HHS Public Access

Author manuscript

Prog Cardiovasc Dis. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Prog Cardiovasc Dis. 2015 ; 58(1): 32–40. doi:10.1016/j.pcad.2015.05.004.

High-Density Lipoprotein Function Measurement in Human Studies: Focus on Cholesterol Efflux Capacity

Anand Rohatgi, M.D., M.S.C.S.

Division of Cardiology, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, U.S.A

Abstract

A low plasma level of high-density lipoprotein (HDL) cholesterol (HDL-C) is a major risk factor for the development of atherosclerotic cardiovascular disease (ASCVD). However, several observations have highlighted the shortcomings of using cholesterol content as the sole reflection of HDL metabolism. In particular, several large randomized controlled trials of extended release niacin and cholesteryl-ester transfer protein (CETP) inhibitors on background statin therapy have failed to show improvement in ASCVD outcomes despite significant increases in HDL-C. Reverse cholesterol transport (RCT) is the principal HDL function that impacts macrophage foam cell formation and other functions such as endothelial activation of endothelial nitric oxide synthase, monocyte adhesion, and platelet aggregation. Cholesterol efflux from macrophages to plasma/serum reflects the first critical step of RCT and is considered a key anti-atherosclerotic function of HDL. Whether this function is operative in humans remains to be seen, but recent studies assessing cholesterol efflux in humans suggest that the cholesterol efflux capacity (CEC) of human plasma or serum is a potent marker of ASCVD risk. This review describes the methodology of measuring CEC *ex vivo* from human samples and the findings to date linking CEC to human disease. Studies to date confirm that CEC can be reliably measured using stored human blood samples as cholesterol acceptors and suggest that CEC may be a promising new biomarker for atherosclerotic and metabolic diseases. Further studies are needed to standardize measurements and clarify the role CEC may play in predicting risk of developing disease and response to therapies.

Keywords

HDL; Lipoprotein; Function; Cholesterol Efflux; coronary disease; heart disease; atherosclerosis

Correspondence: Anand Rohatgi, MD, MSCS, Assistant Professor, Department of Internal Medicine, Division of Cardiology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-8830, (214) 645-7500 (O), (214) 645-2480 (F), Anand.rohatgi@utsouthwestern.edu.

Disclosures: Anand Rohatgi is supported by the National Heart, Lung, and Blood Institute of the NIH under Award Number K08HL118131.

Research grant, Merck, Significant.

Advisory Board, Astra Zeneca, modest.

Introduction

A low plasma level of high-density lipoprotein (HDL) cholesterol (HDL-C) is a major risk factor for the development of atherosclerotic cardiovascular disease (ASCVD).[1] However, several observations have highlighted the shortcomings of using cholesterol content as the sole reflection of HDL metabolism. HDL-C is lower in the insulin resistance state, which also confers increased ASCVD risk. This is evidenced by the fact that the association between low HDL-C and ASCVD is attenuated by adjustment for total low-density lipoprotein (LDL) particle concentration.[2] In addition, genetic studies of low or high high-density lipoprotein (HDL) cholesterol (HDL-C) have not shown association with increased or decreased risk, respectively.[3,4] Lastly, several large randomized controlled trials of extended release niacin and cholesteryl-ester transfer protein (CETP) inhibitors on background statin therapy have failed to show improvement in ASCVD outcomes despite significant increases in HDL-C.[5–8] These observations on the shortcomings of using cholesterol content of HDL as a marker of risk have focused attention on other parameters of HDL metabolism to improve prediction of ASCVD risk and response to therapy.

HDL exerts several key anti-atherosclerotic functions related to cholesterol transport, endothelial and vascular function, and inflammation. Reverse cholesterol transport (RCT), the ability of HDL to accept cholesterol from the periphery and deliver it to the liver for excretion, is the principal method for HDL biogenesis from nascent lipid-poor particles to mature cholesteryl-ester laden spherical particles;[9] RCT is also considered the principal HDL function that impacts macrophage foam cell formation and other functions such as endothelial activation of endothelial nitric oxide synthase (eNOS), monocyte adhesion, and platelet aggregation.[10] Therefore, RCT is the overriding action of HDL on multiple cell types.

