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Low HDL cholesterol and particle concentrations are associated with greater levels of endothelial activation markers in Multi-Ethnic Study of Atherosclerosis participants

Brian T. Steffen¹, Suzette J. Bielinski², Paul A. Decker³, Cecilia Berardi^{2,4}, Nicholas B. Larson³, James S. Pankow⁵, Erin D. Michos⁶, Naomi Q. Hanson¹, David M. Herrington⁷, and Michael Y. Tsai¹

¹Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN

²Division of Epidemiology, Department of Health Sciences Research, Mayo Clinic, Rochester, MN

³Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN

⁴Department of Internal Medicine, Albert Einstein College of Medicine, Bronx, NY

⁵Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN

⁶Division of Cardiology, Johns Hopkins University School of Medicine, Baltimore, MD

⁷Heart and Vascular Center of Excellence, Wake Forest University School of Medicine, Winston-Salem, NC

Abstract

Background—High density lipoproteins (HDL) are well-characterized for their role in reverse cholesterol transport but may confer other cardiovascular benefits—specifically, HDL may suppress the endothelial activation cascade in the initiating stages of atherogenesis.

Corresponding Author: Dr. Michael Y. Tsai, 420 Delaware St. SE, Mayo Mail Code 609, Minneapolis, MN 55455-0392, Phone: 612-626-3629, Fax: 612-625-1121, tsai001@umn.edu.

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CONTRIBUTIONS

Brian T Steffen: composed the manuscript text; contributed to the study design; interpreted results; expedited revisions

Suzette J Bielinski: contributed to study design; interpreted results; assisted with revisions

Paul A Decker conducted the statistical analysis; interpreted results; assisted with revisions

Cecilia Berardi: assisted with interpreting results; assisted with revisions

Nicholas B Larson assisted with the statistical analysis and contributed to the manuscript text; assisted with revisions

James S Pankow: assisted with the statistical analysis, manuscript text, interpreting results; assisted with revisions

Erin D Michos: assisted with interpreting results; assisted with revisions

Naomi Q Hanson: assisted with interpreting results; assisted with revisions

David M Herrington: assisted with interpreting results; assisted with revisions

Michael Y Tsai: contributed to the text of the manuscript and study design; interpreted results; assisted with revisions

DISCLOSURES

The authors have no disclosures. All authors have approved the final article.

Objective—It was the primary aim of this study to examine the relations of HDL-cholesterol (HDL-C), total HDL-particle (HDL-P) concentrations, and HDL-P subclasses with circulating levels of endothelial activation markers in a subcohort of Multi-Ethnic Study of Atherosclerosis participants.

Methods—HDL-C was measured by enzymatic assay, and total HDL particle and subclass concentrations were assessed by nuclear magnetic resonance spectroscopy. Concentrations of circulating endothelial activation markers were determined through immunoassay. Multivariable linear regression was used to determine the cross-sectional associations between HDL variables and endothelial markers with statistical adjustment for age, race/ethnicity, sex, education, systolic blood pressure, hypertension medication use, BMI, smoking status, lipid lowering medication use, serum creatinine, diabetes, LDL-C, and coronary artery calcium.

Results—HDL-C and HDL-P were found to be inversely associated with soluble vascular cell adhesion molecule-1 (sVCAM-1), intracellular adhesion molecule-1 (sICAM-1), sL-selectin, and sP-selectin; HDL-P was additionally inversely associated with sE-selectin. Participants with low levels of HDL-C (<40 mg/dL) or HDL-P (<25th percentile) showed 3–12% higher mean levels of sVCAM-1 and sICAM-1 compared to those above these levels (all $p<0.01$).

Conclusion—Coupled with previous evidence, our findings suggest a modest to moderate relation of HDL and circulating levels of endothelial activation markers in humans. Whether this relationship may have clinical implications in suppressing atherogenesis or coronary heart disease development requires additional research.

