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Cholesterol Mass Efflux Capacity, Incident Cardiovascular Disease, and Progression of Carotid Plaque

The Multi-Ethnic Study of Atherosclerosis

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Objective—To assess the role of HDL (high-density lipoprotein)-mediated cholesterol mass efflux capacity (CMEC) in incident cardiovascular disease and carotid plaque progression.

Approach and Results—We measured CMEC in 2 cohorts aged 45 to 84 years at baseline derived from the MESA (Multi-Ethnic Study of Atherosclerosis). Cohort 1 comprised 465 cases with incident cardiovascular disease events during 10 years of follow-up and 465 age- and sex-matched controls; cohort 2 comprised 407 cases with progression of carotid plaque measured by ultrasonography at 2 exams >10 years and 407 similarly matched controls. Covariates and outcome events were ascertained according to the MESA protocol. CMEC level was modestly correlated with HDL cholesterol (R=0.13; P<0.001) but was not associated with age, sex, race/ethnicity, body mass index, diabetes mellitus, alcohol use, smoking status, or statin use. Higher CMEC level was significantly associated with lower odds of cardiovascular disease (odds ratio, 0.82 per SD of CMEC [95% CI, 0.69–0.98; P=0.031] in the fully adjusted model) in cohort 1 but higher odds of carotid plaque progression (odds ratio, 1.24 per SD of CMEC [95% CI, 1.04–1.48; P=0.018] in the fully adjusted model) in cohort 2 but without dose-response effect. In subgroup analysis within cohort 1, higher CMEC was associated with lower risk of incident coronary heart disease events (odds ratio, 0.72 per SD of CMEC (95% CI, 0.5–0.91; P=0.007) while no association was found with stroke events.

Conclusions—These findings support a role for HDL-mediated cholesterol efflux in an atheroprotective mechanism for coronary heart disease but not stroke.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2019;39:89-96. DOI: 10.1161/ATVBAHA.118.311366.)**

Key Words: atherosclerosis ■ cardiovascular diseases ■ cholesterol ■ lipoproteins ■ stroke

In population studies plasma HDL (high-density lipoprotein) cholesterol levels show an inverse relationship to cardiovascular disease (CVD) risk, independent of other risk factors.[1] Studies in cell culture and animal models suggest that HDL has the ability the reduce atherosclerosis by promoting the removal of cholesterol from macrophage foam cells, reducing macrophage-related inflammatory processes in atherosclerotic plaques.[2,3] The relevance of HDL-mediated macrophage cholesterol efflux to human atherosclerosis was suggested by the discovery that the cholesterol efflux capacity (CEC) of HDL was inversely related to coronary atheroma burden in subjects undergoing coronary angiography.[4] Moreover, in a longitudinal design from the Dallas Heart Study[5] and a nested case-control design from the Epic-Norfolk study,[6] the CEC of HDL was inversely related to incident coronary heart disease (CHD). Importantly, these relationships were seen even after adjustment for HDL-cholesterol level, suggesting that CEC is measuring a key function of HDL relevant to CHD. However, the inverse relationship of CEC to CHD has not always been observed. In one study increased HDL-mediated cholesterol efflux was associated with an increased incidence of CHD.[7] In the JUPITER study (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) of rosuvastatin, on treatment CEC measurements only showed a weak inverse correlation with CHD, while baseline samples did not show any relationship.[8] The reasons for these discrepancies are unknown but could be related to sample size, study design, or methods for measuring CEC. Therefore, to further assess the relationship between CEC and incident CHD, we performed a nested case-control study in the MESA (Multi-Ethnic Study of

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Nonstandard Abbreviations and Acronyms	
ABCA1	ATP binding cassette transporter subfamily ABCA member 1
ABCG1	ATP binding cassette transporter subfamily G member 1
CEC	cholesterol efflux capacity
CETP	cholesteryl ester transfer protein
CHD	coronary heart disease
CMEC	cholesterol mass efflux capacity
CVD	cardiovascular disease
eNOS	endothelial nitric oxide synthase
HDL	high-density lipoprotein
HOMA-IR	homeostatic model assessment–insulin resistance
LDL	low-density lipoprotein
LXR	liver X receptor
MESA	Multi-Ethnic Study of Atherosclerosis
SR-B1	scavenger receptor class B type 1

Atherosclerosis) cohort, measured CEC in baseline samples, and then related these measurements to incident CVD.

