Review Article

Is high-density lipoprotein a modifiable treatment target or just a biomarker for cardiovascular disease?

JRSM Cardiovascular Disease
Volume 8: 1–8

DOI: 10.1177/2048004019869736
journals.sagepub.com/home/cvd
SAGE

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Abstract

Epidemiological data strongly support the inverse association between high-density lipoprotein cholesterol concentration and cardiovascular risk. Over the last three decades, pharmaceutical strategies have been partially successful in raising high-density lipoprotein cholesterol concentration, but clinical outcomes have been disappointing. A recent therapeutic class is the cholesteryl ester transfer protein inhibitor. These drugs can increase circulating high-density lipoprotein cholesterol levels by inhibiting the exchange of cholesteryl ester from high-density lipoprotein for triacylglycerol in larger lipoproteins, such as very low-density lipoprotein and low-density lipoprotein. Recent trials of these agents have not shown clinical benefit. This article will review the evidence for cardiovascular risk associated with high-density lipoprotein cholesterol and discuss the implications of the trial data for cholesteryl ester transfer protein inhibitors.

Keywords

Atherosclerosis, cardiology, lipid and lipoprotein metabolism

Received 22 August 2017; Revised received 10 July 2019; accepted 11 July 2019

Introduction

Following the publication of several trials showing little to no clinical improvement from raising high-density lipoprotein cholesterol (HDL-C) with the cholesteryl ester transfer protein inhibitor (CETPi) class of drugs,[1–4] it has been questioned whether HDL-C can ever be a therapeutic target in its own right or whether it simply serves as a biomarker of broader dysfunction of the lipoprotein system. This article will review the evidence for cardiovascular risk associated with HDL and discuss the findings of the CETPi trials in light of what we know of HDL functionality.

Outline of HDL structure and metabolism

The formation of HDL begins with the secretion of lipid-poor apolipoprotein A-I (apoA-I) from the liver and intestine. Once released into plasma, apoA1 rapidly acquires free cholesterol and phospholipids from the liver via the receptor ATP-binding cassette transporter A1 (ABCA1), to form the discoidal, pre-β (nascent) HDL particles (Figure 1 and Table 1). There are a variable number of apoA-I proteins per HDL particle and so it must be remembered that apoA-I concentration is not necessarily a one-to-one surrogate marker for HDL particle number.[5] High levels of apoA-I associates with a reduced risk of cardiovascular disease.[6]

The enzyme lecithin–cholesterol acyltransferase (LCAT) is carried by HDL and esterifies the cholesterol (acquired by ABCA1) to form cholesterol ester (CE). The CE then moves into the centre of the HDL particles as a hydrophobic core, altering the discoidal pre-β HDL to form small, spherical, α-HDL particles (HDL_3). The α-HDL can continue to accept cholesterol (for instance from macrophages within the vessel wall) via ABCA1 and via ATP-binding cassette sub-family G member 1 (ABCG1) which are further esterified by

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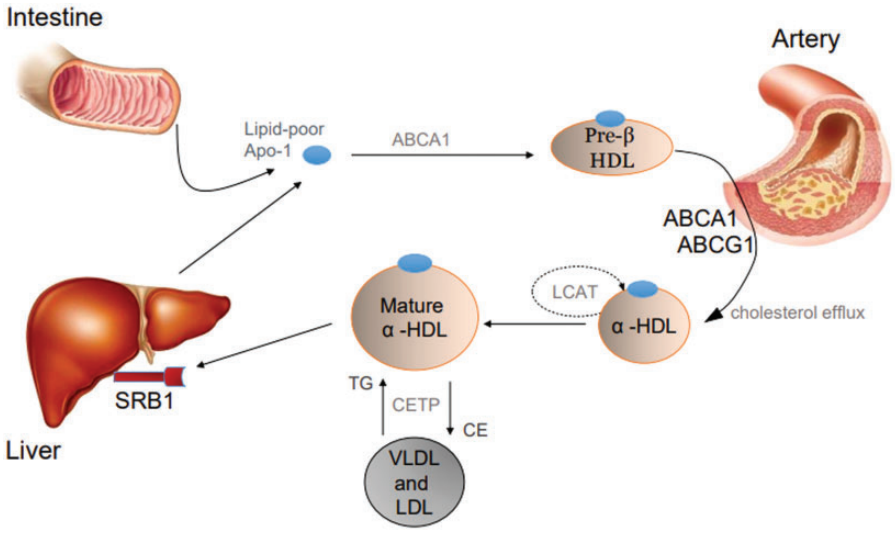

