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ORIGINAL RESEARCH

Anti-Inflammatory HDL Function, Incident Cardiovascular Events, and Mortality: A Secondary Analysis of the JUPITER Randomized Clinical Trial

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BACKGROUND: High-density lipoprotein (HDL) cholesterol has inverse association with cardiovascular disease. HDL possesses anti-inflammatory properties in vitro, but it is unknown whether this may be protective in individuals with inflammation.

METHODS AND RESULTS: The functional capacity of HDL to inhibit oxidation of oxidized low-density lipoprotein (ie, the HDL inflammatory index; HII) was measured at baseline and 12 months after random allocation to rosuvastatin or placebo in a nested case-control study of the JUPITER (Justification for the Use of Statins in Prevention: An Intervention Evaluating Rosuvastatin) trial. There were 517 incident cases of cardiovascular disease and all-cause mortality compared to 517 age- and sex-matched controls. Multivariable conditional logistic regression was used to examine associations of HII with events. Median baseline HII was 0.54 (interquartile range, 0.50–0.59). Twelve months of rosuvastatin decreased HII by a mean of 5.3% (95% CI, −8.9% to −1.7%; P=0.005) versus 1.3% (95% CI, −6.5% to 4.0%; P=0.63) with placebo (P=0.22 for between-group difference). HII had a nonlinear relationship with incident events. Compared with the reference group (HII 0.5–1.0) with the lowest event rates, participants with baseline HII ≤0.5 had significantly increased risk of cardiovascular disease/mortality (adjusted hazard ratio, 1.53; 95% CI, 1.06–2.21; P=0.02). Furthermore, there was significant (P=0.002) interaction for HDL particle number with HII, such that having more HDL particles was associated with decreased risk only when HDL was anti-inflammatory.

CONCLUSIONS: In JUPITER participants recruited on the basis of chronic inflammation, HII was associated with incident cardiovascular disease/mortality, with an optimal anti-inflammatory HII range between 0.5 and 1.0. This nonlinear relationship of anti-inflammatory HDL function with risk may account in part for the HDL paradox.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT00239681.

Key Words: cardiovascular disease risk factors ■ HDL function ■ HDL inflammatory index ■ HDL particle number ■ high-density lipoprotein

Multiple studies have shown that high-density lipoprotein cholesterol (HDL-C) has an inverse relationship with cardiovascular disease (CVD) risk.[1–3] However, evidence from several Mendelian randomization studies and clinical trials of multiple drugs that increased HDL-C levels did not demonstrate cardiovascular benefit.[4–11] This contradiction has been described as the "HDL paradox,"[12] and has called into question the role of HDL-C as a clinical surrogate marker of HDL.

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CLINICAL PERSPECTIVE

What Is New?

- In the JUPITER (Justification for the Use of Statins in Prevention: An Intervention Evaluating Rosuvastatin) trial, the anti-inflammatory function of high-density lipoprotein (HDL) measured with the HDL inflammatory index had a nonlinear association with incident cardiovascular events and mortality, with the lowest risk observed for subjects with HII from 0.5 to 1.0 independent of cardiovascular disease risk factors and statin therapy.
- There was significant interaction for HDL particle number with the HDL inflammatory index, such that having more HDL particles was associated with decreased risk only when HDL was anti-inflammatory.

What Are the Clinical Implications?

- HDL functionality may provide insight into the complex role of HDL, in particular among individuals with chronic inflammation.

Nonstandard Abbreviations and Acronyms

Apo AI	apolipoprotein AI
AUC	area under receiver operating characteristic curve
GlycA	glycosylation inflammation biomarker
HDL-C	high-density lipoprotein cholesterol
HDL-P	high-density lipoprotein particle number
HII	HDL inflammatory index
HR	hazard ratio
hsCRP	high-sensitivity C-reactive protein
JUPITER	Justification for the Use of Statins in Prevention: An Intervention Evaluating Rosuvastatin
LDL-C	low-density lipoprotein cholesterol
NMR	nuclear magnetic resonance
oxLDL	oxidized low-density lipoprotein
SAA	serum amyloid A

The pleiotropic functions of HDL, such as promoting reverse cholesterol transport and its anti-inflammatory, antithrombotic, and nitric oxide effects in vitro and in vivo, taken together with the lack of a causal relationship of HDL-C with CVD, make HDL a complex therapeutic target. As a consequence, focus has shifted away from HDL-C to alternative HDL measures of cardiovascular risk such as HDL particle number (HDL-P), HDL size, apolipoprotein AI (apo AI) modifications, and cholesterol efflux capacity that show promise for cardiovascular risk estimation beyond HDL-C.[13,14] In the JUPITER (Justification for the Use of Statins in Prevention: An Intervention Evaluating Rosuvastatin) trial, we previously reported that among several HDL-related biomarkers of risk, HDL-P measured by nuclear magnetic resonance (NMR) spectroscopy had the strongest inverse association with incident CVD and mortality at baseline and during potent rosuvastatin therapy, which persisted after adjustment for chemically measured HDL-C.[15,16] By comparison, the association of cholesterol efflux capacity with CVD was significant with statin therapy, but did not persist after further adjustment for HDL-C or HDL-P.[16] However, less is known about other functions of HDL, in particular the anti-inflammatory quality of HDL, and whether anti-inflammatory HDL function may protect against clinical events in patients with chronic systemic inflammation. This is important given the conceptual change in our understanding of CVD as a disease that stems not only from atherogenic lipoproteins, but also from chronic inflammation.

The HDL inflammatory index (HII) has been proposed to quantify HDL anti-inflammatory function in vitro, but few prior studies have examined its relationship with incident events. It has been previously reported that HII measured within hours of a cardiac event (a period that is now understood to be of intense inflammatory activity) was associated with increased CVD risk.[17–20] Accordingly, for these studies, HII was as high as 2.9, but with sparse data for HII ≤0.5. HII above 1.0 was defined as dysfunctional with proinflammatory capacity and was associated with a higher risk of CVD. Conversely, HII <1.0 in patients with acute coronary syndromes indicated anti-inflammatory HDL that was associated with lower risk of CVD. Importantly, among patients with no prior CVD, the predictive value of HII for clinical events remains unknown, in particular for HII in the anti-inflammatory range <1.0. To our knowledge, this is the first study to examine HII in the range below 1.0 among individuals with no prior CVD. We therefore hypothesized that HII would be associated with incident CVD/mortality in JUPITER trial participants, in particular as they were recruited on the basis of chronic inflammation. Furthermore, recognizing that potent statin therapy with its anti-inflammatory properties may attenuate the observed associations, we aimed to examine the effects of randomized statin treatment on HII and its associated CVD risk relationship.

METHODS

The data supporting the findings of this study are available to researchers on request from the JUPITER Data Usage Review Committee. Institutional review board approval for this study was obtained from Partners HealthCare (Boston, MA), and all participants provided written informed consent. The first and last authors had full access to all data in the study and take responsibility for their integrity and data analysis.

Study Population

This case-control study was nested in the JUPITER trial (ClinicalTrial.gov No.: NCT00239681),[21] as previously described.[16] In brief, JUPITER was a randomized, double-blind, placebo-controlled trial of rosuvastatin 20 mg daily versus placebo in the primary prevention of CVD in 17 802 asymptomatic men ≥50 years and women ≥60 years with low-density lipoprotein cholesterol (LDL-C) <130 mg/dL and hsCRP (high-sensitivity C-reactive protein) ≥2.0 mg/L who were monitored for a median follow-up period of 1.9 years (maximum 5 years). Exclusion criteria for JUPITER included previous or current use of lipid-lowering therapy, triglycerides >500 mg/dL, diabetes mellitus, use of postmenopausal hormonal therapy, and specific inflammatory conditions such as severe arthritis, lupus or inflammatory bowel disease, or treatment with immunosuppressant medications. The trial protocol required measuring standard lipids and hsCRP at baseline and after 12 months of study treatment. Additional phenotyping was also done on samples that were voluntarily provided by 11 953 (67%) of the participants.

This nested case-control cohort sample of 1034 individuals with available baseline blood samples is comprised of 517 incident cases of myocardial infarction, stroke, hospitalization for unstable angina, arterial revascularization, CVD death, and all-cause mortality matched in a 1:1 ratio based on age (±2 years) and sex to controls who were selected using risk set sampling.[15,22,23] Secondary analysis excluded 209 cases with non-CVD death resulting in 308 pairs. In exploratory analyses, we also examined non-CVD and all-cause mortality.