RCT is a complex process that has been well worked out in animal models.[11] As summarized in Figure 1, lipid-poor apolipoprotein (apo) A-I (apoA-I) interacts with the ABCA1 receptor on hepatocytes and macrophages in the periphery to accept cellular cholesterol. This cholesterol is esterified by lecithin:cholesterol acyltransferase (LCAT), leading to the formation of spherical HDL particles with a hydrophobic cholesteryl ester core. These enlarging HDL particles can continue to accept cholesterol from the periphery via other pathways and deliver it back to the liver for excretion into bile and feces. In addition, cholesterol within HDL particles can be exchanged with LDL and very low-density lipoprotein (VLDL) particles for triglycerides (TGs), leading to catabolism of HDL particles by lipases and either excretion via the kidney or re-participation in accepting cholesterol from the periphery.

Macrophage-specific cholesterol efflux is the key initial step in RCT and has been shown in genetic and pharmacologic animal studies to be more closely associated with atherosclerosis than circulating levels of HDL-C.[9] Whether this function is operative in humans remains to be seen, but recent studies assessing cholesterol efflux in humans suggest that the cholesterol efflux capacity (CEC) of human plasma or serum is a potent marker of ASCVD risk. This review serves to describe the methodology of measuring CEC *ex vivo* from human samples and the findings to date linking CEC to human disease.

Measuring Cholesterol Efflux Capacity (CEC) in Humans

There is no standardized method for measuring CEC in humans and protocols vary considerably; however, they all measure the movement of labeled cholesterol from cells to an extracellular acceptor (Figure 2).[12] In general, most studies in humans have only tested the cholesterol acceptor aspect of efflux, specifically, the differential capacity of human serum/plasma to accept cholesterol from cells in a unidirectional manner. This approach does not take into account the ability of a patient's own macrophages to efflux cholesterol and does not assess cholesterol influx, or net efflux.

Macrophages are the most relevant cell type for studies of atherosclerosis given the central role of macrophage "foam" cells in disorders of lipid accumulation. Macrophages efflux cholesterol via several transporters, including adenosine tri-phosphate (ATP)-binding cassette transporters ABCA1 and ABCG1, scavenger receptor SRB1, as well as via aqueous diffusion. CEC assays can reflect all of these pathways in aggregate or can be modified to interrogate a specific transporter. Choice of cholesterol acceptor can have significant impact on assessment of CEC and is the largest source of variation across studies. Cholesterol acceptor mediums can range in specificity for HDL from isolated pure HDL to apo B-depleted plasma/serum to whole plasma/serum. The use of ApoB-depleted plasma eliminates the role of LDL and VLDL in assessing cholesterol efflux, making it more specific for HDL-mediated CEC. When whole or apoB-depleted plasma/serum is used, other cholesterol acceptors and shuttles such as albumin can also play a role in CEC; however, studies have shown that apoA-I, the main protein constituent of HDL particles, is responsible for ~75–80% of the CEC from macrophage cell lines with amplified ABCA1 transporter pathways.[13,14] In one small study, CEC to apoB-depleted plasma moderately correlated with CEC to isolated HDL ($r=0.46$, $p<0.02$) but was not correlated at all with CEC to whole plasma ($p>0.2$).[15] Ascertaining the specific methodology used to assess CEC is critical when evaluating the reported findings in human studies. Correlations between CEC and other lipid markers can vary widely whether using whole vs. apoB-depleted plasma/serum as the cholesterol acceptor.[15]

CEC and ASCVD

Studies assessing the association between CEC and ASCVD are summarized in Table 1. Perhaps the first reported study of CEC and coronary artery disease (CAD) in humans, a small case-control study in the mid 1990's showed that CEC was lower in patients with prevalent CAD and was the lowest in those with both CAD and diabetes mellitus (DM).[16] Though the vast majority of studies have assessed the cholesterol acceptor capacity aspect of the efflux pathway, one of the earliest studies in humans tested the cholesterol donor capacity of patient-derived peripheral blood mononuclear cells to standardized recombinant HDL2 particles.[17] Macrophages from patients with angiographic CAD had lower CEC than those derived from controls without angiographic CAD and inversely correlated with HDL and LDL particle size. The first large study of CEC in humans utilized a sample of 793 individuals without acute coronary syndrome presenting for coronary angiogram in a case-control design.[18] In this study sample, CEC was positively correlated with HDL-C ($r=0.51$, $p<0.0001$). Increasing CEC was associated with decreased prevalence of angiographic CAD,

