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The Anti-Oxidative Capacity of High-Density Lipoprotein Is Reduced in Acute Coronary Syndrome But Not in Stable Coronary Artery Disease

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Objectives This study examined an anti-inflammatory property of high-density lipoprotein (HDL) in subjects with acute coronary syndrome (ACS) and stable coronary artery disease (CAD) compared with control subjects.

Background HDL has anti-inflammatory properties in vitro, but its relationship to coronary disease in humans is unclear. The high-density lipoprotein inflammatory index (HII) measures the ability of HDL to mitigate oxidation of low-density lipoprotein; this function may be impaired in ACS and/or CAD.

Methods We measured HII in 193 patients undergoing angiography for symptoms of CAD. Control subjects (n = 99) had no angiographic CAD, chronic CAD subjects (n = 51) had ≥70% vessel stenosis, and ACS subjects (n = 43) had ≥20% vessel stenosis and ischemia or infarction. We also examined HII in a cohort of healthy subjects randomly assigned to a statin or placebo.

Results Subjects who had ACS had higher HII (less antioxidative capacity) compared with controls (1.57 vs. 1.17, p = 0.005) or those with chronic CAD (1.57 vs. 1.11, p = 0.006). HII was not different in subjects with stable CAD compared with controls. Furthermore, those subjects with higher HII were more likely to have ACS than no CAD (quartile 4 vs. 1, odds ratio [OR]: 1.74, p = 0.008). In a multivariate logistic regression model, HII was associated with ACS after adjusting for traditional cardiac risk factors (OR: 3.8, p = 0.003). There was a small improvement in HII after statin therapy compared with placebo (−14%, p = 0.03).

Conclusions HDL has less anti-inflammatory capacity as assessed by HII in the setting of ACS compared with controls or subjects with chronic CAD. (J Am Coll Cardiol 2011;58:2068–75) © 2011 by the American College of Cardiology Foundation

The Framingham Heart Study and numerous confirmatory studies have demonstrated a strong inverse correlation between high-density lipoprotein (HDL) level and cardiovascular disease risk (1,2). Despite aggressive lowering of low-density lipoprotein (LDL), low HDL remains a significant cardiovascular risk factor in high-risk patients (3). Furthermore, the most recent trial of cholesteryl ester transfer protein inhibition raised HDL levels by more than 70% but resulted in significant harm (4). Additionally, mutations in HDL metabolism or structure result in varying levels of HDL that do not correlate with atherosclerotic risk as predicted by Framingham (5–7), and modifications to HDL that occur in various disease states seem to attenuate its atheroprotective effect (8–10).

HDL facilitates the efflux of cholesterol from cells such as macrophages, promoting reverse cholesterol transport. A recent study showed that cholesterol efflux capacity, or the ability of HDL to promote reverse cholesterol transport from macrophages, correlated with atherosclerosis, independent of HDL mass (11). Numerous additional functions have been ascribed to HDL, including roles in countering inflammation, oxidation, platelet activation, and promoting endothelial health (8). Many cell-based assays have been developed to measure HDL function, but the high-density lipoprotein inflammatory index (HII) is a measure of how well HDL can prevent the oxidation of LDL in a cell-free environment (12). A few, mostly small, studies have sug-

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gested that patients with underlying inflammatory conditions tend to have HDL that has less anti-inflammatory capacity as measured by the HII (12–20).

Patients with acute coronary syndrome (ACS) have flow-limiting lesions in the coronary arteries. But ACS constitutes a uniquely inflammatory milieu; for example, the proinflammatory cytokine CXCL16 is more highly expressed in subjects with acute myocardial infarction than those with chronic atherosclerosis (21). Given that even small changes in the microenvironment can alter HDL structure and function (22,23), we hypothesized that HDL has impaired anti-inflammatory capacity in patients with ACS, but not in those with stable CAD, as measured by the ability of HDL to prevent oxidation of LDL.