Laboratory Measurements

Using a cell-free assay, we measured HII as the functional ability of HDL to promote or inhibit oxidation of oxidized low-density lipoprotein (oxLDL; Vascular Strategies LLC, Plymouth Meeting, PA).[24] The assay was performed essentially as previously described,[17] with a modification of LDL oxidation. LDL-C was isolated by ultracentrifugation, and then dialyzed in PBS. oxLDL was generated from freshly prepared LDL solution that was incubated uncapped at room temperature for 1 to 2 hours. The oxLDL was diluted to a concentration of 100 μg/mL as previously described. The organic phospholipid 2′,7′-dichlorodihydrofluorescein diacetate was prepared as previously described,[25] as it fluoresces when oxidized and exposed to light. After polyethylene glycol precipitation of apolipoprotein B, HDL-containing supernatant was used in the assay. oxLDL (final concentration: 1.4 μg/mL), 2', 7'-dichlorodihydrofluorescein diacetate (final concentration: 2.9 μg/mL), and a fixed volume of apolipoprotein B-depleted serum from study subjects (5 μL) were incubated with PBS to a final volume of 175 μL in individual wells of a 96-well flat-bottom polypropylene microtiter plate (Fisher Scientific, Pittsburgh, PA). The plate was incubated at 37°C in a microplate reader (Spectra Max, Gemini XS; Molecular Devices, Sunnyvale, CA). Serial excitations at 485 nm were performed every 90 seconds, accompanied by automated plate-shaking. Fluorescence at emission wavelength of 525 nm and cutoff of 515 nm was measured after 1 hour of incubation. Samples were plated in duplicate, and mean fluorescence recorded. Participants' apolipoprotein B-depleted serum containing HDL was mixed with oxLDL and assayed to determine the degree to which participants' HDL promotes or inhibits oxLDL-mediated oxidation of a fluorogenic probe (2′-7′-dichlorofluorescin to its fluorescent analog, 2′-7′-dichlorofluorescein).

HII was defined as $\frac{RFU_{oxLDL+HDL}}{RFU_{oxLDL}}$, where $RFU_{oxLDL+HDL}$ is defined as relative fluorescence units in the oxLDL+HDL group, and RFU_{oxLDL} is defined as relative fluorescence units in the oxLDL-alone group. To ensure uniformity of batch effects, baseline and 12-month samples from cases and matched controls were run in tandem, and a pooled human serum control was used to correct for interassay variation across batches. In addition, for quality assessment of the HII assay, samples were run in a blinded fashion in 10 to 14 separate assays for a mean interassay coefficient of variation of 12%. Four serum samples were run 4 times independently to demonstrate a mean intraassay coefficient of variation of 4%.

Fasting lipoproteins, hsCRP, and glucose levels were measured in a core laboratory as previously described.[21,22,26] HDL-C was measured after heparin–manganese precipitation of apolipoprotein B-containing proteins. HDL-P was measured using NMR spectroscopy LipoProfile IV, by LipoScience (now LabCorp, Raleigh, NC).[21] Apo AI was measured by immunonephelometry using a Behring nephelometric assay (Marburg, Germany). Efflux capacity was quantified in diluted apolipoprotein B-depleted plasma samples using a previously validated cell-based ex vivo assay in this nested case-control study.[16,27]

We also measured several additional inflammatory biomarkers at baseline and 12 months beyond hsCRP and HII. Specifically, we assayed the acute-phase glycosylation inflammation biomarker (GlycA) using an automated proton (H^1) NMR that generated signal amplitudes from the N-acetyl methyl group protons of the N-acetylglucosamine moieties located on specific serum acute phase proteins.[28,29] Also, group IIA secretory phospholipase A_2 was measured with a commercially available enzyme immunoassay (Cayman assay; Cayman Chemical Co., Ann Arbor, MI) using a double-antibody sandwich technique that does not cross react with Group I, IV, V, or X phospholipase A_2 enzymes or other inflammatory mediators (Quest Diagnostics Nichols Institute, San Juan Capistrano, CA).[30] Lipoprotein-associated phospholipase A_2 activity levels were measured using an automated enzyme assay system,[31] and lipoprotein-associated phospholipase A_2 mass concentration was quantified by a latex particle–enhanced turbidimetric immunoassay.[31]

Statistical Analysis

Medians with interquartile range and mean±SD were reported for continuous variables according to the distribution; counts and percentages were reported for categorical variables. Triglycerides and hsCRP were skewed, and therefore were log transformed. We investigated the variation in HII across categorical participant characteristics using Fisher exact test. *T* tests and chi-squared tests were used to examine continuous variables across HII categories defined below. To compare the distribution of biomarkers across strata of HII categories of cases and controls, we used 2-way ANOVA or Cochran–Mantel–Haenszel tests for continuous and categorical biomarkers, respectively. Spearman correlation was used to calculate the magnitude and direction of correlation between continuous biomarkers and HII. The effect of statin treatment on HII and other HDL-related biomarkers was tested by 2-sample *t* test for absolute and percent change from baseline to 12 months. *Z* tests of equality of row proportions were performed to investigate transitions from one HII category to the other within treatment arms over the 12-month follow-up period.

Multivariable conditional logistic regression models evaluated associations of HII with the prespecified outcomes. Exploratory analyses also examined associations with non-CVD mortality and all-cause mortality. The hazard ratio (HR) is an appropriate effect measure to use when risk-set sampling is employed to match controls to cases. It can be estimated with odds ratios reported in conditional logistic regression models.[32] Model 1 was adjusted for age (years), race, randomized treatment group, systolic blood pressure, body mass index, fasting glucose, smoking status, LDL-C, log-transformed triglycerides, and family history of premature coronary artery disease. Model 2 adjusted for model 1 variables and additionally adjusted for HDL-related biomarkers: cholesterol efflux capacity, HDL-C, HDL-P, and apo AI. An additional model further adjusted for hsCRP together with model 1 variables.

To account for possible non-linear relationships between HII and CVD risk, we included higher order (up to fourth degree) polynomial terms of HII in the models. In plots of HR as a function of baseline HII, we excluded observations outside 2.5 SDs of the median HII to avoid regions with sparse data. To accommodate non-monotone relationships between HII and CVD outcomes, we categorized HII into 3 categories. We used the threshold of 1.0 previously reported in the literature as the upper threshold. Then, to find the optimal lower threshold between 0 and 1.0, we identified the HII value that maximized the discrimination of cases from controls in model 1. Discrimination was assessed by area under receiver operating characteristic curve (AUC).[33] To avoid overoptimism in AUC estimation, we used out-of-bag bootstrap to train the model by performing sampling with replacement and then evaluating AUCs in ≈37% of observations not selected by bootstrap resampling algorithm.[34]

In models analyzing associations of on-treatment biomarkers with outcomes, we adjusted for 12-month values of LDL-C, log-triglycerides, and hsCRP. To preserve power while maintaining the matched case-control design, associations of HII with clinically relevant subgroups were analyzed by adding appropriate interaction terms to the models to evaluate for possible effect modification. Subgroup-specific regression parameters, HRs, and 95% CIs were reported by adding interaction terms in the models. All *P* values were 2-sided, with type 1 error rate α=0.05. Analyses were performed using R version 3.5 software (The R Project for Statistical Computing).

RESULTS

Cases and controls were well-balanced on most demographic and lipid parameters with some exceptions (Table 1). Compared with controls, cases that experienced CVD/mortality were significantly more likely to have received placebo, be non-White participants, report current smoking, and have significantly lower baseline HDL-P and apo AI. In addition, cases had slightly lower body mass index and LDL-P, but higher levels of inflammatory biomarkers hsCRP and GlycA. For the CVD-only end point, differences were also observed for treatment group, smoking, GlycA, baseline

