High density lipoprotein functionality and cardiovascular events and mortality. A systematic review and meta-analysis

Maria T Soria-Florido, PhD[a,b,c], Helmut Schröder, PhD[a,d], María Grau, MD, PhD [e,f,g], Montserrat Fitó, MD, PhD [a,h*], Camille Lassale, PhD[a,c,h*]

 a. Cardiovascular Risk and Nutrition Research Group, Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain
 b. PhD Program in Food Sciences and Nutrition, Department of Nutrition, Food Science and Gastronomy, School of Pharmacy and Food Science, Campus de l'Alimentació Torribera, University of Barcelona, Barcelona, Spain
 c. Department of Behavioural Science and Health, University College London, London, United Kingdom
 d. CIBER of Epidemiology and Public Health (CIBERESP), Institute of Health Carlos III, Madrid, Spain
 e. Registre Gironí del COR. Group, Cardiovascular, Epidemiology and Genetics Research Group, Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain
 f. CIBER of Cardiovascular Diseases (CIBERCV), Institute of Health Carlos III, Madrid, Spain
 g. Department of Medicine, University of Barcelona, Barcelona, Spain
 h. CIBER of Physiopathology of Obesity and Nutrition (CIBEROBN), Institute of Health Carlos III, Madrid, Spain

* Address for correspondence

Camille Lassale, PhD, Hospital del Mar Medical Research Institute, Carrer Doctor Aiguader 88, 08003, Barcelona, Spain
Telephone: +34 933 16 17 19   Email: classale@imim.es
Montserrat Fitó, MD, PhD, Hospital del Mar Medical Research Institute, Carrer Doctor Aiguader 88, 08003, Barcelona, Spain
Telephone: (+34) 933 16 07 20 Email: mfito@imim.es

Running title: HDL-function, CVD and mortality; a Meta-analysis

Word count: 4091

Keywords: HDL-function; cardiovascular risk; mortality risk; cholesterol efflux capacity; antioxidant capacity; anti-inflammatory capacity

Total number of figures and tables: 4 figures

ABSTRACT

Background and aims

The aim of this systematic review and meta-analysis was to synthesize studies assessing the associations between high-density lipoprotein functionality and risk of cardiovascular disease and mortality.

Methods

We searched Medline and Embase for the identification of observational studies meeting the inclusion criteria. This meta-analysis was conducted following the PRISMA statement and was registered in. We pooled risk estimates with a random-effect model separately for fatal or non-fatal cardiovascular disease and all-cause mortality.
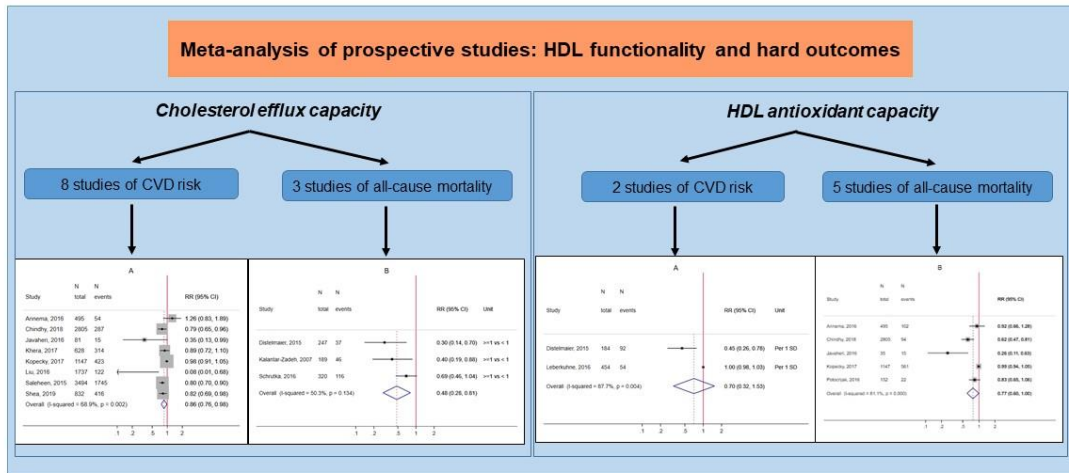
Results

Out of 29 manuscripts, 20 articles investigated cholesterol efflux capacity (13 prospective and 7 cross-sectional), 10 antioxidant capacity (7 prospective and 3 cross-sectional) and two (1 prospective and 1 cross-sectional) anti-inflammatory capacity of high-density lipoprotein. A greater cholesterol efflux capacity was associated with lower risk of cardiovascular disease in 8 comparable prospective studies (RR for 1SD increase: 0.86; 95% CI: 0.76-0.98) and mortality in 5 studies (RR for 1SD increase: 0,77; 0.60-1.00). Better antioxidant capacity was non-significantly associated with lower cardiovascular disease risk in 2 studies (pooled RR for 1SD 0.70; 0.32-1.53) and significantly with mortality in 3 studies (pooled RR for 1SD 0.58; 0.28-0.81). High-density lipoprotein anti-inflammatory ability was associated with a lower cardiovascular disease risk in the only prospective study.

Conclusions

Greater high-density lipoprotein cholesterol efflux capacity and antioxidant/anti-inflammatory capacities were associated with lower risk of cardiovascular disease. However, the heterogeneity between studies and evidence of publication bias warrant caution and highlight the need for larger prospective studies with standardized assays and specific outcomes.

GRAPHICAL ABSTRACT

Meta-analysis of prospective studies: HDL functionality and hard outcomes

Cholesterol efflux capacity
- 8 studies of CVD risk
- 3 studies of all-cause mortality

HDL antioxidant capacity
- 2 studies of CVD risk
- 5 studies of all-cause mortality

INTRODUCTION

Inconsistent results on the causal effect of high-density lipoprotein cholesterol (HDL-C) on CVD risk [1,2] have led to consider that the protective role of HDL might lie in the functional traits of the lipoprotein rather than its cholesterol content.

HDL is a highly heterogeneous particle exhibiting a variety of functions that may contribute to its cardioprotective profile [3]. This lipoprotein plays a key role in the efflux of cholesterol from peripheral cells [4], a primary mechanism in the development of atherosclerotic lesions [5]. Furthermore, HDLs display anti-inflammatory, anti-oxidative, anti-apoptotic, anti-thrombotic and vasodilatory properties which potentially contribute to its atheroprotective nature [6].

The number of articles evaluating the association between HDL functionality and CVD outcomes in human populations is still low, and the available evidence has never been systematically summarized. Therefore, our aim was to conduct a systematic review of observational studies that assessed HDL functionality in relation to CVD events and all-cause mortality, and to perform a meta-analysis of the estimates of longitudinal studies.

MATERIAL AND METHODS

This meta-analysis was conducted following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement [7] and was registered in PROSPERO International Prospective Register of Systematic Reviews under the identifier CRD42017065857 (www.crd.york.ac.uk/PROSPERO).

Search strategy

Two investigators (CL and MSF) independently searched for the relevant studies on Medline and Embase up to 3rd April 2019. The keywords, index terms and Boolean operators used for the literature search are described in Online material. The search was limited to articles published in the English language and to full-text journal articles. Disagreements were discussed and resolved by consensus.