Abbreviations and Acronyms

ACS = acute coronary syndrome(s)
AIP = atherogenic index of plasma
apo = apolipoprotein
BMI = body mass index
CAD = coronary artery disease
CI = confidence interval
HDL = high-density lipoprotein
HII = high-density lipoprotein inflammatory index
LDL = low-density lipoprotein
OR = odds ratio
TG = triglycerides

Methods

Study population. The PennCATH (University of Pennsylvania Catheterization) study examined associations between biochemical and genetic markers in predominantly white patients undergoing catheterization to evaluate coronary anatomy (11,24,25). The study is institutional review board approved (Hospital of the University of Pennsylvania), and all subjects have provided written consent. We selected a new 3-arm, nested case-control sample from this population in a manner previously described (21). Briefly, 193 patients were chosen: 1) control subjects (n = 99) with no angiographic coronary artery disease (CAD); 2) chronic CAD cases (n = 51, >1 vessel with ≥70% stenosis); and 3) ACS cases (n = 43, any vessel with ≥20% stenosis in the setting of elevated cardiac enzymes (creatinine kinase [CK] and CK-MB, troponin I, or troponin T) and/or dynamic electrocardiogram changes). This nested case-control study included a case/control ratio of 1:2, with cases being either ACS or chronic CAD compared with control subjects. Subjects were selected consecutively from the median enrollment period, and the conductors of the HII assay were blinded to group assignment.

HII was additionally tested in available samples from subjects who were enrolled in a previously reported trial of statin therapy (26). Briefly, 99 subjects with high-normal cholesterol levels (LDL 130 to 220 mg/dl) but otherwise healthy, on no lipid-lowering therapies, were randomized to placebo (n = 24), pravastatin 40 mg daily (n = 23), atorvastatin 10 mg daily (n = 27), or atorvastatin 80 mg daily (n = 24). Serum was collected prior to treatment and after 16 weeks of therapy.

Measurement of HDL anti-inflammatory function. HII measures the ability of apolipoprotein (apo)B-depleted serum to inhibit or enhance the oxidation of LDL in the presence of a fluorescent organic substrate. The assay was performed essentially as previously described (12). After polyethylene glycol precipitation of apo B, HDL containing supernatant was used in the assay (Online Fig. 1). We directly measured HDL concentration and controlled for it with post hoc analysis. Additionally, pooled purified LDL was obtained from the Core Lipid Laboratory at the Children's Hospital of Philadelphia (Philadelphia, Pennsylvania); to optimize the redox reaction, LDL was then oxidized by $CuSO_4$ dialysis (27). Oxidized LDL was then dialyzed again in phosphate-buffered saline (PBS) and diluted in PBS to final concentration of 100 μg/ml prior to use as described (12).

The organic phospholipid 2′,7′-dichlorodihydrofluorescein diacetate (DCF) fluoresces when oxidized and exposed to light. This substrate was prepared as described previously (12). Oxidized LDL (final concentration: 1.4 μg/ml), DCF (final concentration: 2.9 μg/ml), and a fixed volume of apo 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 round-bottom polypropylene microtiter plate (Fisher Scientific, Pittsburgh, Pennsylvania). The plate was incubated at 37°C in a microplate reader (Spectra Max, Gemini XS, Molecular Devices, Sunnyvale, California). Serial excitations at 485 nm were performed every 90 s, accompanied by automated plate shaking. Fluorescence at emission wavelength of 530 nm and cutoff of 515 nm was measured after 1 h of incubation. Samples were plated in duplicate, and mean fluorescence recorded. The mean intra-assay coefficient of variation for all samples was 4.1%.

HII was calculated by the following formula: optical density of DCF incubated with apo B–depleted serum minus the optical density of DCF incubated alone. To correct for inter-assay variability across different plates, a pooled serum control from 3 healthy volunteers was included on each plate, and values for samples from subjects in the study were normalized by this pooled value. Additional tests performed to validate the measurement of the HDL inflammatory index are described in the Online Appendix (Online Figs. 2 and 3).