Table 1. Baseline Characteristics

Participant Characteristics	CVD/Mortality		CVD	
	Controls (N=517)	Cases (N=517)	Controls (N=308)	Cases (N=308)
Rosuvastatin arm	243 (47%)	199 (38%)**	137 (44%)	107 (35%)*
Demographic Information				
Age, y	70 (64–75)	70 (63–75)	70 (64–75)	70 (63–75)
Women	146 (28%)	146 (28%)	84 (27%)	84 (27%)
White race	467 (90%)	430 (83%)***	284 (92%)	269 (87%)†
Clinical cardiovascular risk factors				
Body mass index, kg/m^2	28 (26–32)	27 (24–31)***	28 (26–32)	28 (25–31)
Systolic blood pressure, mm Hg	135 (124–144)	134 (126–145)	135 (124–145)	136 (128–146)
Current smoker	55 (11%)	120 (23%)***	29 (9%)	65 (21%)***
Family history of premature CHD	73 (14%)	71 (14%)	41 (13%)	49 (16%)
Metabolic syndrome	198 (39%)	187 (37%)	119 (39%)	129 (42%)
Laboratory/biomarkers cardiovascular risk factors				
hsCRP, mg/L	4.3 (2.9–7.1)	4.8 (3.0–8.7)**	4.3 (2.8–7.0)	4.5 (2.9–7.7)
GlycA, µmol/L	409 (368–453)	423 (380–477)**	410 (371–444)	423 (379–467)*
Lp-PLA$_2$ activity, nmol/min per mL	198 (167–230)	203 (173–233)	198 (168–225)	202 (177–238)*
LpPLA$_2$ mass, µg/L	297 (241–361)	308 (252–373)	296 (245–360)	309 (254–367)
sPLA$_2$ levels, ng/mL	3.8 (2.5–6.2)	4.3 (2.7–6.5)†	3.8 (2.5–6.2)	4.2 (2.7–6.5)
Fasting glucose, mg/dL	95 (89–101)	95 (89–103)	95 (89–101)	95 (89–103)
Hemoglobin A1c, mg/dL	5.6 (5.4–5.8)	5.7 (5.4–5.9)†	5.7 (5.4–5.8)	5.7 (5.4–5.9)
Lipids, mg/dL				
LDL cholesterol	109 (95–119)	106 (92–119)	111 (97–120)	110 (94–120)
Triglycerides	116 (83–165)	116 (87–166)	112 (82–161)	122 (93–171) *
HDL cholesterol	49 (40–61)	47 (40–60)†	49 (40–62)	47 (40–58) *
LDL particle number, nmol/L	1530 (1346–1719)	1517 (1324–1712)*	1524 (1339–1726)	1535 (1358–1743)
Small LDL-P number, nmol/L	1078 (816–1389)	1067 (828–1340)	1036 (804–1342)	1096 (865–1415)†
HDL particle number, µmol/L	22 (19–24)	21 (19–24)***	22 (19–25)	21 (19–24) **
Apolipoproteins, mg/dL				
Apolipoprotein B	88 (77–99)	87 (77–98)	88 (78–100)	90 (79–100)
Apolipoprotein AI	130 (112–153)	123 (107–146)***	130 (113–154)	125 (107–146)**
CEC, %	15.0 (12.6–17.5)	14.8 (12.4–17.1)	15.3 (12.9–18.0)	15.2 (12.8–17.6)
HII	0.54 (0.50–0.59)	0.54 (0.50–0.58)	0.54 (0.50–0.59)	0.54 (0.50–0.58)

Values represent n (%) or medians (25%–75%). Family history of premature coronary heart disease was defined as diagnosis of the disease in a male first-degree relative before the age of 55 years or in a female first-degree relative before the age of 65 years. CEC indicates cholesterol efflux capacity; CHD, coronary heart disease; GlycA, glycoprotein acetylation; HDL, high-density lipoprotein; HDL-P, high-density lipoprotein particle number; HII, HDL inflammatory index; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; LDL-P, low-density lipoprotein particle number; Lp-PLA$_2$, lipoprotein-associated phospholipase A$_2$; and sPLA$_2$, secretory phospholipase A$_2$.

†0.1, *<0.05, **<0.01, and ***<0.001 for *P* values comparing baseline characteristics across controls and cases for both outcomes.

HDL-C, HDL-P, and apo AI. They were more likely to have higher baseline triglycerides and Lp-PLA$_2$ activity (not mass). No significant differences were noted in baseline HII, efflux capacity, or hsCRP for cases and controls in these unadjusted analyses.

Among the controls, median baseline HII was 0.54 (interquartile range, 0.50–0.59; Table 1). Apo AI and HDL-P were significantly higher in controls than cases, whereas GlycA was lower in controls than in cases as expected. In this case-control study nested in the JUPITER trial population, HII was <1.0 for 97% of participants at baseline (Figure S1). Baseline HII was higher in non-White participants, particularly in Blacks (Table S1), without significant differences by sex, treatment group, family history of premature coronary artery disease, body mass index, smoking status, or efflux capacity, hsCRP, and HDL-C levels categorized as above or below the median. Baseline HII correlated weakly and inversely with HDL-C (Spearman correlation coefficient, *r*=−0.06; *P*=0.04), but not with HDL-P or efflux capacity (Table S2 and Figure S2). The strongest correlation of HII was observed inversely with selected biomarkers of vascular inflammation such as hsCRP, GlycA, and IIA group IIA secretory phospholipase A$_2$

(*r*=−0.13, −0.18, and −0.14, respectively; *P*<0.0001 for each), but not with others (eg, lipoprotein-associated phospholipase A_2 activity or mass, *P*=0.07 and *P*=0.53, respectively). HII also correlated weakly and inversely with LDL-C (*r*=−0.08; *P*=0.008). A 12-month change in HII and changes in other HDL-related biomarkers were not significantly correlated (*P*>0.10).

In models that adjusted for potential confounders (age [years], treatment group, race, systolic blood pressure, cigarette smoking, body mass index, fasting glucose, LDL-C, family history of premature coronary disease, and log triglycerides), there was a nonlinear relationship of baseline HII with incident CVD/mortality, with statistically significant higher-order (up to third degree) polynomial terms. The shape of this relationship was not altered by including traditional CVD risk factors and hsCRP in the model (Figure 1A and 1B).

To capture the non-monotone relationship and categorize HII as high and low risk, we divided HII into 3 categories (see Statistical Analysis subsection). Previous studies reported HII above 1.0 to be associated with increased risk in patients with clinically evident CVD, so we used 1.0 as the upper cut point supported by existing literature.[17–20] To estimate the lower cut point for HII in the range 0 to 1.0, which has not been studied before, we used out-of-bag bootstrap to find the cutoff that optimizes AUC. The HII cut point of 0.5 produced the highest median AUC after conducting multiple validations using out-of-bag bootstrap. Coincidentally, this statistically determined cut point of 0.5 corresponded approximately to a tertile. For HII within 0 to 1, which accounted for 97% of the data in our sample, we observed a non-linear and decreasing relationship with CVD risk. The observed relationship of HII 0 to 1 persisted after 2 sensitivity analyses (the first, using log transformed HII, and the second by removing outliers more than 2.5 SD from median HII). We also report similar associations of baseline HII with non-CVD and all-cause mortality (Figure S3). The relationship within the full range of HII (ie, 0–1.39) was J-shaped, and persisted in sensitivity analyses, as HII was associated with increased risk of CVD in the lowest (<0.5) and highest (>1.0) HII categories, although data for HII >1.0 were sparse. To acknowledge this sparsity, we report associations for HII 0 to 1 for meaningful interpretation.

HII between 0.5 and 1.0 was associated with the lowest risk for incident CVD/mortality, whereas HII 0 to 0.5 showed a 1.3-fold increase in the risk of incident CVD and/or mortality. Compared with the reference category of HII >0.5 to 1.0, participants with the lower HII range of 0 to 0.5 had 53% significant increased risk for the CVD/mortality (model 1 adjusted HR, 1.53; 95% CI, 1.06–2.21; *P*=0.02), and 28% nonsignificant increased risk for the CVD only end point (model 1 adjusted HR, 1.28; 95% CI, 0.80–2.05; *P*=0.31; Table 2). In exploratory secondary analyses, baseline HII 0 to 0.5 was also associated with a 2-fold increase in the risk for both non-CVD and all-cause mortality (model 1 adjusted HR, 2.13; 95% CI, 1.15–3.93; *P*=0.02 and model 1 adjusted HR, 2.08, 95% CI, 1.17–3.69; *P*=0.01, respectively). Adjustment for hsCRP in the models attenuated observed estimates that became nonsignificant. Adjustment for HDL-related biomarkers (HDL-C, HDL-P, and efflux) also attenuated the observed associations of baseline HII 0 to 0.5 versus the reference category of HII >0.5 to 1.0 (Table S3).

