Pathology (February 2019) **51(2)**, pp. 142–147

LIPIDS AND CARDIOVASCULAR DISEASE

HDL and cardiovascular disease

Stephen J. Nicholls[1,2], Adam J. Nelson[1]

[1]*South Australian Health and Medical Research Institute, Adelaide, SA, Australia;* [2]*Monash University, Adelaide, SA, Australia*

Summary

High-density lipoprotein (HDL) has received increasing interest due to observations of an inverse relationship between its systemic levels and cardiovascular risk and targeted interventions in animal models that have had favourable effects on atherosclerotic plaque. In addition to its pivotal role in reverse cholesterol transport, HDL has been reported to possess a range of functional properties, which may exert a protective influence on inflammation, oxidation, angiogenesis and glucose homeostasis. This has led to the development of a range of HDL targeted therapeutics, which have undergone evaluation in clinical trials. The current state of HDL in cardiovascular prevention will be reviewed.

Key words: HDL; lipids; atherosclerosis; cardiovascular risk; clinical trials.

Received 3 October, revised 28 October, accepted 28 October 2018
Available online 3 January 2019

INTRODUCTION

On the basis of their clinical benefit in large cardiovascular outcomes trials, the use of statins for lowering of low-density lipoprotein (LDL) cholesterol has become widespread in cardiovascular risk reduction strategies.[1–6] The finding of a substantial residual clinical risk in statin-treated patients suggests that additional therapeutic strategies will be required to achieve more effective risk reduction.[7] For more than 50 years, increasing interest has focused on the potentially protective effects of high-density lipoprotein (HDL), by virtue of observations of an inverse relationship between systemic measures of both HDL cholesterol and its major protein, apolipoprotein A-I (apoA-I), and prospective cardiovascular risk.[8,9]

STRUCTURAL AND COMPOSITIONAL CONSIDERATIONS

HDL represents a variety of lipoproteins, isolated within the 1.05–1.21 g/mL density fraction of plasma.[10] These particles differ widely in terms of size, shape and lipid composition.[11] The fundamental structure of an HDL particle includes a central core of esterified cholesterol, surrounded by a surface monolayer of phospholipid, free cholesterol and apolipoproteins.[12] HDL spans the spectrum from small, lipid deplete, discoidal particles, to larger, cholesterol rich, spherical particles. The protein composition of the surface monolayer is predominantly populated by apoA-I and apoA-II (Fig. 1). Shotgun proteomic studies have identified a large number of additional proteins that can be carried on HDL particles.[13] Whether the specific protein cargo on an individual HDL particle modifies its functional properties remains to be determined.

LESSONS FROM PATHOLOGY AND GENETIC STUDIES

Early studies from coronary care units suggested that patients with myocardial infarction appeared to have less HDL cholesterol.[14] This was subsequently confirmed by a number of large population studies, which consistently demonstrated an inverse association between HDL cholesterol levels and the prospective incidence of coronary heart disease.[8,9,15] This relationship is curvilinear, largely driven by an increase in cardiovascular risk in individuals with low HDL cholesterol levels. More recently, low HDL cholesterol levels continued to be associated with an increase in cardiovascular events when LDL cholesterol levels were below 70 mg/dL (1.8 mmol/L) in clinical trials of statin therapy.[16] Genetic studies, in contrast, do not demonstrate a clear association between HDL cholesterol and cardiovascular risk.[17] Mendelian randomisation studies do not report any relationship between polymorphisms influencing HDL cholesterol levels and cardiovascular event rates.[18–20] This is in contrast to findings with LDL cholesterol, which provided strong support for its causal role in atherosclerotic cardiovascular disease.[21–23] As a result, there is a disconnect between the observational data from population and genetic studies.[24] Whether this suggests that HDL cholesterol is more important as an epiphenomenal risk factor as opposed to a causal factor, and therefore a pharmacologic target, remains uncertain.