Study selection

The inclusion criteria was as follows: 1) Exposure: Functionality tests performed on isolated HDL, ApolipoproteinB (ApoB) depleted serum (ABDS) or ApoB-depleted plasma (ABDP); 2) Outcome: (i) Any major adverse cardiovascular event (MACE), including CVD mortality, alone or in combination as a composite outcome, or (ii) all-cause mortality; 3) Design: observational study (cohort, case-control); 4) Population: inpatients or community dwelling, free-living setting, adults (aged ≥18 years); 5) Association estimate: available information on *multivariate or univariate model with relative risk estimate (hazard ratio (HR), odds ratio (OR), relative risk (RR)) and associated standard error (SE) or 95%confidence intervals (95% CI). If the study had any of the following characteristics, it was excluded: 1) Exposure: measurement carried out on any other biological specimen than isolated HDL, ABDS or ABDP; 2) Outcome: studies assessing the relationship between HDL functionality and any other outcome different from those defined in the inclusion criteria; 3) Design: retrospective study, reviews or meta-analysis; 4) Population: pregnant or lactating women, individuals aged under 18y.

After a first screening of titles and abstracts, full texts were retrieved and a second evaluation was carried out. When relevant information was missing, we contacted the corresponding author.

Data extraction and quality assessment

Data were recorded in a standardized extraction form. When the study authors built more than one statistical model, we kept the estimates adjusted for the greatest number of factors. When the same model was adjusted for a set of factors plus HDL-C or ApolipoproteinA-I (ApoA-I), we only kept the estimate adjusted for HDL-C.

The quality of the included articles was assessed by two independent investigators (CL and MSF) using the Newcastle-Ottawa Scale [8]. To be included in the meta-analysis, a study had to meet our quality criteria of rating 5 stars (out of 9) or more.

Statistical analysis

For prospective studies, and when at least two studies with estimates on comparable scales were available, we performed random-effect meta-analysis [9] to provide an overall estimate of the association between HDL functionality and the incidence of CVD events or all-cause mortality. We pooled the results separately for each HDL functionality and for each outcome. We combined only comparable estimates (per 1SD, or per category) together. When data from the same cohort was used in different manuscripts, we included the results with the longest follow-up time. We express all estimates of the relationship between a greater functionality performance and CVD or mortality risk so that an estimate lower than 1 means that better HDL-related function is associated with a lower risk of outcome.

We assessed between-study heterogeneity using the Q statistic and the I^2 statistic method [10]. A value of I^2 of 0-40% was regarded as low, 30-60% as moderate, 50-90% as substantial, and 75-100% as high [11]. Presence of heterogeneity was tested by a chi-square test. A p-value <0.10 and high estimates of I^2 indicated substantial and significant heterogeneity [11].

We conducted a series of sensitivity analysis, stratifying by: a) quality score, combining results for studies with a score ≤7 and with a score >7; b) methodology of cholesterol efflux capacity assays (J774 cell lines vs BODIPY-cholesterol) to better understand the nature of the variability disclosed in this meta-analysis. We also planned to include two further sub-analysis on manuscripts adjusted for c) albumin and d) systemic markers of inflammation. Analysis c) aimed at the study of the predictive traits of HDL-functionality independently of the potential anti-oxidative and anti-inflammatory properties of the albumin protein; analysis d) aimed to disentangle whether HDL function can predict CVD risk independently of low-grade inflammation, and is not merely a surrogate marker of a pro-inflammatory atherogenic milieu. Any evidence of publication bias was visually diagnosed using a funnel plot and

quantified with Egger's test [12]. We considered that there was likely presence of publication bias if the two-sided p-value of Egger's test was lower than 0.05. The commands metan, confunnel and metabias in Stata version 14 (Stata Corp, College Station, TX, USA) were used.

RESULTS

Search results and study characteristics

The detailed workflow of the search process and study selection is shown in eFigure 1. The primary search yielded 4,503 papers. Of these, 2,644 were excluded because the exposure and/or the outcome were irrelevant and 1,703 did not meet our inclusion criteria regarding language or study design. The full texts of the 156 potential studies were retrieved for further evaluation and 29 were finally included. The studies characteristics are shown in eTable 1, 2, and 3. Data not shown in three manuscripts were provided by the authors on request [13–15].

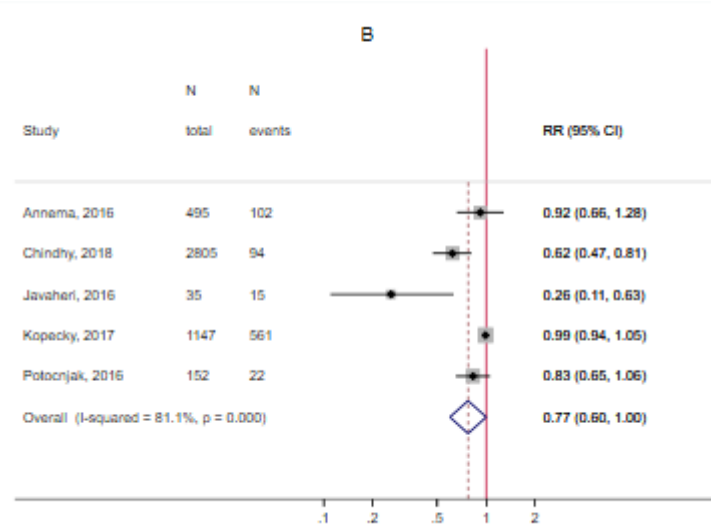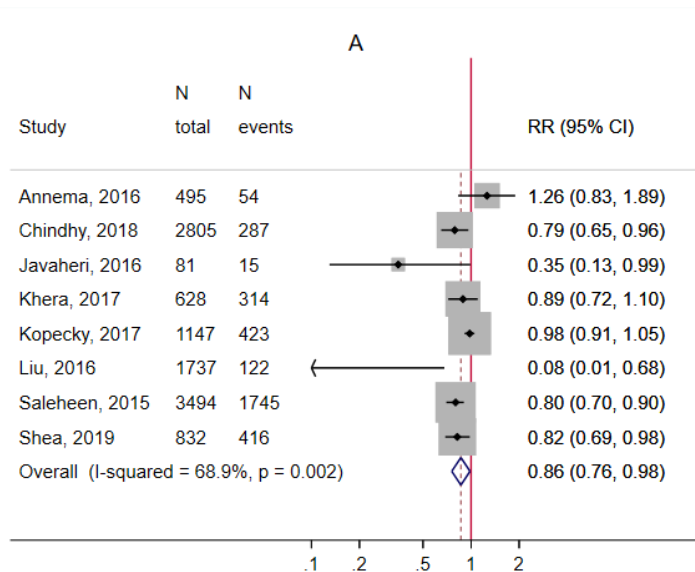### Cholesterol efflux capacity (CEC)

We identified 20 manuscripts appraising the capacity of HDL to remove lipids from cells – and, in one case, from a lipid matrix - in relation with hard events (CVD or mortality, eTable 1). A total of 21,022 participants aged 30-75.2y were included, of which 47.8% were men. The 20 articles reported on 10 different longitudinal cohorts [14–24] and 3 case-controls nested within cohorts [25–27], and 7 cross-sectional or case-control studies [14,28–33]. Six studies [15,16, 18, 21,23,24] reported on the risk of death of all-cause. Two papers analyzed all-cause mortality within a combined endpoint, therefore we did not include them in the meta-analysis focusing on all-cause mortality [18,26]. One article was excluded from the meta-analysis as the OR was comparing the highest quartile of CEC with a combination of the three lowest quartiles together [30]. In another study [33], a β-coefficient was provided without a standard error or confidence interval. Three articles analyzed the relationship between CEC and the risk of CVD in the same sample from the Dallas Heart Study [19–21] so we only include in the meta-analysis estimates with the longest follow-up [20]. In 18 articles, the authors included HDL-C as a covariate [14–25, 27–31,33].