Moreover, only one study in 203 healthy White subjects has reported an inverse relationship between CEC and carotid-intima thickness,[4] whereas another study found no relationship after multivariate adjustment between CEC and lipid-rich necrotic cores as determined by carotid magnetic resonance imaging.[9] Therefore, in a second subsample from the MESA cohort, we investigated the relationship of CEC to progression of carotid artery plaque. To date, all studies have used a radioactive or fluorescent cholesterol tracer to measure efflux of cholesterol from cultured macrophages to HDL over the course of several hours. Because there is a bidirectional exchange of cholesterol between cells and HDL, the efflux of labeled cholesterol from cells may be partly or wholly counter-balanced by the uptake of nonlabeled cholesterol from HDL. Thus, the efflux of labeled cholesterol is not an accurate measure of the net movement (efflux minus influx) of cholesterol between cells and HDL. We used a cholesterol mass efflux assay in which the change in cholesterol mass in media is directly measured[10] to circumvent this problem. We determined the cholesterol mass efflux capacity (CMEC) of the HDL fraction in serum samples from subjects in the MESA cohort. Our studies provide confirmation of the inverse relationship between CEC and CHD. Unexpectedly they also revealed a positive relationship between CMEC and carotid plaque progression and no relationship to nonhemorrhagic stroke.

Methods

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Participants and Baseline Measures

The MESA is a population-based study of 6814 men and women aged 45 to 85 years, without known clinical CVD at time of entry, recruited from 6 US communities (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; northern Manhattan, NY; and St Paul, MN). Sampling and recruitment procedures have been reported.[11] Questionnaires were used to assess age, sex, race/ethnicity, educational and income levels, occupational information, smoking status, and medication use for diabetes mellitus, lipid lowering, and hypertension. Classification of race/ethnicity was based on self-identification using questions based on the United States 2000 census questionnaire. Physical activity,[12] alcohol intake, and diet[13] were measured using questionnaires. Height and weight were measured, and body mass index was computed as kg/m^2. Blood pressure was measured 3 times at 1 minute intervals after a standardized protocol.[14] Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or self-reported high blood pressure and on treatment with medication for hypertension.[15] Diabetes mellitus was defined as being on treatment with insulin or oral medication for diabetes mellitus or fasting glucose ≥126 mg/dL.[16] The baseline exam was conducted between August 1, 2000 and July 30, 2002. Follow-up at 10 years (MESA exam 5) was 76% (n=4655) of those alive. Centrally trained and certified study staff performed all participant measurements. Institutional Review Board approval was obtained at all MESA sites. Consent was obtained from all participants.

Laboratory Measurements

Fasting blood specimens were analyzed for serum glucose, total cholesterol, HDL cholesterol, and triglyceride levels. LDL (low-density lipoprotein) cholesterol was calculated in plasma specimens having a triglyceride value <400 mg/dL using the Friedewald formula.[17]

Plasma HDL Preparation

ApoB-containing particles were precipitated from serum by adding 100 µL of serum to 40 µL of 20% polyethylene glycol (Sigma P-2139 in 200 mmol/L glycine, pH10) solution. This mixture was incubated at room temperature for 15 minutes then was centrifuged at 4000 rpm for 20 minutes. The supernatant, containing HDL fractions, was removed and used for experiments as previously described.[10]

Cholesterol Mass Efflux Measurements

Cholesterol efflux measurements were performed at Columbia University in a completely blinded fashion and data transmitted to the University of Washington for unblinding. THP-1 monocytes (ATCC TIB-202, Manassas, VA) were cultured in RPMI (Roswell Park Memorial Institute) 1640 medium supplemented with 10% fetal bovine serum at 37°C in 5% Co_2. Cells were treated with 100 nmol/L Phorbol myristate acetate for 24 hours to facilitate differentiation into macrophages. Then, adherent macrophages were incubated with 50 µg/mL acetyl-LDL and 3 µmol/L LXR (liver X receptor) agonist (TO901317) for 24 hours before cholesterol efflux studies.