FUNCTIONAL PROPERTIES

The best characterised function of HDL involves its central role in reverse cholesterol transport.[25] HDL has been identified as a major recipient of free cholesterol, effluxed from cells. Once present on the HDL particle surface, this cholesterol is rapidly esterified by lecithin:cholesterol acyltransferase (LCAT) and then subsequently stored within the particle core.[26] This maintains a relatively low cholesterol concentration on the particle surface, which permits ongoing movement of lipid from cell to HDL. It also changes HDL morphology from a nascent, lipid-deplete, discoidal particle, to one that is larger, cholesterol rich and spherical in shape. Cholesterol is subsequently taken up by the liver, facilitated

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: https://doi.org/10.1016/j.pathol.2018.10.017

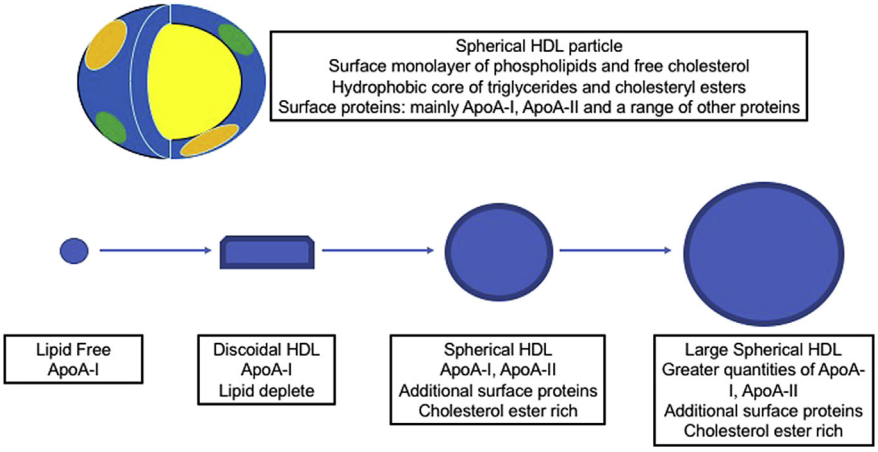

Fig. 1 Heterogeneity of high-density lipoprotein (HDL) particles in the systemic circulation span from lipid-free apolipoprotein (apo)A-I and lipid-deplete, discoidal HDL particles, through to cholesterol rich, spherical HDL particles. Increasing particle size generates a larger surface area, permitting carriage of more proteins.

by binding of circulating HDL to the scavenger receptor-BI (SR-BI) on the hepatocyte surface.[27] Cholesterol within HDL particles can alternatively be transferred to LDL and very low-density lipoprotein (VLDL) particles, in exchange for triglyceride, a process promoted by cholesteryl ester transfer protein (CETP).[28] The fate of this transferred cholesterol is uncertain. Given that LDL particles are ultimately taken up by the liver, it is possible that this CETP-mediated transfer may present an additional pathway for reverse cholesterol transport.[29] Changes in HDL morphology influence interactions with different cellular cholesterol transporter proteins. Lipid free apoA-I and discoidal, lipid-deplete HDL particles preferentially accept cholesterol effluxed via the ATP binding cassette A1 (ABCA1), while spherical, lipid enriched HDL particles accept cholesterol via ABCG1 and SR-BI.[30,31] This may have potential implications for different therapeutic strategies aimed at enhancing lipid mobilisation as an approach to reducing cardiovascular risk.

Laboratory studies have revealed that HDL particles possess a number of additional functional properties beyond their role in lipid mobilisation. HDL have been demonstrated to exert favourable effects on inflammatory and oxidative pathways implicated in cardiovascular disease.[32] This involves inhibition of lipid peroxidation, cytokine-induced upregulation of proinflammatory adhesion molecule and chemokine expression by endothelial cells, and promotion of macrophage phenotype switching from a pro-inflammatory to anti-inflammatory form.[33–35] This is likely to play an important role at all stages of atherosclerosis, from early plaque formation through to a more vulnerable setting. The role of HDL inhibiting apoptosis, and thrombosis factors[36,37] involved in plaque rupture, are also likely to confer a protective effect at the level of the artery wall. HDL has also been reported to exert favourable effects on new vessel formation, which is pivotal for successful angiogenesis in the setting of vascular disease.[38] Central to these properties involves the favourable impact of HDL on nitric oxide bioavailability, as evidenced by an increase in cellular synthesis with HDL co-incubation and improvements in non-invasive measures of vascular reactivity with HDL infusions.[39] Whether additional proteins carried on HDL particles confer these activities is uncertain, although there is evidence that factors such as paraoxonase, which is carried on HDL and inhibits lipid oxidation, is associated with reduced systemic measures of oxidative stress and cardiovascular risk.[40,41] Similarly, microRNA species found associated with HDL particles have been demonstrated to play an important role in lipid mobilisation and angiogenesis activities of HDL.[42,43] Beyond its effects on the vessel wall, HDL has also been demonstrated to promote islet cell function and potentially improve glycaemic control in the setting of diabetes.[44,45]