Figure 1. Outline of HDL metabolism.
ABCA1: ATP-binding cassette transporter A1; ABCG1: ATP-binding cassette sub-family G member 1; CE: cholesterol ester; CETP: cholesteryl ester transfer protein; HDL: high-density lipoprotein; LCAT: lecithin–cholesterol acyltransferase; LDL: low-density lipoprotein; SR-B1: scavenger receptor B type 1; TAG: triacylglycerol; VLDL: very low-density lipoprotein.

Table 1. Classifications of the HDL molecule.

	Gel fractionation	Nuclear magnetic resonance spectroscopy	Surface charge
Smaller particles	HDL3c	H1	Pre-beta (1,2,3)
	HDL3b	H2	Pre-alpha (1,2,3)
	HDL3a	H3	
			alpha 4
	HDL2a	H4	alpha 3
			alpha 2
Larger particles	HDL2b	H5	alpha 1

Note: Illustrative only – equivalence of position between columns should not be assumed.
HDL: high-density lipoprotein.

LCAT, leading to the conversion of HDL_3 to the larger HDL_2 (Table 1).

Subsequently, in the 'direct pathway', HDL_2 binds to the extracellular domain of scavenger receptor B type 1 (SR-B1) and CE then taken up by hepatocytes via a lipophilic channel, for subsequent excretion in the bile (this is the basis of the concept of reverse cholesterol transport).[7] The 'indirect pathway' involves the transport of CE to the apolipoprotein B (apoB)-containing lipoproteins. Cholesterol ester transfer protein (CETP) is mainly bound to HDL. This facilitates the exchange of CE in mature HDL particles with triacylglycerol (TAG) in chylomicrons and very low-density lipoprotein (VLDL). Hepatic lipase then leads to the release of TAG from the HDL particle, dramatically reducing its size and releasing lipid-poor apoA-I, which can be degraded by the kidney.

This is a highly simplified account of the HDL molecule and its metabolism. In fact, the HDL molecule is highly complex and contains over 80 proteins, more of which are acute-phase proteins than proteins involved in lipid metabolism – giving credence to the idea that HDL is involved in inflammation.[8]

Depending on the methodology used, HDL particles may be classified in a number of ways which can make discussion of HDL confusing (Table 1).

HDL may be classified by:

a. apolipoprotein content. Lipoprotein A-I contains only apoA-I on its surface, whilst lipoprotein A-I/A-II is an HDL with apoA-I plus A-II on its surface.
b. ultracentrifugation: separated by density which is proportional to the protein and lipid composition: the more protein and less lipid, the denser the particle.

Association of HDL with cardiovascular disease

The HDL 'story' starts in the 1970s and the Framingham cohort: when it was shown that HDL-C had a strong association with coronary artery disease (CAD) (Figure 2). Later, post hoc evaluation of the Treating to New Targets study showed that even

