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ORIGINAL RESEARCH ARTICLE

High-Density Lipoprotein Anti-Inflammatory Capacity and Incident Cardiovascular Events

Editorial, see p 1946

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BACKGROUND: The role of high-density lipoprotein (HDL) function in cardiovascular disease represents an important emerging concept. The present study investigated whether HDL anti-inflammatory capacity is prospectively associated with first cardiovascular events in the general population.

METHODS: HDL anti-inflammatory capacity was determined as its ability to suppress TNFα (tumor necrosis factor α)–induced VCAM-1 (vascular cell adhesion molecule-1) mRNA expression in endothelial cells in vitro (results expressed as achieved percent reduction by individual HDL related to the maximum TNFα effect with no HDL present). In a nested case-control design of the PREVEND (Prevention of Renal and Vascular End Stage Disease) study, 369 cases experiencing a first cardiovascular event (combined end point of death from cardiovascular causes, ischemic heart disease, nonfatal myocardial infarction, and coronary revascularization) during a median of 10.5 years of follow-up were identified and individually matched to 369 controls with respect to age, sex, smoking status, and HDL cholesterol. Baseline samples were available in 340 cases and 340 matched controls.

RESULTS: HDL anti-inflammatory capacity was not correlated with HDL cholesterol or hsCRP (high-sensitivity C-reactive protein). HDL anti-inflammatory capacity was significantly lower in cases compared with controls (31.6% [15.7–44.2] versus 27.0% [7.4–36.1]; $P<0.001$) and was inversely associated with incident CVD in a fully adjusted model (odds ratio [OR] per 1 SD, 0.74 [CI, 0.61–0.90]; $P=0.002$). Furthermore, this association was approximately similar with all individual components of the cardiovascular disease end point. The HDL anti-inflammatory was not correlated with cholesterol efflux capacity ($r=-0.02$; $P>0.05$). When combining these 2 HDL function metrics in 1 model, both were significantly and independently associated with incident cardiovascular disease in a fully adjusted model (efflux: OR per 1 SD, 0.74; $P=0.002$; anti-inflammatory capacity: OR per 1 SD, 0.66; $P<0.001$). Adding HDL anti-inflammatory capacity improved risk prediction by the Framingham risk score, with a model likelihood-ratio statistic increase from 10.50 to 20.40 ($P=0.002$).

CONCLUSIONS: The HDL anti-inflammatory capacity, reflecting vascular protection against key steps in atherogenesis, was inversely associated with incident cardiovascular events in a general population cohort, independent of HDL cholesterol and HDL cholesterol efflux capacity. Adding HDL anti-inflammatory capacity to the Framingham risk score improves risk prediction.

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ORIGINAL RESEARCH ARTICLE

Clinical Perspective

What Is New?

- The anti-inflammatory capacity of high-density lipoprotein (HDL) is associated with incident cardiovascular disease events in the general population.
- This association was independent of a number of established cardiovascular biomarkers, notably including HDL cholesterol and hs-CRP (high-sensitivity C-reactive protein).
- The HDL anti-inflammatory capacity is associated with cardiovascular disease events independent of cholesterol efflux capacity.
- Adding HDL anti-inflammatory capacity to the Framingham risk score or replacing HDL cholesterol with this functional metric of HDL particles improves risk prediction.

What Are the Clinical Implications?

- HDL anti-inflammatory capacity has the potential to provide independent clinical information for cardiovascular disease risk assessment.
- The HDL anti-inflammatory capacity could represent a novel pharmacological intervention target for improving cardiovascular disease risk.

Although circulating levels of high-density lipoprotein (HDL) cholesterol are inversely associated with cardiovascular disease (CVD) risk in the general population,[1] outcomes of pharmacological intervention trials aiming to increase plasma HDL cholesterol have been disappointing and largely negative.[2–5] Together with genetic studies that also show that lifelong low or high levels of HDL cholesterol do not relate as anticipated to CVD outcomes,[6] focus has shifted from HDL cholesterol as a biomarker to the measurement of HDL function metrics.[7] A much-researched functionality of HDL particles is its ability to mediate cholesterol efflux from macrophage foam cells, the first step in the atheroprotective reverse cholesterol transport pathway.[8,9] It has been demonstrated that HDL cholesterol efflux capacity is inversely associated with incident CVD events in the general population independent and even irrespective of HDL cholesterol levels.[10–12] However, cholesterol efflux still tracks considerably with HDL cholesterol and apolipoprotein (apo) A1 levels,[10–13] while an HDL function metric that is less dependent on the plasma HDL cholesterol concentration would be more interesting, both from the perspective of intervention and from the perspective of risk prediction. Given that another key biological role of HDL is protection against inflammation,[14] and given the inflammatory nature of the atherosclerotic process,[15] anti-inflammatory properties of HDL might have particularly high clinical significance. In agreement with this hypothesis, a small study in patients who experienced acute myocardial infarction showed that a lower anti-inflammatory capacity of HDL relates to a higher incidence of new major cardiac events.[16] Whether a similar prospective association exists in the general population is not currently known. Therefore, the present study aimed to prospectively determine whether the anti-inflammatory capacity of HDL associates with incident CVD events in a general population cohort when taking established CVD risk factors into account. To ascertain the relevance of this metric of HDL function, irrespective of plasma HDL cholesterol, a nested case-control study design was chosen, matching participants not only for age and sex but also for HDL cholesterol.