More recent data have suggested that these functional activities of HDL may vary in different states. In particular, there is evidence that HDL is less protective in the setting of diabetes mellitus,[46,47] acute coronary syndromes,[48] systemic inflammation,[49] smoking[50] and chronic kidney disease.[51] Differences in cholesterol efflux capacity associate with altered levels of cardiovascular risk in large case cohort studies.[52,53] While it has been suggested that therapeutic agents may enhance or impair HDL functionality, this remains to be fully elucidated. Similarly, whether additional markers of HDL functionality or characterisation of its protein cargo can be used to stratify cardiovascular risk is unknown.

These properties all contribute to a potential favourable effect at the level of the artery wall. Animal studies have demonstrated that promoting HDL functionality, via direct infusion or transgenic expression of its major protein, apoA-I, has a favourable effect on plaque burden and composition,[54] in stent restenosis,[55,56] vascular inflammation,[33] and myocardial injury in the setting of ischaemic injury.[57] All of these findings support the concept that targeting HDL may have a protective influence on cardiovascular risk in humans.

EFFECTS OF ESTABLISHED LIPID MODIFYING THERAPIES

Existing approaches to lipid modulation in cardiovascular prevention have mild to modest effects on HDL. Lifestyle changes can associate with modest HDL raising, in the presence of loss of abdominal adiposity.[58] Statins increase HDL cholesterol by 3–15%, with evidence that these changes associate with their benefits on plaque progression and cardiovascular events.[59–61] Fibrates similarly raise HDL

cholesterol by 5–20% and in small studies have shown improvement in coronary[62] and carotid atherosclerosis,[63] although not consistently.[64] Outcome studies have failed to show efficacy in the unselected patient, however, in those with elevated triglycerides or low baseline HDL, there is a consistent signal for benefit,[65–68] possibly mediated through elevations in the concentration of small HDL particles.[69] Niacin is the most effective HDL-raising agent currently used in clinical practice. While early studies demonstrated that niacin exerted a favourable effect on progression of coronary[70] and carotid atheroma,[71,72] in addition to cardiovascular events,[73] more recent studies have failed to demonstrate any clinical benefit of empirical niacin use in the setting of background statin therapy.[74,75] HDL raising also associates with the benefits of the PPAR-γ agonist, pioglitazone, on progression of coronary[76] and carotid[77] atherosclerosis in patients with type 2 diabetes, although the outcome trial failed to show a significant reduction in the composite endpoint.[78]

EMERGING THERAPIES

Considerable efforts have been undertaken to develop novel approaches to targeting HDL in order to achieve more effective reductions in cardiovascular risk. Infusing HDL mimetics provides the opportunity to directly administer lipid deplete HDL particles, with early evidence of favourable effects on lipid transport[79] and endothelial function.[80] The first mimetic studied in clinical trials, containing recombinant apoA-I_{Milano}, was reported to produce rapid regression of coronary atherosclerosis in patients following an acute coronary syndrome.[81] However, subsequent imaging trials of other mimetics have failed to demonstrate incremental regression with infusion of mimetics containing wild type apoA-I (CSL-111)[82] or apoA-I and sphingomyelin (CER-001)[83] in intensive statin treated patients. In contrast, a small imaging study demonstrated a trend towards plaque regression in acute coronary syndrome patients who received autologous infusions of HDL which had undergone selective delipidation.[84] With refinements to the production process, CSL-112 has demonstrated tolerability in a large safety study[85] and has advanced to evaluation in a large cardiovascular outcomes trial.