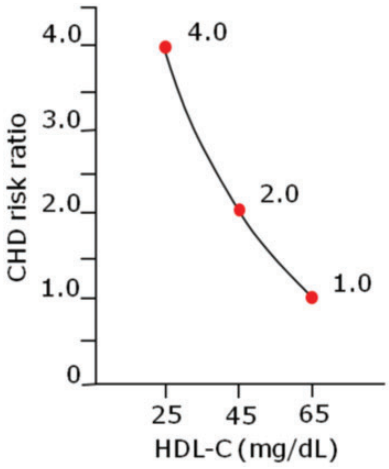

Figure 2. Relative risk of CHD risk according to HDL-C concentration, from the Framingham study. Equivalent values for HDL-C (in mmol/l): 25 mg/dl = 0.65 mmol/l; 45 mg/dl = 1.17 mmol/l; 65 mg/dl = 1.68 mmol/l. Source: Modified with permission from Kannel.[9]
CHD: coronary heart disease; HDL-C: high-density lipoprotein cholesterol.

when LDL-cholesterol (LDL-C) was tightly controlled, there was still an association between low HDL-C and increased risk for cardiovascular events.[10] Overall, increasing HDL-C by 2–3% was associated with a reduction in the risk of cardiac events of 2–4%, independent of the LDL-C level.[11] However, more recent data, from the Secondary Manifestations of Arterial Disease study as well as the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) study have suggested that the inverse association between HDL-C and vascular events can be abolished in patients who are well-controlled on statin therapy.[12,13]

The Framingham data showed that patients with diabetes had lower plasma HDL-C and higher TAG concentrations. In these patients, HDL-C was a less powerful prognosticator than non-HDL-C. The metabolic syndrome is characterized by abdominal obesity, low HDL-C, raised TAG, elevated blood pressure, and impaired glucose tolerance.[14,15] The National Cholesterol Education Program (NCEP) Adult Treatment Panel III adopted HDL-C levels of <1.0 mmol/l in men and <1.3 mmol/l in women to indicate the cutoff for low HDL-C in metabolic syndrome.[15] This recognizes the differential risk that HDL-C has between genders. When NCEP III criteria were retrospectively applied to the National Health and Nutrition Examination Survey II Mortality Study, there was a near linear relationship between the number of metabolic syndrome criteria present and mortality from cardiovascular disease.[16] However, in this study HDL-C was not independently associated with death.

Since the Framingham study, HDL-C has been incorporated into a number of cardiovascular risk prediction models. These models frequently utilize HDL-C in a ratio with LDL-C, or with total cholesterol, as this provides greater discriminatory and predictive power for coronary heart disease than lipoproteins considered in isolation.[17] ApoB may also be usefully measured as it represents the total number of potentially atherogenic lipoproteins. As a result, apoB may improve coronary heart disease (CHD) risk assessment by identifying more high-risk individuals than the usual lipid profile alone.[18] The correlation between apoA-I concentration and HDL particle number is less accurate, with coefficients of 0.54 and 0.69.[5] At a population level, the information gained from measurement of these apolipoproteins only adds moderately to the information derived from traditional cholesterol measurements (as used in the validated European Systematic Coronary Risk Evaluation classification, for example) and so is not advocated for routine use.[19,20]

First intervention trials

In the late 1980s–1990s, therapeutic trials held promise that raising HDL-C could reduce vascular burden. In the Familial Atherosclerosis Treatment Study, patients with CAD were randomized to niacin and the colestipol (bile acid resin), statin monotherapy, colestipol monotherapy, or placebo. After 2.5 years, HDL-C in the niacin–colestipol group increased by 43%. Multivariate analysis indicated that an increase in HDL-C correlated independently with regression of coronary lesions on angiography.[21] Ten years later the HDL-Atherosclerosis Treatment Study was published. This was a study of 160 men with CAD and low HDL-C and showed that coronary stenosis *progressed* by 3.9% over three years in patients randomized to placebo but *regressed* by 0.4% with simvastatin–niacin.[22] These promising results – showing that raising HDL-C (with niacin) could improve surrogate markers of cardiovascular disease – raised expectations for the AIM-HIGH study.[23] This was the first well-powered trial (3414 participants) targeting HDL-C increment, with a primary endpoint of cardiovascular events. All patients received simvastatin, 40–80 mg/day, plus ezetimibe, 10 mg/day, if needed, to maintain an LDL-C level of 1.03–2.07 mmol/l. The trial was stopped early, after a mean follow-up period of three years, owing to a lack of efficacy. At two years, niacin therapy had significantly increased the median HDL-C level from 0.91 to 1.08 mmol/l. LDL-C fell from 1.91 to 1.60 mmol/l. This was followed three years later by the publication of the Heart Protection Study 2 Treatment of HDL to Reduce the Incidence of Vascular Events.[24] This study evaluated extended-release niacin in

