Assessment of HDL Cholesterol Removal Capacity: Toward Clinical Application

Ryuji Toh

Division of Evidence-based Laboratory Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

While there is a controversy regarding the causal relationship between high-density lipoprotein cholesterol (HDL-C) and cardiovascular disease (CVD), recent studies have demonstrated that the cholesterol efflux capacity (CEC) of HDL is associated with the incidence of CVD. However, there are several limitations to current assays of CEC. First, CEC measurements are not instantly applicable in clinical settings, because CEC assay methods require radiolabeled cholesterol and cultured cells, and these procedures are time consuming. Second, techniques to measure CEC are not standardized. Third, the condition of endogenous cholesterol donors would not be accounted for in the CEC assays. Recently, we established a simple, high-throughput, cell-free assay system to evaluate the capacity of HDL to accept additional cholesterol, which is herein referred to as “cholesterol uptake capacity (CUC)”. We demonstrated that CUC represents a residual cardiovascular risk in patients with optimal low-density lipoprotein cholesterol control independently of traditional risk factors, including HDL-C. Establishing reproducible approaches for the cholesterol removal capacity of HDL is required to validate the impact of dysfunctional HDL on cardiovascular risk stratification in the “real world”.

Key words: High-density lipoprotein (HDL), Cardiovascular disease, HDL cholesterol (HDL-C), Cholesterol efflux capacity (CEC), Cholesterol uptake capacity (CUC)

Copyright©2019 Japan Atherosclerosis Society
This article is distributed under the terms of the latest version of CC BY-NC-SA defined by the Creative Commons Attribution License.
especially in the setting of cholesterol enrichment. ABCA1 mediates the cellular binding of apoAI, the major structural protein of HDL, and the unidirectional export of cholesterol and phospholipids (PLs) to lipid-free/-poor apoAI, leading to nascent HDL formation23-25).

On the other hand, SR-BI and ATP-binding cassette transporter G1 (ABCG1) contribute to the maturation of HDL. SR-BI mediates bidirectional cholesterol exchange22, 26). ABCG1 does not mediate the cellular binding of HDL, but promotes the transport of free cholesterol from the cell interior to the plasma membrane27). The cholesterol translocated by ABCG1 in the cell membrane is exported to HDL via an aqueous diffusion pathway22). Lecithin: cholesterol acyltransferase (LCAT) on HDL prevents the return of accepted cholesterol to the cell by esterification, because cholesteryl esters are more hydrophobic than free cholesterol and move to the core of HDL28).

Mature HDL particles can be remodeled to smaller particles with the release of apoAI by the actions of hepatic lipase, endothelial lipase, and phospholipase A2, which hydrolyze TG and PLs in HDL29-36).

HDL-C, but also accelerates atherosclerosis17).

On the contrary, recent large cohort studies have demonstrated that the cellular cholesterol efflux capacity (CEC) of HDL, a dynamic rate of initial step in RCT, is associated with both the prevalence and the incidence of CVD, and is a better predictor than steady-state circulating HDL-C levels18-20). However, at present, CEC measurements are not instantly applicable as a high-throughput assay in clinical settings. In this review, we discuss the limitations of current CEC assays and future directions.

### Cellular Cholesterol Efflux

As most types of cells are unable to catabolize cholesterol, RCT is indispensable for homeostasis. The efflux of cholesterol from cells to serum is an initial step in the RCT pathway. HDL is the component of serum responsible for mediating cholesterol efflux21). Cholesterol-enriched macrophages can release cholesterol to HDL using several pathways22).

First, ATP-binding cassette transporter A1 (ABCA1) is an important player in HDL biogenesis, especially in the setting of cholesterol enrichment. ABCA1 mediates the cellular binding of apoAI, the major structural protein of HDL, and the unidirectional export of cholesterol and phospholipids (PLs) to lipid-free/-poor apoAI, leading to nascent HDL formation23-25).

On the contrary, recent large cohort studies have demonstrated that the cellular cholesterol efflux capacity (CEC) of HDL, a dynamic rate of initial step in RCT, is associated with both the prevalence and the incidence of CVD, and is a better predictor than steady-state circulating HDL-C levels18-20). However, at present, CEC measurements are not instantly applicable as a high-throughput assay in clinical settings. In this review, we discuss the limitations of current CEC assays and future directions.

### Cellular Cholesterol Efflux

As most types of cells are unable to catabolize cholesterol, RCT is indispensable for homeostasis. The efflux of cholesterol from cells to serum is an initial step in the RCT pathway. HDL is the component of serum responsible for mediating cholesterol efflux21). Cholesterol-enriched macrophages can release cholesterol to HDL using several pathways22).

First, ATP-binding cassette transporter A1 (ABCA1) is an important player in HDL biogenesis, especially in the setting of cholesterol enrichment. ABCA1 mediates the cellular binding of apoAI, the major structural protein of HDL, and the unidirectional export of cholesterol and phospholipids (PLs) to lipid-free/-poor apoAI, leading to nascent HDL formation23-25).