CMEC Analysis

CMEC was analyzed in DMEM containing 0.2% BSA in the presence of polyethylene glycol-HDL matched by volume (ratio 7:1). After 6 hours incubation with HDL, the mass of total cholesterol was determined from the collected media by colorimetric assay. The HDL-mediated CMEC was calculated by subtraction of cholesterol mass of the medium cultured with or without cells. This allows the determination of the net cholesterol efflux driven by HDL particles reflecting the ability of HDL to remove cellular cholesterol.[10] The assay was run in triplicate; the intra-assay coefficient of variation was 4.6%.

Cardiovascular Events

Participants were followed for incident CVD events for a median of 10.2 years from their baseline examinations. In addition to 5 follow-up MESA examinations, a telephone interviewer contacted each participant every 9 to 12 months to inquire about interim hospital admissions, cardiovascular outpatient diagnoses, and deaths. To verify self-reported diagnoses, copies were requested of all death certificates and medical records for all hospitalizations and outpatient cardiovascular diagnoses. Next of kin interviews for out of hospital cardiovascular deaths were obtained.[18] Medical records were obtained for ≈99% of reported hospitalized cardiovascular events and information on 97% of reported outpatient cardiovascular diagnostic encounters. Follow-up telephone interviews were completed in 90% of living participants. Trained personnel abstracted medical records suggesting possible cardiovascular events. Two physicians independently reviewed all abstracted medical records for end point classification and assignment of incidence dates, using prespecified criteria.

Carotid Ultrasonography

B-mode ultrasound images of the right and left common carotid, bifurcation, and internal carotid artery segments were recorded on Super-VHS videotape with a Logiq 700 ultrasound system using the M12L transducer (General Electric Medical Systems, CCA frequency 13 MHz) at MESA exams 1 and 5 as described previously.[19] Carotid plaque score (range 0–12) was defined as the number of carotid plaques in the internal, bifurcation, and common segments of both carotid arteries.[19,20] Carotid plaque was defined as a discrete, focal wall thickening ≥1.5 cm or focal thickening at least 50% greater than the surrounding intima-media thickening.[21] Progression was defined as an increase in the carotid plaque score from exam 1 to 5, which almost always was because of the appearance of a new plaque.[19] For carotid plaque presence and score, intrareader reproducibility was κ=0.83 (95% CI, 0.70–0.96) and inter-reader reproducibility was κ=0.89 (95% CI, 0.72–1.00).[19]

Statistical Analysis

Cases, defined as participants who had an incident CVD event between exams 1 and 5, were matched by age (5-year intervals) and sex to controls, defined as those who did not have a CVD event (cohort 1; Figure). Controls were required to have at least as much event-free follow-up time as cases. Similarly, cases with carotid plaque progression were matched by the same factors to controls, defined as participants who did not have any increase in carotid plaque score between MESA exams 1 and 5 (cohort 2). All CVD cases were used. Of the total 1923 cases of carotid plaque progression, a random sample was selected based on age- and sex-matching to controls. Bivariate displays of values for the predictor of interest and covariates were used to compare case versus control groups. Conditional logistic regression models (conditional on the matching) were used to examine the relationship between cholesterol efflux and case-control status. A series of staged models were used to control for potential confounders. Model 1 adjusted for age, sex, race/ethnicity, body mass index, and study site. Model 2 additionally adjusted for diabetes mellitus, current smoking, total and HDL cholesterol, statin and antihypertensive medication use, and systolic blood pressure. Model 3 additionally adjusted for pack-years of smoking, alcohol use, intentional exercise, and Mediterranean diet score.[22]

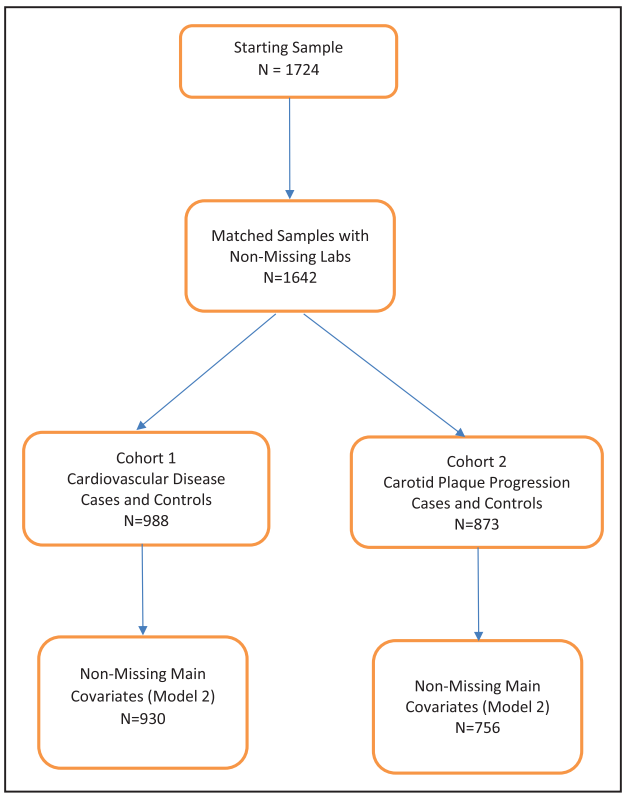

Figure. Flowchart.

Results

After exclusion of matched cases and controls with missing values for laboratory data or other key covariates, 930 subjects (465 cases and matched 465 controls) were available for analysis for incident CVD and 814 subjects (407 cases and 407 matched controls) for progression of carotid artery plaque (Figure). Cases and controls did not differ with respect to age and sex because they were matched on these criteria (Table 1). As expected, cases had higher prevalence or levels of body mass index, diabetes mellitus, smoking, hypertension, systolic and diastolic blood pressure, and lower level of intentional exercise and HDL cholesterol (48.5 versus 50.6 mg/dL for the CVD cases and controls, respectively; 49.9 versus 51.8 mg/dL for the carotid plaque progression cases versus controls). Mean CMEC was lower in the CVD cases versus controls (2.9 versus 3.1 mg/dL) but higher in the carotid artery plaque progression cases versus controls (3.1 versus 2.8 mg/dL; Table 1).

Table 2 shows mean CMEC levels in the control group classified by demographic and clinical characteristic. There was no statistically significant difference by age category, sex, race/ethnicity, body mass index category, diabetes mellitus category, alcohol use, smoking status, or statin use. CMEC was modestly correlated with HDL cholesterol (R=0.13; P<0.001), total cholesterol (R=0.08; P=0.036), and age (R=0.02; P=.009) but not with triglyceride level or homeostatic model assessment–insulin resistance (Tables I and II in the online-only Data Supplement).

In conditional logistic regression models examining the association of CMEC with incident CVD, higher level of CMEC was associated with lower odds of incident CVD, with little difference in magnitude of the effect size or P value with adjustment for covariates (Table 3, top), with odds ratio of 0.82 per SD of CMEC (95% CI, 0.69–0.98; P=0.031) in the fully adjusted model. In contrast, a higher level of CMEC was associated with greater odds of carotid artery plaque progression (Table 3, bottom), with an odds ratio of 1.24 per SD of CMEC (95% CI, 1.04–1.48; P=0.018) in the fully adjusted model.

We further examined the association between higher level of CMEC and lower risk of incident CVD events by separately analyzing CHD events and stroke events. As shown in Table 4, higher CMEC level was associated with lower odds of CHD events (odds ratio, 0.72 per SD of CMEC (95% CI, 0.56–0.91; P=0.007) in the fully adjusted model while no association was found with stroke events or with stroke events after exclusion of hemorrhagic strokes (odds ratio, 1.01 per SD of CMEC (95% CI, 0.70–1.45; P=0.978). These findings in cohort 1, in a separate sample of cases and controls, were consistent with the lack of protection of higher CMEC level against carotid plaque progression in cohort 2. For both incident CVD and plaque progression, we tested for potential interactions between CMEC and sex, race/ethnicity, diabetes mellitus, hypertension, chronic kidney disease, smoking, and statin use; none was significant at the P<0.05 level. Additionally,

Table 1. Participant Characteristics for Incident CVD and Carotid Plaque Progression by Case and Control Status

	CVD (N=465) CHD=270, Stroke=195	No CVD (N=465)	Plaque Progression (N=407)	No Plaque Progression (N=407)
	Mean±SD or N (%)	Mean±SD or N (%)	Mean±SD or N (%)	Mean±SD or N (%)
Age, y	67.8±9.8	67.3±9.7	63.2±9.0	63.0±9.2
Male	271 (58.3%)	271 (58.3%)	203 (49.9%)	203 (49.9%)
Race/ethnicity				
White	181 (38.9%)	178 (38.3%)	167 (41.0%)	136 (33.4%)
Chinese American	38 (8.2%)	70 (15.1%)	39 (9.6%)	73 (17.9%)
Black	130 (28.0%)	124 (26.7%)	93 (22.9%)	117 (28.7%)
Hispanic	116 (24.9%)	93 (20.0%)	108 (26.5%)	81 (19.9%)
Body mass index, kg/m^2	28.6±5.2	27.7±5.1	28.3±5.0	28.0±5.2
Diabetes mellitus				
Normal	288 (61.9%)	335 (72.0%)	292 (71.7%)	319 (78.4%)
IFG	72 (15.5%)	69 (14.8%)	58 (14.3%)	56 (13.8%)
Untreated diabetes mellitus	18 (3.9%)	20 (4.3%)	14 (3.4%)	5 (1.2%)
Treated diabetes mellitus	87 (18.7%)	41 (8.8%)	43 (10.6%)	27 (6.6%)
Smoking status				
Never	214 (46.0%)	235 (50.5%)	195 (47.9%)	234 (57.5%)
Former	172 (37.0%)	175 (37.6%)	155 (38.1%)	146 (35.9%)
Current	79 (17.0%)	55 (11.8%)	57 (14.0%)	27 (6.6%)
Pack-years smoking	15.5±24.8	11.2±20.0	14.0±26.0	8.4±16.7
Second hand smoke, h/wk	3.5±12.0	2.7±9.9	4.2±12.9	3.3±13.2
Alcohol use				
Never	101 (21.8%)	111 (23.9%)	80 (19.7%)	90 (22.4%)
Former	145 (31.3%)	96 (20.7%)	99 (24.3%)	85 (21.2%)
Current	218 (47.0%)	257 (55.4%)	228 (56.0%)	226 (56.4%)
Cholesterol, mg/dL				
Total	193.5±35.4	192.1±35.9	195.7±34.3	191.8±32.4
LDL	117.9±30.6	115.6±29.6	119.4±30.7	115.8±28.5
HDL	48.5±14.5	50.6±15.0	49.9±14.3	51.8±15.0
Efflux mass, mg cholesterol/dL serum	2.9±1.9	3.1±1.8	3.1±1.9	2.8±1.8
Statin use	84 (18.1%)	84 (18.1%)	59 (14.5%)	59 (14.5%)
Hypertension	311 (66.9%)	254 (54.6%)	197 (48.4%)	167 (41.0%)
Hypertension medication use	243 (52.3%)	206 (44.3%)	161 (39.6%)	156 (38.3%)
Systolic blood pressure	137.2±23.2	130.9±21.2	128.4±20.9	124.0±20.2
Diastolic blood pressure	74.1±11.0	72.9±9.7	72.3±10.5	72.1±10.3
Intentional exercise (MET-min/wk)	1283±1904	1660±2178	1418±1933	1492±2048

CHD indicates coronary heart disease; CVD, cardiovascular disease; HDL, high-denstiy lipoprotein; IFG, impaired fasting glucose; LDL, low-density lipoprotein; and MET, metabolic equivalent.

statin use was not associated with CMEC level in our controls (Table 2). For these reasons, we did not pursue additional sensitivity analyses stratified by statin use. Models with results for all covariates are shown for carotid plaque progression, incident CVD, incident CHD, and incident stroke in Tables III through VI in the online-only Data Supplement, respectively. There was no evidence of a dose-response relationship in the association between CMEC and plaque progression (Table VII in the online-only Data Supplement).

Discussion

Using a novel assay based on the ability of HDL to stimulate the efflux of cholesterol from cholesterol-loaded macrophages, we have shown a strong, independent relationship