combination with laropiprant (a prostaglandin receptor antagonist to reduce facial flushing) to effective statin-based LDL-C lowering treatment in 25,673 high-risk patients with prior vascular disease. Over 3.9 years follow-up, participants assigned to extended-release niacin–laropiprant had an LDL-C level 0.25 mmol/l lower and an HDL-C level 0.16 mmol/l higher than in those assigned to placebo. Despite this biochemical improvement, there was no significant effect on the incidence of major vascular events (13.2 and 13.7% of participants with an event, respectively).

Niacin is the most effective treatment to raise HDL-C on the market[15] and yet has not been conclusively shown to improve clinical outcomes. Limited clinical benefit was considered a consequence of successfully treating LDL-C with statins, as thereafter only marginal incremental gains in clinical endpoints may occur from raising HDL-C. Furthermore, the increase in HDL-C was small in both of these studies.

CETP inhibition

Observational data of four families in Japan with high HDL-C showed a shared mutation in the gene encoding CETP.[25] CETP inhibition offered the prospect of much greater HDL-C increment than that seen with niacin and could therefore address some of the concerns with the AIM-HIGH and HPS2 studies. CETP reduces circulating HDL-C levels by transferring cholesteryl ester (CE) from HDL to larger lipoproteins, such as chylomicrons, VLDL, and low-density lipoprotein (LDL), in exchange for TAG. The rational for inhibition of CETP was that the CE would remain within the HDL particle and be delivered to the liver for uptake and clearance, thus completing the final step of reverse cholesterol transport. CETPi is undoubtedly effective at raising HDL-C. The first CETP inhibitor evaluated in clinical trials was torcetrapib in the ILLUMINATE study.[1] Just over 15,000 patients at high risk of CHD were randomized to treatment with torcetrapib (60 mg) plus atorvastatin versus atorvastatin alone (10–80 mg). After 12 months of torcetrapib therapy, there was an increase of 72.1% in HDL-C and a decrease of 24.9% in LDL-C. The trial was terminated early because of excess in deaths in the torcetrapib/atorvastatin versus atorvastatin groups (82 versus 51, respectively). Increases in heart failure, angina, and revascularization procedures were also observed. Were detrimental outcomes due to the class of drug or specific to the molecule? With torcetrapib there was increased blood pressure and increased plasma levels of aldosterone. The picture was more complex still as post hoc analysis indicated that lower rates of major cardiovascular events occurred in those with greater increases in HDL-C,[1] holding out the prospect of future success with CETPi.

Another CETPi, dalcetrapib, was also in development (Dal-HEART programme). Although no adverse effects were seen on endothelial function or vascular structure,[26,27] a large phase 3 clinical trial of 15,871 patients was terminated early after an interim analysis showed no benefit despite increasing HDL-C by 31–40%.[4]