On the contrary, recent large cohort studies have demonstrated that the cellular cholesterol efflux capacity (CEC) of HDL, a dynamic rate of initial step in RCT, is associated with both the prevalence and the incidence of CVD, and is a better predictor than steady-state circulating HDL-C levels18-20). However, at present, CEC measurements are not instantly applicable as a high-throughput assay in clinical settings. In this review, we discuss the limitations of current CEC assays and future directions.

![Fig. 1. Post-transcriptional modification of apoAI and CEC](image-url)
Current Understanding of CEC

HDL is a heterogeneous population of particles, and ranges in size from <7 to >14 nm\(^37,38\). Subspecies of HDL are considered to be involved in different pathways of cellular cholesterol efflux\(^39,40\). The smallest apoAI-containing HDL (pre-β-1) and small, dense HDL subfractions (HDL3b and 3c) are principal transporters via the ABCA1 pathway\(^38,41\). On the other hand, PL-rich HDL2 is an efficient acceptor of cholesterol because it provides a larger target for effective collisions with diffusing free cholesterol\(^42-44\). The fatty acid composition of PLs in HDL has also been proposed to affect CEC. We have demonstrated that the oral administration of eicosapentaenoic acid (EPA) improved CEC in patients with dyslipidemia\(^45\), and confirmed the beneficial effects of EPA-rich HDL on cholesterol efflux using reconstituted HDL\(^46\). Increases in unsaturated PL acyl chains in HDL particles have been demonstrated to result in more efficient cholesterol acceptors\(^47\). Omega 3 fatty acids have also been shown to increase LCAT activity\(^48\).

On the other hand, the oxidation of several specific amino acids in apoA1 could result in a reduction in CEC\(^49\,50\)(Fig. 1). Granular leukocyte-derived myeloperoxidase (MPO) promotes the oxidation of apoA1, while paraoxonase 1 (PON1) has antioxidant properties for HDL\(^52\,53\). We have demonstrated that patients with high MPO levels and low PON1 activity in serum exhibit impaired CEC\(^54\).

Limitations of Conventional CEC Assays

Fig. 2 shows the conventional procedure for a CEC assay. Macrophages are exposed by radiolabeled cholesterol. Then, they are stimulated by HDL, and the excreted cholesterol is measured by a scintillation counter\(^59\).

Although CEC has been used as a marker for CVD, there are several limitations to current CEC assays for clinical application\(^60\). First, this method requires radiolabeling and cultured cells, and these procedures are time consuming. Second, procedures for CEC measurement are not standardized, which makes it difficult to compare different CEC studies. Varied systems to measure CEC exist, as shown in Fig. 3\(^56,57\). Several donor cells are employed for CEC measurements. Fu5AH rat hepatoma cells, which express high levels of SR-BI but lack functional ABCA1, are used to assess SR-BI-mediated efflux\(^58\). On the other hand, J774 mouse macrophage cells express low levels of SR-BI, and stimulation with cyclic adenosine monophosphate (cAMP) can upregulate its ABCA1 expression\(^59,60\). In the Dallas Heart
The third limitation is that the status of endogenous cholesterol donors would not be accounted for in in vitro CEC assays. Changes of in vivo macrophage cellular function resulting from various conditions have been reported as follows: phenolic acids increased ABCG1 and SR-BI expression\(^66\); on the other hand, xanthine oxidoreductase suppressed ABCA1 and ABCG1 expression in macrophages\(^67\); while we have demonstrated that EPA could improve CEC\(^45, 46\), another group has reported that EPA might reduce ABCA1 functionality in macrophages\(^68\).

Curiously, ABCA-1 dependent CEC was reported to be enhanced rather than impaired in patients with high TG levels\(^69\). In those patients, a reduction in large HDL particles and an increase in pre-β-1 particles were observed. Concomitantly, SR-BI-dependent efflux, which is mediated mainly by large HDL, decreased. On the other hand, accompanied by an increase in pre-β-1 particles, ABCA-1-dependent efflux was also augmented\(^69\). However, ABCA1-dependent efflux was determined using J744 cells as described above\(^69\). The lack of the macrophage ability assessment in an individual might cause overestimation.