METHODS

Study Population

The data that support the findings of this study are available from the corresponding author on reasonable request. We performed a nested case-control study among participants in the PREVEND study (Prevention of Renal and Vascular End Stage Disease). PREVEND was initiated to investigate the association of renal damage with CVD in a large cohort from inhabitants in the city of Groningen in Northern Netherlands. Details of the study have been described elsewhere.[10,17] In the period between 1997 and 1998, all inhabitants of the city of Groningen 28 to 75 years of age (85 421 total participants) were sent a questionnaire containing information about the presence of CVD risk factors and morbidity, as well as a vial to collect an early-morning urine sample. In total, 40 856 participants (47.8%) responded. The questionnaire collected information about the presence of risk factors of CVD and of CVD morbidity. We excluded pregnant women from the study; we also excluded persons with diabetes who were using insulin. All participants with a urinary albumin concentration ≥10 mg/L were invited to the clinic together with randomly selected participants with a urinary albumin concentration <10 mg/L. The study population comprised 8592 predominantly White participants who completed the total screening program. The study was approved by the medical ethics committee of the University Medical Center Groningen in The Netherlands (approval number MEC96/01/022). All participants gave written informed consent.

Study Design

First, participants who had experienced a CVD event before the baseline evaluation were excluded. Cases were identified as participants who had a first CVD event before the end of follow-up (January 1, 2009). Cases were then divided into quartiles on the basis of HDL cholesterol and stratified according to sex and current smoking behavior at baseline. Each case was matched to a control participant of the same sex, same smoking status, age (within 5 years), and HDL cholesterol (maximal difference, 3.9 mg/dL [0.1 mmol/L]). Of the 8267 eligible participants, all 369 cases that had been recorded during follow-up and 369 matched controls were initially identified. Blood samples for HDL anti-inflammatory

capacity determinations were available in 357 controls and 352 cases. This resulted in 340 matched case-control pairs, which were included in the current analysis. The results of the study questionnaire indicated that, at the time of blood sampling, no participant had experienced a recent acute illness, HIV infection, cancer, or any other inflammatory condition.

Outcome Measures

The combined end point of our study was incident CVD, defined as death from CVD, hospitalization for myocardial infarction (MI), percutaneous transluminal coronary angioplasty, ischemic heart disease, or coronary artery bypass graft. From the time of inclusion in the study, the vital status of the participants was checked through the municipal registry. The cause of death was obtained by linking the number of the death certificates to the primary cause of death, as coded by a physician from the Central Bureau of Statistics (Voorburg/Heerlen, The Netherlands). Causes of death were coded according to the *International Classification of Diseases, Ninth Revision* (*ICD-9*). Information on MI (*ICD-9* code 410), percutaneous transluminal coronary angioplasty (*ICD-9* code 45), ischemic heart disease (*ICD-9* code 411), and coronary artery bypass graft (*ICD-9* code 414) was obtained from the national hospital information system (Prismant, Utrecht, The Netherlands). The censoring date was the date obtained from the municipal registry or date of death.

Clinical Measures, Procedures, and Definitions

Body mass index (BMI) was calculated as the ratio between weight and height squared (kg/m^2). Blood pressure was measured using an automatic Dinamap XL model 9300 series device (Johnson & Johnson, Tampa, FL). Hypertension was defined as systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg or the use of antihypertensive drugs. Microalbuminuria was defined as urinary albumin excretion (UAE) between 30 and 300 mg per 24 hours based on 2 24-hour urine collections. Type 2 diabetes was defined as a fasting glucose ≥126 mg/dL (7.0 mmol/L), a random glucose ≥200 mg/dL (11.1 mmol/L), self-report of a physician diagnosis, or the use of glucose lowering drugs. Alcohol consumption was recorded assuming 1 drink to contain 10 g of alcohol. Smoking was categorized into "current," "former," and "never." Estimated glomerular filtration rate (eGFR) was calculated applying the combined creatinine–cystatin C equation, which is considered to provide a more accurate estimate of GFR compared to the eGFR equation by creatinine alone.[18,19] Information on medication use was combined with data from a pharmacy-dispensing registry, which has complete information on drug use of >95% of participants in the PREVEND study.

Laboratory Methods

Venous blood samples were obtained after 15 minutes rest after an overnight fast. Plasma glucose was measured directly after blood sampling. Plasma total cholesterol and glucose were assessed using Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY). Triglycerides were measured enzymatically. HDL cholesterol was measured with a homogeneous method (direct HDL; Aeroset System; Abbott Laboratories, Abbott Park, IL).[20] Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. Low-density lipoprotein cholesterol was calculated by the Friedewald formula if triglycerides were ≤399 mg/dL (4.5 mmol/l). ApoA1 and apoB were determined by nephelometry with reagents for Dade Behring nephelometer systems (BN II; Siemens, Marburg, Germany). HsCRP (high-sensitivity C-reactive protein) was assayed by nephelometry with a lower limit of 0.175 mg/L (BNII Nephelometer). Serum creatinine was measured by an enzymatic method on a Roche Modular analyzer (Roche Diagnostics, Mannheim, Germany) and serum cystatin C by Gentian Cystatin C Immunoassay (Gentian AS, Moss, Norway) on a Modular analyzer (Roche Diagnostics). UAE was measured by nephelometry with a threshold of 2.3 mg/L. Cholesterol efflux capacity was quantified using human THP-1–derived macrophage foam cells and apoB-depleted plasma as published previously.[10]