The background to the ACCELERATE trial was therefore not auspicious. The ACCELERATE trial comprised 12,092 patients who had at least one of the following conditions: an acute coronary syndrome within the previous 30–365 days, cerebrovascular atherosclerotic disease, peripheral vascular arterial disease, or diabetes mellitus with CAD. At three months, LDL-C had decreased by 31% with evacetrapib versus a 6% increase with placebo, and HDL-C had increased by 133.2% with evacetrapib versus a 1.6% increase with placebo. Despite this, the trial was terminated early due to no difference in primary outcome of macrovascular event (hazard ratio, 1.01; 95% confidence interval, 0.91–1.11; $P = 0.91$).[3] The REVEAL study assessed the efficacy of adding anacetrapib or placebo to effective LDL-lowering treatment with atorvastatin among patients with pre-existing atherosclerotic vascular disease.[2] Anacetrapib led to a 1% absolute risk reduction in coronary event rate, over a median of four years, compared to placebo (10.8% versus 11.8%; $P = 0.004$). However, there was no difference in all-cause mortality or death from coronary disease. It is possible that anacetrapib uniquely impacts HDL structure and function compared to other CETPi,[28] which could lead to improved clinical outcomes. Although statistically significant, the trial was clinically disappointing and well below that of a competitor drug class targeting LDL receptor degradation (proprotein convertase subtilisin-kexin type 9 inhibitors)[29] and so regulatory approval was not pursued.

At this point, it appears the idea that the lack of clinical effect of niacin was due to inadequate elevation of HDL-C was incorrect. It is looking more likely that an increase in the cholesterol content of HDL does not necessarily affect plaque biology. As an example, preclinical studies in SR-B1 transgenic mice have shown a disconnect between plasma HDL-C and the level of reverse cholesterol transport and athero-protection.[30] The principle that the predominant action of HDL is the reverse transport of cholesterol (from tissues to the liver) may therefore be wrong. It may well be that one or more of HDL's pleiotropic actions (including maintenance of endothelial function, preventing lipoprotein oxidation, anti-inflammatory and anti-thrombotic functions) is responsible for any clinical benefit of HDL.[31] It is important to note that none of these actions is mediated by the cholesterol content but

rather by the multitude of proteins and microRNAs carried on the HDL molecule.

Can HDL have a therapeutic action?

ApoA-I Milano is a naturally occurring variant of the ApoA-I HDL lipoprotein, due to the replacement of arginine by cysteine at position 173, which was first described in a population in northern Italy. This mutation is characterized by low plasma HDL-C, likely mediated by reduced LCAT activation.[32] Despite low levels of HDL-C, carriers of this variant have minimal burden of coronary atherosclerosis leading to speculation that this is a gain-of-function mutation.[32]

Cholesterol efflux promoters are molecules that mimic pre-β HDL structure by containing ApoA-I Milano and phospholipid. Initial studies of its use were promising.[33] These included a study in patients with acute coronary syndromes whereby weekly infusion of apoA-I Milano, for five weeks, produced significant regression of coronary atherosclerosis, as assessed by intravascular ultrasound.[34]

However, phase 2 trials of MDCO-216 in the MILANO-pilot study,[35] and CER-001 in the Atherosclerosis Regression Acute Coronary Syndrome Trial,[36] have not been able to confirm these effects on intracoronary atherosclerotic plaque. A third compound (CSL-112) has shown promising effects on cholesterol efflux capacity.[37,38]

The rationale for the infusion of apoA-I Milano was to provide pre-β-HDL-like particles to promote cholesterol efflux from atherosclerotic lesions. A possible limitation of this approach may be that pre-β-HDL levels are often already elevated in patients with CHD, together with low levels of large, CE-rich HDL (α_1-HDL).[39,40] It may be that therapeutic agents are required to promote the evolution of pre-β-HDL to mature α-HDL, not least because many of the beneficial functions of HDL are performed by larger, spherical particles. A drug class that may achieve HDL maturation is recombinant LCAT.