Study\(^{19}\) and the European Prospective Investigation of Cancer (EPIC)-Norfolk study\(^{20}\), J774 cells treated with cAMP were used to measure CEC. On the contrary, Ogura, et al. have recently demonstrated that CEC determined using J774 cells without cAMP treatment was also inversely associated with the presence of atherosclerotic CVD in patients with familial hypercholesterolemia\(^61\). In cases requiring the assessment of ABCA1-dependent CEC, the basal CEC (without cAMP) is subtracted from the total CEC (with cAMP)\(^62\). Because the ultracentrifugation procedure for HDL isolation requires several days, most of the recent reports employed apoB-depleted serum as the cholesterol acceptor. However, apoB-depleted serum has been reported to contain not only HDL and apoA1 but also other components, such as albumin, that can accept the cholesterol released from macrophages\(^63\). Moreover, HDL composition and/or size distribution might vary depending on the apoB depletion methods\(^64\). Li, et al. even reported that cholesterol efflux to apoB-depleted serum was paradoxically associated with an increased prospective risk of CVD\(^65\). While a protocol using radiolabeled cholesterol does not lend itself to the development of a high-throughput assay, fluorescence-labeled cholesterol is alternatively available for CEC measurements. Fractional efflux rates obtained with BODIPY-cholesterol were reported to be greater than those with tritium-labeled cholesterol\(^65\).

The third limitation is that the status of endogenous cholesterol donors would not be accounted for in in vitro CEC assays. Changes of in vivo macrophage cellular function resulting from various conditions have been reported as follows: phenolic acids increased ABCG1 and SR-BI expression\(^66\); on the other hand, xanthine oxidoreductase suppressed ABCA1 and ABCG1 expression in macrophages\(^67\); while we have demonstrated that EPA could improve CEC\(^45, 46\), another group has reported that EPA might reduce ABCA1 functionality in macrophages\(^68\).
Cholesterol Uptake Capacity, A New Measure for HDL Functionality

In order to break through this situation, we have recently established a simple, high-throughput, cell-free assay system to evaluate the “cholesterol uptake capacity (CUC)” as a novel concept for HDL functionality\(^70\).

The procedural schema of our new assay is shown in Fig. 2. After removing apoB, serum is incubated with fluorescence-labeled cholesterol, HDL is captured by specific antibodies for apoAI coated on a microplate, and then the amount of the labeled cholesterol in the HDL is measured using a plate reader. This assay system does not require radiolabeling and cultured cells, and the procedures are simple, with a short turnaround time. Moreover, the application of the anti-apoAI antibody allows a specific evaluation of the ability of HDL to accept cholesterol.

We revealed that CUC was suppressed by MPO treatment, indicating that CUC has the potential to evaluate the oxidation-induced inactivation of HDL\(^70\). Furthermore, we found that CUC correlated inversely with the requirement for revascularization because of the recurrence of coronary lesions in patients with optimal control of LDL-C. A multivariate analysis adjusted for traditional coronary risk factors, including HDL-C, showed that only CUC remained significant\(^70\).

Difference between CEC and CUC

Since CUC was determined by a cell-free assay, CUC does not reflect ABCA1-mediated efflux (Fig. 4). On the other hand, we demonstrated that CUC was associated with CEC determined using J774 cells without cAMP (non-ABCA1-mediated, basal CEC)\(^70\), which was employed in the study conducted on patients with familial hypercholesterolemia\(^61\). As the CUC assay is an aqueous diffusion-dependent system, it appears to mainly reflect the contribution of PL-rich, matured HDL to cholesterol efflux (Fig. 4). As expected, HDL particle concentration (HDL-P) measurements using nuclear magnetic resonance spectroscopy demonstrated that large HDL-P showed a more prominent association with CUC than small HDL-P, suggesting that CUC is influenced predominantly by the concentration of matured HDL particles (Fig. 5).

Since the facilitation of HDL biogenesis is a potential therapeutic approach, small HDL particles that promote cholesterol efflux by the ABCA1 path-
has been used as a marker for CVD, there is no established method for its measurement for use in routine practice. On the other hand, the cell-free assay system to measure CUC will allow a high-throughput characterization of HDL functionality. Establishing reproducible, standardized approaches for HDL-cholesterol removal capacity is required for further validation of its impact on cardiovascular risk stratification in the “real world.”

Conflicts of Interest

The Division of Evidence-based Laboratory Medicine, Kobe University Graduate School of Medicine, was established by an endowment fund from the Sysmex Corporation.

Acknowledgments

This research was supported by a Grant-in-Aid for Scientific Research (C) JP18K08073 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1) Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs DR, Jr., Bangdiwala S, Tyroler HA: High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation, 1989; 79: 8-15
2) Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, Patsch W and Atherosclerosis Risk in Communities Study G: Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communi-

Fig. 5. Correlations between CUC and HDL particle concentration (HDL-P)
Small (diameter range: 7.3–8.2 nm) and large (diameter range: 9.4–14 nm) HDL-P were determined at LipoScience/LabCorp (Burlington, NC) using nuclear magnetic resonance spectroscopy and the LipoProfile-3 algorithm.