even when adjusted for traditional risk factors and HDL-C. CEC was also inversely associated with severity of angiographic CAD and, in a separate cohort, with carotid intima media thickness. This study established the relevance of measuring CEC in humans with regard to ASCVD, but its cross-sectional design limited the ability to determine whether impaired efflux occurred prior to the onset of CAD. In a letter to the editor in response to this study, another group reported their observation using a slightly different assay in a nested case-control design that CEC was not associated with incident ASCVD events, suggesting that choice of assay methodology may affect findings.[19]

The second large study assessed both the cross-sectional association of CEC and CAD as well as the longitudinal association between CEC and incident ASCVD events.[13] Two convenience samples were used: one included 577 patients enrolled from outpatient clinics with CAD defined as a history of CAD, MI, or coronary revascularization, the second included 1150 patients presenting for coronary angiogram with CAD defined as at least 50% coronary stenosis. CEC was inversely associated with prevalent CAD in the outpatient cohort but not in the angiographic cohort. The angiographic cohort was followed prospectively for 3 years and CEC measured at the time of angiogram was surprisingly positively associated with incident cardiovascular (CV) events (MI, stroke, or death). One explanation of this observation could be the use of a convenience sample with a mix of patients both with and without angiographic CAD.[20] It is also interesting to note the lack of a dose-response relationship across increasing tertiles of CEC (HR for tertile 2: 0.9; tertile 3: 1.9), suggesting a threshold effect. Regardless, it remains possible that the association between CEC and CV disease (CVD) may vary depending on the population being studied.

Our assessment of CEC in the Dallas Heart Study was the third large study of CEC and CAD and the first longitudinal study among an unselected healthy population.[21] Of note, this study employed fluorescent-labeled cholesterol instead of radiolabeled cholesterol used in most other studies. Among 2924 healthy individuals free from cardiovascular disease, CEC was inversely associated with incident CV events (MI, stroke, coronary revascularization, or CV death). The inverse association was graded across increasing quartiles of CEC, was not attenuated by adjustment for CAD risk factors, HDL-C, or HDL-P, and was similar when CEC was analyzed as a continuous variable (HR 1SD: 0.68 [95% CI 0.55–0.84]). Furthermore, as the first multiethnic study of HDL function, the Dallas Heart Study showed no interaction by race/ethnicity, an important observation as therapies targeting HDL and HDL function are developed.

Another study of CEC and incident CAD was conducted in the EPIC-Norfolk cohort and is also a longitudinal study among an unselected population free from CVD.[22] Using a nested case-control design with almost 1900 events, the top tertile of CEC, as compared to the bottom tertile, was inversely associated with incident CV events in fully adjusted analyses (OR 0.75, 95%CI 0.53–0.97). This study is consistent with the prior cross-sectional inverse associations with prevalent CAD and the longitudinal inverse association with incident CV events among a healthy population. Overall, these studies have established that CEC can be measured in high-throughput fashion in thousands of human subjects and exhibits a validated inverse association with incident CAD among healthy individuals.

CEC and Chronic Kidney Disease(CKD)

Patients with CKD and end-stage renal disease (ESRD) on dialysis are at marked increased risk for CVD events and CV death. Several studies have shown alterations in HDL function in this population including CEC (Table 1). A small case-control study of adults with ESRD on hemodialysis showed that isolated HDL from patients with ESRD on dialysis elicited significantly less cholesterol efflux than matched controls but did not correlate with inflammation.[23] Using a similar methodology, a more recent case-control study in children showed that there was a trend toward lower CEC in children with CKD or ESRD on dialysis vs. controls (did not meet statistical significance).[24] Both of these studies used gas chromatography instead of labeled cholesterol to quantify efflux. In the largest case-control study to date, CEC was worse among children with CKD compared to controls and was inversely associated with worsening CKD stage.[25] Interestingly, in a subset of children followed prospectively after kidney transplantation, CEC did not improve. These reports suggest that CEC can be impaired in children before the onset of traditional ASCVD risk factors in adulthood. Although not the subject of this review, all of these studies also assessed HDL action on endothelial function and vascular inflammation, showing consistent worsening of these HDL functions among adults and children with CKD and on dialysis.