LCAT

LCAT is an enzyme produced by the liver that converts cholesterol to CE. The CE can be sequestered into the core of the lipoprotein particle, eventually making the HDL spherical (Figure 1). Therefore, LCAT is thought to play a role in reverse cholesterol transport, and hence may protect against the development of CHD.[40]

Recombinant LCAT has been evaluated in phase 1 studies. Six hours after a single infusion, HDL-C rose by up to 42% and remained elevated up to four days later. Pre-β-HDL also rapidly decreased and was undetectable within 12 h.[41]

It may also be possible to activate LCAT with 'small molecules' which would not require intravenous administration. One such catalyst is a small heterocyclic amine called 'Compound A' or (3–(5-(ethylthio)-1,3,4-thiadiazol-2-ylthio)pyrazine-2-carbonitrile).[42]

HDL diagnostics – What should we be measuring?

Measurements of HDL that are currently available in routine clinical practice include HDL-C and apoA-I quantification. Given the data outlined above, these HDL measures, although useful biomarkers of cardiovascular risk, do not reflect HDL functionality or reflect the ability to prevent atherosclerosis. At present, functional measures have yet to be validated for clinical use. In light of the pleiotropic actions of HDL, choosing what to measure may be fraught with difficulty. For instance, a measure of cholesterol efflux capacity may be suitable if it is felt that reverse cholesterol transport is a key component of HDL function.[43,44] Total efflux mediated by pathways of cholesterol efflux from macrophages (as described earlier: ABCA1 and ABCG1, SR-B1) may be quantified. However, other pathways may prove to be clinically more relevant, and so worthy of measurement (Figure 3).

Once a critical, clinically relevant, function of HDL is identified, the question arises as to what component of the HDL molecule drives this functional benefit and whether it may itself be measured? Proteomic techniques have shown that over 80 proteins exist on the HDL molecule.[8] Examples of candidate proteins that are currently under investigation include serum amyloid A1 – this may displace apoA-I thereby reducing the ability of HDL to promote cholesterol efflux as well as reducing its anti-inflammatory ability[46]: Sphingosine-1-phosphate (S1P) is carried on the HDL molecule and binds to G-protein receptors in the endothelium, through which it elicits anti-inflammatory effects and may mediate ischaemic preconditioning.[47]

HDL subclass

Analysis of the JUPITER data suggested that HDL particle number had a greater association with incident cardiovascular disease than HDL-C levels, apoA-I, or cholesterol efflux capacity.[44]

As discussed earlier in this review, many of the beneficial functions of HDL are performed by larger, spherical particles. It is conceivable therefore that concentrations of HDL subpopulations could demonstrate stronger associations with cardiovascular risk compared to total HDL particle number. However, data are conflicting with regard to relationships between HDL size and vascular disease. For instance, small HDL particles have been positively associated with