Conclusion

Although CEC as an indicator of HDL function way are emphasized as cardioprotective species of HDL. In such a context, CEC was determined using ABCA1-upregulated cells in recent large cohort studies. However, all patients with Tangier disease, which is caused by null-mutations in ABCA1, may not necessarily be at high risk of CVD despite marked deficiencies in HDL-C and apoAI. Therefore, the impact of ABCA1-mediated cholesterol efflux on anti-atherosclerotic process is still controversial. On the other hand, most evidence suggests that levels of large HDL particles are inversely related with the risk of CVD, whereas levels of small HDL particles are positively correlated with this risk. In dyslipidemic conditions, such as diabetes and insulin resistance, a remodeling of HDL toward smaller particles is assumed to occur due to activity alterations of lipases involved in HDL metabolism and CETP. However, an increase in small HDL particles might seemingly cause an enhancement of CEC, as described above. On the contrary, CUC has the potential utility of cardiovascular risk stratification through the monitoring of disturbances in HDL maturation. Further trials are required to compare the clinical usefulness between “cell-based” CEC and “cell-free” CUC assays.

In terms of the reproducibility of CUC, the intra-assay coefficient of variation (within-run precision) was less than 5% and the inter-assay coefficient of variation (between-run precision) was 7%, both of which are comparable to those of CEC reported in the EPIC-Norfolk study. We are currently developing a completely automated system to measure CUC.

Although CEC as an indicator of HDL function...
HDL Cholesterol Removal Capacity Assessment

ties (ARIC) Study. Circulation, 2001; 104: 1108-1113

3) Emerging Risk Factors C, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A, Wood AM, Lewington S, Sattar N, Packard CJ, Collins R, Thompson SG, Danesh J: Major lipids, apolipoproteins, and risk of vascular disease. JAMA, 2009; 302: 1993-2000

4) Rosenson RS: The High-Density Lipoprotein Puzzle: Why Classic Epidemiology, Genetic Epidemiology, and Clinical Trials Conflict? Arterioscler Thromb Vasc Biol, 2016; 36: 777-782

5) Karathanasak SK, Freeman LA, Gordon SM, Remaley AT: The Changing Face of HDL and the Best Way to Measure It. Clin Chem, 2017; 63: 196-210

6) Investigators A-H, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desgivres-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W: niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med, 2011; 365: 2255-2267

7) Barter PJ, Caulfield M, Eriksson M, Grundy SM, Kastelein JJ, Komajda M, Lopez-Sendon J, Mosca L, Tardif JC, Waters DD, Shear CL, Revkin JH, Buhr KA, Fisher MR, Tall AR, Porte J, Investigators I: Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med, 2007; 357: 2109-2122

8) Schwartz GG, Olsson AG, Abdulla WE, Probstfield JL, Anderson T, Chaitman BR, Desgivres-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W: niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. N Engl J Med, 2011; 365: 2255-2267

9) Lincoff AM, Nichols SJ, Riesmeyer JS, Barter PJ, Brewer HB, Fox KA, Gibson CM, Granger C, Menon V, Montalescot G, Rader D, Tall AR, Meir E, Wolski K, Rutotolo G, Vangerow B, Weerakkody G, Goodman SG, Conde D, McGuire DK, Nicolau JC, Leiva-Pons JL, Pesant Y, Li W, Kandath D, Kozou S, Tahir Kheli N, Mason D, Nissen SE, Investigators A: Evacetrapib and Cardiovascular Outcomes in High-Risk Vascular Disease. N Engl J Med, 2016; 375: 42-51

10) Group HTRC, Bowman L, Hopewell JC, Chen F, Wallendzus K, Stevens W, Collins R, Wiviott SD, Cannon CP, Braunwald E, Sammons E, Landray MJ: Effects of Anacetrapib in Patients with Atherosclerotic Vascular Disease. N Engl J Med, 2017; 376: 2478-2486

11) Tall AR, Rader DJ: Trials and Tribulations of CETP Inhibitors. Circ Res, 2018; 122: 106-112

12) Voigt BF, Pelosi GM, Orho-Melander M, Frikke-Schmidt R, Barbic M, Jensen MK, Hindy G, Holm H, Ding EL, Johnson T, Schunkert H, Samani NJ, Clarke R, Hopewell JC, Thompson PJ, Li M, Thorleifsson G, Newton-Cheh C, Munusuru K, Pircull CEO, Saleheen D, Chen L, Stewart A, Schillert A, Thorsteinsdottir U, Thorgerisson G, Anand S, Engel J, Morgan T, Spertus J, Stoll M, Berger K, Martilloni N, Gibb A, McKeown PP, Peterson CC, Epstein SE, Devaney J, Burnett MS, Mooser V, Rippati S, Surakka I, Nienhuis MN, Sinisalo J, Lokki ML, Perola M, Hauvulinna A, di Faire U, Gigante B, Ingelsson E, Zeller T, Wild P, de Bakker PI, Klungel OH, Maitland-van der Veen AH, Peters BJ, de Boer A, Grobbee DE, Kamphuisen PW, Even DNH, Elbers GJ, Kjeldsen N, Hoffmann H, Villumsen J, Hjiri A, D'Heur A, Blomberg B, Scharf A, Danesh J, Blankenberg S, Roberts R, McPherson R, Watkins H, Hall AS, Overvad K, Rimm E, Boerwinkle E, Tybjaerg-Hansen A, Cupples LA, Reilly MP, Melander O, Mannucci PM, Ardissino D, Siscovick D, Eloumu R, Stefanosk K, D'Onofrio CJ, Salomaa V, Rader DJ, Peltonen L, Schwartz SM, Alshuler D, Kihrespan J: Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. Lancet, 2012; 380: 572-580

