HDL Cholesterol Efflux Capacity

Subjects: Cell Biology

Contributor: Francesca Zimetti

Over the years, the relationship between high-density lipoprotein (HDL) and atherosclerosis, initially highlighted by the Framingham study, has been revealed to be extremely complex, due to the multiple HDL functions involved in atheroprotection. Among them, HDL cholesterol efflux capacity (CEC), the ability of HDL to promote cell cholesterol efflux from cells, has emerged as a better predictor of cardiovascular (CV) risk compared to merely plasma HDL-cholesterol (HDL-C) levels. HDL CEC is impaired in many genetic and pathological conditions associated to high CV risk such as dyslipidemia, chronic kidney disease, diabetes, inflammatory and autoimmune diseases, endocrine disorders, etc.

reverse cholesterol transport | high density lipoprotein | cholesterol efflux capacity | atherosclerosis | cardiovascular disease

1. Introduction

High-density lipoprotein cholesterol (HDL-C) has been considered for years the “good” cholesterol, as suggested by the inverse correlation between its plasma levels and the cardiovascular (CV) risk, as shown by the epidemiology [1][2]. However, the relationship between HDL and atherosclerosis was found to be much more complex compared, for example, to that observed for low density lipoproteins (LDL). Firstly, a recent study has revealed a paradoxical association between very high HDL-C levels and increased CV disease (CVD) [3]. In addition, the failure of the trials testing the HDL-C raising drugs, together with the results of genetic studies on several polymorphisms or rare mutations, have led to some shadow being cast on the “HDL hypothesis” [4][5][6].

In parallel, a growing interest pointed to the importance of the HDL function in the context of atheroprotection. In facts, HDL possess several functions, among which the best known is the ability to promote the first step of the reverse cholesterol transport (RCT) process, namely the cholesterol efflux from the macrophages of the arterial wall [7][8].

Cell cholesterol efflux may occur through multiple mechanisms including aqueous diffusion, as well as facilitated and active transport both promoted by specific membrane proteins, such as the scavenger receptor class B, type I (SR-BI) and members of the ATP binding cassette (ABC) transporter family, ABCG1 and ABCA1, respectively [9]. The ability of HDL to promote cell cholesterol efflux (cholesterol efflux capacity, CEC) appears to be more related to HDL size and composition in terms of proteins and lipids, than to HDL-C plasma levels. CEC is usually evaluated by radioisotopic or fluorimetric bioassays in which cells are exposed to the HDL serum fraction, obtained by

depletion of serum of the apolipoprotein (apo)B-containing lipoproteins. Such evaluation has emerged as a potentially accurate estimate of the RCT efficiency in humans [10].

The clinical relevance of HDL CEC is well highlighted by many studies, in which an inverse relationship was detected between CEC and the prevalence of atherosclerosis, as well as the incidence of CV events, occurring independently of plasma HDL-C levels [11]. In addition, CEC has been found to be impaired in several genetic disorders leading to dyslipidemia, as well as in specific pathological conditions associated to higher CV risk, such as chronic kidney disease, diabetes, inflammatory and autoimmune diseases, endocrine disorders etc.

2. HDL and RCT

HDL was discovered in the 1950s and in 1960s the Framingham Heart Study reported the inverse relationship between plasma levels of these lipoproteins and atherosclerosis [1]. This milestone discovery encouraged investigations of the mechanism by which HDL could exert their anti-atherogenic properties, leading to the identification of the RCT process [12]. RCT, proposed for the first time by Glomset, is the key physiological pathway by which excess cholesterol is removed from the peripheral tissues to the liver for the final excretion into the bile and feces [12]. RCT may prevent the formation and progression of atherosclerotic plaque through the HDL-mediated removal of cholesterol from the arterial wall, identified as the main anti-atherogenic mechanism of HDL.

This process could be outlined in three phases:

- Cellular cholesterol efflux from macrophages;
- HDL remodeling in plasma;
- Cholesterol hepatic uptake and excretion.