CETP plays an important role in remodelling of lipid particles, by virtue of its role in facilitating transfer of esterified cholesterol from HDL to VLDL and LDL particles, in exchange for triglycerides. Support for developing CETP inhibitors was provided from evidence that these agents would substantially elevate HDL cholesterol levels,[86] in addition to observations from genetic studies that polymorphisms associated with low CETP activity associate with lower cardiovascular event rates[28,87,88] and that CETP inhibition has favourable effects on animal models of atherosclerosis.[89–92] While these agents produce robust HDL cholesterol raising, in addition to LDL cholesterol lowering with more potent agents, their impact has been variable on coronary[93] and carotid atheroma,[94,95] as well as cardiovascular event rates in large clinical trials. Large scale trials have observed evidence of off-target toxicity with torcetrapib,[96] clinical futility with dalcetrapib[97] and evacetrapib,[98] and finally modest clinical efficacy with long term administration of anacetrapib.[99] Subsequent genetic analyses have provided some insights into factors that may associate with potential benefit of CETP inhibitors. Pharmacogenomic analysis of the dalcetrapib clinical trial demonstrated efficacy in those patients who harboured the AA genotype of the *ADCY9* gene on chromosome 16.[100] This also associated with greater increases in cholesterol efflux activity and more favourable effects on inflammatory markers. This has led to the initiation of a new clinical trial, which is directly evaluating the impact of dalcetrapib in high risk patients with the AA genotype. Similar findings could not be definitively replicated with evacetrapib, posing the question of whether this is a dalcetrapib-specific phenomenon.[101] Mendelian randomisation has also interrogated the potential impact of CETP deficiency and confirmed an association with cardiovascular protection, which associates with the degree of apoB reduction.[102] This relationship appeared to be stronger in the absence of polymorphisms reducing HMG-CoA reductase, the target of statins. This suggests that CETP inhibitors may be more effective when administered as monotherapy, although this has not been prospectively evaluated in a clinical trial. Accordingly, there remains uncertainty regarding the clinical utility of this target.[103] While originally on the basis of their ability to raise HDL cholesterol, it may be that their ultimate benefit is derived from other properties.

Within the systemic circulation, HDL particles are subject to a vast range of remodelling factors, which influence their size, shape and chemical composition. Given their influence on global lipid transport and additional HDL properties, these factors provide additional targets for therapeutic modification. LCAT facilitates esterification of cholesterol on the HDL particle surface. This enables transfer of cholesterol to the particle core and permits ongoing cholesterol efflux to the particle surface. LCAT agonists have the potential to elevate HDL cholesterol and promote lipid transport, but the impact of this therapeutic approach remains to be determined.[104]

CONCLUSION

Evidence continues to accumulate supporting the concept that HDL plays an important role in the prevention of atherosclerotic disease. Whether this continues to provide HDL quantity and functional quality with an essential place in risk prediction, or whether it ultimately provides an additional target for risk reduction, will only be answered by ongoing clinical trials in this space.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Address for correspondence: Stephen Nicholls, South Australian Health and Medical Research Institute, PO Box 11050, Adelaide, SA, 5001, Australia. E-mail: stephen.nicholls@sahmri.com

References

1. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; 344: 1383–9.
2. Downs JR, Clearfield M, Weis S, *et al*. Primary prevention of acute coronary events with lovastatin in men and women with average cholesterol levels: results of AFCAPS/TexCAPS. Air Force/Texas Coronary Atherosclerosis Prevention Study. *JAMA* 1998; 279: 1615–22.
3. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002; 360: 7–22.

4. Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) Study Group. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N Engl J Med* 1998; 339: 1349–57.
5. Sacks FM, Pfeffer MA, Moye LA, *et al*. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N Engl J Med* 1996; 335: 1001–9.
6. Shepherd J, Cobbe SM, Ford I, *et al*. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med* 1995; 333: 1301–7.
7. Libby P. The forgotten majority: unfinished business in cardiovascular risk reduction. *J Am Coll Cardiol* 2005; 46: 1225–8.
8. Gordon DJ, Probstfield JL, Garrison RJ, *et al*. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 1989; 79: 8–15.
9. Gordon T, Castelli WP, Hjortland MC, *et al*. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am J Med* 1977; 62: 707–14.
10. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955; 34: 1345–53.
11. Rye KA, Clay MA, Barter PJ. Remodelling of high density lipoproteins by plasma factors. *Atherosclerosis* 1999; 145: 227–38.
12. Heinecke JW. The HDL proteome: a marker–and perhaps mediator–of coronary artery disease. *J Lipid Res* 2009; 50 (Suppl): S167–71.
13. Vaisar T, Pennathur S, Green PS, *et al*. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. *J Clin Invest* 2007; 117: 746–56.
14. Barr DP, Russ EM, Eder HA. Protein-lipid relationships in human plasma. II. In atherosclerosis and related conditions. *Am J Med* 1951; 11: 480–93.
15. Assmann G, Schulte H, von Eckardstein A, *et al*. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis* 1996; 124 (Suppl): S11–20.
16. Barter P, Gotto AM, LaRosa JC, *et al*. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *N Engl J Med* 2007; 357: 1301–10.
17. Vitali C, Khetarpal SA, Rader DJ. HDL Cholesterol metabolism and the risk of CHD: new insights from human genetics. *Curr Cardiol Rep* 2017; 19: 132.
18. Frikke-Schmidt R, Nordestgaard BG, Stene MC, *et al*. Association of loss-of-function mutations in the ABCA1 gene with high-density lipoprotein cholesterol levels and risk of ischemic heart disease. *JAMA* 2008; 299: 2524–32.
19. Haase CL, Tybjaerg-Hansen A, Qayyum AA, *et al*. LCAT, HDL cholesterol and ischemic cardiovascular disease: a Mendelian randomization study of HDL cholesterol in 54,500 individuals. *J Clin Endocrinol Metab* 2012; 97: E248–56.
20. Voight BF, Peloso GM, Orho-Melander M, *et al*. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet* 2012; 380: 572–80.
21. Consortium CAD, Deloukas P, Kanoni S, *et al*. Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet* 2013; 45: 25–33.
22. Ference BA, Yoo W, Alesh I, *et al*. Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis. *J Am Coll Cardiol* 2012; 60: 2631–9.
23. Schunkert H, Konig IR, Kathiresan S, *et al*. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet* 2011; 43: 333–8.
24. Nicholls SJ, Psaltis PJ. Will Big data shine light at the end of the tunnel for HDL? *J Am Coll Cardiol* 2016; 68: 2084. –5.
25. Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. *J Lipid Res* 1995; 36: 211–28.
26. Calabresi L, Franceschini G. Lecithin:cholesterol acyltransferase, high-density lipoproteins, and atheroprotection in humans. *Trends Cardiovasc Med* 2010; 20: 50–3.
27. Lambert G, Chase MB, Dugi K, *et al*. Hepatic lipase promotes the selective uptake of high density lipoprotein-cholesteryl esters via the scavenger receptor B1. *J Lipid Res* 1999; 40: 1294–303.
28. Barter PJ, Brewer Jr HB, Chapman MJ, *et al*. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. *Arterioscler Thromb Vasc Biol* 2003; 23: 160–7.
29. Barter P, Rye KA. Cholesteryl ester transfer protein: its role in plasma lipid transport. *Clin Exp Pharmacol Physiol* 1994; 21: 663–72.
30. Cavelier C, Rohrer L, von Eckardstein A. ATP-Binding cassette transporter A1 modulates apolipoprotein A-I transcytosis through aortic endothelial cells. *Circ Res* 2006; 99: 1060–6.
31. Rohrer L, Ohnsorg PM, Lehner M, *et al*. High-density lipoprotein transport through aortic endothelial cells involves scavenger receptor BI and ATP-binding cassette transporter G1. *Circ Res* 2009; 104: 1142–50.
32. Barter PJ, Nicholls S, Rye KA, *et al*. Antiinflammatory properties of HDL. *Circ Res* 2004; 95: 764–72.