13) Bartlet J, Predazzi IM, Williams SM, Bush WS, Kim Y, Havas S, Toth PP, Fazio S, Miller M: Is Isolated Low High-Density Lipoprotein Cholesterol a Cardiovascular Disease Risk Factor? New Insights From the Framingham Offspring Study. Circ Cardiovasc Qual Outcomes, 2016; 9: 206-212

14) Madsen CM, Varbo A, Nordestgaard BG: Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. Eur Heart J, 2017; 38: 2478-2486

15) Hirata A, Sugiyama D, Watanabe M, Tamakoshi A, Iso H, Kotani K, Kiyama M, Yamada M, Ishikawa S, Murakami Y, Miura K, Ueshima H, Okamura T, Evidence for Cardiovascular Prevention from Observational Cohorts in Japan Research G: Association of extremely high levels of high-density lipoprotein cholesterol with cardiovascular mortality in a pooled analysis of 9 cohort studies including 43,407 individuals: The EPOCH-JAPAN study. J Clin Lipidol, 2018; 12: 674-684 e675

16) Hirano K, Yamashita S, Nakajima N, Arai T, Maruyama T, Yoshida Y, Ishigami M, Sakai N, Kameda-Takemura K, Matsuzawa Y: Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. Marked hyperalphalipoproteinemia caused by CETP gene mutation is not associated with longevity. Arterioscler Thromb Vasc Biol, 1997; 17: 1053-1059

17) Zannou P, Khetarpal SA, Larach DB, Hancock-Cerutti MF, MILLIS S, Cuchel M, DerOhanessian S, Kontush A, Surendran P, Troup L, Saleheen D, Trompet S, Toth PP, Fazio S, Miller M: Is Isolated Low High-Density Lipoprotein Cholesterol a Cardiovascular Disease Risk Factor? New Insights From the Framingham Offspring Study. Circ Cardiovasc Qual Outcomes, 2016; 9: 206-212

18) Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafari K, French BC, Phillips JA, Muckavage
ML, Wilensky RL, Mohler ER, Rothblat GH, Rader DJ: Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med, 2011; 364: 127-135

19) Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neelaid J, Yuhanna IS, Rader DR, de Lemos JA, Shaul PW: HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med, 2014; 371: 2383-2393

20) Saleheen D, Scott R, Javad S, Zhao W, Rodrigues A, Picataghi A, Lukmanova D, Muckavage ML, Luben R, Billheimer J, Kastelien JJ, Boekholt SM, Khaw KT, Wareham N, Rader DJ: Association of HDL cholesterol efflux capacity with incident coronary heart disease events: a prospective case-control study. Lancet Diabetes Endocrinol, 2015; 3: 507-513

21) Rothblat GH, de la Llera-Moya M, Atger V, Kellner-Weibel G, Williams DL, Phillips MC: Cell cholesterol efflux: integration of old and new observations provides new insights. J Lipid Res, 1999; 40: 781-796

22) Phillips MC: Molecular mechanisms of cellular cholesterol efflux. J Biol Chem, 2014; 289: 24020-24029

23) Wang S, Gulshan K, Brubaker G, Hazen SL, Smith JD: ABCA1 mediates unfolding of apolipoprotein A-I terminus on the cell surface before lipidation and release of nascent high-density lipoprotein. Arterioscler Thromb Vasc Biol, 2013; 33: 1197-1205

24) Wang S, Smith JD: ABCA1 and nascent HDL biogenesis. Biofactors, 2014; 40: 547-554

25) Gulshan K, Brubaker G, Conger H, Wang S, Zhang R, Hazen SL, Smith JD: PI(4,5)P2 Is Translocated by ABCA1 to the Cell Surface Where It Mediates Apolipoprotein A1 Binding and Nascent HDL Assembly. Circ Res, 2016; 119: 827-838

26) Zannis VI, Chroni A, Krieger M: Role of apoA-I, ABCA1, LCAT, and SR-BI in the biogenesis of HDL. J Mol Med (Berl), 2006; 84: 276-294

27) Sankaranarayanan S, Oram JF, Asztalos BF, Vaughan AM, Lund-Katz S, Adorni MP, Phillips MC, Rothblat GH: Effects of acceptor composition and mechanism of ABCG1-mediated cellular free cholesterol efflux. J Lipid Res, 2009; 50: 275-284