2.1. Cellular Cholesterol Efflux from Macrophages

In the first step, cholesterol is initially moved from arterial macrophages to extracellular HDL [13]. This is the rate-limiting step of the entire RCT process [13] and plays a pivotal role in the maintenance of intracellular cholesterol homeostasis, crucial for macrophage function and viability. The excess of free cholesterol (FC) is toxic for cells, and this may be a very important factor considering that most peripheral cells and tissues (except for those of steroidogenic organs) cannot catabolize cholesterol. For this reason, macrophages protect themselves against FC accumulation by either transforming it to cholesteryl esters (CE) for intracellular storage or by effluxing it to extracellular acceptors such as HDL [14]. Cholesterol efflux mainly depends on macrophage cholesterol content, on the expression of various macrophage cholesterol transporters that mediate efflux and also on the features of the HDL acting as extracellular acceptors, mainly in terms of lipid and protein composition, as well as size [15][16].

2.2. HDL Remodeling in Plasma

In the subsequent RCT steps, cholesterol-enriched HDL may undergo remodeling in size and composition through the activity of the two enzymes lecithin-cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP). LCAT, synthetized in the liver, is the only enzyme able of esterifying FC of HDL to CE, transforming nascent discoidal particles in spherical HDL. The preferential lipoprotein substrates for LCAT are the discoidal preβ-HDL, produced through the initial interaction of lipid-free apolipoprotein A-I (apoA-I) with the ABCA1 transporter, with consequent cellular efflux of FC and phospholipids [17]. By esterifying HDL cholesterol, the enzyme LCAT helps to maintain the unesterified cholesterol gradient between the cell membrane and the extracellular acceptors, determining a constant flux of cholesterol from periphery to circulating lipoproteins and avoiding cholesterol reuptake by cells [18]. Despite its central function in HDL remodeling and maturation, the role of LCAT in the pathogenesis of atherosclerosis is still debated. In mice, the overexpression of human LCAT increased circulating HDL levels, but did not enhance macrophage RCT. Moreover, a significant macrophage RCT emerged in LCAT-deficient mice despite extremely low plasma levels of HDL-C [19]. Existing data in humans are controversial, but markedly support the idea that decreased LCAT concentration and activity, despite reducing HDL levels, are not related with the pathogenesis of atherosclerosis [17]. In particular, a study by our group demonstrated that serum from LCAT deficiency patients display increased ABCA1-mediated capacity to promote cholesterol efflux compared to control subjects, due to the presence of high levels of preβ-HDL [20].

The second enzyme playing a key role in RCT is CETP. This enzyme is an hydrophobic glycoprotein mainly produced by the liver, circulating in plasma largely bound to HDL [21]. It promotes the net mass transfer of CE from anti-atherogenic HDL to pro-atherogenic apoB)containing lipoproteins, in exchange with triglycerides (TG). Human studies have generally supported the idea that CETP deficiency, associated with increased HDL and apoA-I levels, is anti-atherogenic. This hypothesis has paved the way for developing CETP inhibitors as a possible strategy to raise HDL levels in humans, to reduce atherosclerosis and to treat CVD [22]. In human studies, CETP inhibition indeed induced an increase in HDL-C together with a decrease in the non-HDL-C. Nevertheless, clinical studies have failed in proving their capacity to reduce CV risk [23].

2.3. Cholesterol Hepatic Uptake and Excretion

In the third step of RCT, free and esterified HDL-derived cholesterol are uptaken by hepatocytes through the transporter SR-BI, generating cholesterol-poor HDL that can be recycled [24]. Moreover, in addition to the major pathway via SR-BI, under some conditions, HDL can be recognized also by LDL receptors (LDLr) in the liver, as observed in mice [25]. In addition, a recent study supported a novel concept suggesting that, at least in mice, a shift in macrophage-derived unesterified cholesterol from HDL to LDL provides a significant route for macrophage cholesterol to reach the liver via the hepatic LDLr [26].