The HII-CVD/mortality association was different across levels of HDL-P with significant interaction. The observed nonlinear, decreasing relationship of HII with CVD/mortality risk was modified by HDL-P, whereby the contribution of dysfunctional HII (category 0–0.5) to CVD/mortality risk was only apparent when HDLP was above the median (*P*

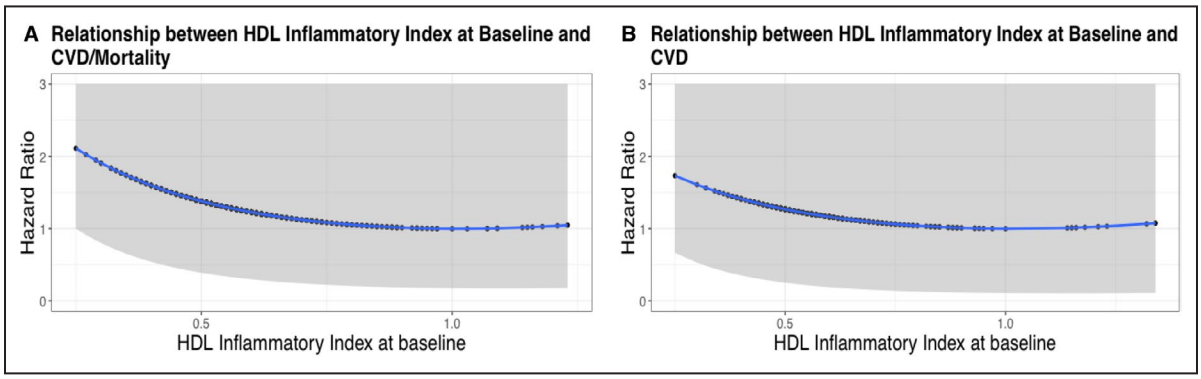

Figure 1. Relationship between HDL inflammatory index at baseline and CVD /all-cause mortality (A) and CVD only (B). Conditional logistic regression was used to estimate CVD hazard ratios as a function of baseline HDL inflammatory index adjusted for age, drug (statin vs placebo), race, systolic blood pressure, cigarette smoking, body mass index, glucose level, low-density lipoprotein cholesterol, family history of premature coronary disease, triglycerides, and high-sensitivity C-reactive protein. Regions with sparse data are not displayed (ie, < and >2.5 SD). CVD indicates cardiovascular disease; HDL, high-density lipoprotein.

Table 2. Association Between Baseline HDL Inflammatory Index and Incident Events

	HII 0 to 0.5	HII >0.5 to 1.0
CVD/mortality		
N (N cases/N controls)	287 (151/136)	720 (348/372)
Adj. HR (95% CI)	1.53 (1.06–2.21)	Reference
P value	0.02	
CVD		
N (N cases/N controls)	175 (90/85)	427 (208/219)
Adj. HR (95% CI)	1.28 (0.80–2.05)	Reference
P value	0.31	
Non-CVD mortality		
N (N cases/N controls)	112 (61/51)	293 (140/153)
Adj. HR (95% CI)	2.13 (1.15–3.93)	Reference
P value	0.02	
All-cause mortality		
N (N cases/N controls)	123 (67/56)	330 (158/172)
Adj. HR (95% CI)	2.08 (1.17–3.69)	Reference
P value	0.01	

Hazard ratios (HRs) were obtained from conditional logistic regression models adjusted for the following CVD risk factors: age, treatment group, race, smoking status, systolic blood pressure, body mass index, fasting glucose, baseline LDL cholesterol level, baseline log-transformed triglyceride level, and family history of premature coronary heart disease. CVD indicates cardiovascular disease; HDL, high-density lipoprotein; and HII, HDL inflammatory index.

interaction=0.03). In addition, baseline HII also modified the inverse association of HDL-P with incident CVD/mortality (*P* interaction=0.002; Figure 2). CVD/mortality risk decreased with higher levels of HDL-P (measured separately by NMR spectroscopy) as expected only among participants with anti-inflammatory HDL (ie, HII between 0.5 and 1.0). By contrast, higher levels of HDL-P were significantly associated with increased risk of CVD/mortality in high-risk HII category 0 to 0.5. In HII category >1.0, risk of incident CVD/mortality increased with higher levels of HDL-P, but not significantly so. No other significant modification of effect estimates was noted by randomization to rosuvastatin treatment versus placebo, race, or other groups (Figure 2).

To further understand the clinical characteristics of cases in the high-risk HII category of 0 to 0.5, we compared their baseline characteristics with controls and those in the reference HII category of >0.5 to 1.0 (Table 3). Compared with controls, cases with high-risk dysfunctional HII (HII ≤0.5) were more likely to have received placebo, whereas cases with low-risk HII >0.5 to 1.0 were more likely to be non-White. There were significantly more women and White cases that had high-risk dysfunctional HII. In addition, among cases, inflammatory markers-hsCRP and IIA group IIA secretory phospholipase A_2 were higher, and hemoglobin was lower, in the high-risk dysfunctional HII group.

Twelve-month HII measurements were available in 586 of 1034 (57%) study participants. HII decreased significantly from baseline over 12 months in the statin therapy arm (–5.3%; 95% CI, –8.9% to –1.7%), with a nonsignificant decrease in the placebo arm (–1.3%; 95% CI, –6.5% to 4.0%; Table 4, Table S4), and without a statistically significant difference across treatment arms (*P*=0.22). We report transitions between HII categories after 12 months of rosuvastatin therapy in Table S5. No significant shifts across HII categories were noted within treatment arms over 12 months (all *P*>0.60 using the *z* test of equality of row proportions).

No significant associations for on-statin HII (Table S6) or for change in HII levels with events were detected.

DISCUSSION

In this nested case-control study of the JUPITER trial, we present evidence of the complex biology of HDL in CVD risk and add to existing data on the pleiotropic functional effects of HDL. Although few studies have examined the HII–CVD relationship, to our knowledge, this present study is the first to investigate the anti-inflammatory metric of HDL in a primary prevention population, and one that is enriched with inflammation. We observed a non-linear, decreasing relationship between baseline HII and risk of CVD events and mortality that was independent of traditional risk factors and statin therapy. A novel finding is the association of HII 0 to 0.5 with increased risk of incident CVD/mortality compared with HII >0.5 to 1.0 despite normal LDL-C levels. Furthermore, there was significant (*P*=0.002) interaction for HDL particle number with HII, such that having more HDL particles was associated with decreased risk only when HDL was anti-inflammatory.

Similar to other studies conducted in secondary prevention populations that investigated HII in relation to prevalent CVD,[17,18] HII correlated inversely and weakly with HDL-C. Besides significant inverse correlation of HII with inflammatory markers (ie, hsCRP, GlycA, and IIA group IIA secretory phospholipase A_2), participants in the high-risk HII category 0 to 0.5 had a higher inflammatory biomarker burden than those in the low-risk HII category >0.5 to 1.0. This finding might clarify the conflicting correlation of HII and hsCRP reported in prior studies[17,20] and explain the lower levels of HDL-C and apo AI observed in the high-risk HII category 0 to 0.5.[35,36]

Rosuvastatin had a small, but favorable impact on HDL-C, HDL-P, and apo AI as previously