28) Czarnecki H, Yokoyama S: Regulation of cellular cholesterol efflux by lecithin:cholesterol acyltransferase reaction through nonspecific lipid exchange. J Biol Chem, 1996; 271: 2023-2028

29) Perret B, Mabile L, Martinez L, Terce F, Barbaras R, Collet X: Hepatic lipase: structure/function relationship, synthesis, and regulation. J Lipid Res, 2002; 43: 1163-1169

30) Jin W, Marchadier D, Rader DJ: Lipases and HDL metabolism. Trends Endocrinol Metab, 2002; 13: 174-178

31) McCoy MG, Sun GS, Marchadier D, Maugais C, Glick JM, Rader DJ: Characterization of the lipolytic activity of endothelial lipase. J Lipid Res, 2002; 43: 921-929

32) Ishida T, Choi S, Kundu RK, Hirata K, Rubin EM, Cooper AD, Quertermous T: Endothelial lipase is a major determinant of HDL level. J Clin Invest, 2003; 111: 347-355

33) Sun L, Ishida T, Miyashita K, Kinoshita N, Mori K, Yasuda T, Toh R, Nakajima K, Imamura S, Hirata K: Plasma activity of endothelial lipase impacts high-density lipoprotein metabolism and coronary risk factors in humans. J Atheroscler Thromb, 2014; 21: 313-321

34) de Beer FC, de Beer MC, van der Westhuysen DR, Castellani LW, Luisis AJ, Swanson ME, Grass DS: Secretory non-pancreatic phospholipase A2: influence on lipoprotein metabolism. J Lipid Res, 1997; 38: 2232-2239

35) de Beer FC, Connell PM, Yu J, de Beer MC, Webb NR, van der Westhuysen DR: HDL modification by secretory phospholipase A(2) promotes scavenger receptor class B type I interaction and accelerates HDL catabolism. J Lipid Res, 2000; 41: 1849-1857

36) Tietge UJ, Maugais C, Cain W, Grass D, Glick JM, de Beer FC, Rader DJ: Overexpression of secretory phospholipase A(2) causes rapid catabolism and altered tissue uptake of high density lipoprotein cholesterol ester and apolipoprotein A-I. J Biol Chem, 2000; 275: 10077-10084

37) Asztalos BF, Tani M, Schafer EJ: Metabolic and functional relevance of HDL subspecies. Curr Opin Lipidol, 2011; 22: 176-185

38) Heinecke JW: Small HDL promotes cholesterol efflux by the ABCA1 pathway in macrophages: implications for therapies targeted to HDL. Circ Res, 2015; 116: 1101-1103

39) Rothblat GH, Phillips MC: High-density lipoprotein heterogeneity and function in reverse cholesterol transport. Curr Opin Lipidol, 2010; 21: 229-238

40) Camont L, Chapman MJ, Kontush A: Biological activities of HDL subpopulations and their relevance to cardiovascular disease. Trends Mol Med, 2011; 17: 594-603

41) Du XM, Kim MJ, Hou L, Le Goff W, Chapman MJ, Van Eck M, Curtiss LK, Burnett JR, Cartland SP, Quinn CM, Kocka M, Kontush A, Rye KA, Krithairades L, Jessup W: HDL particle size is a critical determinant of ABCA1-mediated macrophage cellular cholesterol export. Circ Res, 2015; 116: 1133-1142

42) Davidson WS, Rodrigueza WV, Lund-Katz S, Johnson WJ, Rothblat GH, Phillips MC: Effects of acceptor particle size on the efflux of cellular free cholesterol. J Biol Chem, 1995; 270: 17106-17113

43) Fournier N, de la Llera-Moya M, Burkey BF, Swaney JB, Paterniti J, Jr., Moatti N, Atger V, Rothblat GH: Role of HDL phospholipid efflux of cell cholesterol to whole serum: studies with human apoA-I transgenic rats. J Lipid Res, 1996; 37: 1704-1711

44) Yancey PG, de la Llera-Moya M, Swarnakar S, Monzo P, Klein SM, Connelly MA, Johnson WJ, Williams DL, Rothblat GH: High density lipoprotein phospholipid composition is a major determinant of the bi-directional flux and net movement of cellular free cholesterol mediated by scavenger receptor BI. J Biol Chem, 2000; 275: 36596-36604

45) Tanaka N, Ishida T, Nagao M, Mori T, Monguchi T, Sasaki M, Mori K, Kondo K, Nakajima H, Honjo T, Inro Y, Toh R, Shinohara M, Hirata K: Administration of high dose eicosapentaenoic acid enhances anti-inflammatory properties of high-density lipoprotein in Japanese patients with dyslipidemia. Atherosclerosis, 2014; 237: 577-583