After hepatic uptake, cholesterol can be eliminated into the bile as neutral sterols via the transporters ABCG5/ABCG8 or through ABCA1, after conversion into bile acids, and excreted via faeces [27]. For many years, the hepatobiliary route has been considered the only way for cholesterol excretion. Conversely, in recent years another metabolic pathway has been discovered. This mechanism, known as trans-intestinal cholesterol excretion,

takes place directly from plasma to the lumen of the intestine and markedly contributes to the total fecal neutral sterol excretion

References

1. Peter W. Wilson; Robert J. Garrison; William P. Castelli; Manning Feinleib; Patricia M. McNamara; William B. Kannel; Prevalence of coronary heart disease in the framingham offspring study: Role of lipoprotein cholesterols. *The American Journal of Cardiology* **1980**, *46*, 649-654, 10.1016/0002-9149(80)90516-0.
2. D J Gordon; J L Probstfield; R J Garrison; J D Neaton; W P Castelli; J D Knoke; D R Jacobs; S Bangdiwala; H A Tyroler; High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies.. *Circulation* **1989**, *79*, 8-15, 10.1161/01.cir.79.1.8.
3. Christian M. Madsen; Anette Varbo; Børge G. Nordestgaard; Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. *European Heart Journal* **2017**, *38*, 2478-2486, 10.1093/eurheartj/ehx163.
4. Gregory G. Schwartz; Anders G. Olsson; Markus Abt; Christie M. Ballantyne; Philip J. Barter; Jochen Brumm; Bernard R. Chaitman; Ingar M. Holme; David Kallend; Lawrence A. Leiter; et al.Eran LeitersdorfJohn J.V. McMurrayHardi MundlStephen J. NichollsPrediman K. ShahJean-Claude TardifR. Scott Wright Effects of Dalcetrapib in Patients with a Recent Acute Coronary Syndrome. *New England Journal of Medicine* **2012**, *367*, 2089-2099, 10.1056/nejmoa1206797.
5. A. Michael Lincoff; Stephen J. Nicholls; Jeffrey S. Riesmeyer; Philip J. Barter; H. Bryan Brewer; Keith A.A. Fox; C. Michael Gibson; Christopher Granger; Venu Menon; Gilles Montalescot; et al.Daniel RaderAlan R. TallEllen McErleanKathy WolskiGiacomo RuotoloBurkhard VangerowGovinda WeerakkodyShaun G. GoodmanDiego CondeDarren K. McGuireJose C. NicolauJose L. Leiva-PonsYves PesantWeimin LiDavid KandathSimon KouzNaeem TahirkheliDenise MasonSteven E. Nissen Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. *New England Journal of Medicine* **2017**, *376*, 1933-1942, 10.1056/nejmoa1609581.
6. Benjamin F Voight; Gina M Peloso; Marju Orho-Melander; Ruth Frikke-Schmidt; Maja Barbalic; Majken K Jensen; George Hindy; Hilma Hólm; Eric L Ding; Toby Johnson; et al.Heribert SchunkertNilesh J SamaniRobert ClarkeJemma C HopewellJohn F ThompsonMingyao LiGudmar ThorleifssonChristopher Newton-ChehKiran MusunuruJames P PirruccelloDanish SaleheenLi ChenAlexandre Fr StewartArne SchillertUnnur ThorsteinsdottirGudmundur ThorgeirssonSonia AnandJames C EngertThomas MorganJohn SpertusMonika StollKlaus BergerNicola MartinelliDomenico GirelliPascal P McKeownChristopher C PattersonStephen E EpsteinJoseph

DevaneyMary-Susan BurnettVincent MooserSamuli RipattiIda SurakkaMarkku S NieminenJuha SinisaloMarja-Liisa LokkiMarkus PerolaAki HavulinnaUlf de FaireBruna GiganteErik IngelssonTanja ZellerPhilipp WildPaul I W De BakkerOlaf H KlungelAnke-Hilse Maitland-Van der ZeeBas J M PetersAnthonius de BoerDiederick E GrobbeePieter W KamphuisenVera H M DeneerClara C ElbersN Charlotte Onland-MoretMarten H HofkerCisca WijmengaWm Monique VerschurenJolanda Ma BoerYvonne T van der SchouwAsif RasheedPhilippe FrossardSerkalem DemissieCristen WillerRon DoJose M OrdovasGonçalo R AbecasisMichael BoehnkeKaren L MohlkeMark J DalyCandace GuiducciNoël P BurttAarti SurtiElena GonzalezShaun PurcellStacey GabrielJaume MarrugatJohn PedenJeanette ErdmannPatrick DiemertChristina WillenborgInke R KönigMarcus FischerChristian HengstenbergAndreas ZieglerIan BuysschaertDiether LambrechtsFrans Van de WerfKeith A FoxNour Eddine El MokhtariDiana RubinJürgen SchrezenmeirStefan SchreiberArne SchäferJohn DaneshStefan BlankenbergRobert RobertsRuth McPhersonHugh WatkinsAlistair S HallKim OvervadEric RimmEric BoerwinkleAnne Tybjaerg-HansenL Adrienne CupplesMuredach P ReillyOlle MelanderPier M MannucciDiego ArdissinoDavid SiscovickRoberto ElosuaKari StefanssonChristopher J O'DonnellVeikko SalomaaDaniel J RaderLeena PeltonenStephen M SchwartzDavid AltshulerSekar Kathiresan Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *The Lancet* **2012**, *380*, 572-580, 10.1016/s0140-6736(12)60312-2.

7. Elda Favari; Angelika Chroni; Uwe J. F. Tietge; Ilaria Zanotti; Joan Carles Escolà-Gil; Franco Bernini; Cholesterol Efflux and Reverse Cholesterol Transport. *Snake Venoms* **2014**, *224*, 181-206, 10.1007/978-3-319-09665-0_4.
8. Mireille Ouimet; Tessa J. Barrett; Edward A. Fisher; HDL and Reverse Cholesterol Transport. *Circulation Research* **2019**, *124*, 1505-1518, 10.1161/circresaha.119.312617.
9. Michael C. Phillips; Molecular Mechanisms of Cellular Cholesterol Efflux. *Journal of Biological Chemistry* **2014**, *289*, 24020-24029, 10.1074/jbc.r114.583658.
10. Marina Cuchel; Anand Rohatgi; Frank M. Sacks; John R. Guyton; JCL roundtable: High-density lipoprotein function and reverse cholesterol transport. *Journal of Clinical Lipidology* **2018**, *12*, 1086-1094, 10.1016/j.jacl.2018.09.005.
11. Maria T Soria-Florido; Helmut Schröder; María Grau; Montserrat Fitó; Camille Lassale; High density lipoprotein functionality and cardiovascular events and mortality: A systematic review and meta-analysis. *Atherosclerosis* **2020**, *302*, 36-42, 10.1016/j.atherosclerosis.2020.04.015.
12. J.A. Glomset; E.T. Janssen; R. Kennedy; J. Dobbins; Role of plasma lecithin:cholesterol acyltransferase in the metabolism of high density lipoproteins. *Journal of Lipid Research* **1966**, *7*, 639-648, 10.1016/s0022-2275(20)39245-2.
13. Ilaria Zanotti; Cellular Cholesterol Efflux Pathways: Impact on Intracellular Lipid Trafficking and Methodological Considerations. *Current Pharmaceutical Biotechnology* **2011**, *999*, 1-10, 10.2174/

1389211214701242010.

14. Marina Cuchel; Daniel J. Rader; Macrophage Reverse Cholesterol Transport. *Circulation* **2006**, *113*, 2548-2555, 10.1161/circulationaha.104.475715.
15. Charlotte P.J. Talbot; Jogchum Plat; Andreas Ritsch; Ronald P. Mensink; Determinants of cholesterol efflux capacity in humans. *Progress in Lipid Research* **2018**, *69*, 21-32, 10.1016/j.plipres.2017.12.001.
16. Katrin Niisuke; Zsuzsanna Kuklenyik; Katalin V. Horvath; Michael S. Gardner; Christopher A. Toth; Bela F. Asztalos; Composition-function analysis of HDL subpopulations: influence of lipid composition on particle functionality. *Journal of Lipid Research* **2020**, *61*, 306-315, 10.1194/jlr.ra119000258.
17. Alice Ossoli; Sara Simonelli; Cecilia Vitali; Guido Franceschini; Laura Calabresi; Role of LCAT in Atherosclerosis. *Journal of Atherosclerosis and Thrombosis* **2016**, *23*, 119-127, 10.5551/jat.32854.
18. Helena Czarnecka; Shinji Yokoyama; Regulation of Cellular Cholesterol Efflux by Lecithin:Cholesterol Acyltransferase Reaction through Nonspecific Lipid Exchange. *Journal of Biological Chemistry* **1996**, *271*, 2023-2028, 10.1074/jbc.271.4.2023.
19. Hiroyuki Tanigawa; Jeffrey T. Billheimer; Jun-Ichiro Tohyama; Ilia V. Fuki; Dominic S. Ng; George H. Rothblat; Daniel J. Rader; Lecithin: Cholesterol Acyltransferase Expression Has Minimal Effects on Macrophage Reverse Cholesterol Transport In Vivo. *Circulation* **2009**, *120*, 160-169, 10.1161/CIRCULATIONAHA.108.825109.
20. Laura Calabresi; Elda Favari; Elsa Moleri; Maria Pia Adorni; Matteo Pedrelli; Sara Costa; Wendy Jessup; Ingrid C. Gelissen; Petri T. Kovanen; Franco Bernini; et al.Guido Franceschini Functional LCAT is not required for macrophage cholesterol efflux to human serum. *Atherosclerosis* **2009**, *204*, 141-146, 10.1016/j.atherosclerosis.2008.08.038.
21. Tall, A.; Plasma cholesteryl ester transfer protein. *J Lipid Res* **1993**, *34* , 1255-1274.
22. Philip J. Barter; H. Bryan Brewer; M. John Chapman; Charles H. Hennekens; Daniel J. Rader; Alan R. Tall; Cholesteryl Ester Transfer Protein. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2003**, *23*, 160-167, 10.1161/01.atv.0000054658.91146.64.
23. Philip J. Barter; Mark Caulfield; Mats Eriksson; Scott M. Grundy; John J. P. Kastelein; Michel Komajda; Jose Lopez-Sendon; Lori Mosca; Jean-Claude Tardif; David D. Waters; et al.Charles L. ShearJames H. RevkinKevin A. BuhrMarian R. FisherAlan R. TallBryan Brewer Effects of Torcetrapib in Patients at High Risk for Coronary Events. *New England Journal of Medicine* **2007**, *357*, 2109-2122, 10.1056/nejmoa0706628.
24. Daniel J. Rader; Eric T. Alexander; Ginny L. Weibel; Jeffrey Billheimer; George H. Rothblat; The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis.

Journal of Lipid Research **2009**, *50*, S189-S194, 10.1194/jlr.r800088-jlr200.

25. Alexander C. Bashore; Mingxia Liu; Chia-Chi C. Key; Elena Boudyguina; Xianfeng Wang; Caitlin M. Carroll; Janet K. Sawyer; Adam E. Mullick; Richard G. Lee; Shannon L. Macauley; et al.John S. Parks Targeted Deletion of HepatocyteAbca1Increases Plasma HDL (High-Density Lipoprotein) Reverse Cholesterol Transport via the LDL (Low-Density Lipoprotein) Receptor. *Arteriosclerosis, Thrombosis, and Vascular Biology* **2019**, *39*, 1747-1761, 10.1161/atvbaha.119.312382.
26. Lidia Cedo; Jari Metso; David Santos; Annabel García-León; Nuria Plana; Sonia Sabate-Soler; Noemi Rotllan; Andrea Rivas-Urbina; Karen A Mendez-Lara; Mireia Tondo; et al.Josefa GironaJosep JulveVictor PallarèsAleyda Benitez-AmadoVicenta Llorente CortesAntonio PérezDiego Gómez-CoronadoAnna-Kaisa RuotsalainenAnna-Liisa LevonenJose Luis Sanchez-QuesadaLuis MasanaPetri T KovanenMatti Sakari JauhiainenMiriam Lee-RueckertFrancisco Blanco-VacaJoan Carles Escola-Gil LDL Receptor Regulates the Reverse Transport of Macrophage-Derived Unesterified Cholesterol via Concerted Action of the HDL-LDL Axis: Insight from Mouse Models. *Circulation Research* **2020**, *127*, 778, 10.1161/circresaha.119.316424.
27. Liqing Yu; Robert E. Hammer; Jia Li-Hawkins; Klaus Von Bergmann; Dieter Lutjohann; Jonathan C. Cohen; Helen H. Hobbs; Disruption of Abcg5 and Abcg8 in mice reveals their crucial role in biliary cholesterol secretion. *Proceedings of the National Academy of Sciences* **2002**, *99*, 16237-16242, 10.1073/pnas.252582399.