Other biochemical and clinical markers. We examined the association of HII with other markers of HDL function and clinical or biochemical markers of atherosclerosis. HDL-mediated cholesterol efflux capacity was calculated as previously described, and primary data were re-analyzed in this study (11). The atherogenic index of plasma (AIP) is the logarithmic transformation of the ratio of plasma triglyceride (TG) level to HDL level. This measure correlates with angiographic CAD in a population of subjects at risk for atherosclerosis (28). We calculated AIP = log([TG]/[HDL]) for our study population.

Statistical analysis. Results are expressed as mean and SD for continuous variables, or median and interquartile range if data were skewed. HII and TG level showed a non-normal distribution in our population, similar to previous findings (28); this corrected after logarithmic transformation, which was used in all subsequent analyses (Online Fig. 4). Study groups were compared using analysis of variance and Student *t* test, and the association of HII with clinical variables was

assessed with the use of Pearson's correlation coefficients. Chi-square or Fisher exact test was employed for comparisons between nominal variables. For non-Gaussian data, nonparametric tests were employed, including Wilcoxon/Kruskal-Wallis rank sum tests and Van der Waerden test for group differences, and Spearman rank correlation coefficients for linear correlations. Multinomial logistic regression was used to examine the association between HII and coronary artery disease status after adjustment for age, sex, smoking status, diabetes, hypertension, cholesterol level, triglyceride level, LDL level, and HDL mass. Significant p values were <0.05 on 2-tailed analysis. Analysis of HII and other variables before and after statin treatment was performed using matched pairs *t* tests. Data were analyzed using JMP 8.0 statistical software (SAS Institute, Cary, North Carolina).

Results

Association of HII with traditional cardiac risk factors. We examined the relationship between HII and traditional cardiac risk factors in this cohort of high-risk patients (Fig. 1). Sex, age, smoking status, diabetes status, hypertension status, total cholesterol, and LDL concentration did not correlate with HII ($p > 0.05$). However, HDL concentration was inversely

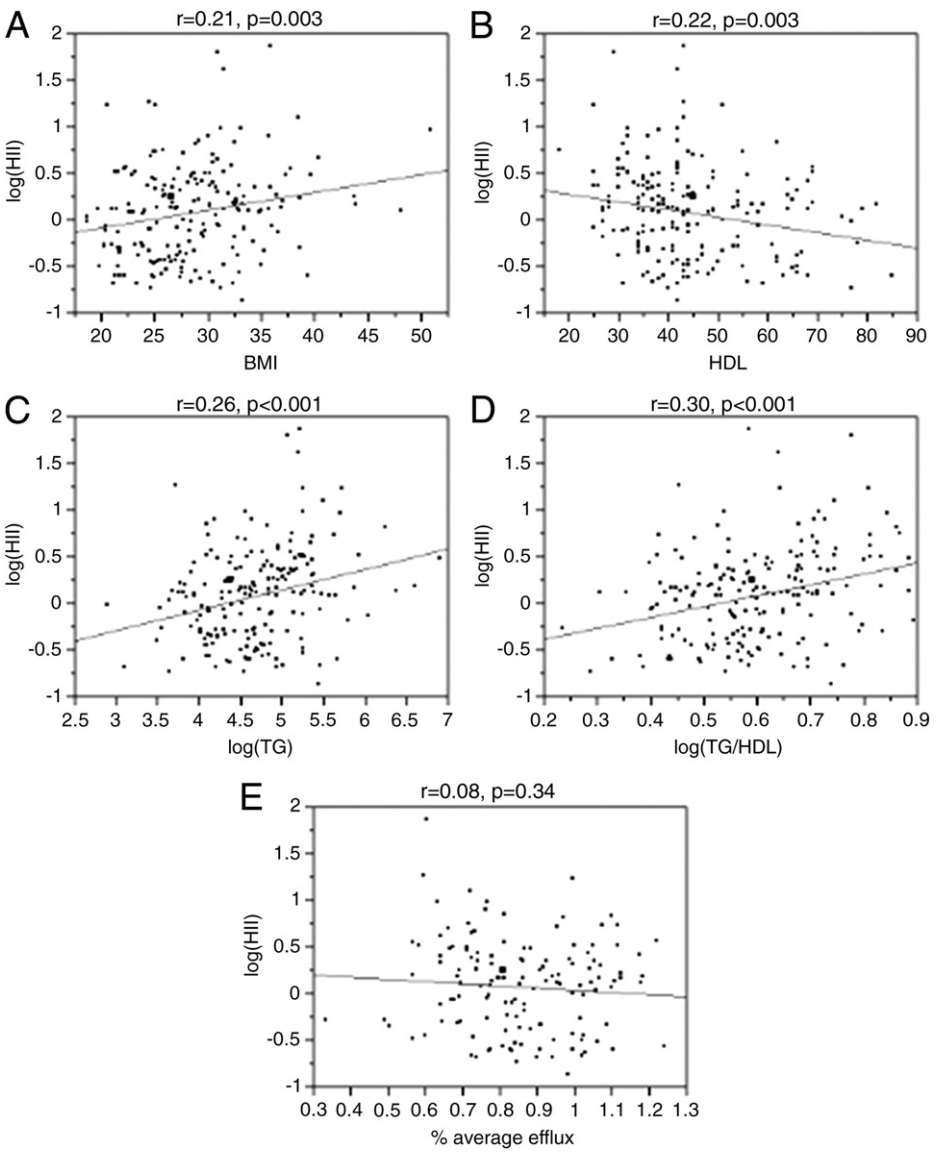

Figure 1 Association of HII With Traditional Cardiac Risk Factors

On bivariate analysis, body mass index (BMI) **(A)**, high-density lipoprotein (HDL) concentration **(B)**, triglyceride (TG) level **(C)**, and atherogenic index of plasma (AIP, log[TG/HDL]) **(D)** all correlated significantly with high-density lipoprotein inflammatory index (HII). HDL-mediated cholesterol efflux capacity (percent average efflux) **(E)** did not correlate with HII. Values for HII and triglyceride level were logarithmically transformed to achieve a normal distribution prior to analysis.

Table 1 Baseline Clinical Characteristics of CAD Subjects and Controls

	Control (n = 99)	Chronic CAD (n = 51)	Acute Coronary Syndrome (n = 43)
Male	59 (60%)	40 (78%)*	31 (72%)*
Age, yrs	61.8 ± 10.2	55.9 ± 7.9†	59.1 ± 11.5‡
BMI, kg/m^2	28.3 ± 5.8	29 ± 5.6	29 ± 4.6
Smokers	43 (45%)	28 (55%)	21 (50%)
Diabetic patients	10 (10%)	8 (16%)	9 (20%)
Hypertensive patients	48 (49%)	30 (59%)	25 (60%)
Log(triglycerides)	4.77 (4.62–4.93)	4.61 (4.48–4.75)	4.81 (4.62–5.00)
Total cholesterol, mg/dl	174 ± 37	177 ± 37	170 ± 40
HDL, mg/dl	46 ± 15	44 ± 12	42 ± 8.7
LDL, mg/dl	103 ± 30	106 ± 30	99 ± 33

Values are density (%), mean ± SD, or mean (95% confidence interval). *p = 0.05 by Pearson chi-square test, compared to control; †p < 0.01 by 2-tailed Student *t* test, chronic CAD compared with control. ‡There was no significant difference in age between the chronic CAD and ACS subjects (p = 0.11) or the control and ACS subjects (p = 0.15).

BMI = body mass index; CAD = coronary artery disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

correlated with HII, as expected (p = 0.003). Additionally, body mass index (BMI) and TG correlated positively with HII (p = 0.003 and p < 0.001, respectively). HII and AIP, which incorporates both HDL and TG concentration, also correlated positively (p < 0.001). HII did not correlate with cholesterol efflux capacity.

Correlation of HII with CAD. The baseline characteristics of the case-control cohort are presented in Table 1. Distribution of HII in the study population is shown in Online Figure 4.

After log transformation of HII values, subjects with ACS had significantly higher average HII than control subjects, despite having no difference in average HDL cholesterol (Fig. 2). Mean HII for acute CAD subjects was 1.57 ± 1.15, and mean HII for control subjects was 1.17 ± 0.58 (p = 0.005; Online Fig. 5). There was no difference between mean HII in chronic CAD subjects (1.11 ± 0.70) and control subjects (p = 0.6). However, there was a significant difference between ACS subjects and chronic CAD subjects (p = 0.006). Furthermore, these results persisted after controlling for HDL concentration (acute vs. control, p = 0.02; acute vs. chronic, p = 0.03; control vs. chronic, p = 0.6). Results were not different upon non-parametric analysis using non–log-transformed values for HII (Online Fig. 5).

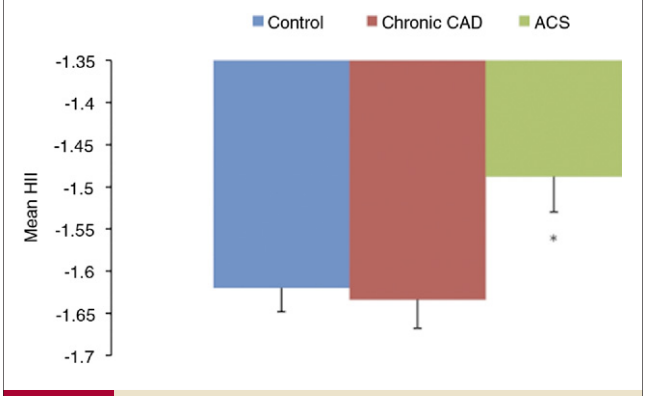

Figure 2 Mean HII Is Different Among Cases and Controls

The high-density lipoprotein inflammatory index (HII) was measured in 99 control subjects, 51 chronic coronary artery disease (CAD) subjects, and 43 subjects with angiographic CAD and either dynamic electrocardiogram changes or elevation of cardiac enzymes. Data are presented here after log transformation of HII (for non-parametric results, see Online Fig. 5). **Error bars** represent the standard error of the mean. There was no significant difference between mean HII in control subjects and those with chronic CAD. *p = 0.005, *t* test comparison of individual means, acute coronary syndrome (ACS) versus control.

The proportion of subjects with ACS increases as HII increases (Fig. 3). In the multivariate logistic regression model, higher HII was associated with an increased risk of ACS compared with control subjects (odds ratio [OR] per 1 SD increase in HII was 3.8 [95% confidence interval (CI): 3.35 to 4.25]; p = 0.003). Additionally, subjects with higher HII had an increased risk of having ACS compared with having chronic CAD (OR: 4.8 [95% CI: 4.3 to 5.2]; p = 0.002). Comparison of chronic CAD subjects with controls revealed only age as a predictive risk factor (OR: 0.94 [95% CI: 0.92 to 0.96]; p = 0.005). Higher age was not significantly predictive of chronic CAD compared with ACS (OR: 1.1 [95% CI: 0.8 to 1.4]; p = 0.055).

We further divided our cohort into quartiles of HII to provide additional support for the correlation between HII and ACS status (Table 2). Subjects who were in quartile 4 of HII were more likely to have ACS compared with either controls or chronic CAD subjects (OR: 1.74 [95% CI: 1.52 to 1.96]), and this risk persisted after controlling for traditional cardiovascular risk factors, including HDL cholesterol (OR: 1.23 [95% CI: 1.15 to 1.31]). The same findings were seen in ACS subjects compared with chronic CAD subjects before and after risk factor adjustment (OR: 1.91 [95% CI: 1.69 to 2.13]; after adjustment OR: 1.17 [95% CI: 1.12 to 1.22]). Being in quartile 2 or 3 of HII did not significantly increase the risk of having ACS compared with controls or chronic CAD before or after risk factor adjustment.

Statin treatment and HII. Treatment of healthy subjects with statin therapy produced expected decreases in total cholesterol, triglycerides, C-reactive protein (CRP), and an insig-

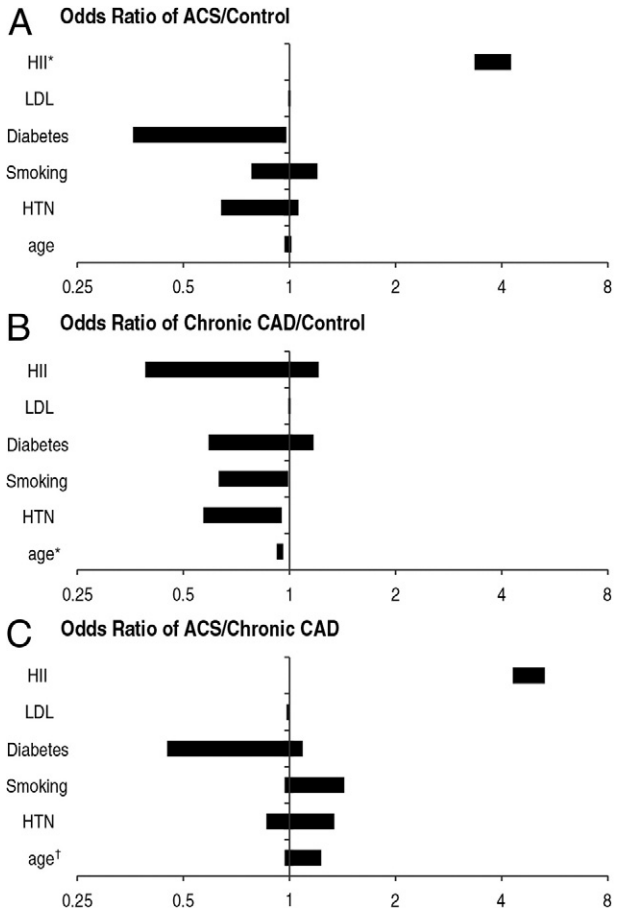

Figure 3 ORs for CAD According to HII and Selected Risk Factors

The multivariate logistic regression model was also adjusted for HDL concentration. HII is a significant risk factor for ACS compared with no CAD **(A)** or chronic CAD **(C)**, but HII does not confer risk of chronic CAD compared with controls **(B)**. **Bars** represent 95% confidence intervals for each odds ratio. *p $<$ 0.01; †p = 0.055. HTN = hypertension; LDL = low-density lipoprotein; other abbreviations as in Figures 1 and 2.

nificant increase in HDL compared with treatment with a placebo (Table 3). Additionally, the decrease in cholesterol was significant and consistent across all statin treatments compared with baseline. Mean HII did not change in the placebo group, but there was a trend for decreased HII with individual statin therapy compared with baseline. Taken in aggregate, treatment with any statin produced a significant decrease in HII compared with placebo (Fig. 4). Interestingly, on bivariate analysis after log transformation, baseline CRP value correlated significantly with HII (r = 0.25, p = 0.02), but this correlation did not persist after treatment.

Discussion

In this study, we found that the ability of HDL to prevent oxidation of LDL correlated strongly with ACS status. There was no association between HII and chronic CAD, but there was a significant difference between subjects with ACS and those with chronic CAD. Furthermore, all of these associations persisted in a prediction model after adjustment for traditional cardiovascular disease risk factors including HDL mass. Our results are the first to our knowledge to demonstrate decreased HDL anti-inflammatory capacity in the setting of ACS.

HDL inflammatory index measures one aspect of HDL's many purported atheroprotective properties. HDL is a chimeric molecule that changes its structure and composition with small changes in the environment; even slightly altered diets can produce different HDL molecules (29). And minor changes in microenvironment especially alter the anti-inflammatory properties of HDL. For example, in chronic inflammatory conditions such as systemic lupus erythematosis and rheumatoid arthritis, atherosclerosis correlates with HII (30). ACS radically changes the circulating environment for HDL, involving both acute inflammation and oxidation, either as part of its pathogenesis or resulting from the ensuing damage (31,32).

Chronic CAD subjects did have significantly less HDL-mediated cholesterol efflux capacity than control subjects in a similar population (11); that study did not, however, include any ACS subjects. There may be a significant difference in the functional assessment of various aspects of the HDL molecule, a concept supported by the lack of association between cholesterol efflux capacity and HII within the same subjects in our study (Fig. 1). In our cohort of ACS patients undergoing angiography, the acute inflammation that occurred with ACS may differ significantly from the chronic inflammation of stable CAD, thus impacting differentially on the HDL inflammatory index.

Table 2 CAD Status According to Quartile of HII

		Unadjusted OR		Adjusted OR for CAD Risk Factors*	
Quartile	n	ACS vs. Control	ACS vs. Chronic CAD	ACS vs. Control	ACS vs. Chronic CAD
1	48	1.00	1.00	1.00	1.00
2	48	4.05 ± 0.63	7.50 ± 0.70	1.30 ± 0.21	1.41 ± 0.17
3	48	1.23 ± 0.34	1.56 ± 0.36	1.09 ± 0.10	1.07 ± 0.08
4	49	1.74 ± 0.21†	1.91 ± 0.22†	1.23 ± 0.081†	1.17 ± 0.047†

Odds ratios (ORs) presented are compared to quartile 1 and represent OR ± SE. *CAD risk factors included age, smoking, diabetes, hypertension, HDL, and LDL. †p $<$ 0.01.
HII = high-density lipoprotein inflammatory index; other abbreviations as in Table 1.

Table 3 Effect of Statin Therapy on HII

Parameter	Placebo			Pravastatin 40 mg			Atorvastatin 10 mg			Atorvastatin 80 mg			Any Statin		
	Baseline	16 Weeks	% Change	Baseline	16 Weeks	% Change	Baseline	16 Weeks	% Change	Baseline	16 Weeks	% Change	Baseline	16 Weeks	% Change
Total cholesterol	239 ± 35	244 ± 4.00	2.0 ± 8.1	243 ± 24	194 ± 21	−19.7 ± 9.6*†	244 ± 26	188 ± 34	−22.6 ± 14.0*†	244 ± 35	161 ± 33	−32.3 ± 18.0*†			
Log(triglycerides)	4.91 ± 0.51	4.95 ± 0.66	13 ± 54	4.84 ± 0.39	4.76 ± 0.41	−2.3 ± 35.0†	4.93 ± 0.45	4.70 ± 0.43	−14.4 ± 27.0*	4.72 ± 0.65	4.43 ± 0.47	−19.1 ± 35.0*			
HDL	47.0 ± 9.8	46.3 ± 42.0	0.13 ± 17.00	49 ± 12	50.3 ± 12.0	4 ± 13	46.0 ± 9.7	51 ± 13	5.8 ± 26.0	48 ± 13	49 ± 15	2.87 ± 16.00			
Log(HII)	0.61 ± 0.23	0.63 ± 0.28	7.8 ± 69.0	0.60 ± 0.29	0.49 ± 0.26	−21 ± 62	0.55 ± 0.26	0.50 ± 0.22	−10 ± 31	0.57 ± 0.19	0.50 ± 0.21	−11 ± 39	0.57 ± 0.25	0.50 ± 0.21	−14.0 ± 5.0‡§
CRP	2.48 ± 2.8	2.28 ± 2.10	31.4 ± 98.0	1.9 ± 1.9	1.85 ± 1.80	9.8 ± 72.0	2.39 ± 0.19	1.92 ± 1.90	−11 ± 46†	1.95 ± 1.50	1.45 ± 1.30	−25 ± 49*			

Values are mean ± SD. *p < 0.01 compared with placebo; †p < 0.001 compared with baseline; ‡p <0.05 compared with placebo; §p = 0.053 compared with baseline.
CRP = C-reactive protein; other abbreviations as in Tables 1 and 2.

Figure 4 HII Improves After Statin Therapy

Subjects were treated with placebo, 40-mg pravastatin, 10-mg atorvastatin, or 80-mg atorvastatin daily. Percent change reflects high-density lipoprotein inflammatory index (HII) in serum samples obtained at the baseline visit and after 16 weeks of therapy, and **error bars** represent SEM: −21 ± 13%, p = 0.07 (pravastatin); −10 ± 6%, p = 0.16 (atorvastatin 10 mg); −11 ± 8%, p = 0.12 (atorvastatin 80 mg); −14 ± 5%, p = 0.03 (any statin). *p = 0.03.

We further examined associations between biomarkers of cardiovascular disease and HII. The AIP correlates with angiographic CAD, lipoprotein size, and lipoprotein composition, and in type 2 diabetic patients, reduces after treatment with pioglitazone (22,28,33–36). There was a clear and direct association between AIP and HII. AIP tracks with changes in lipoprotein composition, which in turn is influenced by an inflammatory milieu. Although we did not see a correlation between subjects with AIP and ACS compared with chronic CAD subjects and controls (data not shown), the positive association between AIP and HII in all subjects suggests that HDL particle composition, for which AIP is a surrogate, can influence the anti-inflammatory properties of HDL.

We speculate here a role for HDL in ACS. HDL cholesterol levels can be lowered in the short term in ACS (37–39). Additionally, HDL does have many interactions that promote endothelial and myocardial health. For example, in rats with induced cardiac ischemia, HDL infusion in the acute setting enhances myocardial recovery (40). Furthermore, infusion of apo A1 particles in human subjects results in short-term HDL increase and immediate restoration of endothelial function as measured by forearm venous occlusion plethysmography (41,42). The question remains, then, whether HDL with reduced anti-inflammatory capacity is a contributor or innocent bystander in acute CAD.

Another finding from our study was that BMI correlated positively with HII. Others have shown that obstructive sleep apnea associates with impaired HDL, perhaps because of increased oxidative stress from periods of hypoxia (43). The strong association between BMI and obstructive sleep apnea may explain our findings of increased HII in subjects with higher BMI. High BMI and high TG levels correlate in metabolic syndrome, which may involve, not only low HDL, but more inflammatory HDL as well. Indeed, others have

demonstrated smaller, denser, and more dysfunctional HDL in subjects with metabolic syndrome (44,45). HII can be an important measure of HDL function in these patients.

Treatment with statins decreased mean HII compared with placebo. One intriguing possibility is that statins exert an anti-inflammatory effect through changes in HDL composition and function (46). However, statin therapy did not affect cholesterol efflux capacity (11), once again providing evidence that there may be many pathways to influence different aspects of HDL function.

Conclusions

HDL's function as an anti-inflammatory molecule can be measured reproducibly and reliably using the HII assay applied to human serum after depletion of apoB lipoproteins. HII is higher (reflecting reduced anti-inflammatory capacity of HDL) in the setting of ACS, but not in the setting of chronic CAD. HDL's anti-inflammatory function is not correlated with cholesterol efflux capacity, suggesting that the HII provides orthogonal information to the efflux assay. Improved HDL function remains an important therapeutic target, and our findings may aid in assessing therapies that increase HDL mass and also improve HDL function, in both chronic disease states and acute clinical syndromes.

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Key Words: acute coronary syndrome(s) ■ coronary artery disease ■ HDL function ■ HDL inflammatory index ■ oxidized LDL.

APPENDIX

For supplemental figures, please see the online version of this paper.