46) Tanaka N, Inro Y, Shinohara M, Tsuda S, Mori T, Nagao M, Oshita T, Mori K, Hara T, Toh R, Ishida T, Hirata K:
Eicosapentaenoic acid-enriched high-density lipoproteins exhibit anti-atherogenic properties. Circ J, 2018; 82: 596-601
47) Davidson WS, Gigliotto KL, Lund-Katz S, Johnson WJ, Rothblat GH, Phillips MC: The effect of high density lipoprotein phospholipid acyl chain composition on the efflux of cellular free cholesterol. J Biol Chem, 1995; 270: 5882-5890
48) Kasbi Chadli F, Nazih H, Krempf M, Nguyen P, Ougueram K: Omega 3 fatty acids promote macrophage reverse cholesterol transport in hamster fed high fat diet. PLoS One, 2013; 8: e61109
49) Shao B, Tang C, Sinha A, Mayer PS, Davenport GD, Brot N, Oda MN, Zhao XQ, Heinecke JW: Humans with atherosclerosis have impaired ABCA1 cholesterol efflux and enhanced high-density lipoprotein oxidation by myeloperoxidase. Circ Res, 2014; 114: 1733-1742
50) DiDonato JA, Aulak K, Huang Y, Wagner M, Gersteneker G, Topbas C, Gogonea V, DiDonato AJ, Tang WH, Meld RA, Fox PL, Plow EF, Smith JD, Fisher EA, Hazen SL: Site-specific nitration of apolipoprotein A-I at tyrosine 166 is both abundant within human atherosclerotic plaque and dysfunctional. J Biol Chem, 2014; 289: 10276-10292
51) Huang Y, DiDonato JA, Levison BS, Schmitt D, Li L, Wu Y, Buffa J, Kim T, Gersteneker GS, Gu X, Kadiyala CS, Wang Z, Culley MK, Hazen JE, Didonato AJ, Fu X, Berisha SZ, Peng D, Nguyen TT, Liang S, Chuang CC, Cho L, Plow EF, Fox PL, Gogonea V, Tang WH, Parks JS, Fisher EA, Smith JD, Hazen SL: An abundant dysfunctional apolipoprotein A1 in human atheroma. Nat Med, 2014; 20: 193-203
52) Nicholls SJ, Hazen SL: Myeloperoxidase, modified lipoproteins, and atherogenesis. J Lipid Res, 2009; 50 Suppl: S346-351
53) Huang Y, Wu Z, Riwanto M, Gao S, Levison BS, Gu X, Fu X, Wagner MA, Besler C, Gersteneker G, Zhang R, Li XM, DiDonato AJ, Gogonea V, Tang WH, Smith JD, Plow EF, Fox PL, Shih DM, Lusis AJ, Fisher EA, DiDonato JA, Landmesser U, Hazen SL: Myeloperoxidase, paraoxonase-1, and HDL form a functional ternary complex. J Clin Invest, 2013; 123: 3815-3828
54) Haraguchi Y, Töhr T, Hasokawa M, Nakajima H, Honjo T, Otsui K, Mori K, Miyamoto-Sasaki M, Shinozaka M, Nishimura K, Ishida T, Hiraoka K: Serum myeloperoxidase/paraoxonase ratio as potential indicator of dysfunctional high-density lipoprotein and risk stratification in coronary artery disease. Atherosclerosis, 2014; 234: 288-294
55) Khera AV, Rader DJ: Cholesterol efflux capacity: full steam ahead or a bump in the road? Arterioscler Thromb Vasc Biol, 2013; 33: 1449-1451
56) Anastasiou M, Kock M, Jessup W, Sullivan D, Kraya K, Kritharides L: Cholesterol efflux capacity: An introduction for clinicians. Am Heart J, 2016; 180: 54-63
57) Talbot CPJ, Plat J, Risch A, Mensink RP: Determinants of cholesterol efflux capacity in humans. Prog Lipid Res, 2018; 69: 21-32
58) Miwa K, Inazu A, Kashwiri M, Nohara A, Higashikata T, Kobayashi J, Koizumi J, Nakajima K, Nakano T, Nii M, Mabuchi H, Yamagishi M: Cholesterol efflux from J774 macrophages and Fu5AH hepatoma cells to serum is preserved in CETP-deficient patients. Clin Chim Acta, 2009; 402: 19-24
59) Bortnick AE, Rothblat GH, Stoudt G, Hoppe KL, Royer LJ, McNeish J, Francone OL: The correlation of ATP-binding cassette I mRNA levels with cholesterol efflux from various cell lines. J Biol Chem, 2000; 275: 28634-28640
60) Santamarina-fojo S, Remaley AT, Neufeld EB, Brewer HB, Jr.: Regulation and intracellular trafficking of the ABCA1 transporter. J Lipid Res, 2001; 42: 1339-1345
61) Ogura M, Hori M, Harada-Shiba M: Association Between Cholesterol Efflux Capacity and Atherosclerotic Cardiovascular Disease in Patients With Familial Hypercholesterolemia. Arterioscler Thromb Vasc Biol, 2016; 36: 181-188