CEC and DM/Metabolic Syndrome(MetS)

DM and MetS are characterized by insulin resistance and dyslipidemia, in particular, high TG and low HDL-C levels, and confer increased risk for ASCVD; CEC has been tested in several studies in these populations (Table 1). The same first case-control study of CEC and CAD in humans was also one of the first to show that CEC was lower in patients with DM, and lowest in those with both DM and CAD.[16] Additional case-control studies, however, have revealed that CEC is, in contrast, often higher in patients with DM compared to controls in states of hypertriglyceridemia or prevalent MetS but is not different with respect to DM status absent these conditions.[15,26–30] This suggests that plasma/serum from individuals with insulin resistance may confer increased efflux *ex vivo*, perhaps due to increased CETP activity (whole plasma)[29] or increased circulating levels of lipid-poor pre-beta HDL particles (whole and apoB-depleted plasma).[28] In contrast to these studies, pathway-specific analyses revealed that ABCA1-mediated CEC was shown to be lower in those with DM but only those with urinary protein, and SRB1-mediated CEC was lower in all patients with DM regardless of proteinuria status.[31] In the only true cohort study, which consisted of 439 Japanese-Americans, oral glucose tolerance testing revealed 8.6% with a new diagnosis of DM and 16% with a new diagnosis of impaired glucose tolerance. Efflux was lower in this newly diagnosed, treatment-naïve glucose intolerance group compared to those with normal glucose tolerance.[32] These studies suggest that factors such as duration of DM and degree of proteinuria and insulin resistance may impact associations with CEC.

CEC and Auto-immune Disorders

Many auto-immune disorders are associated with increased atherosclerosis and ASCVD events. In a moderately-sized case-control study, CEC was lower in patients with psoriasis compared to controls and correlated with larger HDL particle size among those with

psoriasis.[33] Another case-control study reported that CEC was inversely correlated with psoriasis disease severity but PON-1 activity and HDL anti-oxidative capacity were no different by disease status.[34] Among patients with rheumatoid arthritis, one study suggested an inverse correlation between CEC and disease severity[35] and another reported lower ABCG1-mediated CEC but not ABCA1- or SRB1-mediated CEC as compared to controls. In systemic lupus patients, ABCA1- and ABCG1- but not SRB1-mediated CEC was lower as compared to controls.[36] Taken together, these initial studies suggest that CEC is inversely related to auto-immune disease severity, but the associations may be cholesterol-transporter specific and may not be consistent with other HDL function measurements in this population.

CEC and Lifestyle Interventions

With regard to the effect of lifestyle changes on CEC, very few studies with active comparators or placebo controls have been published (Table 2). Several have suggested increased CEC with diets enriched in polyphenols[37] and alcohol intake[38] and no effect with unsaturated fat.[39,40] Only one small study testing exercise and CEC had a control arm,[41] suggesting no improvement with exercise training among patients with DM. Other uncontrolled published studies on exercise and CEC have been summarized in a recent review.[42] The largest of these included 100 overweight women and found that reduction in caloric intake by 500 kcals and increase in physical activity by 5000 steps/day led to a 6% decrease in HDL-C and a 10% decrease in ABCA1-CEC (p=0.006) which was attenuated when adjusted for apoA-I levels.[43]

Conclusion

In summary, cholesterol efflux from macrophages to plasma/serum reflects the first critical step of RCT and is considered a key anti-atherosclerotic function of HDL. Studies to date confirm that CEC can be reliably measured using stored human blood samples as cholesterol acceptors and suggest that CEC may be a promising new biomarker for atherosclerotic and metabolic diseases. Further studies are needed to standardize measurements and clarify the role CEC may play in predicting risk of developing disease and response to various therapies.