Keywords

Epidemiology; HDL; lipoproteins; NMR; risk factors; endothelial activation; cellular adhesion

INTRODUCTION

High density lipoprotein cholesterol (HDL-C) is a well-described lipid measure used in cardiovascular (CV) risk assessment and has been consistently associated with lower incidence of coronary heart disease (CHD) (1–3) and stroke (4). The CV benefit of HDL particles has historically been attributed to their role in reverse cholesterol transport; however, HDL is now recognized as a functionally diverse lipoprotein population that reduces disease incidence by suppressing inflammation, oxidative stress, and, potentially, endothelial activation (5, 6). Of these, the relation of HDL with endothelial activation remains the least well-studied in large cohort populations.

Endothelial activation is a critical component of the immune response but is also an initiating event in atherogenesis. It has been suggested that HDL serves as a negative regulator of endothelial activation by reducing expression of leukocyte adhesion molecules that facilitate transport across the endothelium. Indeed, numerous studies using experimental models have shown that HDL suppresses the expression of vascular cellular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) (7–17), although null findings have also been reported (18, 19). By contrast, evidence in human studies is equivocal with some showing that HDL is inversely associated with circulating levels of soluble VCAM-1 (sVCAM-1), sICAM-1, and sE-selectin (20, 21), while others have shown

no correlations (22, 23). Taken together, experimental studies have documented an HDL-mediated reduction in endothelial activation protein expression, but reports in humans are currently inconclusive. A larger cohort study may provide further insight in to whether HDL levels are related to these markers of vascular inflammation.

A number of variables will be considered in this examination of HDL. First, there is an ongoing controversy as to whether total HDL particle number (HDL-P) serves as a more accurate measure of the lipoprotein pool or better describes relations with outcomes than total HDL-C levels (6). It is also important to recognize that HDL is a heterogeneous pool of small, medium, and large lipoproteins, and these subclasses may differentially affect expression of endothelial activation proteins (7, 24). Therefore, total HDL-P and particle size subclasses will be tested alongside HDL-C. In addition, a non-linear relationship between HDL and endothelial activation has been previously observed in which clinically low levels of HDL-C (<40 mg/dL) promote expression of endothelial activation proteins that is not observed in individuals above this level (20, 21). Such a threshold-based model may provide a more clinically relevant description of HDL and endothelial activation than examining HDL as a continuous variable and will be evaluated here. Overall, cohort-based research of HDL and endothelial activation markers remains limited, and the current analysis of HDL-C, total HDL-P, HDL-P subclasses, and six markers of endothelial activation in Multi-Ethnic Study of Atherosclerosis participants (MESA) represents one of the largest and most comprehensive studies conducted so far.

MATERIALS AND METHODS

Study Population

The study design and methods of MESA have been previously documented (25), and information regarding study protocol is available online (www.mesa-nhlbi.org). Briefly, 6,814 men and women aged 45 to 84 years without clinical evidence of CV disease were recruited from six communities in the United States (Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; New York, NY; and St. Paul, MN). All participants gave informed consent, and Institutional Review Board approval was obtained at all MESA sites.

Recruitment and the baseline examination of MESA (Exam 1) were conducted between the years of 2000–2002. The 2nd Examination of MESA (Exam 2) was conducted between the years of 2002–2004. At all MESA visits, information regarding age, sex, race/ethnicity, education, medication usage, and lifestyle factors was obtained by questionnaires. Height, weight, and blood pressure were measured by trained staff according to standard procedures. Body mass index (BMI) was calculated as weight (kg)/height (m²). Adjustments were also made for systolic blood pressure, anti-hypertensive medication use, and diabetes as defined by use of diabetes medications, fasting blood glucose >126, or physician diagnosis.

Laboratory measurements

Endothelial markers were measured in two different MESA subsets. Each subset was randomly selected from all race/ethnicities (to avoid selection bias) from either Exam 1 or

Exam 2. As a result, the number of MESA participants within analyses was dependent upon the availability of the endothelial activation marker exposure variable: sVCAM-1 (n=2,386); sICAM-1 (n=2,373); sE-selectin (n=978); sL-selectin (n=2397); MMP-1 (n=2378); sP-selectin (n=6,017).