62) de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJ, Rothblat GH: The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein to remove cholesterol from macrophages. Arterioscler Thromb Vasc Biol, 2010; 30: 796-801
63) Li XM, Tang WH, Mosior MK, Huang Y, Wu Y, Matter W, Gao V, Schmitt D, Didonato JA, Fisher EA, Smith JD, Hazen SL: Paradoxical association of enhanced cholesterol efflux with increased incident cardiovascular risks. Arterioscler Thromb Vasc Biol, 2013; 33: 1696-1705
64) Davidson WS, Heinik A, Sexmith H, Melchior JT, Gordon SM, Kuklenyik Z, Woollett L, Barr JK, Jones JJ, Toth CA, Shah AS: The effects of apolipoprotein B depletion on HDL subspecies composition and function. J Lipid Res, 2016; 57: 674-686
65) Sankaranarayanan S, Kellner-Weibel G, de la Llera-Moya M, Phillips MC, Asztalos BF, Bittman R, Rothblat GH: A sensitive assay for ABCA1-mediated cholesterol efflux using BODIPY-cholesterol. J Lipid Res, 2011; 52: 2322-2340
66) Uto-Kondo H, Ayaoi M, Ogura M, Nakaya K, Ito M, Suzuki A, Takiguchi S, Yakushiji E, Terao Y, Ozasa H, Hisada T, Sasaki M, Ohsuzu F, Ikewaki K: Coffee consumption enhances high-density lipoprotein-mediated cholesterol efflux in macrophages. Circ Res, 2010; 106: 779-787
67) Kushiyama A, Okubo H, Sakoda H, Kikuchi T, Fujihiro M, Sato H, Kushiyama S, Ishida T, Hiraoka K: Site-specific nitration of apolipoprotein A-I at tyrosine 166 is both abundant within human atheroma. Nat Med, 2014; 20: 193-203
56) Anastasiou M, Kock M, Jessup W, Sullivan D, Rye KA, Kritharides L: Cholesterol efflux capacity: An introduction for clinicians. Am Heart J, 2016; 180: 54-63
57) Talbot CPJ, Plat J, Risch A, Mensink RP: Determinants of cholesterol efflux capacity in humans. Prog Lipid Res, 2018; 69: 21-32
58) Miwa K, Inazu A, Kashwiri M, Nohara A, Higashikata T, Kobayashi J, Koizumi J, Nakajima K, Nakano T, Nii M, Mabuchi H, Yamagishi M: Cholesterol efflux from J774 macrophages and Fu5AH hepatoma cells to serum is preserved in CETP-deficient patients. Clin Chim Acta, 2009; 402: 19-24
65) Ogura M, Hori M, Harada-Shiba M: Association Between Cholesterol Efflux Capacity and Atherosclerotic Cardiovascular Disease in Patients With Familial Hypercholesterolemia. Arterioscler Thromb Vasc Biol, 2016; 36: 181-188
66) de la Llera-Moya M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJ, Rothblat GH: The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein to remove cholesterol from macrophages. Arterioscler Thromb Vasc Biol, 2010; 30: 796-801
67) Kushiyama A, Okubo H, Sakoda H, Kikuchi T, Fujihiro M, Sato H, Kushiyama S, Ishida T, Hiraoka K: Site-specific nitration of apolipoprotein A-I at tyrosine 166 is both abundant within human atheroma. Nat Med, 2014; 20: 193-203
58) Miwa K, Inazu A, Kashwiri M, Nohara A, Higashikata T, Kobayashi J, Koizumi J, Nakajima K, Nakano T, Nii M, Mabuchi H, Yamagishi M: Cholesterol efflux from J774 macrophages and Fu5AH hepatoma cells to serum is preserved in CETP-deficient patients. Clin Chim Acta, 2009; 402: 19-24
69) Asztalos BF, Horvath KV, Mehain M, Yokota Y, Schaefer EJ: Influence of HDL particles on cell-cholesterol efflux under various pathological conditions. J Lipid Res, 2017; 58: 1238-1246
70) Harada A, Töhr T, Murakami K, Kiriyama M, Yoshikawa
High-density lipoprotein particle subclass heterogeneity and incident coronary heart disease. Circ Cardiovasc Qual Outcomes, 2014; 7: 55-63
71) Serfaty-Lacrosniere C, Civeira F, Lanzberg A, Isaia P, Berg J, Janus ED, Smith MP, Jr., Pritchard PH, Frohlich J, Lees RS, et al.: Homozygous Tangier disease and cardiovascular disease. Atherosclerosis, 1994; 107: 85-98
72) Maeda S, Nakanishi S, Yoneda M, Awaya T, Yamane K, Hirano T, Kohno N: Associations between small dense LDL, HDL subfractions (HDL2, HDL3) and risk of atherosclerosis in Japanese-Americans. J Atheroscler Thromb, 2012; 19: 444-452
73) Akinkuolie AO, Paynter NP, Padmanabhan L, Mora S: