



# Cholesterol efflux capacity, HDL cholesterol, and risk of coronary heart disease: a nested case-control study in men<sup>S</sup>

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**Abstract** The capacity of HDLs to accept cholesterol effluxing from macrophages has been proposed as a new biomarker of HDLs' anti-atherogenic function. Whether cholesterol efflux capacity (CEC) is independent of HDL cholesterol (HDL-C) as a biomarker for coronary heart disease (CHD) risk in a generally healthy primary-prevention population remains unanswered. Therefore, in this nested case-control study, we simultaneously assessed CEC (using J774 cells) and plasma HDL-C levels as predictors of CHD in healthy middle-aged and older men not receiving treatment affecting blood lipid concentrations. We used risk-set sampling of participants free of disease at baseline from the Health Professionals Follow-Up Study, and matched cases (n = 701) to controls 1:1 for age, smoking, and blood sampling date. We applied conditional logistic regression models to calculate the multivariable relative risk and 95% CIs of CHD over 16 years of follow-up. CEC and HDL-C were correlated ( $r = 0.50$ ,  $P < 0.0001$ ). The risk (95% CI) of CHD per one SD higher CEC was 0.82 (0.71–0.96), but completely attenuated to 1.08 (0.85–1.37) with HDL-C in the model. The association per one SD between HDL-C and CHD (0.66; 0.58–0.76) was essentially unchanged (0.68; 0.53–0.88) after adjustment for CEC. **These findings indicate that CEC's ability to predict CHD may not be independent of HDL-C in a cohort of generally healthy men.**—Cahill, L. E., F. M. Sacks, E. B. Rimm, and M. K. Jensen. **Cholesterol efflux capacity, HDL cholesterol, and risk of coronary heart disease: a nested case-control study in men.** *J. Lipid Res.* 2019. 60: 1457–1464.

**Supplementary key words** epidemiology • atherosclerosis • cholesterol metabolism • cholesterol/efflux • high density lipoprotein • myocardial infarction • prospective study

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Cholesterol efflux capacity (CEC) has gained attention as a novel biomarker proposed to reflect function of HDL and improve risk prediction of coronary heart disease (CHD) (1, 2). CEC measures the ability of HDL to receive cholesterol from macrophages, which is the first key step of reverse cholesterol transport, the process in which peripherally deposited cholesterol is taken up and carried by HDL to the liver for excretion (3–5). Reverse cholesterol transport is considered a primary function of HDL, but other functions likely also contribute to its cardioprotection, including macrophage foam cell formation, endothelial activation of endothelial nitric oxide synthase, monocyte adhesion, and platelet aggregation (4, 6).

Low plasma levels of cholesterol in HDL [HDL cholesterol (HDL-C)] are strongly associated with CHD risk in observational studies (7), yet solid evidence of causality is lacking. For example, therapeutics that raise total HDL-C do not substantially lower CHD risk (8–11). Further, human genetic studies of inherited variation that predispose to elevated HDL-C levels do not support a causal relationship between HDL-C and CHD (12). Therefore, understanding of the relationship between CHD risk and the traditional measures of HDL-C or apoA-I concentration could be advanced by biomarkers such as CEC that reflect functional properties of HDL.

CEC has a strong inverse association with prevalent carotid intima-media thickness (13) and also with risk of incident CVD (14, 15). However, some studies, especially those with longitudinal designs, have reported contrary

Abbreviations: CEC, cholesterol efflux capacity; CHD, coronary heart disease; CV, coefficient of variation; HDL-C, HDL cholesterol; HPFS, Health Professionals Follow-up Study; LDL-C, LDL cholesterol; MI, myocardial infarction; RR, relative risk; TG, triglyceride.

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findings (16, 17). Most studies on CEC to date have been conducted in populations on lipid-altering medications and with HDL-C concentrations only weakly associated with risk of CHD or CVD mortality (13, 15, 18–20). Prospective examination of the association between CEC and risk of incident CHD in a generally healthy population sample where HDL-C has the expected strong inverse association with risk of CHD is imperative. Until such assessment, it remains unresolved to what extent the association between CEC and risk of CHD is independent of HDL-C levels. The objective of the present study was to investigate the relationship between CEC and risk of incident CHD alone and in conjunction with HDL-C in a prospective nested case-control study of middle-aged and older men who were free of CVD at blood sample collection and represent a sample not confounded by therapeutics affecting blood lipid concentrations.