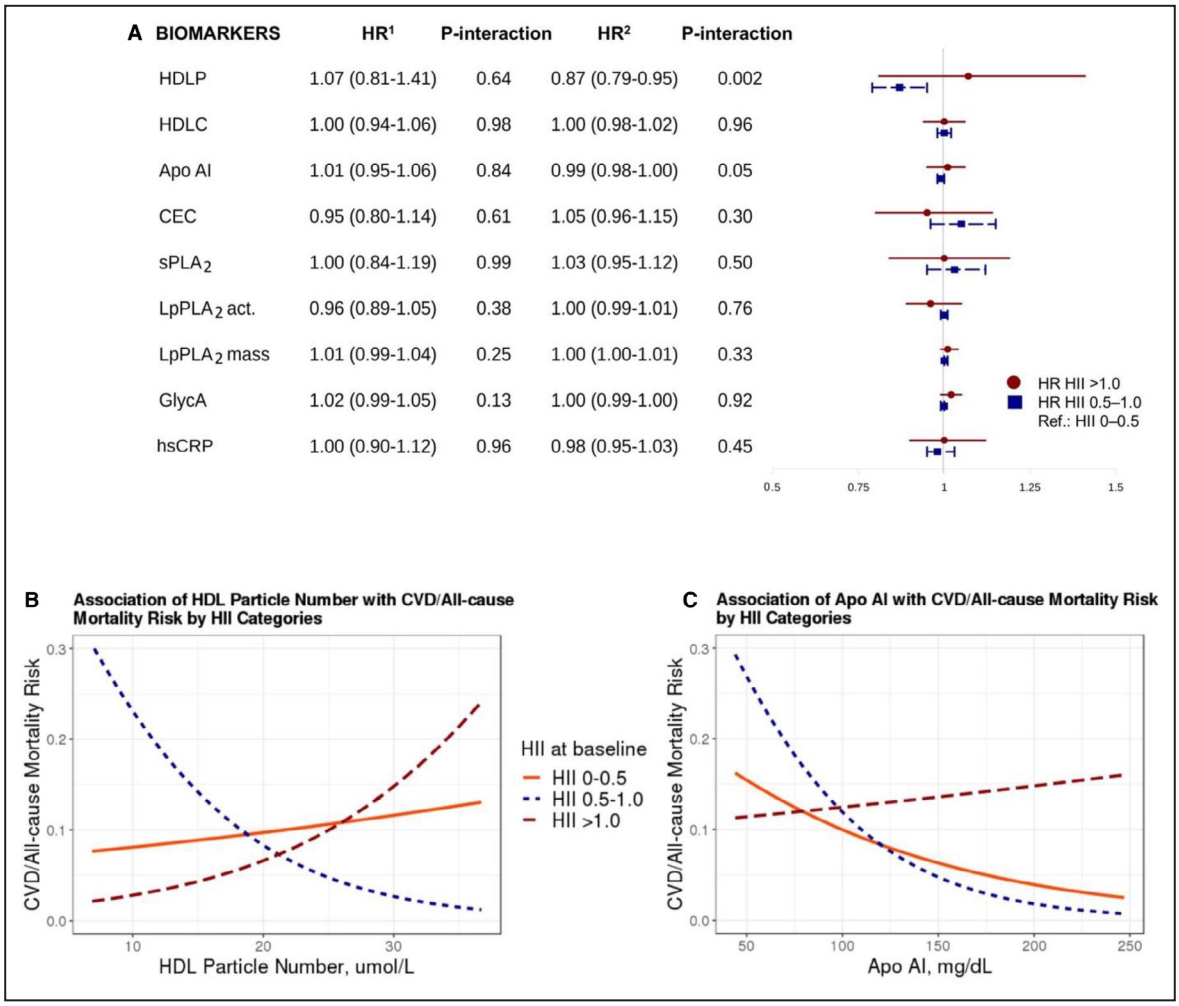

Figure 2. Association of biomarkers with CVD/all-cause mortality risk by HII categories at baseline.
HR[1]–Hazard ratio comparing risk in HII category >1.0 to HII category 0 to 0.5; HR[2]–hazard ratio comparing risk in HII category 0.5 to 1.0 to HII category 0 to 0.5; and *P* for interactions between HII categories and biomarkers of cardiovascular risk at baseline. HRs were obtained from conditional logistic regression models. (**A**) Shows the relationship of HDL-related biomarkers and inflammatory markers with CVD/all-cause mortality risk by HII categories at baseline. (**B**, **C**) Association of HDL-P and Apo AI with CVD/all-cause mortality risk within the three categories of HII. Apo AI indicates apolipoprotein AI; CEC, cholesterol efflux capacity; CVD, cardiovascular disease; GlycA, glycoprotein acetylation; HDL, high-density lipoprotein; HDLC, high-density lipoprotein cholesterol; HDLP, high-density lipoprotein particle number; HII, HDL inflammatory index; hsCRP, high-sensitivity C-reactive protein; Lp-PLA$_2$ act., lipoprotein-associated phospholipase A$_2$ activity; and sPLA$_2$, secretory phospholipase A$_2$.

documented.[15] Rosuvastatin slightly decreased HII over 12 months in the statin arm, but did not significantly decrease HII as compared to placebo, which suggests that statins may not have as robust an effect on normal-range HII.

Our findings support current evidence that links HII to CVD risk.[19,20] More importantly, they extend the a priori hypothesis that HII >1.0 denotes dysfunctionality, with evidence to suggest that HII 0 to 0.5 is associated with increased risk in a primary prevention trial population that was recruited based on chronic inflammation. In keeping with other studies, HII in the range >1.0 suggested increased risk, although not significantly, possibly because of limited power.

These results are particularly interesting, considering that existing data on HII are sparse, and limited to studies conducted among patients with higher ranges of HII and a high-grade, acute inflammatory setting of recent acute coronary syndrome and heart failure.[19,37,38] We observed a lower range of HII, mainly between 0 and 1.0, in our case-control subsample of the primary prevention JUPITER trial. This may be explained by the healthier JUPITER trial population in stable condition as compared with populations in other studies.[21,35,36] Therefore, our observation of HII values of 0 to 0.5 conferring increased risk compared with HII >0.5 to 1.0 is important and shows a longitudinal relationship as we examined baseline samples collected in participants free of CVD

Table 3. Baseline Characteristics of Participants According to HDL Inflammatory Index Categories

	Cases			Controls		
	HII 0 to 0.5	HII >0.5 to 1.0	*P* Value	HII 0 to 0.5	HII >0.5 to 1.0	*P* Value
N	151	348		136	375	
Rosuvastatin arm*	53 (35%)	140 (40%)	0.33	73 (54%)	167 (45%)	0.08
Demographic information						
Age, y	70 (65–75)	69 (63–75)	0.31	71 (66–76)	69 (64–74)	0.03
Women	54 (36%)	89 (26%)	0.03	45 (33%)	99 (27%)	0.17
White race*	138 (91%)	280 (81%)	0.004	128 (94%)	332 (89%)	0.09
Clinical cardiovascular risk factors						
BMI, kg/m^2	27 (24–31)	27 (24–30)	0.52	29 (26–32)	28 (26–32)	0.71
SBP, mm Hg	135 (125–143)	137 (126–148)	0.22	136 (124–145)	135 (124–144)	0.43
Current smoker	32 (21%)	85 (25%)	0.50	17 (13%)	38 (10%)	0.55
FH of premature CHD	22 (15%)	44 (13%)	0.66	17 (13%)	56 (15%)	0.58
Metabolic syndrome	56 (38%)	125 (36%)	0.88	50 (37%)	143 (39%)	0.86
Laboratory/biomarkers cardiovascular risk factors						
hsCRP, mg/L	5.6 (3.3–13)	4.5 (2.9–7.6)	<0.001	4.8 (3.0–8.5)	4.1 (2.8–6.5)	0.004
GlycA, μmol/L	431 (390–487)	419 (377–471)	0.08	421 (379–477)	407 (366–445)	0.01
Lp-PLA$_2$ act., nmol/min per mL	201 (160–237)	207 (178–236)	0.32	198 (164–226)	200 (168–231)	0.80
Lp-PLA$_2$ mass, μg/L	296 (239–354)	309 (254–375)	0.55	302 (244–382)	296 (240–359)	0.25
sPLA$_2$, ng/mL	4.8 (2.8–8.1)	4.1 (2.6–6.1)	0.006	5.1 (2.9–8.0)	3.6 (2.4–5.5)	<0.0001
Fasting glucose, mg/dL	96 (89–104)	96 (89–103)	0.65	95 (90–100)	96 (89–102)	0.38
Hemoglobin A1c, mg/dL	5.7 (5.4–5.9)	5.7 (5.4–5.9)	0.06	5.5 (5.5–5.9)	5.3 (5.3–5.8)	0.31
CEC, %	15 (13–17)	15 (12–17)	0.95	15 (13–18)	15 (12–17)	0.41
Lipids, mg/dL						
LDL-C	110 (97–118)	106 (90–119)	0.14	110 (96–120)	109 (94–119)	0.53
Triglycerides	109 (80–135)	101 (81–128)	0.29	99 (77–126)	102 (81–132)	0.29
HDL-C	48 (40–59)	47 (40–60)	0.80	49 (39–60)	49 (40–62)	0.68
LDL-P, nmol/L	1566 (1348–1706)	1542 (1315–1718)	0.45	1501 (1303–1682)	1552 (1363–1722)	0.10
sLDL-P, nmol/L	1087 (865–1343)	1047 (809–1320)	0.16	1047 (812–1352)	1091 (820–1401)	0.47
HDL-P, μmol/L	21 (19–24)	21 (18–23)	0.08	21 (19–23)	22 (20–25)	0.002
Apolipoproteins, mg/dL						
Apo B	89 (78–98)	88 (76–97)	0.42	87 (76–99)	89 (78–99)	0.34
Apo AI	128 (107–146)	125 (106–145)	0.39	129 (107–147)	135 (114–154)	0.07

Categorical variables are presented as n (%). Test of binomial proportions was used to test for homogeneity of categorical variables across HII categories within and across cases and controls. Continuous variables are presented as median with interquartile range. Student *t* test was used to compare continuous variables across HII categories within cases and controls. ANOVA was performed to compare means of continuous variables across cases and controls within the 2 categories of HII (nonnormally distributed variables were log-transformed). Family history of premature coronary heart disease was defined as diagnosis of the disease in a male first-degree relative before the age of 55 years or in a female first-degree relative before the age of 65 years. BMI indicates body mass index; CEC, cholesterol efflux capacity; FH of premature CHD, family history of premature coronary heart disease; GlycA, glycoprotein acetylation; HDL, high-density lipoprotein; HDL-P, high-density lipoprotein particle number; HII, HDL inflammatory index; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; LDL-P, low-density lipoprotein particle number; Lp-PLA$_2$ act., lipoprotein-associated phospholipase A$_2$ activity; SBP, systolic blood pressure; sLDL-P, small low-density lipoprotein particle number; and sPLA$_2$, secretory phospholipase A$_2$.

