phase 3 trials). Their effects on HDL function are not fully understood, although some data suggest that they have the potential to promote cholesterol efflux capacity (a conclusion highly dependent on the method used to measure it). Both drugs substantially reduce LDL-C concentrations (>30%) and also reduce concentrations of Lp(a). Therefore, a positive outcome in their phase 3 programmes will not prove that the increase in HDL-C per se provided the benefit. However, a negative outcome with these drugs will permanently bury the formal HDL cholesterol hypothesis and cast a general pall over HDL-targeted therapeutics in general. In this case in particular, answers to whether these CETP inhibitors improve HDL function (or not) will be critical.

Some classes of HDL-targeted therapeutics are focused on promoting aspects of the reverse cholesterol transport pathway rather than on raising HDL-C (figure). For example, infusion of apoA-I containing recombinant HDL particles or of lipid-poor HDL particles is an approach that continues to progress in the clinic. ApoA-I can be isolated from human plasma or made recombinantly. In either case, the apoA-I is recombined with phospholipids to form a recombinant particle that prevents the apoA-I from being rapidly catabolised and provides substantially improved pharmacokinetics. In several preclinical studies, there has been documented benefit on atherosclerosis. For two of these approaches (one involving apoA-I isolated from human plasma and the other a recombinant mutant form called apoA-I_{Milano}) encouraging data in phase 2 trials using coronary intravascular ultrasound were reported.[72,73] A third approach to recombinant apoA-I–phospholipid particles was reported to have no significant effect in a coronary intravascular ultrasound trial.[74] The reasons for these discrepant results are not understood, but all of these trials were small phase 2 trials using a surrogate imaging endpoint. An alternative approach is to delipidate autologous HDL and then re-infuse the lipid-poor HDL; a small coronary intravascular ultrasound study suggested some plaque regression.[75] Infusion of recombinant HDL particles promotes cholesterol mobilisation due to enhancing the macrophage efflux capacity of the plasma in a dose-dependent manner.[76] There is substantial enthusiasm for this approach, which is the closest in concept to the HDL-targeted approaches that have been most successful in animal models.

In theory, a therapeutic approach targeted towards upregulating efflux pathways in macrophages could be atheroprotective.[77] For example, liver X receptors are nuclear receptors that transcriptionally upregulate ABCA1 and ABCG1 in macrophages, and liver X receptor agonists have been shown to promote macrophage reverse cholesterol transport and reduce or even regress atherosclerosis in animals. Liver X receptors remain an attractive therapeutic target. However, the liability of liver X receptor activation in the liver causing increased lipogenesis and liver fat has held back clinical development of compounds for what is otherwise a highly attractive target. Another approach being explored is antagonism of miR-33; this microRNA targets and reduces ABCA1 and ABCG1 expression in macrophages. Antagonising miR-33 results in upregulation of these transporters, and it was shown that an anti-sense oligomer to miR-33 resulted in increased reverse cholesterol transport and reduced atherosclerosis in mice,[78] and increased HDL-C concentrations in non-human primates.[79] Increasing LCAT activity has the potential to promote reverse cholesterol transport and, in theory, reduce atherosclerosis.[34] Infusion of recombinant LCAT, or activation of endogenous LCAT, is an approach that has been of interest to the academic and biopharmaceutical communities alike.

Conclusion

HDL-C is a highly effective biomarker for predicting cardiovascular risk and its use for this purpose is undisputed. Whether other related biomarkers, such as apoA-I or HDL-P, will be unequivocally better remains to be seen. However, the classic HDL hypothesis—defined as the concept that intervention to raise HDL-C concentrations will reduce cardiovascular risk—is questionable and is increasingly difficult to defend in its simplest form. The HDL cholesterol hypothesis is gradually being replaced by the HDL function hypothesis, but the latter remains exactly that—a hypothesis awaiting formal testing and validation. HDL-C and other measures of HDL mass are not adequate surrogates for HDL function. The methods for measuring HDL function and related physiological processes such as reverse cholesterol transport in human beings are in the early stages and are much more complex than a simple measure of HDL mass. New tests of HDL function should be reproducible, straightforward to measure, and show an association with coronary heart disease outcome measures. If successful, the CETP inhibitors in clinical development will not formally prove either the HDL cholesterol or the HDL function hypothesis, and if they fail the HDL function hypothesis will not have been disproven. Ultimately, we will need cardiovascular outcome studies of interventions that unequivocally improve aspects of HDL function to test this new hypothesis. The question is whether the community will remain focused on HDL long enough to allow these critical therapies to reach fruition and be tested in appropriately powered randomised clinical trials.

Contributors

DJR was responsible for the first draft of the report, critcal editing of the report, and editing of the figure. GKH was responsible for the literature search, the intial draft of the figure, and critical editing of the report.

Declaration of interests

DJR reports grants from AstraZeneca, BristolMyersSquibb, and National Institutes of Health, during the conduct of the study. DJR is a founder of VascularStrategies, which performs assays of HDL function as a service, and is an inventor on a patent method for reverse cholesterol transport in human beings that is licensed to VascularStrategies by the University of Pennsylvania. GKH reports grants from the Netherlands Organisation for Scientific Research, outside the submitted work.

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