Abbreviations

Apo	Apolipoprotein
ASCVD	Atherosclerotic cardiovascular disease
ATP	Adenosine triphosphate
CAD	Coronary artery disease
CEC	Cholesterol efflux capacity
CETP	Cholesterol Ester Transport Protein
CKD	Chronic kidney disease

CV	Cardiovascular
CVD	Cardiovascular disease
DM	Diabetes mellitus
eNOS	Endothelial nitric oxide synthase
ESRD	End-stage renal disease
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
LCAT, Lecithin	cholesterol acyltransferase
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
MetS	Metabolic syndrome
MI	Myocardial infarction
RCT	Reverse cholesterol transport
TGs	Triglycerides
VLDL	Very low-density lipoprotein

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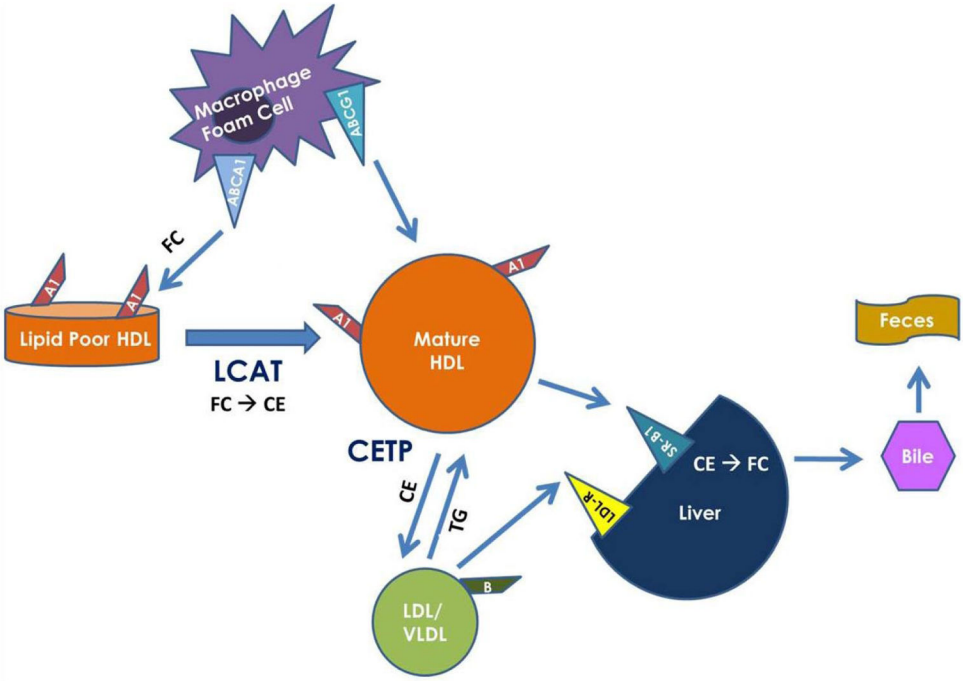

Figure 1.

Reverse Cholesterol Transport. LCAT: Lecithin:Cholesterol Acyltransferase; CETP: Cholesteryl Ester Transfer Protein; FC: Free Cholesterol; CE: Cholesteryl Ester; TG: Triglycerides; LDL-R: Low-density lipoprotein receptor.

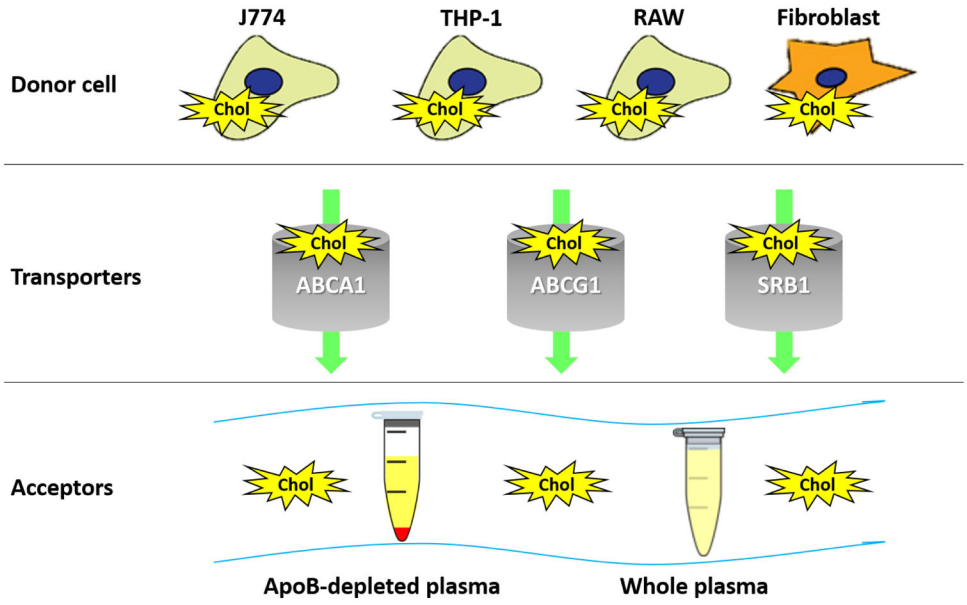

Figure 2.