Table 2. Average Efflux Mass Among Controls, by Baseline Characteristics

	Efflux Mass (mg cholesterol/dL serum) for Controls (N=769)		
	N	Mean (SD)	*P* Value*
Age, y			
45–54	123	3.2 (1.7)	0.077
55–64 (N=208)	204	3.1 (1.7)	
65–74 (N=325)	269	2.9 (1.8)	
75–84 (N=285)	173	2.7 (2.0)	
Sex			
Female	358	3.1 (2.0)	0.109
Male	411	2.9 (1.7)	
Race/ethnicity			
White	279	3.0 (1.8)	0.975
Chinese American	122	2.9 (1.7)	
Black	210	2.9 (1.9)	
Hispanic	158	3.0 (1.8)	
Body mass index (WHO categories)			
Normal	230	3.0 (1.8)	0.880
Grade 1 overweight	324	2.9 (1.8)	
Grade 2 overweight	193	2.9 (1.8)	
Grade 3 overweight	22	3.1 (2.2)	
Diabetes mellitus			
Normal	576	3.0 (1.8)	0.213
IFG	110	2.7 (1.8)	
Untreated diabetes mellitus	23	2.7 (1.7)	
Treated diabetes mellitus	60	3.1 (1.6)	
Smoking status			
Never	409	3.0 (1.9)	0.442
Former	284	2.9 (1.8)	
Current	76	3.0 1.4)	
Alcohol use			
Never	174	2.8 (1.9)	0.309
Former	170	2.9 (1.8)	
Current	419	3.0 (1.8)	
Statin use			
No	642	3.0 (1.8)	0.550
Yes	127	2.9 (1.8)	

IFG indicates impaired fasting glucose.
**P* values from ANOVA models.

between CMEC and incident CVD, and specifically CHD, in the MESA cohort. Our study involving 465 cases and 465 controls in a population-based prospective study with a 10-year follow-up, provides powerful support for the protective role of HDL-mediated cholesterol efflux in CVD, consistent with a majority of previous studies. This relationship was entirely driven by an inverse relationship between efflux and CHD, whereas there was no relationship with stroke or with nonhemorrhagic stroke. Although we found a positive relationship between efflux and carotid plaque progression overall, we did not find a dose-response relationship, possibly suggesting that the positive association is minimal and of unclear significance.

Our study supports the relationship between CEC and CHD, although finding a distinct relationship to carotid plaque progression and stroke. The findings on carotid plaque progression and stroke were found in different sub-cohorts, suggesting these were not chance observations. Mutharasan et al[9] found no association after multivariate adjustment between cholesterol efflux and presence by carotid magnetic resonance imaging of plaque with lipid-rich necrotic core, in 402 individuals in the Chicago Aging Study, while in the Dallas Heart Study CEC was inversely associated with a composite CVD end point that included thrombotic stroke and an inverse relationship with thrombotic stroke considered as a subgroup (n=37).[5] The reasons for the different results are uncertain but could include sample size, imaging modality, study design, and different efflux assays. The differences between the inverse relationship of CMEC to CHD and the variable relationship to carotid plaque progression and nonhemorrhagic/nonembolic stroke points to differences in these disease processes. Myocardial infarction reflects rupture or erosion of coronary plaques, leading to thrombotic occlusion whereas nonhemorrhagic stroke represents several processes, a minority of which represent carotid plaque ulceration and thrombus formation. In addition, in the largest epidemiological studies plasma HDL-cholesterol levels were inversely related to CHD but have no clear relationship to stroke.[1] Interestingly, in the REVEAL study (Randomized Evaluation of the Effects of Anacetrapib Through Lipid-Modification), the CETP (cholesteryl ester transfer protein) inhibitor anacetrapib, which dramatically raised HDL and moderately reduced non-HDL cholesterol, benefited CHD but had no impact on nonhemorrhagic stroke.[23] In contrast, in FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk trial), a PCSK9 (proprotein convertase subtilisin/kexin type 9) neutralizing antibody markedly lowered LDL and slightly raised HDL, with benefit for both CHD and stroke.[24] Statin-mediated LDL lowering is generally associated with reduced nonhemorrhagic stroke.[25] A limitation of our study is that there were too few stroke events (N=28) in cohort 2 to assess the association with carotid plaque. However, in a previous publication from the full MESA cohort, carotid plaque but not intima-media thickening was associated with incident stroke or transient ischemic attack.[26]

The evidence that HDL mediates an atheroprotective effect, at least in part by promotion of cholesterol efflux, has been strongly supported by animal studies. Infusion of HDL[27] or increased expression of the main HDL protein, apoA-1,[28,29] consistently reduces atherosclerosis. Cholesterol efflux from macrophages to HDL is mediated primarily by the ATP binding cassette transporters ABCA1 (ATP binding cassette transporter subfamily ABCA member 1) and ABCG1 (ATP binding cassette transporter subfamily G member 1). Knocking out these transporters in macrophages or endothelial cells results in an increase in atherosclerosis, driven in part by increased