33. Nicholls SJ, Dusting GJ, Cutri B, *et al*. Reconstituted high-density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. *Circulation* 2005; 111: 1543–50.
34. Tedgui A, Mallat Z. Anti-inflammatory mechanisms in the vascular wall. *Circ Res* 2001; 88: 877–87.
35. Xia P, Vadas MA, Rye KA, *et al*. High density lipoproteins (HDL) interrupt the sphingosine kinase signaling pathway. A possible mechanism for protection against atherosclerosis by HDL. *J Biol Chem* 1999; 274: 33143–7.
36. Li D, Weng S, Yang B, *et al*. Inhibition of arterial thrombus formation by ApoA1 Milano. *Arterioscler Thromb Vasc Biol* 1999; 19: 378–83.
37. Suc I, Escargueil-Blanc I, Troly M, *et al*. HDL and ApoA prevent cell death of endothelial cells induced by oxidized LDL. *Arterioscler Thromb Vasc Biol* 1997; 17: 2158–66.
38. Tan JT, Ng MK, Bursill CA. The role of high-density lipoproteins in the regulation of angiogenesis. *Cardiovasc Res* 2015; 106: 184–93.
39. Yuhanna IS, Zhu Y, Cox BE, *et al*. High-density lipoprotein binding to scavenger receptor-BI activates endothelial nitric oxide synthase. *Nat Med* 2001; 7: 853–7.
40. Watson AD, Berliner JA, Hama SY, *et al*. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest* 1995; 96: 2882–91.
41. White CR, Anantharamaiah GM. Cholesterol reduction and macrophage function: role of paraoxonases. *Curr Opin Lipidol* 2017; 28: 397–402.
42. Hourigan ST, Solly EL, Nankivell VA, *et al*. The regulation of miRNAs by reconstituted high-density lipoproteins in diabetes-impaired angiogenesis. *Sci Rep* 2018; 8: 13596.
43. Rayner KJ, Moore KJ. MicroRNA control of high-density lipoprotein metabolism and function. *Circ Res* 2014; 114: 183–92.
44. Kruit JK, Brunham LR, Verchere CB, *et al*. HDL and LDL cholesterol significantly influence beta-cell function in type 2 diabetes mellitus. *Curr Opin Lipidol* 2010; 21: 178–85.
45. von Eckardstein A, Widmann C. High-density lipoprotein, beta cells, and diabetes. *Cardiovasc Res* 2014; 103: 384–94.
46. Ohgami N, Miyazaki A, Sakai M, *et al*. Advanced glycation end products (AGE) inhibit scavenger receptor class B type I-mediated reverse cholesterol transport: a new crossroad of AGE to cholesterol metabolism. *J Atheroscler Thromb* 2003; 10: 1–6.
47. Quintao EC, Medina WL, Passarelli M. Reverse cholesterol transport in diabetes mellitus. *Diabetes Metab Res Rev* 2000; 16: 237–50.
48. Alwaili K, Bailey D, Awan Z, *et al*. The HDL proteome in acute coronary syndromes shifts to an inflammatory profile. *Biochim Biophys Acta* 2012; 1821: 405–15.
49. Mani P, Uno K, Duong M, *et al*. HDL function and subclinical atherosclerosis in juvenile idiopathic arthritis. *Cardiovasc Diagn Ther* 2016; 6: 34–43.
50. Ueyama K, Yokode M, Arai H, *et al*. Cholesterol efflux effect of high density lipoprotein is impaired by whole cigarette smoke extracts through lipid peroxidation. *Free Radic Biol Med* 1998; 24: 182–90.
51. Yamamoto S, Yancey PG, Ikizler TA, *et al*. Dysfunctional high-density lipoprotein in patients on chronic hemodialysis. *J Am Coll Cardiol* 2012; 60: 2372–9.
52. Khera AV, Demler OV, Adelman SJ, *et al*. Cholesterol efflux capacity, high-density lipoprotein particle number, and incident cardiovascular events: an analysis from the JUPITER Trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin). *Circulation* 2017; 135: 2494–504.
53. Rohatgi A, Khera A, Berry JD, *et al*. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med* 2014; 371: 2383–93.
54. Nicholls SJ, Cutri B, Worthley SG, *et al*. Impact of short-term administration of high-density lipoproteins and atorvastatin on atherosclerosis in rabbits. *Arterioscler Thromb Vasc Biol* 2005; 25: 2416–21.
55. Vanags LZ, Tan JTM, Galougahi KK, *et al*. Apolipoprotein A-I reduces in-stent restenosis and platelet activation and alters neointimal cellular phenotype. *JACC Basic Transl Sci* 2018; 3: 200–9.
56. Vanags LZ, Wong NKP, Nicholls SJ, *et al*. High-density lipoproteins and apolipoprotein A-I improve stent biocompatibility. *Arterioscler Thromb Vasc Biol* 2018; 38: 1691–701.
57. Marchesi M, Booth EA, Davis T, *et al*. Apolipoprotein A-IMilano and 1-palmitoyl-2-oleoyl phosphatidylcholine complex (ETC-216) protects the in vivo rabbit heart from regional ischemia-reperfusion injury. *J Pharmacol Exp Ther* 2004; 311: 1023–31.