With regards to the main technical characteristics of the assays, we report the donor cell line, the lipid tracer and the lipid acceptor. In 15 articles, the assay was performed on differentiated-macrophage cell lines, including J774 cell lines [14–17, 19–23, 25,26, 28,29,31,32] and

RAW264.7 [14] In four studies, THP-1 monocytes were differentiated in situ into macrophages [18, 24, 27,30]. The preferred tracer was tritium-labeled cholesterol, and one study used ^{14}C-cholesterol [14]. In four articles, the assay was performed with BODIPY-cholesterol [16,20–22]. We included one study [33] describing the ability of HDL to accept cholesterol, phospholipids and triglycerides from a lipid emulsion in a cell-free assay, and another study [27] in which the authors did not use any tracer. Regarding the lipid acceptor, all studies used ABDS [14–17, 19, 23, 25, 27–29,31,32] or ABDP [18, 20–22, 24, 26,30]. Higher values indicated greater cholesterol efflux expressed as the normalized value to a control pool of serum/plasma or ABDS/ABDP except in four studies [14,15, 27,33] where the raw values were used.

The pooled RR of the association between 1SD increase in CEC and incident CVD was 0.86 (95% CI, 0.76 to 0.98), and with all-cause mortality was 0.77 (95% CI, 0.60 to 1.00) (Figure 1, A for CVD and B for all-cause mortality). Three longitudinal studies were excluded from the analysis [14, 17,19] as the estimates were not reported in a comparable scale. All studies but two [14,17] showed an inverse association of CEC with cardiovascular events. Meta-analysis of cross-sectional studies for the association of CEC with CVD for a 1SD increase is available in eFigure 3. Two cross-sectional studies were not included in the analysis as they did not provide an estimate for 1SD increase[14,30]. The comparison of the highest vs the lowest categories of CEC were associated with a higher risk for cardiovascular outcomes in only one study [14](eTable 1).

Figure 1. Meta-analysis of prospective studies investigating the association between HDL cholesterol efflux capacity and major adverse cardiovascular events (MACE) and all-cause mortality risk. Estimates are risk ratios for MACE (A) and all-cause mortality (B) for a better compared to poorer CEC function (per 1SD increase)

A

Study	N total	N events		RR (95% CI)
Annema, 2016	495	54		1.26 (0.83, 1.89)
Chindhy, 2018	2805	287		0.79 (0.65, 0.96)
Javaheri, 2016	81	15		0.35 (0.13, 0.99)
Khera, 2017	628	314		0.89 (0.72, 1.10)
Kopecky, 2017	1147	423		0.98 (0.91, 1.05)
Liu, 2016	1737	122		0.08 (0.01, 0.68)
Saleheen, 2015	3494	1745		0.80 (0.70, 0.90)
Shea, 2019	832	416		0.82 (0.69, 0.98)
Overall (I-squared = 68.9%, p = 0.002)				0.86 (0.76, 0.98)

.1   .2   .5   1   2

B

Study	N total	N events		RR (95% CI)
Annema, 2016	495	102		0.92 (0.66, 1.28)
Chindhy, 2018	2805	94		0.62 (0.47, 0.81)
Javaheri, 2016	35	15		0.26 (0.11, 0.63)
Kopecky, 2017	1147	561		0.99 (0.94, 1.05)
Potocnjak, 2016	152	22		0.83 (0.65, 1.06)
Overall (I-squared = 81.1%, p = 0.000)				0.77 (0.60, 1.00)

.1   .2   .5   1   2

We combined odds ratios or hazard ratios with corresponding 95% confidence intervals (CI) from prospective studies with random-effect meta-analysis to provide a pooled risk ratio of the association between HDL-cholesterol efflux capacity and the incidence of CVD events or all-cause mortality. We combined only comparable estimates (per 1standard deviation or per category) together.

The percentage of variation explained by heterogeneity rather than chance across longitudinal studies included in the analysis of CEC (1SD increase) in relation with the risk of a CVD event was high (I^2 68.9%), as well as for all-cause mortality (I^2 81.1%), and the Cochrane Q statistic was significant for both groups (p=0.002 for CVD and p<0.001 for all-cause mortality). Visual evaluation of funnel plots (eFigure 2) and Egger´s test (p=0.01 for

CVD and p=0.01 for all-cause mortality) suggests evidence of publication bias for both outcomes.

Regarding study quality, two longitudinal studies [15,22] were rated only 5 stars out of 9, three prospective study [14, 17,19] and 2 cross-sectional studies [28,29] were granted 6 points and the remaining studies achieved a score of 7 or more (eTables 4 and 5). The stratification by study quality showed a less strong association for CEC with CVD and all cause-mortality for those studies rated of higher quality compared to those rated lower. It should be noted though that there were fewer studies of high quality and heterogeneity between them was lower than among studies of lower quality (eFigure 4, A for CVD and B for all-cause mortality). Meta-analysis of 6 studies evaluating CEC exclusively on J774 cells reveals a stronger association with both CVD and all-cause mortality (eFigure 5, A and B respectively), with lower heterogeneity. In contrast, association of cholesterol efflux capacity measured with a novel fluorescent probe with CVD in 2 studies (eFigure 6) was stronger, but I^2 and Cochrane Q statistics indicate greater heterogeneity. The sub-analysis of manuscripts adjusted for either albumin or systemic markers of inflammation could not be performed due to the insufficient number of studies available.
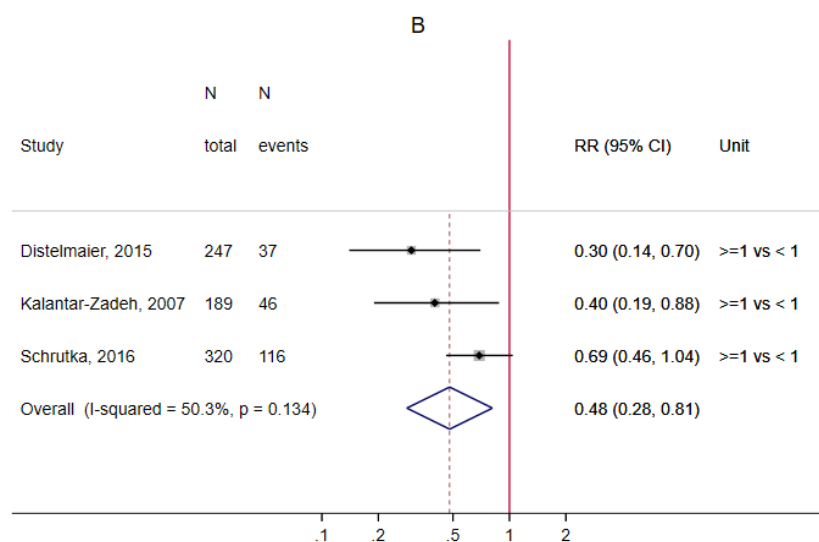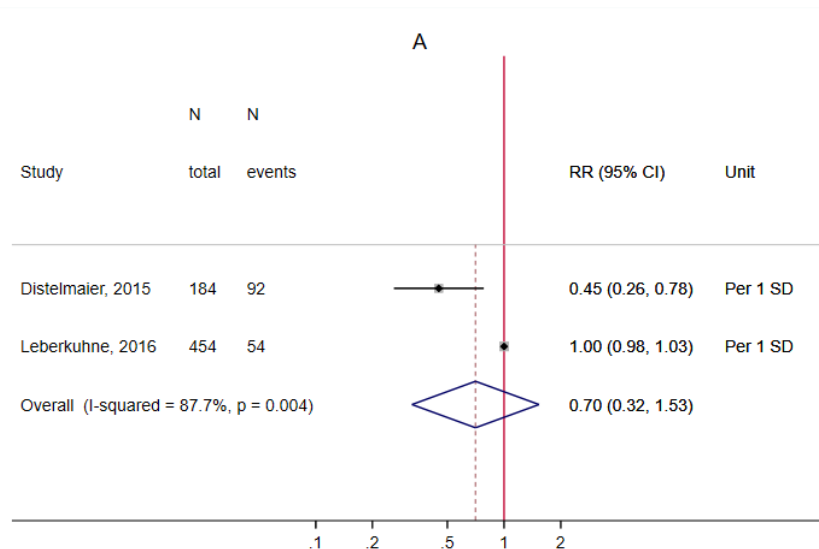
Antioxidant capacity

Ten studies were included that evaluated the anti-oxidative capacity of HDL and the risk of CVD or all-cause mortality (eTable 2): 3 cross-sectional studies [30, 32,34] and 7 longitudinal studies [13,35–40]. Overall, these studies included a total of 2,563 participants aged 35 to 67y, of which 66.2% were men. All studies measured the HDL profile prone to protect low-density lipoprotein (LDL) and other compounds from oxidation. The majority of studies did not adjust for HDL-C (all except three [17,39,40]).

Two major protocols were employed using: (i) HDL, ABDP or ABDS, (ii) native or previously oxidized LDL, (iii) a marker of oxidative degradation. In the first protocol, used in seven studies, the antioxidant action of ABDS or ABDP on pre-oxidized LDL [13, 32, 34–36,39] or on native LDL [37] was assessed in presence of the non-fluorescent dichlorofluorescein diacetate (DCF-DA). The conversion of DCF-DA to its fluorescent form reflects the oxidation of LDL, therefore, higher values were considered to be indicative of poorer protection against oxidation. A variant of this procedure was found in one study [40]: no native or pre-oxidized LDL was involved and the marker of fluorescence was dihydrorhodamine (DHR). Here, a greater percentage of reduction in oxidation reflects a better protective action of HDL. The other protocol, used in two studies[30,38], is based on the production of thiobarbituric acid

10

reactive substances (TBARS) as a measure of the oxidative modification of LDL (native [38] or pre-oxidized[30]). Here, HDL antioxidative capacity is calculated as the percent reduction in TBARS formation obtained relative to a negative control, and lower values indicate better antioxidative protection.

The meta-analysis of two prospective studies shows that higher antioxidant protection exerted by HDL was not statistically significantly associated with lower risk of incident CVD (combined RR estimate for 1SD increase of 0.70 (95% CI, 0.32-1.53), Figure 2, A). The pooled RR of the association with the risk of all-cause mortality from 3 studies was 0.48 (95% CI, 0.28-0.81) (Figure 2, B). Four prospective studies [13,38–40] were not included in the analysis because estimates were not comparable. Only in two cohorts [36,39] the antioxidant protection of the HDL particle was strongly associated with a decreased risk for incident CVD (eTable 2).

Figure 2. Meta-analysis of prospective studies investigating the association between HDL anti-antioxidant capacity and major adverse cardiovascular events (MACE) and all-cause mortality risk. Estimates are risk ratios for MACE (A) and all-cause mortality (B) for a better compared to poorer antioxidant function (1SD increase or categories, as specified)

A

Study	N total	N events		RR (95% CI)	Unit
Distelmaier, 2015	184	92		0.45 (0.26, 0.78)	Per 1 SD
Leberkuhne, 2016	454	54		1.00 (0.98, 1.03)	Per 1 SD
Overall (I-squared = 87.7%, p = 0.004)				0.70 (0.32, 1.53)	

.1 .2 .5 1 2

B

Study	N total	N events		RR (95% CI)	Unit
Distelmaier, 2015	247	37		0.30 (0.14, 0.70)	>=1 vs < 1
Kalantar-Zadeh, 2007	189	46		0.40 (0.19, 0.88)	>=1 vs < 1
Schrutka, 2016	320	116		0.69 (0.46, 1.04)	>=1 vs < 1
Overall (I-squared = 50.3%, p = 0.134)				0.48 (0.28, 0.81)	

.1 .2 .5 1 2

We combined odds ratios or hazard ratios with corresponding 95% confidence intervals (CI) from prospective studies with random-effect meta-analysis to provide a pooled risk ratio of the association between antioxidant capacity of the HDL particle and the incidence of CVD events or all-cause mortality. We combined only comparable estimates (per 1standard deviation or per category) together

Heterogeneity between longitudinal studies for cardiovascular outcomes was high (I^2=87.7%, chi-squared p=0.004) but only moderate for all-cause mortality (I^2=50.3%, chi-squared p<0.134). Funnel plots (eFigure 2) for CVD event (A) and all-cause mortality (B) show asymmetry and Egger´s tests were significant (p=0.073 for CVD and p=0.002 for all-cause mortality), indicating that small studies with non-significant findings are likely not published. In terms of methodology quality, one cohort was rated only 5 stars [35], two cohorts 6 stars [39,40]

and the other seven studies scored 7 or higher (eTables 4 and 5). Sensitivity analysis evaluating the effect of quality of studies on the risk estimate are presented in eFigure 7 and the only study of high quality showed a strong inverse association.

Anti-inflammatory capacity

There were one cohort [41] and one case-control [30] study meeting our inclusion criteria that assessed the anti-inflammatory potential of HDL in relation to CVD events (eTable 3). No study was found that used all-cause mortality as an outcome. The two studies included 204 participants (67% men) in total, aged 63-67y. Both the case-control and cohort studies included HDL-C as an adjustment factor.

The main technical characteristics of the protocols evaluating the anti-inflammatory potential of HDL included the cell line, the use of HDL, ABDS or ABDP, the pro-inflammatory stimuli, and the marker of inflammation. In both papers the technique was *in–vitro*: after the incubation of human umbilical endothelial cell lines with ABDP, the cultured cells were challenged with tumor necrosis factor-α and total RNA was isolated to further measure VCAM-1 mRNA expression levels. Lower values indicated better HDL anti-inflammatory capacity. Results were normalized to control values in the case-control to baseline levels in the cohort study.

In these two studies, individuals presenting the highest anti-inflammatory HDL capacity showed lower CVD prevalence and incidence at 3y compared to low anti-inflammatory capacity, but no meta-analysis was performed due to the insufficient number of studies. Regarding adjustment for albumin, one study showed a strong inverse association between anti-inflammatory capacity and all-cause mortality[37], whereas the second study found no association with incident CVD[40]. Presence of publication bias was revealed by the lack of studies at the right of the funnel plot (eFigure 2, C). Both studies were considered of high quality with 7 stars on the Newcastle Ottawa scale (eTables 4 and 5).

DISCUSSION

We provide the first comprehensive systematic review and meta-analysis of studies assessing the association between different functional properties of HDL and the risk of cardiovascular events and of all-cause mortality. We identified three main domains of functionality: cholesterol efflux capacity, antioxidant and anti-inflammatory capacity, with the latter less studied in comparison with the first two. Results from prospective studies show that, overall, greater levels of cholesterol efflux capacity, and greater anti-inflammatory and

antioxidant capacities exerted by HDL were associated with lower risk of cardiovascular disease. We observed a similar association for the risk of all-cause mortality with CEC and antioxidant activity.

Oxidation of the LDL particle into the endothelium and uncontrolled cholesterol uptake by macrophages are the key triggers for the initiation and progression of the atherosclerotic cardiovascular disease [42] [43].

HDL is under constant remodeling in the plasma which might influence its functionality [44,45]. The variability in HDL particles may help to explain why randomized clinical trials of HDL-C raising drugs have been unsuccessful at decreasing CVD risk [46–48].

Cholesterol efflux capacity

The ability to remove cholesterol from macrophages is considered the main HDL-related atheroprotective function[49]. In our meta-analysis, we found that higher cholesterol efflux was inversely associated with cardiovascular events and all-cause mortality. However, it has not yet been unraveled whether an impaired HDL-cholesterol efflux capacity is the trigger or a consequence of the deleterious effect of a proinflammatory environment and LDL oxidation. Although results from the majority of the studies are consistent, one study by Li et al [14] found opposite results. In that study, levels of ApoA-I were lower and levels of ApolipoproteinA-II higher in patients with CAD. Presence of ApolipoproteinA-II in HDL has been reported to increase cholesterol efflux from macrophages [50]. Moreover, the higher proportion of participants on statin treatment in the CAD group might have positive impact on HDL functions, including cholesterol efflux capacity [51]. We hypothesized that further adjustment of multivariate models would be desirable and might have an impact on the direction of results. Even if this constitutes only partial evidence, the association observed between higher CEC and lower risk of cardiovascular event appears to be independent of inflammatory state, as associations resisted adjustment for C-reactive protein. Of the three longitudinal studies that adjusted for an inflammatory marker (namely, C-reactive protein) [20,21], two report significant HRs, whereas the remaining one, conducted in CKD-Type 2 Diabetes patients [23] reports non significant HRs at all levels of adjustment, except for all cardiac events adjusted for age, sex and CRP.

Antioxidant and anti-inflammatory functions

We identified an inverse association between a better protection against oxidation and inflammation mediated by HDL and decreased risk of CVD. These results are consistent with

current knowledge on the atherosclerosis etiology and with findings from studies supporting that an impaired HDL antioxidant and anti-inflammatory capacities are potential biomarkers of the atherosclerosis processes [52,53].

In addition, oxidation and inflammation state of the HDL particle have a deleterious effect on its cholesterol efflux capacity among other HDL actions [54,55]. Pre-oxidized HDL particles show a poorer ability to remove cholesterol from cells and a greater percentage of cholesteryl esters remaining within the cells compared with non-oxidized HDL [54]. Low-grade inflammation impairs HDL-cholesterol efflux from J774 macrophages [56] and cholesterol efflux has been inversely associated with antioxidant capacity of HDL in a study of patients with rheumatoid arthritis [57]. Our meta-analysis identified ten articles on the antioxidant capacity and two articles on the anti-inflammatory ability of HDL. There is a need for larger longitudinal studies with standardized designs to deeper understand the potential role of HDL functions other than its ability to efflux cholesterol from cells in relation to cardiovascular diseases and mortality.

Limitations

This study has several limitations. Firstly, though this meta-analysis was primarily intended for heart and coronary diseases, we also included broader outcomes such as cardiovascular mortality, stroke or other CVD, because in most studies, the endpoint under evaluation was a composite. Therefore, our meta-analysis is not able to disentangle the specific effects of HDL on different clinical manifestations. Secondly, baseline characteristics of patients, such as age, sex, pre-existing pathologies, vary across studies. The majority of studies report risk estimates adjusted for age, but not all of them considered the effect of sex on results. Moreover, some studies collected only little information on risk factors and related markers, which limited the possible statistical adjustment. Therefore, the great variability of adjustment makes the comparability between studies challenging. Of particular importance if the fact that all assays were conducted on ApoB depleted plasma or serum. The presence of albumin in the analyzed fraction may provide critical contribution to the anti-oxidant or anti-inflammatory properties, over those of HDL, and only a few studies took albuminuria into account[57]. Thirdly, we only kept models that adjusted for HDL-cholesterol, to be interpreted as the relationship between HDL-functional trait and risk of outcome independently of levels of cholesterol in HDL. We used adjustment for HDL-C, and not ApoA1, given its relevance as a clinical lipid marker for cardiovascular events, and we obtained this information in the retrieved studies. Moreover, despite the key role of HDL-bound ApoA1 on the atheroprotective role of HDL, only a minority of articles included information on ApoA1.

Finally, differences in experimental approaches also have a great impact on clinical results. Methods to isolate lipoproteins have consequences on the composition and functionality of HDL subparticles. Extraction by precipitation with polyethylene glycol tends to show better results, but is often accompanied by a considerable decrease in specimen size[59-61]. In relation to cholesterol efflux, the choice of acceptor represents the greatest source of variation since different acceptors are involved in different pathways of cholesterol removal[62]. The use of radio labeled cholesterol still offers some advantages over fluorescent probes, in terms of sensitivity and accuracy[63]. In our meta-analysis, only a minority of studies used BODIPY-cholesterol instead of the traditional radio labeled lipid, despite many logistic and cost advantages. Although some controversy remains on the degree of correlation between results obtained with both probes[59], inter-run variability of the assay with BODIPY-cholesterol has shown high reproducibility, thus switching to fluorescent cholesterol represents many advantages, including safety and efficiency[64-65]. The degree of differentiation of THP-1 monocytes have a marked influence on ApoA-I mediated cholesterol efflux[66]. Moreover, preactivation of cells to enhance ABC transporter expression increases the proportion of efflux through them[67]. In this regard, new cost and time-effective methodologies that are not cell-based are being developed that have the potential to overcome these technical issues[68]. Despite differences in the determination of cholesterol efflux capacity, the majority of studies reported a negative association with cardiovascular disease risk, regardless of the technique employed, as shown in the stratified analysis. However, we advocate that the above issues identified in the present systematic review need to be addressed in future studies in order to advance the knowledge of the relationship between HDL related functions and CVD risk.