P for heterogeneity; obtained from test of binomial proportions for categorical variables and *t* test for continuous variables across HII categories within cases and controls.

*Proportion of demographic characteristics/biomarkers in each category of HII that is significantly different (at 0.05 type 1 error) across cases and controls.

well before any events occurred. Lastly, previous studies were conducted in high-risk patients with chronic diseases like diabetes mellitus and heart failure, which on their own lend to dysfunctionality of HDL[39,40] and higher HII, whereas the present study consisted of participants without diabetes mellitus or heart failure.

The observed relationship of HII with outcomes may reflect compensatory changes in HDL function and/or structure that predate an event despite normal HDL concentration and low-normal LDL-C, in the vascular milieu of chronic inflammation. Our findings suggest that months or years before a CVD event or

Table 4. Impact of Randomized Rosuvastatin Therapy Versus Placebo on HDL Inflammatory Index (HII) Levels Over 12 Months

HII	Rosuvastatin	Placebo	*P* Value vs Placebo
N	234	352	
Mean at baseline	0.58 (0.55, 0.60)	0.60 (0.56, 0.63)	0.36
Mean at 12 mo	0.52 (0.50, 0.55)	0.56 (0.53, 0.59)	0.19*
Absolute change (95% CI)	−0.05 (−0.08, −0.02)	−0.04 (−0.07, −0.002)	0.51
P value vs baseline	0.0003	0.04	
% change (95% CI)	−5.3 (−8.9, −1.7)	−1.3 (−6.5, 4.0)	0.22
P value vs baseline	0.005	0.63	

Analyses were done in participants who had HII levels measured at baseline and 12 months.
*Adjusted for baseline levels.

death, HDL may undergo adaptive changes in its anti-inflammatory role in response to an insult before the decompensation to dysfunctionality associated with an event. This phenomenon of hormesis is a phase of biological adaption to disruption in homeostasis by compensation.[41–45] For example, chronic inflammation results in HDL particles enriched with serum amyloid A (SAA) that displace apo AI. Oxidation of these SAA-containing HDL particles (typical of inflammatory conditions) releases proteins containing SAA that paradoxically delay lipoprotein oxidation in a dose-dependent fashion.[46] This antioxidant effect of SAA is similar to but less efficient than the antioxidant effect of apo AI on LDL, hence compensating partially for the loss of apo AI.[47,48] Nevertheless, the effects of inflammation on HDL function are controversial.[49]

The observed differential effect of HII on the HDL-P–CVD association could be consistent with hormesis because in the HII category 0 to 0.5, HDL's ability to inhibit oxidation of LDL increases. However, in this high-risk category, the compensatory rise in anti-inflammatory HDL function occurs with production of HDL-P that is detrimental to CVD risk (ie, enriched with SAA) suggesting that the particles being produced may be abnormal. Based on these findings of a crucial linkage between the two aspects of HDL on CVD/mortality, we surmise that in the high-risk HII category 0 to 0.5, HDL's anti-inflammatory capacity is impaired, despite increasing particle numbers, and this dysfunctionality predates CVD/mortality. In post hoc exploratory analyses, we also found novel and strong associations for baseline HII 0 to 0.5 in relation to non-CVD mortality that require further evaluation. Inclusion of other measures of HDL structure or function resulted in attenuation of the association between HII and CVD risk, which might indicate the synergistic interplay of several HDL characteristics in relation to CVD risk.

There are strengths and limitations of this study. In this nested study of a randomized trial, various components of HDL functionality and HDL structure (HII, HDL-C, HDL-P, apo AI, and efflux) were ascertained both at baseline and after 12 months of follow-up. Other strengths of the study include random allocation to potent statin therapy or placebo, prospective adjudication of all trial end points, and low-to-normal levels of circulating LDL-C. Potential limitations include a relatively short median follow-up period of 1.9 years as JUPITER was terminated early after proven efficacy of rosuvastatin to reduce incident CVD. The paucity of CVD events may have limited power to detect associations, particularly at 12 months, and in analyses restricted to statin therapy. However, statin therapy did not alter HII significantly. Although this study is nested in a clinical trial, participants were selected based on clinical outcomes and we cannot exclude residual confounding from unmeasured variables such as frailty. The trial's inclusion and exclusion criteria limit generalizability to other populations. Lastly, these results are hypothesis-generating and require validation in other studies.

In conclusion, we present insight into the HDL paradox, and offer a potential explanation. In the JUPITER trial, HII was significantly associated with incident CVD and mortality in a nonlinear relationship, with lowest risk in the HII range between 0.5 and 1.0 independent of CVD risk factors and statin therapy. Furthermore, HII modified the beneficial association of increasing HDL-P with CVD events such that only individuals with optimally functioning anti-inflammatory HDL retain the inverse relationship of HDL-P with events. HDL functionality may provide insight into the complex role of HDL, in particular among individuals with chronic inflammation.

ARTICLE INFORMATION

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Disclosures

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Supplementary Materials

Tables S1–S6
Figures S1–S3

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SUPPLEMENTAL MATERIAL

Table S1. HDL Inflammatory Index, by Demographic and Clinical Subgroups at Baseline.

	N	**Median (25th–75th %)**	***p***
Treatment group			
Placebo	592	0.54 (0.50-0.58)	0.73
Rosuvastatin	442	0.54 (0.50-0.59)	
Sex			
Male	742	0.55 (0.50-0.59)	0.34
Female	292	0.53 (0.49-0.57)	
Race			
White	911	0.54 (0.50-0.58)	0.0002
Black	75	0.59 (0.54-0.69)	
Hispanic	48	0.56 (0.52-0.64)	
BMI, kg/m^2			
<25	273	0.54 (0.50-0.58)	0.75
25-29	429	0.54 (0.50-0.59)	
≥30	329	0.53 (0.50-0.58)	
FH of premature CHD			
No	889	0.54 (0.50-0.59)	0.83
Yes	144	0.54 (0.50-0.58)	
Smoking			
Yes	175	0.54 (0.50-0.58)	0.47
No	858	0.54 (0.50-0.59)	

FH of premature CHD – Family history of premature coronary heart disease.

Table S2. Correlation of HDL Inflammatory Index with other Biomarkers of Cardiovascular Risk at Baseline.

	r	*p*
HDL cholesterol, mg/dL	-0.06	0.04
Cholesterol efflux capacity, %	-0.03	0.27
HDL particle number, µmol/L	-0.02	0.60
HDL size	-0.06	0.06
Apolipoprotein AI, mg/dL	-0.02	0.12
High-sensitivity C-reactive protein, mg/L	-0.13	<0.0001
GlycA, µmol/L	-0.18	<0.0001
Lipoprotein-associated phospholipase A_2 activity, nmol/min/mL	0.07	0.07
Lipoprotein-associated phospholipase A_2 mass, µg/L	-0.02	0.53
Secretory phospholipase A_2, ng/mL	-0.14	<0.0001
Fasting glucose, mg/dL	0.01	0.79
LDL cholesterol, mg/dL	-0.08	0.008
Triglyceride, mg/dL	-0.01	0.63
Apolipoprotein B, mg/dL	-0.05	0.10

r and *p* are Spearman correlation coefficients and the corresponding p-values for HDL inflammatory index.
HDL – High-density lipoprotein; GlycA – Glycoprotein acetylation; LDL – Low-density lipoprotein.

Table S3. Association between Baseline HDL Inflammatory Index and Incident Events (adjusted for HDL-related Biomarkers).

	HII 0–0.5	**HII >0.5–1.0**
CVD/Mortality		
N (N cases/N controls)	287 (151/136)	720 (348/372)
Model 1		
HR (95% CI)	1.53 (1.06–2.21)	Reference
p	**0.02**	
Model 2		
HR (95% CI)	1.44 (0.97-2.14)	Reference
p	**0.07**	
CVD		
N (N cases/N controls)	175 (90/85)	427 (208/219)
Model 1		
HR (95% CI)	1.28 (0.80–2.05)	Reference
p	0.31	
Model 2		
HR (95% CI)	1.29 (0.77-2.16)	Reference
p	0.33	
Non-CVD mortality		
N (N cases/N controls)	112 (61/51)	293 (140/153)
Model 1		
HR (95% CI)	2.13 (1.15–3.93)	Reference
p	**0.02**	
Model 2		
HR (95% CI)	1.80 (0.93-3.46)	Reference
p	**0.08**	
All-cause mortality		
N (N cases/N controls)	123 (67/56)	330 (158/172)
Model 1		
HR (95% CI)	2.08 (1.17–3.69)	Reference

p	**0.01**	
Model 2		
HR (95% CI)	1.71 (0.93-3.16)	Reference
p	**0.08**	

Hazard ratios (HR) were obtained from conditional logistic regression models adjusted for the following CVD risk factors: age, treatment group, race, smoking status, systolic blood pressure, body mass index, fasting glucose, baseline LDL cholesterol level, baseline log-transformed triglyceride level, and family history of premature coronary heart disease. Model 1 was adjusted for the CVD risk factors listed above. Model 2 was further adjusted for HDL-related biomarkers (HDL-C, HDL-P and cholesterol efflux capacity).

Table S4. Effect of Randomized Rosuvastatin Therapy vs. Placebo on HDL-related Biomarker Levels.

HDL Phenotype	Rosuvastatin	Placebo	P Value vs. Placebo
HDL Cholesterol			
N	361	473	
Mean at baseline, mg/dL	51.7	51.0	
Mean at 12 months, mg/dL	55.0	51.8	
Percent Change (95% CI)	7.8 (5.9, 9.7)	2.5 (1.1, 3.9)	<0.00001
P Value vs. Baseline	<0.00001	0.0006	
Apolipoprotein AI			
N	305	421	
Mean at baseline, mg/dL	162.6	161.5	
Mean at 12 months, mg/dL	168.5	163.4	
Percent Change (95% CI)	7.2 (5.0, 9.4)	0.1 (-1.4, 1.6)	<0.00001
P Value vs. Baseline	<0.00001	0.89	
HDL Particle Number			
N	305	421	
Mean at baseline, mg/dL	31.4	31.5	
Mean at 12 months, mg/dL	33.3	31.4	
Percent Change (95% CI)	2.7 (0.9, 4.5)	-1.8 (-3.1, -0.4)	0.0001
P Value vs. Baseline	0.004	0.01	
HDL Inflammatory Index			
N	234	352	
Mean at baseline, mg/dL	0.58	0.60	
Mean at 12 months, mg/dL	0.52	0.56	
Percent Change (95% CI)	-5.3 (-8.9, -1.7)	-1.3 (-6.5, 4.0)	0.22
P Value vs. Baseline	0.005	0.63	

Table S5. Shifts across HDL Inflammatory Index Categories from Baseline to End of Follow-up.

HII Categories at baseline		**HII Categories at 12 months**		**95% CI; *p***
Entire cohort	**N**	**0–0.5**	**>0.5–1.0**	-0.07, 0.10; 0.71
0–0.5	168	115 (68%)	51 (30%)	
>0.5–1.0	399	113 (28%)	275 (69%)	
Statin Arm		**0–0.5**	**>0.5–1.0**	-0.13, 0.13; 0.99
0–0.5	73	50 (68%)	22 (30%)	
>0.5–1.0	154	46 (30%)	104 (68%)	
Placebo Arm		**0–0.5**	**>0.5–1.0**	-0.08, 0.14; 0.63
0–0.5	95	65 (68%)	29 (31%)	
>0.5–1.0	245	67 (27%)	171 (70%)	

Values in brackets represent row percentages

Table S6. Association of On-Statin HDL Inflammatory Index and Incident Events.

	HII 0–0.5	**HII >0.5–1.0**
CVD/Mortality		
N (N cases/N controls)	230 (113/117)	339 (170/169)
Model 1		
HR (95% CI)	1.03 (0.65-1.65)	Reference
p	0.89	
Model 2		
HR (95% CI)	1.16 (0.51-1.44)	Reference
p	0.56	
CVD		
N (N cases/N controls)	169 (83/86)	257 (129/128)
Model 1		
HR (95% CI)	1.18 (0.50-1.46)	Reference
p	0.56	
Model 2		
HR (95% CI)	1.35 (0.41-1.37)	Reference
p	0.34	
Non-CVD mortality		
N (N cases/N controls)	61 (30/31)	82 (41/41)
Model 1		
HR (95% CI)	2.80 (0.83-9.45)	Reference
p	0.10	
Model 2		
HR (95% CI)	1.68 (0.36-7.81)	Reference
p	0.51	
All-cause mortality		
N (N cases/N controls)	70 (34/36)	95 (48/47)
Model 1		
HR (95% CI)	2.09 (0.74-5.90)	Reference
p	0.17	

Model 2		
HR (95% CI)	1.22 (0.36-4.14)	Reference
p	0.74	

Hazard ratios (HR) were obtained from conditional logistic regression models adjusted for the following CVD risk factors: age, treatment group, race, smoking status, systolic blood pressure, body mass index, fasting glucose, baseline LDL cholesterol level, baseline log-transformed triglyceride level, and family history of premature coronary artery disease. Model 1 was adjusted for CVD risk factors. Model 2 was further adjusted for HDL-related biomarkers (HDL-C, HDL-P, cholesterol efflux capacity).

Figure S1. Histogram of HDL Inflammatory Index (HII) at Baseline.

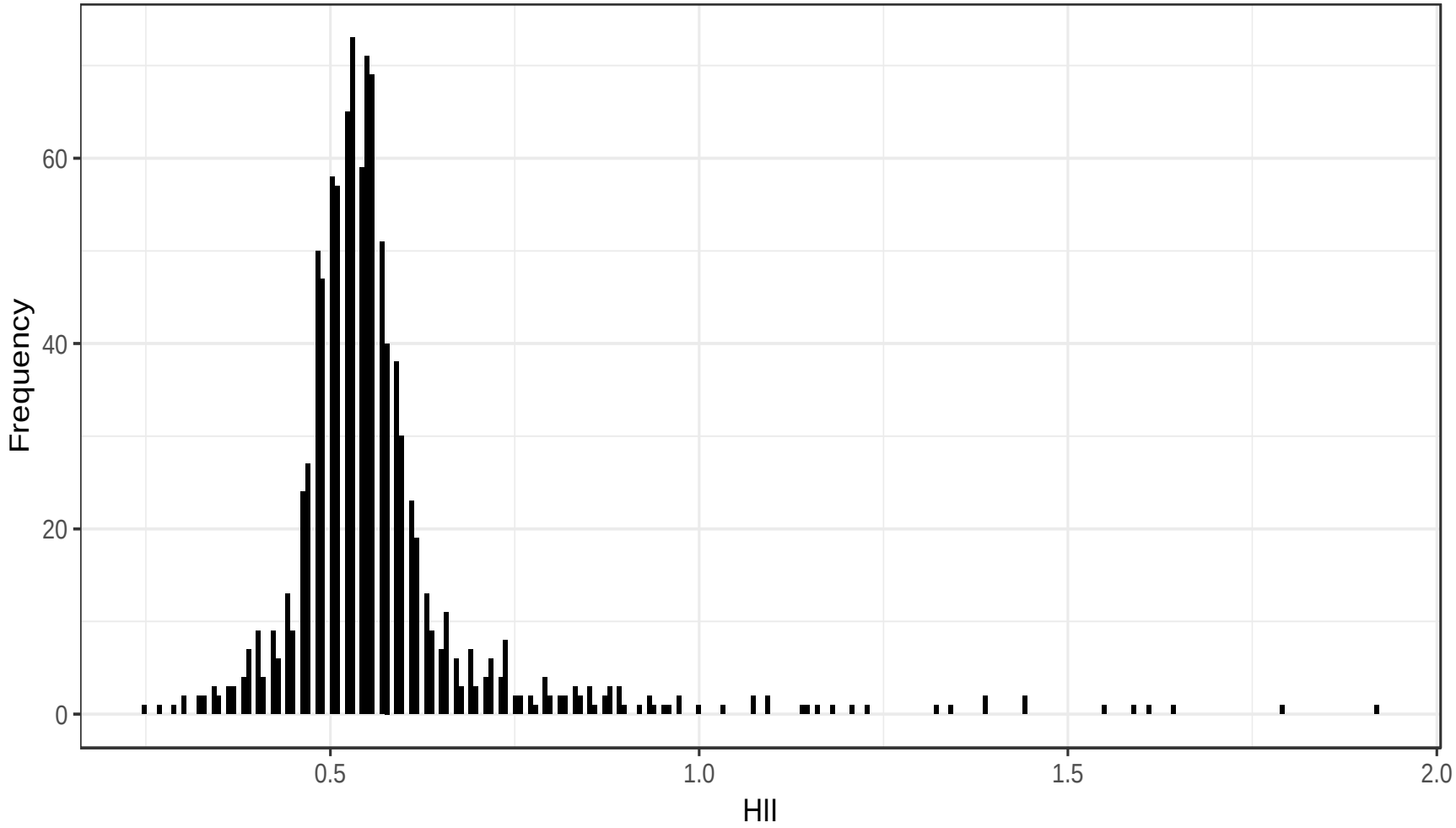
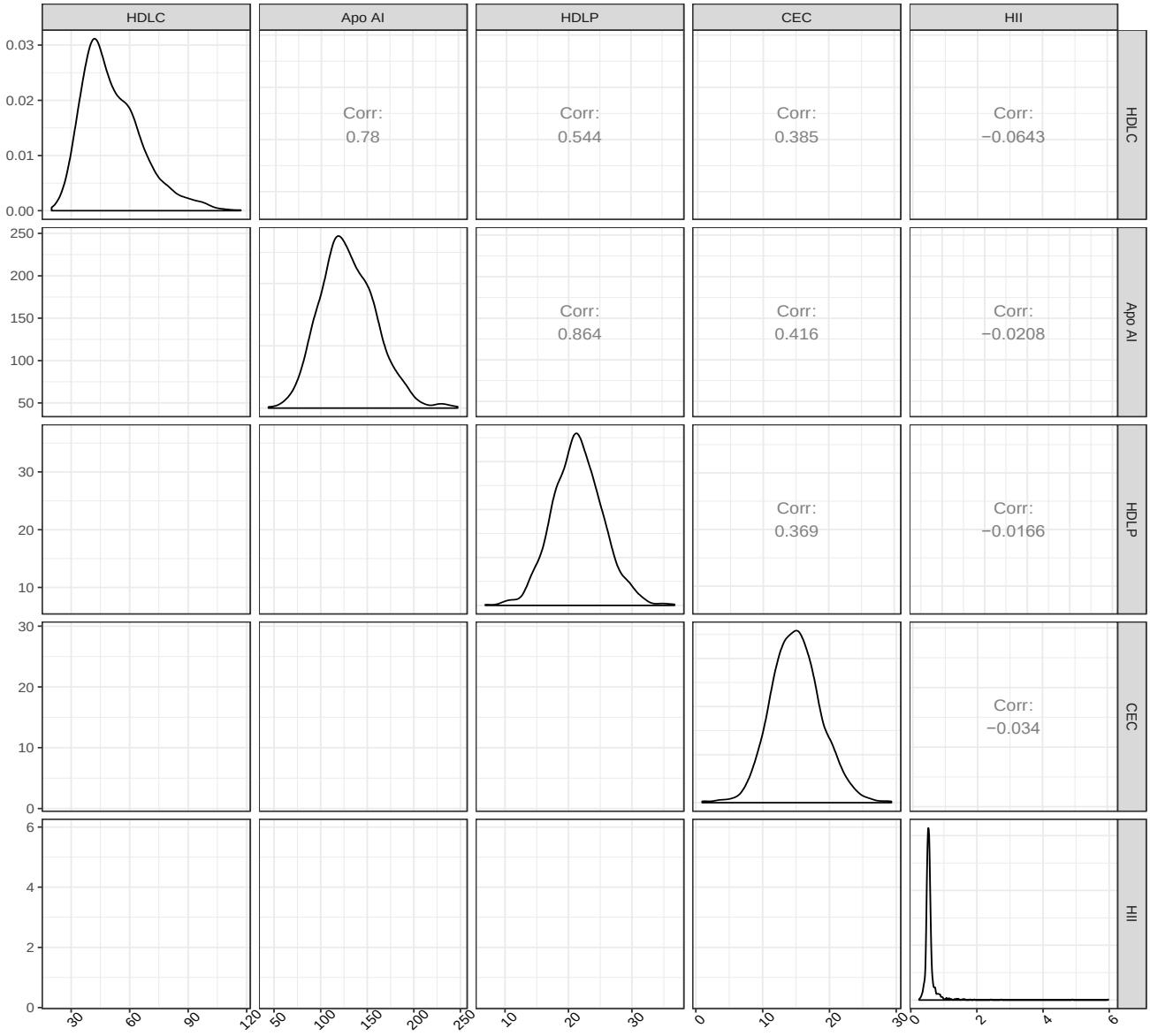
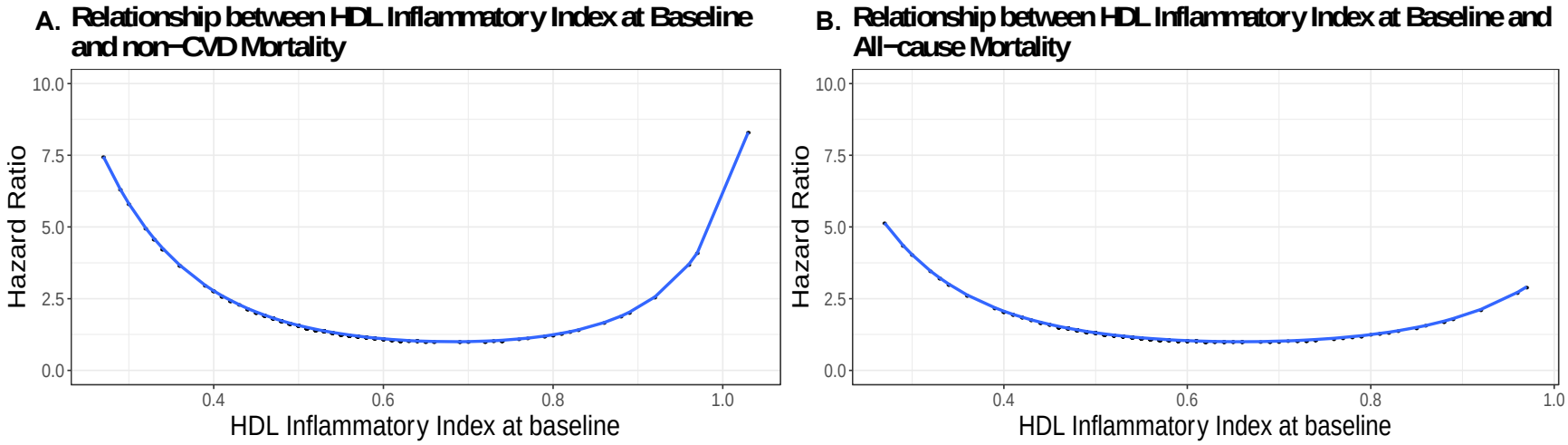


Figure S2. Spearman's Correlation Matrix of HDL-related Biomarkers at Baseline.



HDL-C – High-density lipoprotein cholesterol; Apo AI – Apolipoprotein AI; HDL-P – High-density lipoprotein particle number; CEC – Cholesterol Efflux Capacity; HII – HDL Inflammatory Index.

Figure S3. Relationship between HDL Inflammatory Index at Baseline and Mortality.



Conditional logistic regression was used to estimate CVD hazard ratios as a function of baseline HII adjusted for age, drug (statin vs. placebo), race, systolic blood pressure, cigarette smoking, BMI, glucose level, LDL-C, family history of premature coronary disease, triglycerides, and hsCRP. Regions with sparse data are not displayed (i.e. < and >2.5 SD).

From Figure 2, interactions of HDL-P and HII categories and apo AI and HII categories are significant. In the lowest risk category of HII (0.5 to 1.0), the relationship between HDL-P and CVD and apo AI and CVD is consistent with prior research (risk of CVD is lower with higher levels of these two biomarkers). However interestingly in the HII category 0 to 0.5, apo AI maintains this relationship with risk of CVD but HDL-P reverses its relationship. When HII is between 0 and 0.5, the risk of CVD is lower with lower levels of HDL-P.