Fasting blood was drawn and serum and EDTA-anticoagulant tubes were collected and processed using a standardized protocol (25). Lipids were assayed at a central laboratory (Collaborative Studies Clinical Laboratory at Fairview-University Medical Center, Minneapolis, MN) on thawed EDTA plasma obtained from the baseline exam within two weeks of the sample collection as previously described (26). HDL-C was measured from Exam 1 specimens using the cholesterol oxidase method (Roche Diagnostics) following magnesium/dextran precipitation of non-HDL-C (coefficient of variation= 2.9%). HDL particle concentrations were measured on thawed EDTA plasma specimens (−70 °C) and determined from the amplitude of their unique nuclear magnetic resonance signals (27). Inter-assay reproducibility of total HDL particles was determined from replicate analyses of plasma pools and found to be <5%.

Measurement of endothelial activation markers in this subset of MESA has been described previously (28). Briefly, sE-selectin was measured in a random sample of participants at Exam 1; sVCAM-1, sL-selectin, MMP-1 and sICAM-1 were measured in a random sample of participants at Exam 2. P-selectin was measured on the complete cohort at Exam 2; however, two independent groups of samples were assayed. To address differences in sP-selectin levels across batches, residuals were obtained from a linear regression model with log-transformed raw sP-selectin as the outcomes and batch as the predictor—these residuals were used for all analyses. Soluble VCAM-1, sL-selectin, sE-selectin, and MMP-1 were measured in serum by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA). Soluble ICAM-1 was measured in serum by ELISA using ELISA kits purchased through Bender MedSystems, Vienna. Soluble P-selectin was measured in plasma by ELISA (R&D Systems).

Statistical Analysis

Statistical analysis was conducted using SAS version 9.3 and R version 3.2.3. Baseline characteristics are reported as mean and standard deviation for continuous variables and frequency (%) for categorical variables. Participant characteristics were compared between men and women using the two-sample t-test or chi-square test as appropriate. Adhesion proteins and HDL variables were log-transformed as appropriate. Linear regression models assessed cross-sectional relationships between HDL variables and endothelial markers. For these analyses, HDL measures served as the independent variable, and log transformed proteins served as outcome variables. HDL measures were scaled by dividing by the overall standard deviation for each HDL measure. The effect sizes and 95% confidence intervals were calculated by exponentiating the parameter estimates from the regression model. Assumptions of linearity were assessed using generalized additive models with cubic B-splines. If the linearity assumption did not hold, analyses were performed using splines or cut points as appropriate. Age, race/ethnicity, sex, education, systolic blood pressure, hypertension medication use, BMI, smoking status, lipid lowering medication use, serum

creatinine, diabetes, LDL-C, and baseline coronary artery calcium were included as covariates. Sex interactions were assessed and sex-stratified analyses were performed as appropriate. Covariates measured at exam 1 were used for regression analyses involving proteins measured at exam 1; covariates measured at exam 2 were used for regression analyses involving proteins measured at exam 2.

RESULTS

Demographic characteristics of all MESA participants at Exams 1 and 2 are shown in Table 1. Demographics of the subcohort are shown in Supplemental Table 1. In general, the participant characteristics of the subcohort were very similar to the overall MESA cohort due to random sampling. Lipid and lipoprotein measures are shown in Table 2. Women showed significantly higher levels of HDL-C and total HDL-P as well as greater numbers of medium and large HDL-P compared to men. Concentrations of endothelial activation markers are shown in Table 3. Women showed significantly higher levels of L-selectin and MMP-1 but lower levels of E-selectin and P-Selectin than men.

Table 4 shows associations of HDL measures with circulating levels of soluble endothelial activation markers in a subcohort of MESA participants with further stratification by sex. Multiple adjustments were made to control for other confounding covariates that may influence associations including age, sex, race/ethnicity, education level, systolic blood pressure, hypertension medication use, BMI, smoking status, lipid lowering medication use, serum creatinine, and LDL-C. An additional measure of cardiovascular health, coronary artery calcium, was also included in the model. Supplemental Tables 2a–c show the regression coefficients for all the variables in the adjusted models for comparison of HDL-C with the other included covariates. In the overall subcohort, HDL-C was inversely associated with sVCAM-1 ($p=0.002$), sICAM-1 ($p<0.001$), sL-selectin ($p=0.04$), and sP-selectin ($p<0.001$). HDL-P was similarly inversely associated with sVCAM-1 ($p<0.001$), sICAM-1 ($p<0.001$), sL-selectin ($p=0.001$), and sE-selectin ($p=0.01$). A direct relationship of HDL-P and MMP-1 was observed, although it was borderline in terms of significance ($p=0.05$). No modifying influence of sex was found among the above associations.

In Table 5, associations between HDL-P subclasses and endothelial activation markers are shown. Large HDL-P levels were inversely associated with sICAM-1 ($p<0.001$) and sP-selectin ($p<0.001$). Medium HDL-P levels were inversely associated with sVCAM-1 ($p<0.001$), sICAM-1 ($p<0.001$), sE-selectin ($p=0.03$) and sL-selectin ($p=0.03$). No associations were observed between HDL and small HDL-P levels.

Endothelial marker levels are presented by HDL cutoff values in Table 6. Participants with low HDL-C (<40 mg/dL) compared to normal HDL-C (≥ 40 mg/dL) showed significantly greater levels of sVCAM-1 ($p=0.01$), sICAM-1 ($p<0.001$), and residual sP-Selectin ($p<0.001$). For HDL-P, a clinical cutoff value has not been established and the 25th percentile was designated as a ‘low’ level and corresponded to a concentration of 29.3 nmol/L HDL-P. Participants with HDL-P at or below this cutoff showed greater levels of sVCAM-1 ($p=0.004$) and sICAM-1 ($p<0.001$).

DISCUSSION

In a large multi-ethnic cohort of men and women, HDL-C was found to be independently and inversely associated with endothelial activation markers sVCAM-1, sICAM-1, sL-selectin, and sP-selectin. Relations between endothelial markers and total HDL-P largely matched with those of HDL-C, although an additional association with sE-selectin was observed. Examining HDL-P subclasses showed that medium and large HDL-P levels were differentially associated with sVCAM-1, sICAM-1, and sE-selectin. Finally, stratifying individuals by HDL cutoff values (HDL-C, 40 mg/dL; HDL-P, 25th percentile) revealed that those with lower HDL had significantly greater levels of sVCAM-1 and sICAM-1. These results confirm an inverse relation between HDL and endothelial activation markers, although detected associations were modest in terms of magnitude. Coupled with previous evidence in experimental models, HDL levels within the normal range may contribute to an anti-atherogenic environment by suppressing endothelial activation.

Cell culture studies were among the first to demonstrate that HDL suppresses the expression of VCAM-1, ICAM-1, and E-selectin (7–10) and may do so in a physiological, dose-dependent manner (7). Animal studies further revealed that infusing recombinant HDL suppresses protein expression of carotid VCAM-1 (15) or aortic VCAM-1 and ICAM-1 (16, 17). Potentially explaining this phenomenon, Pan et al. (16) identified a likely pathway using an aortic endothelial cell model. It was demonstrated that HDL reduces surface expression of VCAM-1, ICAM-1, and E-selectin through the activation of annexin A1—a well-described anti-inflammatory mediator (30). Taken together, experimental models have shown that HDL particles act directly upon the endothelium to suppress endothelial activation proteins. However, corresponding studies in humans have yielded mixed results.

Thus far, only a limited number of human studies have examined HDL and endothelial activation. Moreover, statistical models among these studies are varied where some modelled HDL as a continuous variable while others dichotomized HDL in to clinically ‘low’ or ‘normal’ HDL-C levels using 40 mg/dL as a cutoff. In two studies of this latter design, individuals with low HDL-C levels (<40 mg/dL) showed higher levels of sVCAM-1, sICAM-1, or sE-selectin compared to those with normal or high HDL-C (20,21). In contrast, a null finding was reported in a study of 37 normolipidemic CAD patients and 57 healthy controls (31). Our findings are in agreement with the former studies as low HDL-C and low HDL-P were associated with approximately 3–12% higher mean levels of sVCAM-1 and sICAM-1 than those with normal HDL levels.