CONCLUSIONS

The present systematic review and meta-analysis represents a valuable comprehensive summary of the state-of-the-art regarding HDL functionality and its utility as predictor of cardiovascular diseases and mortality. Higher levels of cholesterol efflux capacity and antioxidant/anti-inflammatory capacities promoted by HDL were associated with lower risk of cardiovascular disease. A similar association was observed for the risk of all-cause mortality with cholesterol efflux and antioxidant activity. Results are encouraging but there is a lack of a body of studies, particularly on the anti-inflammatory role of this lipoprotein, adequate enough to draw firm conclusions. Larger studies designed to focus on specific cardiovascular outcomes, instead of combined endpoints, will greatly minimize the variability observed and will help to obtain consistent results on the association between HDL function and cardiovascular outcomes.

HIGHLIGHTS

- This is the first meta-analysis comprehensively summarizing the association between HDL-function and the risk of cardiovascular disease and all-cause mortality

- Higher cholesterol efflux capacity and antioxidant/anti-inflammatory capacities promoted by the HDL particle are associated with lower risk of cardiovascular disease.

- Similarly, the risk of all-cause mortality was associated with lower cholesterol efflux capacity and antioxidant activity

- Larger studies designed to reveal specific cardiovascular outcomes will greatly minimize the variability observed and will help to obtain consistent results on the value of HDL function for the prediction of cardiac outcome

AUTHOR CONTRIBUTION

M.T.S-F. Writing original manuscript, collecting data, performing the analysis; H.S., critical reviewing; M.G., critical reviewing; M.F., critical reviewing; C.L. Writing original manuscript, collecting data, performing the analysis and design of analysis.

REFERENCES

1.   Voight BF, Peloso GM, Orho-Melander M, et al. Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet.* 2012;380(9841):572-580. doi:10.1016/S0140-6736(12)60312-2.

2.   Holmes M V, Asselbergs FW, Palmer TM, et al. Mendelian randomization of blood lipids for coronary heart disease. *Eur Heart J.* 2015;36(9):539-550. doi:10.1093/eurheartj/eht571.

3.   Camont L, Chapman J, Kontush A. Functionality of HDL particles: Heterogeneity and relationships to cardiovascular disease. *Arch Cardiovasc Dis Suppl.* 2011;3(4):258-266. doi:https://doi.org/10.1016/S1878-6480(11)70784-4.

4.   Rader DJ, Alexander ET, Weibel GL, Billheimer J, Rothblat GH. The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis. *J Lipid Res.* 2009;50(Suppl):S189-S194. doi:10.1194/jlr.R800088-JLR200.

5.   Nakagawa K, Nakashima Y. Pathologic intimal thickening in human atherosclerosis is formed by extracellular accumulation of plasma-derived lipids and dispersion of intimal smooth muscle cells. *Atherosclerosis.* 2018;274:235-242. doi:10.1016/j.atherosclerosis.2018.03.039.

6.   Movva R, Rader DJ. Laboratory assessment of HDL heterogeneity and function. *Clin Chem.* 2008;54(5):788-800. doi:10.1373/clinchem.2007.101923.

7.   Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* 2009;6(7):e1000097. doi:10.1371/journal.pmed.1000097.

8.   Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* 2010;25(9):603-605. doi:10.1007/s10654-010-9491-z.

9.   Borenstein M, Hedges L V., Higgins JPT, Rothstein HR. *Introduction to Meta-Analysis.*; 2009.

10.   Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* 2003;327(7414):557 LP - 560. http://www.bmj.com/content/327/7414/557.abstract.

11.   Collaboration TC. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0.* (Higgins JPT GS, ed.).; 2011. www.handbook.cochrane.org.

12.   Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315(7109):629 LP - 634. http://www.bmj.com/content/315/7109/629.abstract.

13.   Schrutka L, Goliasch G, Meyer B, Wurm R, Koller L, Kriechbaumer L, Heinz G, Pacher R, Lang IM, Distelmaier K, Hülsmann M. Impaired high-density lipoprotein antioxidant function predicts poor outcome in critically ill patients. *PLoS One*. 2016;11(3):1-12. doi:10.1371/journal.pone.0151706.

14.   Li XM, Tang WHW, 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(7):1696-1705. doi:10.1161/ATVBAHA.113.301373.

15.   Potočnjak I, Degoricija V, Trbušić M, Terešak SD, Radulović B, Pregartner G, Berghold A, Tiran B, Marsche G, Frank S. Metrics of high-density lipoprotein function and hospital mortality in acute heart failure patients. *PLoS One*. 2016;11(6):1-11. doi:10.1371/journal.pone.0157507.

16.   Liu C, Zhang Y, Ding D, Li X, Yang Y, Li Q, Zheng Y, Wang D, Ling W. Cholesterol efflux capacity is an independent predictor of all-cause and cardiovascular mortality in patients with coronary artery disease: A prospective cohort study. *Atherosclerosis*. 2016;249:116-124. doi:10.1016/j.atherosclerosis.2015.10.111.

17.   Zhang J, Xu J, Wang J, Wu C, Xu Y, Wang Y, Deng F, Wang Z, Chen X, Wu M, Chen Y. Prognostic Usefulness of Serum Cholesterol Efflux Capacity in Patients with Coronary Artery Disease. *Am J Cardiol*. 2016;117(4):508-514. doi:10.1016/j.amjcard.2015.11.033.

18.   Annema W, Dikkers A, de Boer JF, Dullaart RPF, Sanders J-SF, Bakker SJL, Tietge UJF. HDL Cholesterol Efflux Predicts Graft Failure in Renal Transplant Recipients. *J Am Soc Nephrol*. 2016;27(2):595-603. doi:10.1681/ASN.2014090857.

19.   Bauer L, Kern S, Rogacev KS, Emrich IE, Zawada A, Fliser D, Heinemann A, Heine GH, Marsche G. HDL Cholesterol Efflux Capacity and Cardiovascular Events in Patients With Chronic Kidney Disease. *J Am Coll Cardiol*. 2017;69(2):246-247. doi:10.1016/j.jacc.2016.10.054.

20.   Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, Shaul PW. HDL Cholesterol Efflux Capacity and Incident Cardiovascular Events. *N Engl J Med*. 2014;371(25):2383-2393. doi:10.1056/NEJMoa1409065.

21.   Chindhy S, Joshi P, Khera A, Ayers CR, Hedayati SS, Rohatgi A. Impaired Renal Function on Cholesterol Ef fl ux Capacity , HDL Particle Number , and. *J Am Coll Cardiol*. 2018;72(6):698-700. doi:10.1016/j.jacc.2018.05.043.

22.   Mody P, Joshi PH, Khera A, Ayersa CR, Rohatgi A. Beyond Coronary Calcification, Family History, and C-reactive Protein: Cholesterol Efflux Capacity and

Cardiovascular Risk Prediction Purav. *J Am Coll Cardiol*. 2016;2(2):147-185. doi:10.1515/jci-2013-0007.Targeted.

23. Javaheri A, Molina M, Zamani P, et al. Cholesterol efflux capacity of high-density lipoprotein correlates with survival and allograft vasculopathy in cardiac transplant recipients. *J Hear Lung Transplant*. 2016;35(11):1295-1302. doi:10.1016/j.healun.2016.06.022.