Determination of HDL Anti-Inflammatory Capacity

The HDL anti-inflammatory capacity was assessed in vitro as previously described (Figure 1).[21] Baseline EDTA plasma was obtained at time point of inclusion into PREVEND by centrifugation at 4°C, and samples were stored at –80°C until analysis. HDL was isolated from plasma by precipitation of apoB-containing lipoproteins using 36% polyethylene glycol (PEG 6000; Sigma, St. Louis, MO) exactly as published[11,12,21,22] and used directly for the HDL anti-inflammatory assay. Human umbilical vein endothelial cells (provided by the Endothelial Cell Core Facility of the University Medical Center Groningen) were preincubated with either 2% individual apoB-depleted plasma samples or an equal volume of PBS as a control for 30 minutes. Then, 10 ng/mL TNFα ([tumor necrosis factor α] R&D Systems, Abingdon, United Kingdom) was added. After an additional incubation of 5 hours, total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA) and VCAM-1 (vascular cell adhesion molecule-1) mRNA expression levels were determined by quantitative real-time polymerase chain reaction (ABI-Prism 7700; Applied Biosystems, Carlsbad, CA) as described.[21,22] VCAM-1 expression levels were calculated relative to the average of the housekeeping gene cyclophilin and expressed as percent reduction compared with TNFα-stimulated cells without addition of HDL; thus, higher values indicate more efficient anti-inflammatory protection. The intra-assay coefficient of variation (CV) of this assay is 7.6%, the interassay CV is 8.8%. We previously established

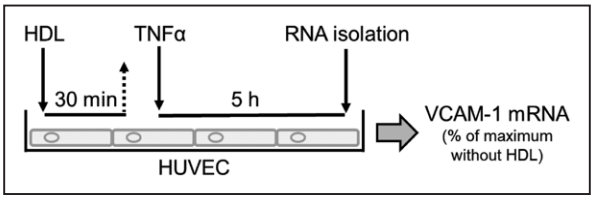

Figure 1. Schematic representation of the HDL anti-inflammatory assay. HUVECs were incubated with HDL for 30 minutes, whereafter TNFα was added for 5 hours. Then, RNA was isolated and VCAM-1 mRNA expression determined. HDL indicates high-density lipoprotein; HUVEC, human umbilical vein endothelial cells; TNFα, tumor necrosis factor α; and VCAM-1, vascular cell adhesion molecule-1.

that freezing of plasma does not affect the results and that the chosen concentration of HDL to be added was within the linear range of the assay[21,22]; sample storage of –80°C at least up to 4 years (n=20) does not affect the anti-inflammatory activity of HDL. Furthermore, using actual samples from this study, we determined that in our assay system less than 16.8% of the overall biological effect of the assay was not attributable to HDL and that it thus largely determines true effects of HDL. In apoB-depleted plasma samples (n=10) the average reduction of the maximum TNFα-induced VCAM-1 mRNA expression was 30.4±11.7% (P=0.002); after removing HDL by ultracentrifugation from the apoB-depleted samples, the remaining anti-inflammatory activity was 5.1±3.6 (not significant compared with the maximum TNFα effect). To limit potential variation because of different assay conditions, measurements of the anti-inflammatory capacity of HDL were carried out at the same time using the same batch of pooled human umbilical vein endothelial cells and the same reagents. Measurements were performed in duplicate.

Statistical Analyses

Differences in baseline characteristics were tested between participants who had experienced a cardiovascular event during follow-up (cases) and those who had not (controls). Categorical variables are expressed as total numbers (%) and differences between groups were tested with a χ^2 test. Normally distributed continuous variables were expressed as mean±SD and differences using *t*-tests; skewed continuous variables were presented as median [25th, 75th quartile] and differences were assessed using Wilcoxon rank-sum test.

Spearman rank correlation coefficients were used to assess relationships between baseline characteristics and anti-inflammatory capacity in crude analysis. Partial correlation coefficients were adjusted for age, sex, and HDL cholesterol. Given the nested case-control design of the study, we used a (multivariable) conditional logistic regression analysis to assess the association of the HDL anti-inflammatory capacity with CVD outcome, with results expressed as odds ratios (ORs) with 95% CI. OR were determined per 1 SD (1 SD=22.2%) increase of anti-inflammatory capacity. Multivariable models were adjusted for established CVD risk factors. In sensitivity analyses, the association of the anti-inflammatory capacity with incident CVD was adjusted for potential confounders individually. Furthermore, the association of the anti-inflammatory capacity with individual CVD endpoints was assessed. The association of the cholesterol efflux capacity with incident CVD was shown as a comparison exposure. In addition, a combination of both HDL anti-inflammatory capacity and HDL cholesterol efflux capacity was assessed.

Effect modification by sex, age, alcohol consumption, smoking status, BMI, diabetic status, hypertension, hsCRP, eGFR, UAE, total cholesterol, triglycerides, and time between blood sampling and incident CVD were tested by inclusion of interaction terms. Subgroup analysis using interaction tests was subsequently performed wherein ORs were determined across categories of baseline characteristics. For continuous variables, the median value was used as the cutoff. The participant characteristics were sex (male versus female), age (<60.8 versus ≥60.8 years), alcohol consumption (<10 or ≥10 g/d), current or former smoking (yes versus no), BMI (<26.9 versus ≥26.9 kg/m^2), diabetes (yes versus no), hypertension (yes versus no), hsCRP (<1.82 versus ≥1.82 mg/L), eGFR (<90.0 versus ≥90.0 mL/min per 1.73 m^2), UAE (<12.2 versus ≥12.2 mg/24 hours), total cholesterol (<229 and ≥229 mg/dL), triglyceride (<121 and ≥121 mg/dL), and time between blood sampling and CVD event (<8.6 and ≥8.6 years). To assess the functional relationship of anti-inflammatory capacity with the probability of CVD events, we used restricted cubic spline analysis with 4 knots placed at recommended percentiles according to Harrell.[23] A logistic regression with the spline term was performed, with adjustment for BMI, diabetes, low-density lipoprotein cholesterol, triglyceride levels, hypertension, and hsCRP.

To further illustrate the relationship of HDL cholesterol levels and the metric of HDL functionality, anti-inflammatory capacity, we investigated the association of both variables with CVD in a mutually adjusted analysis. In this analysis, cases and controls were used that were matched for age, sex, and smoking status but not for HDL cholesterol levels. Analyses were adjusted for CVD risk factors, namely BMI, diabetes, low-density lipoprotein cholesterol, triglyceride levels, hypertension, and hsCRP.

Furthermore, the contribution of the 2 HDL function metrics, anti-inflammatory capacity and cholesterol efflux capacity, to disease prediction was assessed. Because of the nested nature of the analysis, the addition of anti-inflammatory capacity, cholesterol efflux capacity, and both function metrics together to the Framingham risk score was assessed using likelihood ratio statistics.[24] In addition, we used the Akaike information criterion and Bayesian information criterion[25] to estimate whether substitution of the HDL cholesterol level with the HDL function metric anti-inflammatory capacity in the Framingham risk score improved the predictive value of the risk equation.

Two-sided P values <0.05 were considered statistically significant. Statistical analysis was performed using STATA version 15.0 (StataCorp, College Station, TX).

RESULTS

The median follow-up time was not different in cases (IQR, 10.5 [9.9–10.8] years) and in controls (IQR, 10.4 [9.9–10.8] years). As shown in Table 1, cases had a significantly higher prevalence of hypertension and diabetes, used more lipid-lowering drugs, and had higher hsCRP, total cholesterol, low-density lipoprotein cholesterol, triglyceride, and apoB levels. Cases had a significantly lower cholesterol efflux capacity. HDL cholesterol and apoA1 concentrations were similar between groups as a consequence of the matching procedure. It is notable that the HDL anti-inflammatory capacity was significantly lower in cases, despite virtually no difference in HDL cholesterol levels (31.6% [15.7–44.2] versus 27.0% [7.4–36.1]; P<0.001). In (i) univariate, as well as (ii) age- and (iii) age- and HDL cholesterol–adjusted analyses, no clinical or laboratory value was significantly correlated with the HDL anti-inflammatory capacity (Table I in the Data Supplement).

Table 1. Baseline Characteristics of the Study Participants, by Case-Control Status at Follow-Up

Variable	Controls	Cases	*P* value
No. of participants	340	340	
Male sex, n (%)	239 (70)	239 (70)	1.00
Age, y	59.0±10.8	59.2±10.9	0.88
Body mass index, kg/m^2	26.9±4.3	27.5±4.1	0.084
Smoking, n (%)			0.50
Current	71 (20.9)	60 (17.6)	
Former	125 (36.8)	136 (40.0)	
Never	144 (42.4)	144 (42.4)	
Alcohol intake, n (%)			0.60
<10 grams/day	247 (72.9)	253 (74.6)	
≥10 grams/day	92 (27.1)	86 (25.4)	
Hypertension, n (%)	164 (48.2)	206 (60.6)	0.001
Diabetes, n (%)	12 (3.5)	24 (7.1)	0.040
Lipid-lowering drug use, n (%)	9 (2.6)	20 (5.9)	0.037
Antihypertensive medication use, n (%)	69 (20.3)	97 (28.5)	0.012
Systolic blood pressure, mm Hg	136.7±20.4	142.6±22.4	<0.001
Diastolic blood pressure, mm Hg	77.7±9.3	80.0±9.8	0.002
Glucose-lowering drug use, n (%)	4 (1.2)	13 (3.8)	0.027
Fasting glucose, mg/dL	91.4±18.8	93.1±26.9	0.33
High-sensitivity C-reactive protein, mg/L	1.6 (0.7, 3.4)	2.1 (0.9, 4.5)	0.004
Estimated glomerular filtration rate,* mL/(min·1.73 m^2)	89.3±15.6	87.9±15.9	0.26
Urinary albumin excretion, mg/24 h	11.5 (7.0, 24.5)	13.0 (7.7, 28.4)	0.078
Cholesterol, mg/dL			
Total	226.1±43.8	241.3±44.5	<0.001
Low-density lipoprotein	153.2±41.7	166.4±40.1	<0.001
High-density lipoprotein	45.6±13.6	45.1±13.2	0.59
Triglycerides, mg/dL	115.9 (85.0, 160.6)	125.7 (88.5, 181.0)	0.044
Apolipoprotein A1, mg/dL	1.3±0.3	1.3±0.3	0.11
Apolipoprotein B, mg/dL	1.1±0.3	1.2±0.3	0.006
Cholesterol efflux capacity, AU	1.0±0.2	0.9±0.3	0.002
Anti-inflammatory capacity, %	31.6 (15.7, 44.2)	27.0 (7.4, 36.1)	<0.001

Normally distributed continuous variables are presented as mean±SD. Continuous variables with a skewed distribution are presented as median (25th, 75th percentile). Categorical data are summarized by n (%).

*Based on the creatinin–cystatin C equation.

In a univariate conditional logistic regression analysis, the HDL anti-inflammatory capacity showed an inverse association with incident CVD events (OR per 1 SD, 0.77 [95% CI, 0.66–0.91]; *P*=0.002) (Table 2). When adjusting for BMI, alcohol intake, diabetes status, hypertension, and use of lipid lowering drugs, this association remained essentially unaltered (model 1: OR per 1 SD, 0.78 [95% CI, 0.66–0.93]; *P*=0.005). After further adjustment for total cholesterol, apoA1 and triglyceride levels (model 2: OR per 1 SD, 0.75 [95% CI, 0.63–0.90]; *P*=0.002), and hsCRP, UAE, and eGFR (model 3: OR per 1 SD, 0.74 [95% CI, 0.61–0.90]; *P*=0.002), the inverse association of the anti-inflammatory capacity with incident CVD also did not materially change.

In sensitivity analyses adjustment was performed for individual CVD risk factors (Table II in the Data Supplement), which did not significantly alter the association. Analyses were also repeated for individual components of the combined CVD end point. In crude analyses, the HDL anti-inflammatory capacity was suggested to be inversely associated with incident CVD death (n=17; OR per 1 SD, 0.61 [95% CI, 0.25–1.49]; *P*=0.28), ischemic heart disease (n=92; OR per 1 SD, 0.75 [95% CI, 0.55–1.00]; *P*=0.06), hospitalization for MI (n=139; OR per 1 SD, 0.85 [95% CI, 0.65–1.10]; *P*=0.22), percutaneous transluminal coronary angioplasty (n=54; OR per 1 SD, 0.74 [95% CI, 0.48–1.16]; *P*=0.19), and coronary artery bypass graft (n=38; OR

Table 2. Association of the HDL Anti-Inflammatory Capacity With Incident Cardiovascular Events in 340 Control Participants and 340 Matched Case Participants

Variable	Odds ratio per 1 SD increase in anti-inflammatory capacity*	95% CI	*P* value
Crude	0.77	0.66–0.91	0.002
Model 1†	0.78	0.66–0.93	0.005
Model 2‡	0.75	0.63–0.90	0.002
Model 3§	0.74	0.61–0.90	0.002

Data are odds ratios (95% CI) for incident cardiovascular disease events obtained with multivariable conditional logistic regression models. Triglycerides, urinary albumin excretion, and high-sensitivity C-reactive protein values were $\log_e$ transformed. The use of glucose-lowering drugs and antihypertensive medication is included in the definition of diabetes and hypertension, respectively. HDL indicates high-density lipoprotein.

*1 SD=22.2%.

†Model 1: crude + body mass index, alcohol intake (<10 g/day or ≥10 g/day), diabetes status, hypertension, and use of lipid-lowering drugs.

‡Model 2: Model 1 + total cholesterol, apolipoprotein A1, and triglycerides.

§Model 3: Model 2 + high-sensitivity C-reactive protein, urinary albumin excretion, and estimated glomerular filtration rate.

per 1 SD, 0.72 [95% CI, 0.44–1.18]; *P*=0.19). Furthermore, in sensitivity analyses the association between cholesterol efflux capacity and incident CVD events was assessed as a comparison exposure (Table 3). When combining the cholesterol efflux capacity and the anti-inflammatory capacity in 1 model, both HDL function metrics were significantly and independently associated with incident CVD events in a crude (OR per 1 SD, 0.74; *P*=0.002 and OR per 1 SD, 0.69; *P*<0.001, respectively) (Table 3), as well as fully adjusted model (OR per 1 SD, 0.74; *P*=0.002 and OR per 1 SD, 0.66; *P*<0.001, respectively) (Table 3).

As shown in Figure 2, the association of the HDL anti-inflammatory capacity with CVD was different for males and females (*P* for interaction=0.008) and participants with high versus low BMI (*P* for interaction=0.003) and high versus low triglyceride levels (*P* for interaction<0.001) (Figure 2). These interactions were also present with BMI (*P* for interaction=0.003) and triglycerides (*P* for interaction<0.001) as continuous variables.

Restricted cubic spline analysis showed that the probability of a CVD event is approximately constant for values of HDL anti-inflammatory capacity ranging from –40% to 10%, while at higher values of the HDL anti-inflammatory capacity, an increase of anti-inflammatory capacity is directly proportional to a decrease in risk (Figure 3).

When adding anti-inflammatory capacity to the well-established Framingham risk score, the model likelihood ratio statistic increases from 10.50 to 20.40. A significant likelihood-ratio test (*P*=0.002) indicated a statistically significant greater predictive power. With the further addition of cholesterol efflux capacity, the model likelihood-ratio statistic again increased to 32.84, with a significant likelihood-ratio test (*P*=0.0005). When substituting HDL cholesterol with anti-inflammatory capacity in the Framingham risk score equation, the Akaike information criteria decreases from 936 to 542 and the Bayesian information criteria from 945 to 550, again suggesting an increase in model fit.

The HDL anti-inflammatory capacity was inversely associated with risk of CVD events in a CVD risk-adjusted model (OR per 1 SD, 0.74 [95% CI, 0.60–0.92]; *P*=0.007), which remained unchanged after further adjustment for HDL cholesterol (OR per 1 SD, 0.74 [95% CI, 0.60–0.92]; *P*=0.008) (Figure 4). On the other hand, plasma HDL cholesterol was not significantly associated with a lower risk of CVD events in a model adjusted for CVD risk factors (OR per 1 SD, 1.19 [95%

Table 3. Comparison of the Association of Different HDL Function Metrics With Incident Cardiovascular Disease in 340 Matched Control Participants and 340 Case Participants

	Anti-inflammatory capacity			Cholesterol efflux capacity			Combined model anti-inflammatory capacity and cholesterol efflux			
	Odds ratio per 1 SD increase	95% CI	*P* value	Odds ratio per 1 SD increase	95% CI	*P* value		Odds ratio per 1 SD increase	95% CI	*P* value
Crude	0.77	0.66–0.91	0.002	0.70	0.59–0.83	<0.001	Anti-inflammatory capacity	0.74	0.62–0.87	<0.001
							Cholesterol efflux capacity	0.69	0.57–0.82	<0.001
Model 1*	0.78	0.66–0.93	0.005	0.70	0.59–0.84	<0.001	Anti-inflammatory capacity	0.75	0.63–0.90	0.001
							Cholesterol efflux capacity	0.69	0.58–0.83	<0.001
Model 2†	0.75	0.63–0.90	0.002	0.70	0.58–0.85	<0.001	Anti-inflammatory capacity	0.72	0.61–0.87	0.001
							Cholesterol efflux capacity	0.69	0.57–0.84	<0.001
Model 3‡	0.74	0.61–0.90	0.002	0.67	0.55–0.82	<0.001	Anti-inflammatory capacity	0.74	0.61–0.90	0.002
							Cholesterol efflux capacity	0.66	0.54–0.81	<0.001

Data are odds ratios (95% CI) for incident cardiovascular disease events obtained with multivariable conditional logistic regression models. Triglycerides, urinary albumin excretion, and high-sensitivity C-reactive protein values were $\log_e$ transformed. The use of glucose-lowering drugs and antihypertensive medication is included in the definition of diabetes and hypertension, respectively. HDL indicates high-density lipoprotein.

*Model 1: crude + body mass index, alcohol intake (<10 g/day or ≥10 g/day), diabetes status, hypertension, and use of lipid-lowering drugs.

†Model 2: Model 1 + total cholesterol, apolipoprotein A1, and triglycerides.

‡Model 3: Model 2 + high-sensitivity C-reactive protein, urinary albumin excretion, and estimated glomerular filtration rate.

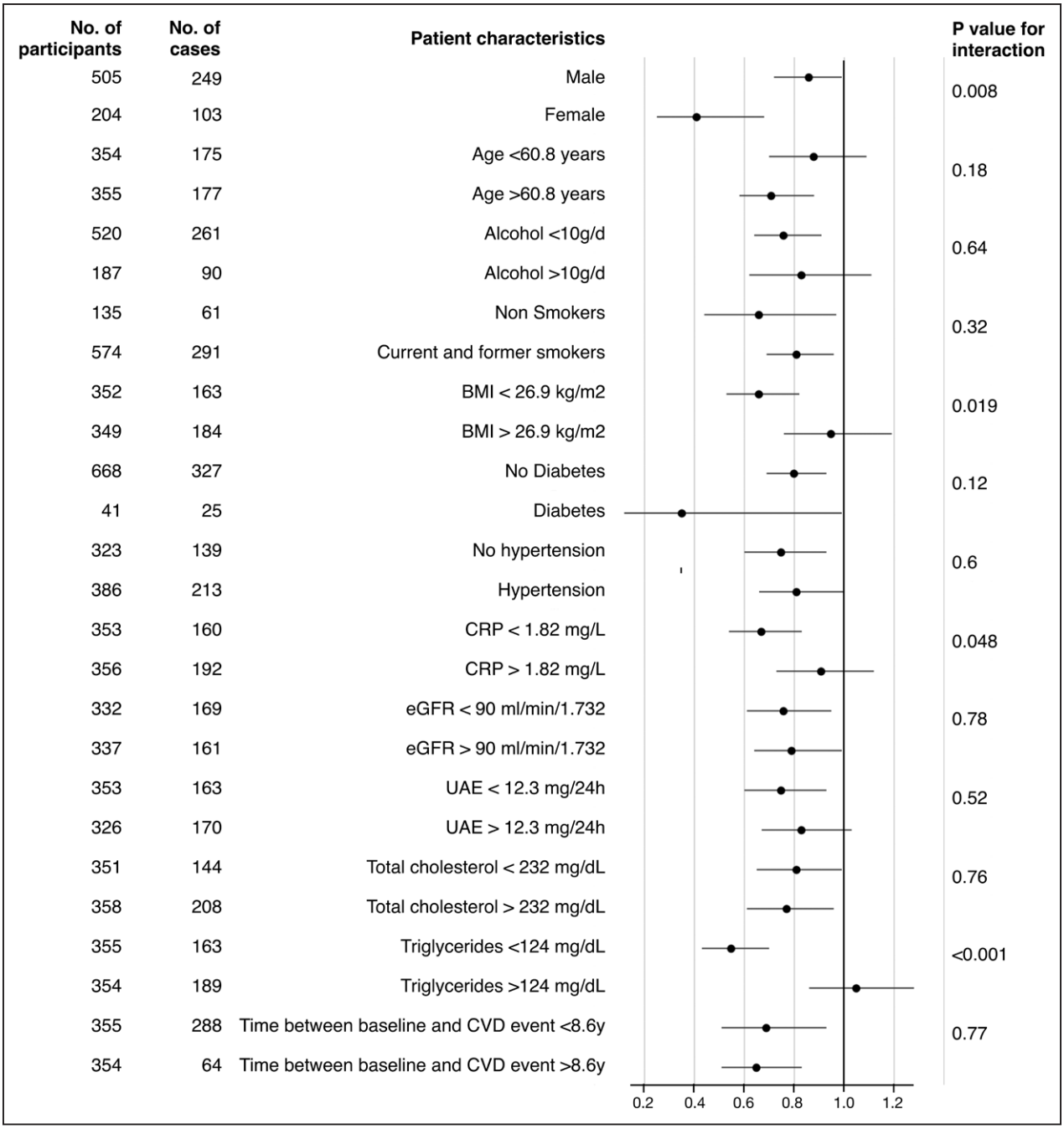

Figure 2. Odds ratios for incident cardiovascular events per 1 SD increase in the HDL anti-inflammatory capacity, by several participant-level characteristics.

Data are odds ratios (95% CI) for incident cardiovascular disease events obtained with logistic regression models. Triglycerides, urinary albumin excretion, and high-sensitivity CRP values were $\log_e$ transformed. BMI indicates body mass index; CRP, C-reactive protein; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate (based on the creatinine–cystatin C equation); and UAE, urinary albumin excretion.

CI, 0.61–2.33]; *P*=0.60). Further adjustment for anti-inflammatory capacity changed the OR slightly (OR per 1 SD, 1.10 [95% CI, 0.55–2.14]; *P*=0.81) (Figure 4).

DISCUSSION

The results of this prospective, individually matched, nested case-control study demonstrate that the anti-inflammatory capacity of HDL is associated with incident CVD events in a general population cohort independent of HDL cholesterol and apoA1 levels. Since only very limited associations with established CVD biomarkers were observed, notably including HDL cholesterol, hsCRP, and HDL cholesterol efflux capacity, we surmise that determining the HDL anti-inflammatory capacity has the potential to provide independent clinical information on risk assessment for patients initially free of clinically manifest CVD. In support of this point, we

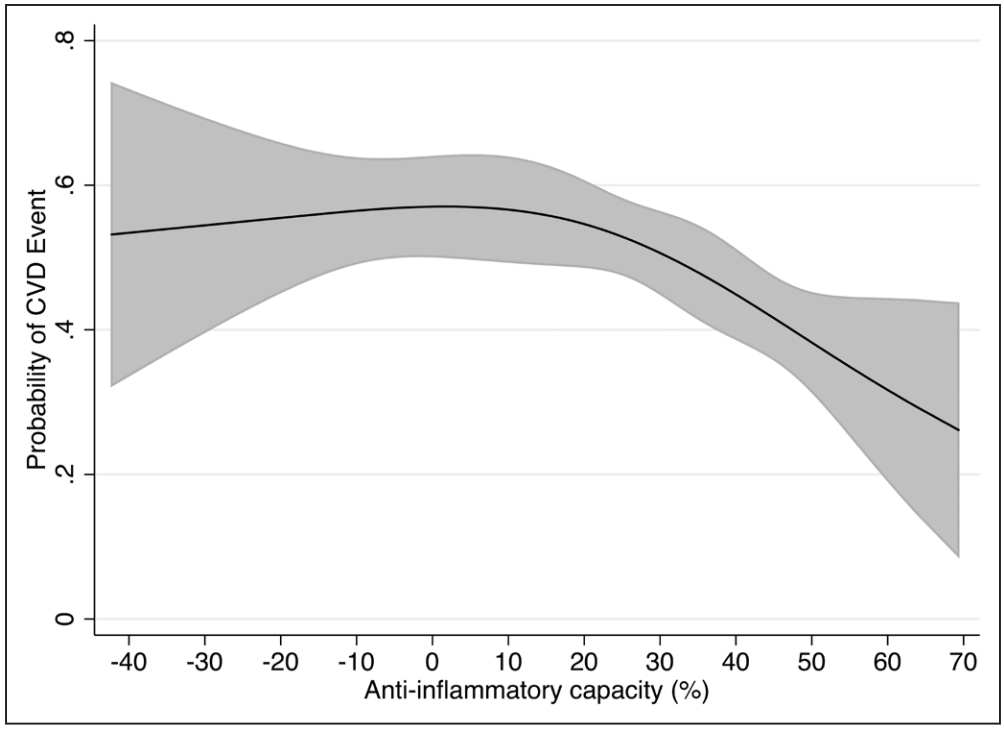

Figure 3. Probability of CVD events according to HDL anti-inflammatory capacity.
Probabilities were obtained by multivariate conditional logistic regression using restricted cubic splines with four knots, adjusted for body mass index, diabetes, low density lipoprotein cholesterol, triglyceride levels, hypertension, and high sensitivity C-reactive protein. CVD indicates cardiovascular disease; and HDL, high density lipoprotein.

found that either adding HDL anti-inflammatory capacity to the Framingham risk score or substituting HDL cholesterol for this functional metric in the Framingham risk score significantly improved risk prediction. It is notable that the association of the HDL anti-inflammatory capacity with incident CVD was also independent of HDL cholesterol efflux capacity.

Atherosclerosis has a strong inflammatory component. Endothelial inflammation, specifically as indicated by increased expression of adhesion molecules such as VCAM-1, is observed in animal models of atherosclerosis as well as in human atherosclerotic plaques.[15] The importance of inflammation is further evidenced by the usefulness of hsCRP or adhesion molecules as CVD biomarkers[26,27] and by the recent results of the CANTOS trial (Canakinumab Anti-Inflammatory Thrombosis Outcome Study) showing that treatment with canakinumab, which targets the interleukin-1β innate immunity pathway, improves CVD outcomes.[28] In addition, decreasing functional VCAM-1 expression either by the use of an antibody[29] or genetically[30] resulted in decreased atherosclerosis development in mouse models. HDL is regarded as a component of the innate immune system and has been shown in in vitro as well as in vivo studies to decrease VCAM-1 expression in vascular tissue next to other markers of inflammation,[31–34] neutralize bacterial lipopolysaccharide,[35] and protect against bacterial infections.[14] Therefore,

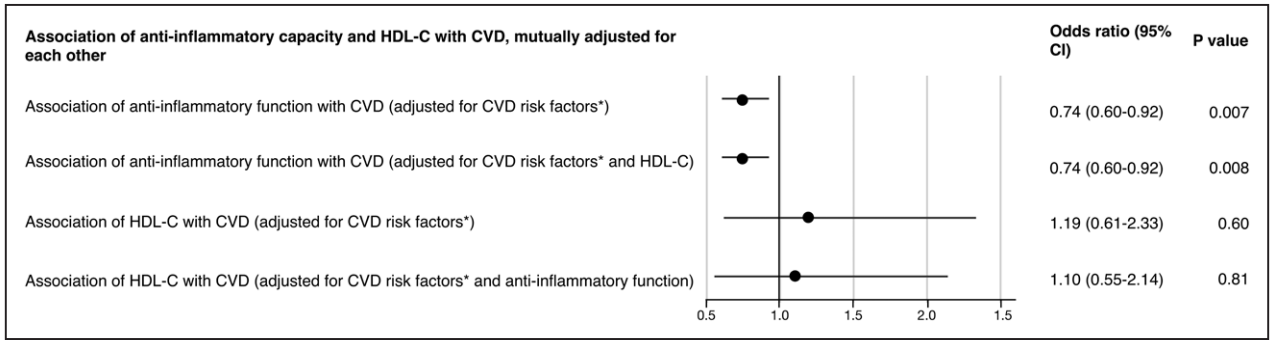

Figure 4. Association of the HDL anti-inflammatory capacity and HDL cholesterol levels with CVD risk mutually adjusted for each other.
Data are odds ratios (95% CI) for incident cardiovascular disease events obtained with multivariable logistic regression models. CVD indicates cardiovascular disease; and HDL-C, high density lipoprotein cholesterol. *Diabetes, hypertension, body mass index, plasma low density lipoprotein cholesterol, triglycerides, and high-sensitivity C-reactive protein.

the anti-inflammatory activity of HDL, as measured in the current study, conceivably represents a reflection of a pathophysiologically relevant process with respect to atherosclerotic CVD. Because the cholesterol content of HDL has not been mechanistically implicated in the anti-inflammatory or anti-infectious properties of these particles, it is biologically plausible that we did not observe a correlation between HDL cholesterol and anti-inflammatory capacity. On the other hand, no correlation of the HDL anti-inflammatory capacity with hsCRP was detected either. These results could potentially indicate that the processes causing systemic inflammation with the secretion of acute phase proteins by hepatocytes are distinct from the ones affected by the action of HDL in the vessel wall. Of note, the association of the HDL anti-inflammatory capacity was less strong in participants with higher BMI and higher triglycerides, suggesting that maybe a higher endogenous inflammatory load, commonly associated with these conditions, attenuates the effect. In contrast, it appears that the HDL anti-inflammatory activity could be of greater predictive utility in subgroups with lower BMI or triglycerides. However, more work and data would be required before such a general conclusion could be formally drawn.

At present, it is incompletely understood which components of the complex HDL particles—carrying a large amount of protein and lipid cargo—govern the anti-inflammatory activity toward endothelial or vascular smooth muscle cells. It has been previously shown that the acute phase protein SAA (serum amyloid A) can replace apoA1 on HDL particles and that enrichment of HDL with SAA can give rise to a proinflammatory HDL particle in end-stage renal disease patients; in addition, SAA can signal through FPR2 (formyl peptide receptor 2).[31] However, such a mechanism does not appear highly likely in our study from the general population, investigating participants with mostly normal renal function and an overall low inflammatory load. Changes in the composition of phospholipid species have also been reported to impact the anti-inflammatory activity of HDL; specifically, an increased phosphatidylserine content was associated with a better anti-inflammatory capacity.[36] Directly,[33] but also in a more indirect fashion through induction of endothelial nitric oxide synthase and the subsequent production of nitric oxide,[37] sphingosine 1-phosphate within HDL can act to decrease inflammation. More mechanistic studies seem warranted to explore HDL components and underlying molecular pathways since an improved understanding could result in the identification of a simple biomarker reflecting HDL (dys)function that would be more suitable for clinical routine than a rather complex assay based on primary cells. In addition, such mechanistic insights would also provide novel intervention targets aimed at reducing CVD risk.

Several methodological aspects and limitations need to be considered. Our study included White participants with a relatively narrow genetic background. Replication is needed before generalizing our findings. In addition, stroke was not included as an end point. The case-control design of this study limits the ability to investigate the association of the HDL anti-inflammatory capacity with other known CVD risk factors. Furthermore, HDL function assays are not yet standardized, thus allowing interpretations only in context of the assay conditions applied. A similar reasoning holds true for the isolation of HDL. It is noteworthy that although precipitation of apoB-containing lipoproteins has the advantage to preserve all HDL subfractions, still as much as approximately 17% of the anti-inflammatory biological activity captured by the assay is contained within the fraction that is rendered devoid of more mature HDL by ultracentrifugation, but conceivably still contains biological activity from preβ-HDL particles. Because these data were generated in subjects from the general population participating in the present study, it is unclear whether such a reasoning would also apply to patients with a high inflammatory load. In contrast, precipitation of apoB-containing lipoproteins, during isolation by ultracentrifugation (cleaner in terms of eliminating plasma components), high centrifugal forces and ionic strengths are applied resulting in the loss of numerous HDL-associated proteins and a relative depletion in preβ-HDL.[9] Furthermore, for large cohorts such as the current one, ultracentrifugation is technically not feasible if refreezing of the isolates is to be avoided. For this reason, apoB-depletion has been largely applied in other cohort studies dealing with HDL function.[11–13]

In summary, the current work identified an impaired HDL anti-inflammatory capacity as a functional metric prospectively associated with increased cardiovascular risk in the general population. The results of the HDL anti-inflammatory assay cover a considerable dynamic range from largely suppressing endothelial cell inflammation (positive values) to capturing proinflammatory HDL particles (negative values). It is notable that the HDL anti-inflammatory capacity was independent of a large number of established cardiovascular biomarkers including hsCRP. In contrast with the cholesterol efflux function of HDL that tracks moderately with HDL cholesterol levels in the case of radiolabel assay (in the current cohort, r=0.439; P<0.001)[10,12,13] and much less in the case of BODIPY-cholesterol (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) efflux assay,[11] the anti-inflammatory capacity did not show any significant correlation with HDL cholesterol or apoAI. Combined, our results place clinical meaning to the potential of testing for anti-inflammatory properties of HDL to achieve improved cardiovascular risk prediction.

ORIGINAL RESEARCH ARTICLE

ARTICLE INFORMATION

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Disclosures

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