Cholesterol Efflux Assay. The movement of labeled cholesterol from within cells to extracellular acceptors is quantified as cholesterol efflux. Choice of donor cells, cholesterol transporters interrogated, type of labeled cholesterol and cholesterol acceptor can affect efflux measurements. Chol = labeled cholesterol. ApoB = Apolipoprotein B. J774, THP-1, RAW = types of macrophage cell lines.

Table 1

Studies Correlating Cholesterol Efflux Capacity with Human Disease

Study	Efflux Specifics	Population	Endpoint	Efflux Result
ASCVD				
Linsel-Nitschke et al., 2009[17]	• Patient-derived PBMCs to standardized HDL2	• Cross-sectional case-control • Presenting for angiogram • N=90 cases • N=52 controls	• >50% angiographic coronary stenosis	**Lower in CAD:** • 46% in CAD vs. 51% in controls, P=0.028
Khera et al., 2011[18]	• J774 macrophages to ApoB-depleted plasma	• Cross-sectional case-control • Presenting for angiogram • N=442 cases • N= 351 controls	• >50% angiographic coronary stenosis	**Lower in CAD:** • Q4 vs. Q1: OR 0.48 [0.30–0.78] • 1 SD: OR 0.75 [0.63–0.90]
De Vries et al., 2011[19]	• Fibroblasts to whole plasma	• Nested case-control • Presenting for angiogram • N=113 cases • N=111 controls	• Incident CVD: CV death, hospitalization for MI, PCI/CABG	**No difference:** • HR 1.06 [0.88–1.29], p=0.52
Li et al., 2013[13]	• RAW264.7 macrophages to ApoB-depleted plasma	• Cross-sectional case-control and longitudinal • Outpatient: N=146 cases, N=431 controls • Angiographic: N=871 cases, N=279 controls	• CAD vs. no CAD for outpatient and angiographic cohort • Incident CVD: MI, stroke, death at 3 years for angiographic cohort	**Lower in outpatient CAD:** • T3 vs. T1: OR 0.2, p<0.05 **No difference in angiographic CAD:** • T3 vs T1: OR 1.2, p=ns **Higher in incident CVD:** • T3 vs. T1: HR 1.9 [1.1–3.1]
Rohatgi et al., 2014[21]	• J774 macrophages to ApoB-depleted plasma	• Longitudinal • Population-based sample free of CVD • N=2924	• Incident CVD: MI, stroke, coronary revascularization, CV death	**Inverse with CVD:** • Q4 vs. Q1: HR 0.33 [0.19–0.55] • 1 SD: HR 0.68 [0.55–0.84]
CKD				
Shroff et al., 2014[25]	• J774 macrophages to ApoB-depleted serum	• Cross-sectional case-control • Children • N=82 cases • N=12 controls	• CKD stages 2–5, Dialysis, transplantation	• **Lower in CKD** • **Inverse with CKD stage** • **No change with transplantation**
Kaseda et al., 2015[24]	• THP-1 monocytes to isolated HDL	• Cross-sectional • Children • N=16 with CKD and 15 on dialysis • N=10 controls	• CKD or dialysis	**No difference**