24. Kopecky C, Ebtehaj S, Genser B, Drechsler C, Krane V, Antlanger M, Kovarik JJ, Kaltenecker CC, Parvizi M, Wanner C, Weichhart T, Säemann MD, Tietge UJF. HDL Cholesterol Efflux Does Not Predict Cardiovascular Risk in Hemodialysis Patients. *J Am Soc Nephrol*. 2017;28(3):769-775. doi:10.1681/ASN.2016030262.

25. Saleheen D, Scott R, Javad S, Zhao W, Rodrigues A, Picataggi A, Lukmanova D, Mucksavage ML, Luben R, Billheimer J, Kastelein JJP, Boekholdt 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(7):507-513. doi:10.1016/S2213-8587(15)00126-6.

26. Khera A V, Demler O V, Adelman SJ, Collins HL, Glynn RJ, Ridker PM, Rader DJ, Mora S. 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(25):2494-2504. doi:10.1161/circulationaha.116.025678.

27. Shea S, Stein JH, Jorgensen NW, Mcclelland RL, Tascau L, Shrager S, Heinecke JW, Yvan-charvet L, Tall AR. Cholesterol Mass Efflux Capacity , Incident Cardiovascular Disease , and Progression of Carotid Plaque The Multi-Ethnic Study of Atherosclerosis. *Atheroscler Thromb Vasc Biol*. 2018;39:89-96. doi:10.1161/ATVBAHA.118.311366.

28. Ishikawa T, Ayaori M, Uto-Kondo H, Nakajima T, Mutoh M, Ikewaki K. High-density lipoprotein cholesterol efflux capacity as a relevant predictor of atherosclerotic coronary disease. *Atherosclerosis*. 2015;242(1):318-322. doi:10.1016/j.atherosclerosis.2015.06.028.

29. Ogura M, Hori M, Harada-shiba M. Association Between Cholesterol Efflux Capacity and Atherosclerotic Cardiovascular Disease in Patients With Familial Hypercholesterolemia. 2016;(36):181-188. doi:10.1161/ATVBAHA.115.306665.

30. Annema W., Willemsen H M., F DRP de BJ, et al. HDL function is impaired in acute myocardial infarction independent of plasma HDL cholesterol levels. *J Clin Lipidol*. 2016;10(6):1318-1328. doi:10.1016/j.jacl.2016.08.003.

31. Khera A V., Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French

BC, Phillips JA, Mucksavage ML, Wilensky RL, Mohler ER, Rothblat GH, Rader DJ. Cholesterol Efflux Capacity, High-Density Lipoprotein Function, and Atherosclerosis. *N Engl J Med*. 2011;364(2):127-135. doi:10.1056/NEJMoa1001689.

32.   Patel PJ, Khera A V., Wilensky RL, Rader DJ. Anti-Oxidative and cholesterol efflux capacities of high-Density lipoprotein are reduced in ischaemic cardiomyopathy. *Eur J Heart Fail*. 2013;15(11):1215-1219. doi:10.1093/eurjhf/hft084.

33.   Sprandel MCO, Hueb WA, Segre A, Ramires JAF, Kalil-Filho R, Maranhão RC. Alterations in lipid transfers to HDL associated with the presence of coronary artery disease in patients with type 2 diabetes mellitus. *Cardiovasc Diabetol*. 2015;14(1):107. doi:10.1186/s12933-015-0270-8.

34.   Patel PJ, Khera A V., Jafri K, Wilensky RL, Rader DJ. The anti-oxidative capacity of high-density lipoprotein is reduced in acute coronary syndrome but not in stable coronary artery disease. *J Am Coll Cardiol*. 2011;58(20):2068-2075. doi:10.1016/j.jacc.2011.08.030.

35.   Distelmaier K, Schrutka L., Seidl V., Winter M P., Wurm R., Mangold A., Perkmann T., Maurer G., Adlbrecht C., Lang I M. Pro-oxidant HDL predicts poor outcome in patients with ST-elevation acute coronary syndrome. *Thromb Haemost*. 2015;114(1):133-138. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L605 025906%5Cnhttp://dx.doi.org/10.1160/TH14-10-0834%5Cnhttp://sfx.library.uu.nl/utrecht?sid=EMBASE&issn=03406245&id=doi:10.11 60/TH14-10-0834&atitle=Pro-oxidant+HDL+predicts+poor+out.

36.   Distelmaier K., Wiesbauer F., Blessberge H., Oravec S., Schrutka L., Binder C., Dostal E., Schillinger M., Wojta J., Lang I M., Maurer G., Huber K., Goliasch G. Impaired antioxidant HDL function is associated with premature myocardial infarction. *Eur J Clin Invest*. 2015;45(7):731-738. doi:10.1111/eci.12466.

37.   Kalantar-Zadeh K, Kopple JD, Kamranpour N, Fogelman AM, Navab M. HDL-inflammatory index correlates with poor outcome in hemodialysis patients. *Kidney Int*. 2007;72(9):1149-1156. doi:10.1038/sj.ki.5002491.

38.   Leberkühne LJ, Ebtehaj S, Dimova LG, Dikkers A, Dullaart RPF, Bakker SJL, Tietge UJF. The predictive value of the antioxidative function of HDL for cardiovascular disease and graft failure in renal transplant recipients. *Atherosclerosis*. 2016;249:181-185. doi:10.1016/j.atherosclerosis.2016.04.008.

39.   Schrutka L, Distelmaier K, Hohensinner P, Sulzgruber P, Lang IM, Maurer G, Wojta J, Hülsmann M, Niessner A, Koller L. Impaired High-Density Lipoprotein Anti-Oxidative Function Is Associated With Outcome in Patients With Chronic Heart Failure. *J Am*

Heart Assoc. 2016;5(12). doi:10.1161/JAHA.116.004169.

40.   Untersteller K, Meissl S, Trieb M, Emrich IE, Zawada AM, Holzer M, Knuplez E, Fliser D, Heine GH, Marsche G. HDL functionality and cardiovascular outcome among non-dialysis chronic kidney disease patients. *J Lipid Res*. 2018.

41.   Dullaart RPF, Annema W, Tio RA, Tietge UJF. The HDL anti-inflammatory function is impaired in myocardial infarction and may predict new cardiac events independent of HDL cholesterol. *Clin Chim Acta*. 2014;433:34-38. doi:10.1016/j.cca.2014.02.026.

42.   Nyyssönen K, Kurl S, Karppi J, Nurmi T, Baldassarre D, Veglia F, Rauramaa R, de Faire U, Hamsten A, Smit AJ, Mannarino E, Humphries SE, Giral P, Grossi E, Tremoli E. LDL oxidative modification and carotid atherosclerosis: Results of a multicenter study. *Atherosclerosis*. 2012;225(1):231-236. doi:https://doi.org/10.1016/j.atherosclerosis.2012.08.030.

43.   Ali M, Girgis S, Hassan A, Rudick S, Becker RC. Inflammation and coronary artery disease: from pathophysiology to Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS). *Coron Artery Dis*. 2018:1-9. doi:10.1097/mca.0000000000000625.

44.   von Hodenberg E, Heinen S, Howell KE, Luley C, Kubler W, Bond HM. Cholesterol efflux from macrophages mediated by high-density lipoprotein subfractions, which differ principally in apolipoprotein A-I and apolipoprotein A-II ratios. *Biochim Biophys Acta*. 1991;1086(2):173-184.