Table 3. Conditional Logistic Regression Models for Incident Hard CVD and Plaque Progression (OR and 95% CI per SD of Efflux Mass)

	Efflux Mass (mg cholesterol/dL serum)	
	OR (95% CI) per SD=1.9	*P* Value
Incident hard CVD		
Model 1 (N=930; events=465)	0.84 (0.72–0.99)	0.032
Model 2 (N=930; events=465)	0.80 (0.68–0.94)	0.006
Model 3 (N=832; events=416)	0.82 (0.69–0.98)]	0.031
Plaque progression		
Model 1 (N=814, events=407)	1.18 (1.01–1.39)	0.039
Model 2 (N=814; events=407)	1.20 (1.02–1.42	0.030
Model 3 (N=728; events=364)	1.24 (1.04–1.48)	0.018

Model 1: adjusted for age, sex, race/ethnicity, body mass index, and site. Model 2: model 1 covariates plus diabetes mellitus status, current smoking, total and HDL cholesterol, statin use, hypertension medication, and systolic blood pressure. Model 3: model 2 covariates plus pack-years smoking, alcohol use, intentional exercise, and Mediterranean diet. No. of subjects and events smaller than in models 1 and 2 because of missing data for covariates. CVD indicates cardiovascular disease; HDL, high-density lipoprotein; and OR, odds ratio.

Table 4. Conditional Logistic Regression for Incident Stroke and CHD Events Separately (OR and 95% CI per SD of Efflux Mass)

	Efflux Mass (mg cholesterol/dL serum)	
	OR (95% CI) per SD=1.9	*P* Value
CHD		
Model 1 (N=540; Events=270)	0.75 (0.60–0.92)	0.006
Model 2 (N=540; Events=270)	0.69 (0.55–0.86)	0.001
Model 3 (N=484; Events=242)	0.72 (0.56–0.91)	0.007
Stroke		
Model 1 (N=390; Events=195)	0.99 (0.77–1.26)	0.921
Model 2 (N=390; Events=195)	0.99 (0.77–1.27)	0.951
Model 3 (N=348; Events=174)	0.99 (0.73–1.36)	0.969
Stroke excluding hemorrhagic		
Model 1 (N=302; Events=151)	1.02 (0.78–1.33)	0.905
Model 2 (N=302; Events=151)	1.01 (0.76–1.35)	0.945
Model 3 (N=268; events=134)	1.01 (0.70–1.45)	0.978
Stroke excluding hemorrhagic and embolic		
Model 1 (N=240; Events=120)	1.03 (0.75–1.42)	0.863
Model 2 (N=240; Events=120)	0.96 (0.67–1.38)	0.837
Model 3 (N=210; Events=105)	1.16 (0.61–2.22)	0.653

Model 1: adjusted for age, sex, race/ethnicity, body mass index, and site. Model 2: model 1 covariates plus diabetes mellitus status, current smoking, total and HDL cholesterol, statin use, hypertension medication, and systolic blood pressure. Model 3: model 2 covariates plus pack-years smoking, alcohol use, intentional exercise, and Mediterranean diet. No. of subjects and events smaller than in models 1 and 2 because of missing data for covariates. CHD indicates coronary heart disease; HDL, high-density lipoprotein; and OR, odds ratio.

inflammatory processes and, for endothelial cells, decreased eNOS (endothelial nitric oxide synthase) activity.[30,31] Differences in flow characteristics between coronary and carotid circulations may modify the response to HDL-mediated CMEC. Our studies were conducted by measuring efflux of cholesterol to apoB-depleted serum and thus not a direct measurement of HDL-mediated efflux. However, previous studies have shown that cholesterol efflux to apoB-depleted serum is largely driven by HDL.[4] Our CMEC assay used human THP-1 macrophages, in which ABCA1 and ABCG1 are strongly upregulated by cholesterol loading and treatment with an LXR activator. ABCA1 and ABCG1 mediate unidirectional net cholesterol efflux from cells to apoA-1 or HDL, and the THP-1 cell assay is probably largely measuring ABCA1/ABCG1-driven cholesterol efflux.[32] Most previous studies have used cAMP-treated murine J774 macrophages in which ABCA1 is upregulated but also with contributions of ABCG1, SR-B1 (scavenger receptor class B type 1), and aqueous diffusion to total cholesterol efflux.[4] The latter 2 processes are bidirectional and thus can result in an increase in radioactive cholesterol in HDL without any change in net cholesterol efflux.[32,33] Thus, the contributions of specific and nonspecific components of the isotopic efflux assay may vary with different samples. Although this can be compensated by large sample size and statistical adjustment for HDL-cholesterol levels, it may reduce the sensitivity and specificity of the CEC measurement.

The so-called HDL hypothesis based largely on the epidemiological relationships—that increasing HDL-cholesterol levels therapeutically will produce a consistent and proportionate reduction in CHD risk—has been called into question. First, clinical trials with agents that raise HDL-cholesterol level, notably niacin[34] and some CETP inhibitors,[35,36] have not shown consistent benefit. Notably, however, these agents while raising HDL cholesterol effectively (especially CETP inhibitors) only modestly increased CEC.[10,37] Most recently, the REVEAL trial with the CETP inhibitor anacetrapib, the largest trial and the first to go to completion, did show a highly significant reduction in CHD.[23] Although the magnitude of the CHD benefit appeared to correlate with the reduction in non-HDL cholesterol, there are likely wide confidence intervals to the CHD assessments, and a role of increased HDL levels and increased CEC cannot be excluded.[38] Second, Mendelian Randomization studies have shown that while single nucleotide polymorphisms affecting LDL cholesterol and triglyceride levels have the expected relationship to CHD, HDL-cholesterol associated single nucleotide polymorphisms did not, perhaps suggesting that HDL cholesterol is not in the causal pathway of atherosclerosis.[39,40]

Our studies strongly support that HDL is directly involved in an atheroprotective mechanism and is not simply a biomarker for other processes more directly related to atherosclerosis. However, because cholesterol efflux measurements generally show weak correlations with HDL-cholesterol measurements they may not be measuring the same factor that was originally discovered in epidemiological studies of HDL-cholesterol levels. Apart from a weak trend of decreasing CMEC with aging and a modest (R=0.13) correlation with HDL-C, we did not detect any relationship to covariates. Prior studies also have not found the strong relationship

of CEC to specific HDL composition or size measurements at least as determined by nuclear magnetic resonance.[41] In addition, a recent study suggests that CEC does not have strong genetic determination.[41] In small studies, HDL particle number measured by ion mobility shift assays does have a relationship both to ABCA1-mediated cholesterol efflux and CHD.[42,43] It will be important in the future studies to define the physical or compositional factors in HDL that are responsible for differences in its ability to promote cholesterol efflux from macrophages.

On a therapeutic level, our study suggests that therapies that increase cholesterol efflux, such as infusions of cholesterol poor reconstituted HDL that are in phase 3 clinical studies, or upregulation of ABCA1/G1, for example, by LXR activator treatment have potential to decrease CHD risk. However, our findings suggest that these treatments may benefit CHD but not stroke.

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Disclosures

None.

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Highlights

- We measured HDL (high-density lipoprotein)-mediated cholesterol mass efflux capacity in samples from the MESA (Multi-Ethnic Study of Atherosclerosis). Higher cholesterol mass efflux capacity level was significantly associated with lower odds of incident cardiovascular disease in fully adjusted multivariate models.
- Higher cholesterol mass efflux capacity level was associated with lower risk of incident coronary heart disease events, but no association was found with risk of stroke.
- Higher cholesterol mass efflux capacity level was associated with higher risk of carotid plaque progression, but no dose-response relationship was found for this association, suggesting that the positive finding is of minimal or unclear significance.
- These findings support a role for HDL-mediated cholesterol efflux in an atheroprotective mechanism for coronary heart disease but not stroke.
- At a therapeutic level, these findings suggest that therapies that increase cholesterol efflux, such as infusions of cholesterol poor reconstituted HDL that are in phase 3 clinical studies, or upregulation of ABCA1/G1 (ATP binding cassette transporter subfamily ABCA member 1/G member 1), for example, by LXR (liver X receptor) activator treatment have potential to decrease coronary heart disease risk but possibly not stroke.