Study	Efflux Specifics	Population	Endpoint	Efflux Result
Yamamoto et al., 2012[23]	• THP-1 monocytes to isolated HDL	• Cross-sectional • Adults • N=29 cases • N=28 controls	• ESRD on dialysis	**Lower in ESRD:** • Absolute 10% reduced efflux, p<0.001.
Auto-immune				
Charles-Schoeman et al., 2012[35]	• RAW264.7 macrophages to isolated HDL	• Cross-sectional • N=40 cases • N=40 controls	• Rheumatoid arthritis	**No difference but inverse with RA disease severity:** • (r=−0.39, p=0.01)
Holzer et al., 2012[34]	• RAW264.7 macrophages to isolated HDL	• Cross-sectional • N=15 cases • N=15 controls	• Psoriasis	**Lower in psoriasis:** • p<0.001 **Inverse with psoriasis disease severity:** • r=−0.52, p<0.05
Mehta et al., 2012[33]	• J774 macrophages to ApoB-depleted serum	• Cross-sectional • N=100 cases • N=100 controls	• Psoriasis	**Lower in psoriasis:** • p=0.006
Ronda et al., 2014[36]	• J774, CHO-K1, FU5AH cells to ApoB-depleted serum	• Cross-sectional • N=30 cases with RA • N=30 cases with SLE • N=30 controls	• Rheumatoid arthritis and systemic lupus erythematosus	**ABCA1 efflux:** • lower in SLE (p<0.05) but not in RA **ABCG1 efflux:** • lower in SLE (p<0.001) and RA (p<0.05), and lower in SLE compared to RA (p<0.01) **SRB1 efflux:** • higher in SLE compared to RA (p<0.01), but neither different from control **ACBG1 efflux:** • inversely correlated with RA disease severity (r= −0.47, p<0.01)
Roe et al., 2014[44]	• J774 macrophages to ApoB-depleted serum	• Cross-sectional • N=115 cases • N=56 controls	• PCOS	**Lower in PCOS:** • p<0.02
Diabetes				

Study	Efflux Specifics	Population	Endpoint	Efflux Result
Cavallero et al., 1995[45]	• Ob 1771 cells to isolated LpA-I	• Cross-sectional • Patients with diabetes • N=14 cases • N=12 controls	• Diabetes vs. controls • Fasting vs. post-prandial	**Lower in DM:** • In both fasting and post-prandial states (p<0.05) **Higher in post-prandial state:** • in controls but not in DM
Syvanne et al., 1996[16]	• Fu5AH cells to whole plasma	• Cross-sectional • Patients with diabetes and/or coronary disease • N=91 with DM (47 with CAD) • N=60 without DM (35 with CAD)	• Diabetes vs. no diabetes • CAD vs. no CAD	**Lower in DM and in CAD:** • p=0.04 for both **Highest in those without DM or CAD and lowest in those with DM and CAD:** • 19.8% vs. 17.3%
Brites et al., 1999[46]	• Fu5AH cells to whole serum	• Cross-sectional • Men with diabetes • N=31 cases • N=12 controls	• Diabetes vs. controls • Fasting vs. post-prandial	**Lower in DM:** • only in post-prandial group (p=0.01)
Zhou et al., 2008[31]	• Fu5AH cells to whole serum	• Cross-sectional • Patients with diabetes • N=60 cases • N=20 controls	• Proteinuria status • Diabetes vs. control	**ABCA1-efflux:** • lower in DM with microalbuminuria or proteinuria (p<0.05) **SRB1-efflux:** • lower in all DM groups (p<0.05)
Dullaart et al., 2008[26]	• Fibroblasts to whole plasma	• Cross-sectional • Patients with Metabolic syndrome (MetSyn) • N=76 cases (55 with DM) • N=94 controls	• Metsyn vs. No metsyn, modified by DM status[29,30]	**Higher in metsyn**: • 8.8% vs. 8.5%, • Highest in metsyn+DM (8.8%) and lowest in DM without metsyn (8.3%).
de Vries et al., 2008[28]	• Fibroblasts to whole plasma	• Cross-sectional • Patients with diabetes • N=88 cases (28=high TG; 56= normal TG) • N=56 controls	• High vs. normal triglycerides (TG)	**Higher in high vs. normal TG:** • 9.0% vs. 8.4%, p<0.01 **No different in DM with normal TG vs. controls**
Zhou et al., 2009[47]	• Fu5AH cells to whole serum	• Cross-sectional • Patients with diabetes • N=137 cases • N=75 controls	• Diabetes vs. control	**Lower in DM:** • p=0.02