58. Kraus WE, Houmard JA, Duscha BD, *et al*. Effects of the amount and intensity of exercise on plasma lipoproteins. *N Engl J Med* 2002; 347: 1483–92.
59. Athyros VG, Mikhailidis DP, Papageorgiou AA, *et al*. Effect of atorvastatin on high density lipoprotein cholesterol and its relationship with coronary events: a subgroup analysis of the GREek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) Study. *Curr Med Res Opin* 2004; 20: 627–37.
60. Cui Y, Watson DJ, Girman CJ, *et al*. Effects of increasing high-density lipoprotein cholesterol and decreasing low-density lipoprotein cholesterol on the incidence of first acute coronary events (from the Air Force/Texas Coronary Atherosclerosis Prevention Study). *Am J Cardiol* 2009; 104: 829–34.
61. Nicholls SJ, Tuzcu EM, Sipahi I, *et al*. Statins, high-density lipoprotein cholesterol, and regression of coronary atherosclerosis. *JAMA* 2007; 297: 499–508.
62. Effect of fenofibrate on progression of coronary-artery disease in type 2 diabetes: the Diabetes Atherosclerosis Intervention Study, a randomised study. *Lancet* 2001; 357: 905–10.
63. Zhu S, Su G, Meng QH. Inhibitory effects of micronized fenofibrate on carotid atherosclerosis in patients with essential hypertension. *Clin Chem* 2006; 52: 2036–42.
64. Hiukka A, Westerbacka J, Leinonen ES, *et al*. Long-term effects of fenofibrate on carotid intima-media thickness and augmentation index in subjects with type 2 diabetes mellitus. *J Am Coll Cardiol* 2008; 52: 2190–7.
65. Bezafibrate Infarction Prevention. Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease. *Circulation* 2000; 102: 21–7.
66. Frick MH, Elo O, Haapa K, *et al*. Helsinki Heart Study: primary-prevention trial with gemfibrozil in middle-aged men with dyslipidemia. Safety of treatment, changes in risk factors, and incidence of coronary heart disease. *N Engl J Med* 1987; 317: 1237–45.
67. Keech A, Simes RJ, Barter P, *et al*. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 2005; 366: 1849–61.
68. Rubins HB, Robins SJ, Collins D, *et al*. Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol. Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N Engl J Med* 1999; 341: 410–8.
69. Asztalos BF, Collins D, Horvath KV, *et al*. Relation of gemfibrozil treatment and high-density lipoprotein subpopulation profile with cardiovascular events in the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Metabolism* 2008; 57: 77–83.
70. Brown BG, Zhao XQ, Chait A, *et al*. Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 2001; 345: 1583–92.
71. Lee JM, Robson MD, Yu LM, *et al*. Effects of high-dose modified-release nicotinic acid on atherosclerosis and vascular function: a randomized, placebo-controlled, magnetic resonance imaging study. *J Am Coll Cardiol* 2009; 54: 1787–94.
72. Taylor AJ, Sullenberger LE, Lee HJ, *et al*. Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2: a double-blind, placebo-controlled study of extended-release niacin on atherosclerosis progression in secondary prevention patients treated with statins. *Circulation* 2004; 110: 3512–7.
73. Canner PL, Berge KG, Wenger NK, *et al*. Fifteen year mortality in Coronary Drug Project patients: long-term benefit with niacin. *J Am Coll Cardiol* 1986; 8: 1245–55.
74. Group HTC, Landray MJ, Haynes R, *et al*. Effects of extended-release niacin with laropiprant in high-risk patients. *N Engl J Med* 2014; 371: 203–12.
75. Investigators A-H, Boden WE, Probstfield JL, *et al*. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med* 2011; 365: 2255–67.
76. Nissen SE, Nicholls SJ, Wolski K, *et al*. Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: the PERISCOPE randomized controlled trial. *JAMA* 2008; 299: 1561–73.
77. Mazzone T, Meyer PM, Feinstein SB, *et al*. Effect of pioglitazone compared with glimepiride on carotid intima-media thickness in type 2 diabetes: a randomized trial. *JAMA* 2006; 296: 2572–81.
78. Dormandy JA, Charbonnel B, Eckland DJ, *et al*. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial in macroVascular Events): a randomised controlled trial. *Lancet* 2005; 366: 1279–89.
79. Angelin B, Parini P, Eriksson M. Reverse cholesterol transport in man: promotion of fecal steroid excretion by infusion of reconstituted HDL. *Atheroscler Suppl* 2002; 3: 23–30.
80. Spieker LE, Sudano I, Hurlimann D, *et al*. High-density lipoprotein restores endothelial function in hypercholesterolemic men. *Circulation* 2002; 105: 1399–402.