45.   Ohta T, Saku K, Takata K, Nakamura R, Ikeda Y, Matsuda I. Different Effects of Subclasses of HDL Containing ApoA-I but Not ApoA-II (LpA-I) on Cholesterol Esterification in Plasma and Net Cholesterol Efflux From Foam Cells. *Arterioscler Thromb Vasc Biol*. 1995;15(7):956 LP - 962. http://atvb.ahajournals.org/content/15/7/956.abstract.

46.   The AIM-HIGH Investigators. Niacin in Patients with Low HDL Cholesterol Levels Receiving Intensive Statin Therapy. *N Engl J Med*. 2011;365(24):2255-2267. doi:10.1056/NEJMoa1107579.

47.   Collaborators of Heart Protection Study 2–Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE). Effects of Extended-Release Niacin with Laropiprant in High-Risk Patients. *N Engl J Med*. 2014;371(3):203-212. doi:10.1056/NEJMoa1300955.

48.   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(21):2109-2122. doi:10.1056/NEJMoa0706628.

49.   Rosenson RS, Brewer HBJ, Davidson WS, Fayad ZA, Fuster V, Goldstein J,

Hellerstein M, Jiang XC, Phillips MC, Rader DJ, Remaley AT, Rothblat GH, Tall AR, Yvan-Charvet L. Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circulation.* 2012;125(15):1905-1919. doi:10.1161/CIRCULATIONAHA.111.066589.

50.   Melchior JT, Street SE, Andrasske AB, Furtado JD, Sacks FM, Shute RL, Greve EI, Swertfeger DK, Li H, Amy S, Lu LJ, Davidson WS. Apolipoprotein A-II alters the proteome of human lipoproteins and enhances cholesterol efflux from ABCA1. *J Lipid Res.* 2017;(513):1-44.

51.   Lee CJ, Choi S, Cheon DH, Kim KY, Cheon EJ, Ann S-J, Noh H-M, Park S, Kang S-M, Choi D, Lee JE, Lee S-H. Effect of two lipid-lowering strategies on high-density lipoprotein function and some HDL-related proteins: a randomized clinical trial. *Lipids Health Dis.* 2017;16(1):49. doi:10.1186/s12944-017-0433-6.

52.   Chen X, Wu Y, Liu L, Su Y, Peng Y, Jiang L, Liu X, Huang D. Relationship Between High Density Lipoprotein Antioxidant Activity and Carotid Arterial Intima-Media Thickness in Patients with Essential Hypertension. *Clin Exp Hypertens.* 2010;32(1):13-20. doi:10.3109/10641960902929487.

53.   McMahon M, Grossman J, Skaggs B, FitzGerald J, Sahakian L, Ragavendra N, Charles-Schoeman C, Watson K, Wong WK, Chen W, Gorn A, Karpouzas G, Weisman M, Wallace DJ, Hahn BH. Dysfunctional Pro-Inflammatory High Density Lipoproteins Confer Increased Risk for Atherosclerosis in Women with Systemic Lupus Erythematosus. *Arthritis Rheum.* 2009;60(8):2428-2437. doi:10.1002/art.24677.

54.   Nagano Y, Arai H, Kita T. High Density Lipoprotein Loses its Effect to Stimulate Efflux of Cholesterol from Foam Cells After Oxidative Modification. *Proc Natl Acad Sci U S A.* 1991;88(15):6457-6461. http://www.jstor.org/stable/2357314.

55.   Vaisar T, Tang C, Babenko I, Hutchins P, Wimberger J, Suffredini AF, Heinecke JW. Inflammatory remodeling of the HDL proteome impairs cholesterol efflux capacity. *J Lipid Res.* 2015;56(8):1519-1530. doi:10.1194/jlr.M059089.

56.   McGillicuddy FC, de la Llera Moya M, Hinkle CC, Joshi MR, Chiquoine EH, Billheimer JT, Rothblat GH, Reilly MP. Inflammation Impairs Reverse Cholesterol Transport in vivo. *Circulation.* 2009;119(8):1135-1145. doi:10.1161/CIRCULATIONAHA.108.810721.

57.   Charles-Schoeman C, Lee YY, Grijalva V, Amjadi S, FitzGerald J, Ranganath VK, Taylor M, McMahon M, Paulus HE, Reddy ST. Cholesterol efflux by high density lipoproteins is impaired in patients with active rheumatoid arthritis. *Ann Rheum Dis.* 2012;71(7):1157-1162. doi:10.1136/annrheumdis-2011-200493.

58. Wiedermann, CJ. Anti-inflammatory activity of albumin. *Critical care medicine*. 2007; 35(3): 981-983.

59. Hafiane A, Genest J. HDL-mediated cellular cholesterol efflux assay method. *Ann Clin Lab Sci*. 2015;45(6):659-668.

60. Davidson WS, Heink A, Sexmith H, Melchior JT, Gordon SM, Kuklenyik Z, Woollett L, Barr JR, Jones JI, Toth CA, Shah AS. The effects of apolipoprotein B depletion on HDL subspecies composition and function. *J Lipid Res*. 2016;57(4):674-686. doi:10.1194/jlr.M066613.

61. Shah AS, Heink A, Davidson W. The Impact of Apolipoprotein B Depletion Methods on HDL Subspecies*. *J Clin Lipidol*. 2015;9(3):430. doi:10.1016/j.jacl.2015.03.037.

62. Rohatgi A. High-Density Lipoprotein Function Measurement in Human Studies: Focus on Cholesterol Efflux Capacity. *Prog Cardiovasc Dis*. 2015;58(1):32-40. doi:10.1016/j.pcad.2015.05.004.

63. Zanotti I, Favari E, Bernini F. Cellular cholesterol efflux pathways: impact on intracellular lipid trafficking and methodological considerations. *Curr Pharm Biotechnol*. 2012;13(2):292-302. doi:10.2174/138920112799095383.

64. 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(12):2332-2340. doi:10.1194/jlr.D018051.

65. Holtta-Vuori M, Uronen R-L, Repakova J, Salonen E, Vattulainen I, Panula P, Li Z, Bittman R, Ikonen E. BODIPY-cholesterol: a new tool to visualize sterol trafficking in living cells and organisms. *Traffic*. 2008;9(11):1839-1849. doi:10.1111/j.1600-0854.2008.00801.x.

66. Yano K, Ohkawa R, Sato M, Yoshimoto A, Ichimura N, Kameda T, Kubota T, Tozuka M. Cholesterol Efflux Capacity of Apolipoprotein A-I Varies with the Extent of Differentiation and Foam Cell Formation of THP-1 Cells. *J Lipids*. 2016;2016:9891316. doi:10.1155/2016/9891316.

67. Low H, Hoang A, Sviridov D. Cholesterol Efflux Assay. *J Vis Exp*. 2012;(61):3810. doi:10.3791/3810.

68. Kuusisto S, Holmes M V., Ohukainen P, Kangas AJ, Karsikas M, Tiainen M, Perola M, Salomaa V, Kettunen J, Ala-Korpela M. Direct estimation of HDL-mediated cholesterol efflux capacity from serum. *Clin Chem*. 2018;65(8). doi:10.1101/396929.