Study	Efflux Specifics	Population	Endpoint	Efflux Result
Nestel et al., 2012[30]	• THP-1 monocytes to plasma	• Cross-sectional • Patients with metabolic syndrome (MetSyn) • N=25 cases • N=22 controls	• Insulin Resistance (IR) vs. insulin sensitivity (IS)	**Higher in IR vs. IS:** • p=0.0005 **Inverse with ApoA-I:** • r=−0.36
Low et al., 2012[15]	• THP-1 monoyctes to: • whole plasma • ApoB-depleted plasma • Isolated HDL	• Cross-sectional • Patients with diabetes (DM) • N=42 cases (26 on statin/17 not on statin) • N=controls	• Diabetes vs. control	**Higher in DM:** Increased by • 30% (whole plasma; p<0.001); • 34% (apoB-depleted plasma; p<0.001); • 100% (isolated HDL; p<0.001)
Dullart et al., 2012[27]	• Fibroblasts to whole plasma	• Cross-sectional • N=22 with T2DM • N=36 with IFG • N=37 with NFG	• T2DM vs. normal IFG vs. normal	**No difference by glycemic status**
Yassine et al., 2014[29]	• BHK cells to ApoB-depleted plasma	• Cross-sectional • Patients with diabetes (DM) • N=45 cases • N=26 controls	• High vs. normal triglycerides (TG)	**ABCA1 efflux:** • higher with high TG (p=0.01) • correlated with CETP and PLTP activity • increased with high fat intake
Kubota et al., 2014[32]	• THP-1 monocytes to whole serum	• Cross-sectional • Japanese-americans without diabetes • N=439	• IGT vs. Normal by OGTT • N=38 with new DM • N=71 new IGT • N=330 normal GT (NGT)	**Lower in glucose intolerance:** • 33.2% vs. 31.4%, p=0.01 **Inverse with OGTT measures:** • r=−0.14 to −0.18, p=0.02 to <0.001

Table 2

Effect of Lifestyle Interventions on Cholesterol Efflux Capacity in Humans

Study	Efflux Assay	Study design/Population	Intervention	Effect on Efflux
Diet				
Beulens et al., 2004[38]	• J774 and FU5AH cells to ApoB-depleted serum	• Cross-over RCT • 24 European men	• 40g/day whisky vs. water	• ABCA1-efflux increased 17.5% with whiskey (p=0.027) • Pre-beta1 Increased by 31% • SRB1-efflux increased by 4.6% (p=0.002)
Buonacorso et al., 2007[39]	• Murine macrophages to isolated HDL and to whole plasma	• RCT • N=30 healthy Brazilians	• Trans-fat (8.3%) vs. polyunsaturated (14.6%) vs. saturated (13.2%)	• No change with any diet
Kralova Lesna et al., 2008[40]	• THP-1 monocytes to whole serum (5%)	• Crossover RCT • N=14 healthy Caucasian men	• High saturated fat diet vs. high polyunsaturated fat (PUFA) • 4 weeks each arm	• No increase or decrease with either diet compared to each other or baseline
Kralova Lesna et al., 2010[48]	• THP-1 monocytes to whole plasma (5%)	• Cross-over RCT • 13 men	• 36g/d beer vs non-alcoholic beverages	• On-treatment trended higher with alcohol (14.9% vs. 13.8%; p=0.059; no baseline measure)
Aicher et al., 2012[43]	• BHK cells to ApoB-depleted serum	• No control • N=100 overweight women	• Lifestyle intervention for all participants to reduce 500kcal and increase 5000 steps/d. • Comparison with baseline values	• ABCA1efflux: 10% decrease, p=0.006; lost significance when adjusted to ApoA-I levels • ABCG1: no change • SRB1: no change • 6% decline in HDL-C
Hernaez et al., 2014[37]	• THP-1 monocytes to isolated HDL	• Crossover RCT • N=47 healthy European men	• High vs. low polyphenol olive oil	• Increased with high polyphenol diet (p=0.043).
Exercise				

Study	Efflux Assay	Study design/Population	Intervention	Effect on Efflux
Ribiero et al., 2008[41]	• Murine macrophages whole serum	• N=21 with DM • N=11 controls	• 4 month exercise protocol vs. sedentary	• No difference • Decline in pre-beta1 with exercise in DM pts.
Blazek et al., 2013[42]				• Review on exercise and HDL