81. Nissen SE, Tsunoda T, Tuzcu EM, *et al*. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA* 2003; 290: 2292–300.
82. Tardif JC, Gregoire J, L'Allier PL, *et al*. Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: a randomized controlled trial. *JAMA* 2007; 297: 1675–82.
83. Nicholls SJ, Andrews J, Kastelein JJP, *et al*. Effect of serial infusions of CER-001, a pre-beta high-density lipoprotein mimetic, on coronary atherosclerosis in patients following acute coronary syndromes in the CER-001 Atherosclerosis Regression Acute Coronary Syndrome Trial: a randomized clinical trial. *JAMA Cardiol* 2018; 3: 815–22.
84. Waksman R, Torguson R, Kent KM, *et al*. A first-in-man, randomized, placebo-controlled study to evaluate the safety and feasibility of autologous delipidated high-density lipoprotein plasma infusions in patients with acute coronary syndrome. *J Am Coll Cardiol* 2010; 55: 2727–35.
85. Gibson CM, Korjian S, Tricoci P, *et al*. Rationale and design of Apo-I Event Reduction in Ischemic Syndromes I (AEGIS-I): a phase 2b, randomized, placebo-controlled, dose-ranging trial to investigate the safety and tolerability of CSL112, a reconstituted, infusible, human apoA-I, after acute myocardial infarction. *Am Heart J* 2016; 180: 22–8.
86. Brousseau ME, Schaefer EJ, Wolfe ML, *et al*. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. *N Engl J Med* 2004; 350: 1505–15.
87. Johannsen TH, Frikke-Schmidt R, Schou J, *et al*. Genetic inhibition of CETP, ischemic vascular disease and mortality, and possible adverse effects. *J Am Coll Cardiol* 2012; 60: 2041–8.
88. Thompson A, Di Angelantonio E, Sarwar N, *et al*. Association of cholesteryl ester transfer protein genotypes with CETP mass and activity, lipid levels, and coronary risk. *JAMA* 2008; 299: 2777–88.
89. Morehouse LA, Sugarman ED, Bourassa PA, *et al*. Inhibition of CETP activity by torcetrapib reduces susceptibility to diet-induced atherosclerosis in New Zealand White rabbits. *J Lipid Res* 2007; 48: 1263–72.
90. Okamoto H, Yonemori F, Wakitani K, *et al*. A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature* 2000; 406: 203–7.
91. Rittershaus CW, Miller DP, Thomas LJ, *et al*. Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis. *Arterioscler Thromb Vasc Biol* 2000; 20: 2106–12.
92. Sugano M, Makino N, Sawada S, *et al*. Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. *J Biol Chem* 1998; 273: 5033–6.
93. Nissen SE, Tardif JC, Nicholls SJ, *et al*. Effect of torcetrapib on the progression of coronary atherosclerosis. *N Engl J Med* 2007; 356: 1304–16.
94. Bots ML, Visseren FL, Evans GW, *et al*. Torcetrapib and carotid intima-media thickness in mixed dyslipidaemia (RADIANCE 2 study): a randomised, double-blind trial. *Lancet* 2007; 370: 153–60.
95. Kastelein JJ, van Leuven SI, Burgess L, *et al*. Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. *N Engl J Med* 2007; 356: 1620–30.
96. Barter PJ, Caulfield M, Eriksson M, *et al*. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med* 2007; 357: 2109–22.
97. Schwartz GG, Olsson AG, Abt M, *et al*. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med* 2012; 367: 2089–99.
98. Lincoff AM, Nicholls SJ, Riesmeyer JS, *et al*. Evacetrapib and cardiovascular outcomes in high-risk vascular disease. *N Engl J Med* 2017; 376: 1933–42.
99. Group HTRC, Bowman L, Hopewell JC, *et al*. Effects of anacetrapib in patients with atherosclerotic vascular disease. *N Engl J Med* 2017; 377: 1217–27.
100. Tardif JC, Rheaume E, Lemieux Perreault LP, *et al*. Pharmacogenomic determinants of the cardiovascular effects of dalcetrapib. *Circ Cardiovasc Genet* 2015; 8: 372–82.
101. Nissen SE, Pillai SG, Nicholls SJ, *et al*. ADCY9 genetic variants and cardiovascular outcomes with evacetrapib in patients with high-risk vascular disease: a nested case-control study. *JAMA Cardiol* 2018; 3: 401–8.

102. Ference BA, Kastelein JJP, Ginsberg HN, *et al*. Association of genetic variants related to CETP inhibitors and statins with lipoprotein levels and cardiovascular risk. *JAMA* 2017; 318: 947–56.
103. Nicholls SJ. CETP-inhibition and HDL-cholesterol: a story of CV risk or CV benefit, or both. *Clin Pharmacol Ther* 2018; 104: 297–300.
104. Gunawardane RN, Fordstrom P, Piper DE, *et al*. Agonistic human antibodies binding to lecithin-cholesterol acyltransferase modulate high density lipoprotein metabolism. *J Biol Chem* 2016; 291: 2799–811.