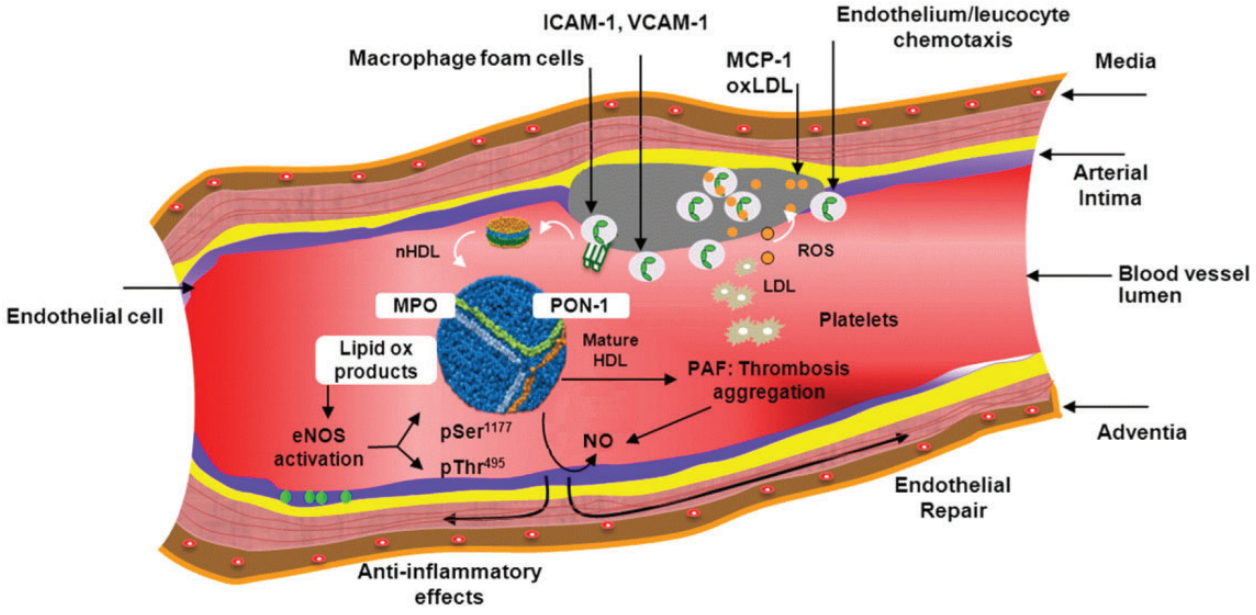

Figure 3. Mechanisms of vascular effects of HDL and associated functional assays. Endothelial adhesion molecules, including intercellular adhesion molecule 1 and vascular cell adhesion protein 1 are induced in response to inflammatory signals.* They facilitate the attachment* and migration of monocytes into the intima, where the monocytes differentiate into macrophages in response to monocyte colony-stimulating factor.* LDL interaction with macrophages and proteoglycans serves to trap the LDL molecule. LDL oxidation proceeds via lipoxygenases,* myeloperoxidase,* and endothelial nitric oxide synthase (eNOS)* that induce nitric oxide release in the endothelium. Regulation of eNOS activity is through phosphorylation of serine and threonine residues ($pSer^{1177}$, $pThr^{495}$). HDL downregulates the production of the pro-inflammatory platelet-activating factor. HDL-associated paraoxonase-1* inhibits macrophage cholesterol biosynthesis and enhances HDL-mediated cholesterol efflux. *Can be measured by functional assay. Source: Modified with permission from Hafiane A and Genest.[45]
HDL: high-density lipoprotein; ICAM-1: intercellular adhesion molecule 1; LDL: low-density lipoprotein; MCP-1: monocyte chemoattractant protein-1; MPO: myeloperoxidase; NO: nitric oxide; PAF: platelet-activating factor; PON-1: paraoxonase-1; ROS: reactive oxygen species; VCAM-1: vascular cell adhesion protein 1.

carotid intima-media thickness[48] – a measure of carotid atherosclerotic vascular disease, and with CAD[49] but inversely associated to coronary calcification.[50] Small HDL size is associated with the metabolic syndrome however and the positive association between small HDL size and CAD risk was abolished after adjustment for apoB and triglyceride levels.[49] Similarly, when adjusted for both apoA-I and apoB, large HDL molecules (measured by NMR) were *adversely* associated with cardiovascular risk[51] further illustrating the difficulties of making inferences of HDL behaviour from measurement of volume or size. It is possible that very large (cholesterol enriched) HDL molecules may switch to become cholesterol donors, rather than acceptors. It has also been hypothesized that in disease states of chronic oxidative stress, rather than HDL being anti-inflammatory, it may become pro-inflammatory.[52]

Conclusion

Epidemiological data strongly support the inverse association between HDL concentration and cardiovascular risk. HDL-C has proved to be a useful biomarker for cardiovascular risk assessment. Pharmaceutical strategies to raise HDL-C concentration have not met with clinical success. Although disappointing, this has led to a much greater understanding of the composition and role of the HDL molecule which holds potential for future therapeutic approaches.

Acknowledgements

The author would like to acknowledge the University of Surrey library service.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Ethical approval

Ethical approval was not required for this work.

Guarantor

Martin B Whyte is the article guarantor and accepts responsibility for the integrity of the manuscript.

Contributorship

MBW was solely responsible for the article.

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