## MATERIALS AND METHODS

### Study population

The Health Professionals Follow-up Study (HPFS) is a prospective cohort of 51,529 male health professionals (dentists, optometrists, osteopathic physicians, pharmacists, podiatrists, and veterinarians) who were 40 to 75 years of age at baseline in 1986. Between 1993 and 1995, blood samples were collected from participants free of CVD and cancer; thus, this blood collection time serves as the baseline of our present prospective nested case-control study of CHD, which is nested within the larger HPFS cohort study. Information on anthropometric and lifestyle factors was obtained through self-administered questionnaires every 2 years and diet every 4 years as part of the larger prospective HPFS cohort study, so baseline anthropometric and lifestyle variables for the present nested case-control study were derived from the 1994 questionnaire (closest to blood collection) with missing information substituted from the two previous questionnaires. Men who had an incident myocardial infarction (MI) or fatal CHD between the date of blood draw and January 2010 were identified as cases in our present nested case-control study. Using risk-set sampling, controls were selected randomly and matched 1:1 on age, smoking, and month of blood draw among participants who were free of CVD at the time CHD was diagnosed in the case patient. The majority (>95%) of participants in this case-control sample were Caucasian. The validity of the questionnaires and the reproducibility of the measurements have been previously reported (21). Three participants were missing cholesterol values, and two had missing alcohol intake data, leaving a final sample of 696 controls and 701 cases. The study protocol was approved by the institutional review boards of the Brigham and Women's Hospital and Harvard T. H. Chan School of Public Health, and those of participating registries as required. Informed consent was obtained and the study abided by the Declaration of Helsinki principles.

### CHD case assessment

Incident CHD in our study was defined as nonfatal MI and fatal CHD. MI was confirmed based on the criteria of the World Health Organization (symptoms plus either diagnostic electrocardiographic changes or altered levels of cardiac enzymes) (22). As previously reported (23), nonfatal events were confirmed through review of medical records by physicians blinded to the participants' questionnaire reports. Deaths were identified from state vital records and the National Death Index or reported by the

participant's next of kin or the postal system. Fatal CHD was confirmed by an examination of hospital or autopsy records (by the listing of CHD as the cause of death on the death certificate or if CHD was the underlying and most plausible cause of death).

### HDL-C and CEC measurement

Participants underwent phlebotomy and returned ethylenediaminetetraacetic acid-preserved tubes of whole blood in a Styrofoam container with an icepack to the Brigham and Women's Hospital/Harvard Cohorts Biorepository of the Brigham and Women's Hospital, Harvard Medical School via overnight courier. Upon arrival, whole blood samples were centrifuged to separate plasma, buffy coat, and red blood cells, which were stored in cryotubes as in the vapor phase of liquid nitrogen freezers at less than  $-130^{\circ}\text{C}$ . Details about the Brigham and Women's Hospital/Harvard Cohorts Biorepository have been described elsewhere (24). The biomarkers in this sample generally showed excellent stability and reproducibility during simulated transport and storage (25).

Standard methods were employed by the Rifai laboratory at Harvard University to measure serum lipids (26). Total plasma cholesterol [coefficient of variation (CV) of 1.8%] was measured by the esterase-oxidase method, and triglycerides (TGs) (5.1% CV) were measured by an enzymatic procedure. LDL cholesterol (LDL-C) (2.7% CV) was determined by a homogenous direct method and HDL-C (2.2% CV) by enzymatic assay (27).

The CEC assay (9.2% CV) was performed by the Rader laboratory at the University of Pennsylvania, using methods similar to those described by Khara et al. (13) with a few small changes to improve throughput and simplify the assay for use in large populations: J774 cells derived from a murine macrophage cell line were labeled with  $2\ \mu\text{Ci}$  of  $^3\text{H}$ -cholesterol (Perkin Elmer) per milliliter of medium overnight and without the presence of an acetyl-CoA acetyltransferase inhibitor, as preliminary data indicated that <4% of total cellular cholesterol was cholesterol ester. ABCA1 was upregulated for 4 h by incubation with 0.3 mM 8-(4-chlorophenylthio)-cyclic AMP. Efflux media containing the equivalent of 2% apoB-depleted plasma were then incubated for 2 h at  $37^{\circ}\text{C}$  in a non- $\text{CO}_2$  incubator. A pooled plasma control was included on each of the 24-well plates for normalization.

The percent cholesterol efflux was calculated by the following formula:  $(\text{cpm of } ^3\text{H}\text{-cholesterol in media} - \text{cpm of } ^3\text{H}\text{-cholesterol in baseline control}) / (\text{cpm of } ^3\text{H}\text{-cholesterol in cells} + \text{cpm of } ^3\text{H}\text{-cholesterol in the media}) \times 100$ , yielding a normalized percentage (15). In our within-person pilot study of 44 participants who provided two measures 1–2 years apart, CEC was well-correlated between draws (Spearman  $r = 0.61$ ) and had strong intraclass correlations ( $r = 0.62$ ).

### Statistical analysis

To account for the potential variation in CEC by batch (because there were 24 batches), values of each CEC exposure were recalibrated to represent the average distribution across batches using the Rosner recalibration method (28, 29). The Rosner method assumes that all batches combined represent an average batch, regresses the values, and calibrates the values within each batch by adding the resulting value of the coefficients for that batch minus the average of the batch coefficients. Therefore, log-transformed CEC variables were regressed on batch using linear regression models, and then we used the back-transformed measures of CEC in our analyses as recalibrated levels accounting for the variability between batches. CEC was examined as a continuous variable (per one SD in CEC) and also in quintiles, the cut-points of which were derived from only the controls.

Participant characteristics were compared between cases and controls using  $t$ -tests for continuous variables and  $\chi^2$  tests for categorical variables. For HbA1c and TGs, we used log-transformation

to normalize the distributions. Relative risks (RRs) of CHD were estimated by incidence rate ratios using conditional logistic regression conditional on matching factors (age in years, smoking, month of blood draw). Analyses were adjusted for fasting status (yes/no), BMI (categories: <20, 20–24, 25–29, 30–34, and  $\geq 35$  kg/m<sup>2</sup>), alcohol intake (categories: nondrinker, 0.1–4.9 g of alcohol per day (g/day), 5.0–14.9 g/day, 15.0–29.9 g/day,  $\geq 30.0$  g/day), parental MI before the age of 60 (yes/no), history of high cholesterol (yes/no), and the history of high blood pressure (yes/no), TG, and LDL-C. Additionally, the multivariable model further included HDL-C to observe the influence of HDL-C on the ability of CEC to predict risk of CHD. Similarly, the association between HDL-C and risk of CHD was determined with and without adjustment for CEC. Although we had collected data on (lipid-lowering, blood pressure-lowering, blood sugar-lowering) medication use, such medication use in this sample of relatively healthy men was uncommon, and adjustment for it did not alter our results. We further ran analyses stratified by established CHD risk factors. All analyses were conducted in SAS version 9.4 at a two-tailed  $\alpha$  level of 0.05.

## RESULTS

CEC was normally distributed in both cases and controls separately and combined. As expected, cases had higher mean BMI, total cholesterol, LDL-C, apoB, TG, and HbA<sub>1c</sub> and a greater prevalence of hypertension, diabetes, hypercholesterolemia, family history of CHD, and medication use at baseline as compared with controls (**Table 1**). Cases also had a lower mean HDL-C and drank less alcohol than controls. CEC (mean, SD; unit is normalized percentage) was significantly lower in cases (0.97, 0.14) as compared with controls (0.99, 0.14). CEC was highly correlated with HDL-C ( $r = 0.50$ ,  $P < 0.0001$  in all participants and  $r = 0.53$ ,  $P < 0.0001$  in controls only) (**Table 2**). CEC was most strongly associated with total cholesterol ( $r = 0.34$ ,  $P < 0.0001$ ), alcohol intake ( $r = 0.25$ ,  $P < 0.0001$ ), and LDL-C ( $r = 0.15$ ,  $P < 0.0001$ ) (in all participants). CEC did not correlate with diabetes, hypertension, TGs, or HbA<sub>1c</sub> (all  $r < 0.10$ ).

Accounting for matching factors (age, smoking, and date of blood draw) and all covariates except blood lipids, the RR (95% CI) of CHD per one SD of CEC was 0.89 (0.77–1.02) (**Table 3**). After progressive covariate adjustment for LDL-C and log-transformed TG, the RR was 0.82 (0.71–0.95), which suggests an important inverse association between CEC and risk of CHD. However, after further adjustment for HDL-C, the inverse RR of CHD for efflux capacity was completely attenuated to 1.08 (0.85–1.37) and no longer significant. In a model that included HDL-C as a covariate, but not LDL-C or TG, the RR (95% CI) of CHD per one SD of CEC was 1.25 (1.04–1.50) ( $P = 0.02$ ), indicating that adjusting for HDL-C can reverse the inverse association between efflux and CHD. In contrast, the inverse association between HDL-C and CHD (0.68; 0.60–0.78) was unchanged by mutual adjustment for CEC, LDL-C, and TG (after adjustment: 0.68; 0.53–0.88) in the fully adjusted model.

When CEC and HDL-C were expressed as quintiles and mutually adjusted (**Fig. 1**), the RR comparing top versus

bottom quintiles of CEC was 1.53 (0.75–3.11,  $P$ -trend of 0.34), whereas the RR was 0.33 (0.17–0.65,  $P$ -trend of 0.008) for HDL-C. Stratifying our CEC results by established CHD risk factors did not reveal any important effect modification (supplemental Table S1).

## DISCUSSION

In our prospective nested case-control study with 16 years of follow-up, CEC and HDL-C were both significantly inversely associated with first coronary event. In models that mutually adjusted for HDL-C and CEC, HDL-C retained its significant inverse prediction of risk of CHD, whereas CEC did not, suggesting that CEC's association with CHD may be outweighed by HDL-C in relatively healthy (and relatively unmedicated) populations in which a strong inverse correlation exists between HDL-C levels and risk of CHD.

Our present findings uniquely align results from both the older cross-sectional studies and the more recent prospective longitudinal studies. For example, Khera et al. (13) reported in a cross-sectional case-control study that the capacity of HDL to stimulate CEC was inversely associated with angiographically confirmed CHD, independently of HDL-C or apoA1. Li et al. (16) also observed that CEC was inversely associated with prevalent CHD cross-sectionally. However, in their prospective analyses among patients with prevalent CVD, Li et al. (16) found greater CEC was associated with higher risk of CVD over 3 years of follow-up (2.19; 1.02–4.74 for highest versus lowest tertile of CEC) even after multivariable adjustment including HDL-C levels. In certain models in the present study that included HDL-C, CEC became significantly associated with higher risk of CHD, as well. Possible interpretations of this finding include that sera from prospective longitudinally studied populations (which are usually relatively healthy samples taken several years before heart disease develops and CHD events occur) may be able to accept more cholesterol or contain increased concentrations of other lipoproteins not associated with HDL than less-healthy cross-sectional samples, leading to a different relationship between CEC (as currently measured by assays) and the risk of CHD over time as lipid profiles change and plaques develop. Certainly, cross-sectional studies can be prone to reverse causation (the CHD status could influence CEC and other plasma biomarkers), which is less likely in prospective longitudinal studies because participants provide samples years before the CHD event.

Some recent studies conducted among prospective cohorts free of underlying CVD have reported strong inverse associations between CEC and cardiovascular events even with adjustment for HDL-C (14, 15, 30). For example, in an analysis of the Dallas Heart Study with black participants comprising 49% of the population, Rohatgi et al. (15) reported a 67% lower risk of a mixed cardiovascular endpoint in the highest versus lowest quartile of CEC (0.33; 0.19–0.55), adjusted for HDL-C. Interestingly, HDL-C itself was not strongly inversely associated with risk of CHD in



TABLE 1. Baseline (1994) characteristics of HPFS CHD case-control study participants

	Controls		Cases		<i>P</i>
	Number of Subjects	Mean (SD) or %	Number of Subjects	Mean (SD) or %	
CEC <sup>a</sup>	696	0.99 (0.14)	701	0.97 (0.14)	0.01
Demographic and anthropometric factors					
Age (years) <sup>b</sup>	696	63.0 (8.7)	701	63.0 (8.7)	0.99
Fasting ≥8 h (yes)	696	419 (60%)	701	415 (59%)	0.70
BMI	696	25.7 (3.4)	701	26.2 (3.3)	0.005
Medical history					
History of diabetes	696	20 (3%)	701	59 (8%)	<0.0001
History of hypertension	696	181 (26%)	701	259 (37%)	<0.0001
History of hypercholesterolemia	696	269 (37%)	701	347 (50%)	<0.0001
Cholesterol-lowering medication use	696	45 (6%)	701	54 (8%)	0.36
Parental CHD before age 60	696	232 (33%)	701	284 (41%)	0.005
Lipid-related markers					
Total cholesterol (mg/dl)	696	199.2 (33.5)	701	205.9 (37.4)	0.0004
HDL-C (mg/dl)	696	46.9 (12.6)	701	42.4 (11.6)	<0.0001
LDL-C (mg/dl)	696	129.0 (30.7)	701	135.0 (33.7)	0.0005
apoB (mg/dl)	418	90.1 (20.4)	423	98.4 (23.5)	<0.0001
TGs (mg/dl) <sup>c</sup>	696	129.1 (65.2)	701	157.0 (92.3)	<0.0001
HbA <sub>1c</sub> (%) <sup>c</sup>	694	5.65 (0.56)	699	5.89 (1.03)	<0.0001
hsCRP (mg/l) <sup>c</sup>	696	2.12 (4.45)	700	2.33 (3.82)	<0.0001
Lifestyle factors					
Activity (MET-hours/week)					
Quintile 1 (≤9.0)		139 (20%)		172 (25%)	
Quintile 2 (9.1–19.1)		140 (20%)		127 (18%)	
Quintile 3 (19.2–33.1)		139 (20%)		128 (18%)	0.33
Quintile 4 (33.2–56.8)		149 (20%)		139 (20%)	
Quintile 5 (>56.8)		139 (20%)		135 (19%)	
Current Smoking <sup>b</sup>	696	44 (6%)	701	51 (7%)	0.46
Alcohol intake					
Nondrinker		147 (21%)		196 (28%)	
0.1–4.9 g/day		145 (21%)		179 (25%)	
5.0–14.9 g/day		200 (29%)		168 (24%)	0.0006
15.0–29.9 g/day		108 (15%)		88 (13%)	
≥30 g/day		96 (14%)		70 (10%)	
Diet quality score <sup>d</sup>					
Quintile 1 (≤44.69)		138 (20%)		162 (23%)	
Quintile 2 (44.70–51.29)		140 (20%)		144 (21%)	
Quintile 3 (51.30–58.12)		140 (20%)		161 (23%)	0.11
Quintile 4 (58.13–64.57)		138 (20%)		122 (17%)	
Quintile 5 (>64.57)		140 (20%)		112 (16%)	

Quintiles are based on the distributions of controls only. hsCRP, high-sensitivity C-reactive protein; MET, metabolic equivalent task.

<sup>a</sup>CEC is calculated as a normalized percentage using the formula: (cpm of <sup>3</sup>H-cholesterol in media – cpm of <sup>3</sup>H-cholesterol in baseline control)/(cpm of <sup>3</sup>H-cholesterol in cells + cpm of <sup>3</sup>H-cholesterol in the media) × 100.

<sup>b</sup>Case-control matching factor.

<sup>c</sup>TGs, hsCRP, and HbA<sub>1c</sub> were the only skewed variables. For all subsequent tables/analyses these three skewed variables will be log-transformed.

<sup>d</sup>Diet quality score is the Alternate Healthy Eating Index 2010.

the Dallas Heart Study (15, 31). The strength of HDL-C as a risk factor for CHD has been questioned in individuals of African American descent (31), which may offer a partial explanation for the discrepancy in findings between the Dallas Heart Study CEC analysis and the present study. Unlike our study of Caucasian participants that measured CEC by radiolabeled cholesterol, CEC and HDL-C were not correlated in the Dallas Heart Study where CEC was measured using fluorescent labeled cholesterol. It is possible that the latter method specifically captures efflux via ABCA1, whereas the radiolabeling method captures all potential pathways.

A correlation ( $r = 0.40$ ) was observed between HDL-C and CEC in the prospective EPIC Norfolk Study (15), which measured CEC by radiolabeled cholesterol and reported a lower risk of incident CHD in the highest versus

lowest tertile of CEC (0.64; 0.51–0.80), adjusted for HDL-C (15). Besides lower mean CEC than in the EPIC Norfolk Study, cohort characteristics that set our study apart include that our generally healthy participants (all men) had a high diet quality, high physical activity, low smoking, and low use of lipid-lowering medications. This may have created a setting in which HDL-C concentrations are so strongly and reliably predictive of incident CHD risk that CEC adds negligible further predictive value. Similar to HDL, CEC has been shown to be significantly lower in men than in women (14, 32), but the extent of the effect of sex on the association between CEC and CHD outcomes remains unknown. Our present study was conducted only in men and cannot shed further light on this important point, and so further studies in women are warranted.

TABLE 2. Cross-sectional Spearman correlates with CEC in all participants and in only controls

	Correlation ( <i>P</i> ) with CEC <sup>a</sup>	
	All Participants	Controls Only
Age (years)	-0.01 (0.57)	0.01 (0.73)
Fasting ≥8 h at blood draw	-0.01 (0.70)	-0.04 (0.33)
BMI	-0.11 (<0.0001)	-0.13 (0.0004)
History of diabetes	-0.04 (0.15)	-0.07 (0.05)
History of hypertension	0.02 (0.54)	-0.02 (0.61)
History of hypercholesterolemia	0.14 (<0.0001)	0.15 (<0.0001)
Cholesterol-lowering medication use (%)	0.07 (0.008)	0.07 (0.08)
Parental CHD before age 60	0.05 (0.09)	0.05 (0.21)
Total cholesterol (mg/dl)	0.34 (<0.0001)	0.34 (<0.0001)
HDL-C (mg/dl)	0.50 (<0.0001)	0.53 (<0.0001)
LDL-C (mg/dl)	0.15 (<0.0001)	0.15 (0.0001)
apoB (mg/dl)	0.14 (<0.0001)	0.08 (0.09)
TGs (mg/dl) <sup>†</sup>	0.02 (0.44)	-0.04 (0.31)
HbA1c <sup>b</sup>	-0.05 (0.06)	-0.07 (0.09)
hsCRP (mg/l) <sup>b</sup>	-0.07 (0.008)	-0.007 (0.85)
Physical activity (quintiles)	0.06 (0.02)	0.10 (0.01)
Current smoker	-0.03 (0.26)	-0.03 (0.51)
Alcohol (categories)	0.25 (<0.0001)	0.28 (<0.0001)
Diet quality score <sup>c</sup> (quintiles)	0.06 (0.02)	0.04 (0.21)

hsCRP, high-sensitivity C-reactive protein.

<sup>a</sup>CEC is calculated as a normalized percentage using the formula: (cpm of <sup>3</sup>H-cholesterol in media - cpm of <sup>3</sup>H-cholesterol in baseline control)/(cpm of <sup>3</sup>H-cholesterol in cells + cpm of <sup>3</sup>H-cholesterol in the media) × 100.

<sup>b</sup>Log-transformed because the variable has a skewed distribution.

<sup>c</sup>Diet quality score is the Alternate Healthy Eating Index 2010.

Similar to our results, CEC was less robust than HDL-C as a predictor of CVD events in a recent nested case-control analysis in the JUPITER primary prevention trial of rosuvastatin (33). HDL-C predicted incident CVD more robustly than CEC, which only significantly predicted CVD events until adjusted for HDL-C (33). Additionally, CEC was prospectively positively associated with hospital mortality in univariate analysis of 152 heart failure patients, but was not significant after adjustment for HDL-C (18).

In a cohort such as ours that has a strong correlation between HDL-C and CEC alongside a strong association between HDL-C and risk of CHD, high CEC may simply represent high HDL-C levels. The discordance between CEC and HDL-C in some populations is poorly understood, but could fluctuate due to (class and dose of) lipid-modifying medication, a setting in which CEC may reflect an entity not captured by HDL-C. The discordance between HDL-C and CEC has been shown to be at least partially

explained by other serum lipids, as elevated TG levels can maintain CEC in individuals with low HDL-C (34). Unlike HDL-C, CEC does not correlate with TG and diabetes; thus in populations where diabetes and hypertriglyceridemia are strong contributors to CHD-risk, efflux may retain more of its predictive value upon adjustments for CHD risk factors.

The precision of the two measures must also be considered, and studies should be interpreted in the context of the relative precision of the measures. If measured well, general population samples of nonblack participants have found HDL-C to be strongly predictive of future CHD risk (35, 36). In the present study's samples, HDL-C was measured well (low CV of 2.2%) in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory while the experimental measure of CEC was less precise (CV of 9.2%), which may have influenced our findings. No standard assay assessing CEC exists yet, and so the applied

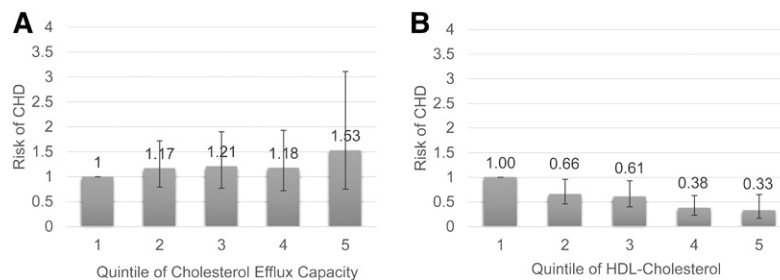
TABLE 3. RRs and 95% CIs for CHD per one SD of CEC and HDL-C

	CEC <sup>a</sup>			HDL-C		
	RR	95% CI	<i>P</i>	RR	95% CI	<i>P</i>
Multivariable model <sup>b</sup>	0.89	0.77-1.02	0.09	0.68	0.60-0.78	<0.0001
+ LDL	0.84	0.73-0.98	0.02	0.68	0.60-0.78	<0.0001
+ logTG	0.82	0.71-0.95	0.01	0.66	0.58-0.76	<0.0001
+ HDL-C or CEC	1.08	0.85-1.37	0.52	0.68	0.53-0.88	0.004
+ HDL-C or CEC	1.25	1.04-1.50	0.02	0.60	0.51-0.72	<0.0001
+ LDL	1.18	0.98-1.43	0.08	0.60	0.51-0.72	<0.0001
+ logTG	1.08	0.85-1.37	0.52	0.68	0.53-0.88	0.004

SD is 0.14 for CEC and 12.60 for HDL-C and is based on controls only.

<sup>a</sup>CEC is calculated as a normalized percentage using the formula: (cpm of <sup>3</sup>H-cholesterol in media - cpm of <sup>3</sup>H-cholesterol in baseline control)/(cpm of <sup>3</sup>H-cholesterol in cells + cpm of <sup>3</sup>H-cholesterol in the media) × 100.

<sup>b</sup>Adjusted for fasting status, history of diabetes and hypertension, parental CHD before age 60, BMI (<20, 20-24, 25-29, 30-34, ≥35 kg/m<sup>2</sup>), and alcohol intake (nondrinker, 0.1-4.9 g of alcohol per day (g/day), 5.0-14.9 g/day, 15.0-29.9 g/day, ≥30.0 g/day). Matching factors (cases and controls were matched on age, smoking, and date of blood draw) are incorporated into the analysis using conditional logistic regression.



**Fig. 1.** Multivariable-adjusted RRs and 95% CIs for CHD by quintile of CEC (A) and quintile of HDL-C (B). *P*-trend is 0.34 for CEC and *P*-trend is 0.0008 for HDL-C. Quintile cut-points are based on controls only. CEC is calculated as a normalized percentage using the formula: (cpm of  $^3\text{H}$ -cholesterol in media – cpm of  $^3\text{H}$ -cholesterol in baseline control)/(cpm of  $^3\text{H}$ -cholesterol in cells + cpm of  $^3\text{H}$ -cholesterol in the media)  $\times$  100. Multivariable model: in addition to mutually matching factors (age, smoking, and month of blood draw) incorporated through the use of conditional logistic regression, the multivariable model is adjusted for BMI (<20, 20–24, 25–29, 30–34,  $\geq 35$  kg/m $^2$ ), fasting status, history of hypertension, history of hypercholesterolemia, parental CHD before age 60, and alcohol intake (nondrinker, 0.1–4.9 g of alcohol per day (g/day), 5.0–14.9 g/day, 15.0–29.9 g/day,  $\geq 0.0$  g/day), LDL-C, and logTG. CEC and HDL-C are mutually adjusted for each other.

methodology varies among studies (15, 37). Although all assays measuring CEC quantify the amount of labeled cholesterol as it moves from specific cell types to a known extracellular acceptor (1), these cell types and acceptors can vary, as do the different mechanisms postulated for cholesterol efflux, and also the considerable heterogeneity in protein and lipid composition of HDL. Two main measurement approaches to measuring CEC have emerged, and a study employing both the original radiolabeled cholesterol assay for total efflux and the newer fluorescent cholesterol labeling method reported a correlation between the two methods of just 0.54 (15). Despite remaining unknowns and the lack of a standardized assay, CEC has now been measured in many different population-based and clinical cohorts, and our overall conclusion is that an inverse relationship exists for CEC with prevalent atherosclerosis and incident CHD, but that may not be independent of HDL-C (38).

### Implications

Results of the present study confirm an inverse relationship between CEC and incident CHD but suggest that CEC's ability to predict CHD may be overshadowed by HDL-C in a population with typical HDL-C levels that are strongly inversely associated with CHD. The present study also identifies gaps in knowledge related to the measurement of CEC, mechanisms of cholesterol efflux, the various components and roles of HDL, and the lack of a direct relationship between circulating HDL-C and CEC in some populations but not others. This lack of a consistently direct relationship between HDL-C and CEC (15) has been suggested to explain the failure of strategies that simply raise HDL-C without accounting for effects on HDL function (38). Despite its firm inverse relationship with risk of CHD, the causal relation between HDL-C and CHD is uncertain (39), and HDL's function as a simple carrier of cholesterol is being challenged (37). The very concept of reverse cholesterol transport has yet to be validated against atherosclerosis and CVD (40). As yet, we do not know whether the CEC measured *in vitro* reflects what occurs in

*vivo* in humans (40). The constellation of HDL itself is a result of many proteins and phospholipids with diverse metabolic actions (not all beneficial), and HDL subspecies are just starting to be understood in terms of function and potential opposing relations to disease (6, 41–43). Further research into the components, properties, mechanisms, and genetic associations of CEC may offer a potential understanding of the relationship between HDL-C and CHD.

### Study strengths and limitations

Strengths of the present analysis include comprehensive data gathered prospectively in a setting of middle-aged participants free of underlying CVD, with a long duration of follow-up and a large number of cases matched to controls of the same age and smoking status in order to reduce the possibility of residual confounding. The main limitation of the present study is that we only had a single measurement of CEC, making us unable to study changes in efflux capacity. Another important limitation was that the participants we studied were all men and predominantly Caucasian, thus we could not generalize our results to women or other racial-ethnic groups. Given the differences in both HDL-C and CEC across sex and ethnicity, further prospective studies of the CEC biomarker in women and other races/ethnicities are needed. Additional limitations included that non-ABCA1 efflux was not able to be measured in this large population study and that further studies are required to better understand and standardize the processes (i.e., cell types and acceptors) used in CEC assays.

### CONCLUSIONS

Our results indicate that CEC's ability to predict CHD is not independent of HDL-C in our cohort of generally healthy men not confounded by therapeutics affecting blood lipid concentrations. Further work is required to understand the biology of CEC and to standardize its

measurement. Whether modulation of CEC would decrease CVD risk remains unknown.<sup>6,16</sup>

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