Similar to analyses that modelled HDL as a dichotomized variable, those that examined HDL as a continuous variable have reported mixed results. A nested case-control study of Epidemiological Study of Myocardial Infarction participants found that HDL-C was inversely associated with sVCAM-1 and sICAM-1 in 613 control subjects (23). In contrast, a case-control study of Atherosclerosis Risk in Communities participants reported that HDL-C was not associated with sVCAM-1 or sICAM-1 in 316 healthy control subjects (22), though an inverse association was observed with E-selectin. In treating HDL-C as a continuous variable, our findings are in agreement with the former. Whether the discrepant findings among studies are due to differences in statistical power or sample differences is unclear.

However, it is possible that true effect sizes are comparatively small and difficult to detect with smaller sample sizes. For example, within these MESA participants, one greater standard deviation in HDL-C (14 mg/dL) was associated with 2% lower concentrations of sVCAM-1 with a confidence interval ranging from 1–3%. This modest effect size coupled with the variation inherent in any analytical laboratory technique may have contributed to previous null findings including in the Atherosclerosis Risk in Communities cohort.

HDL particle subclass concentrations and endothelial activation remain less well-studied than HDL in cohort studies, but associations have been reported with incident CV disease. Generally, large and medium HDL particle concentrations have been shown to be inversely related to CV risk, whereas small particle subclass concentrations have been directly related with CV risk (32–34). However, contradictory findings have also been reported (35). Partially in agreement with the above paradigm, large and medium HDL subclass concentrations were found to be inversely associated with four and two of six endothelial markers, respectively. No associations were observed between small HDL particles and any endothelial marker. Given the novelty of these results, their limited effect sizes, and the ongoing controversy of NMR-derived HDL particle subclasses (36), these findings should be confirmed in other cohorts and interpreted with caution.

Limitations

It is important to acknowledge several limitations within this study. HDL-P was only measured in Exam 1 specimens while endothelial marker levels were assessed at either Exam1 or Exam 2 (a difference of 1.5 – 2 years), depending on the marker. This disparity in temporality may have influenced our findings, although it must be recognized that HDL is a stable population of lipoprotein particles and no change was observed in mean HDL levels between Exams 1 and 2. Apart from this temporality issue, it must be noted that systemic blood levels of these markers may not be representative of the local endothelium. As indicated by experimental studies, a stronger relation of HDL and endothelial proteins may be present at the level of the endothelium where HDL is directly suppressing protein expression. In addition, HDL may have disparate influences in individuals with a greater burden of chronic disease or those with dyslipidemia than the relatively healthy individuals in the MESA cohort at baseline. We are unable to evaluate this in the present cross sectional analysis, and few studies have been conducted to this end. It must also be acknowledged that HDL-C and HDL-P measures are gross estimates of the HDL population, but numerous characteristics of HDL were not assessed including protein and lipid composition as well as physical structure. These characteristics have been shown to regulate HDL function and may be critical in evaluating the potential influence of HDL on endothelial protein and gene expression. The cross-sectional design precludes the ability to establish temporality or causation. Finally, although multiple adjustments were made within the analysis, the possibility of residual confounding cannot be excluded.

Conclusion

In combination with previous evidence from experimental models, the observed associations suggest a modest to moderate beneficial influence of HDL on endothelial function. HDL

may be particularly critical when levels fall below a certain threshold and may result in an environment or predisposition to endothelial activation and nascent atherogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- HDL-C is independently and inversely associated with endothelial activation markers
- HDL-P likewise inversely associates with endothelial activation
- Medium and large HDL-P subclasses inversely associate with endothelial activation
- No relation was found with small HDL-P and endothelial activation markers
- HDL <40mg/dL is associated 3–12% greater levels of endothelial activation markers

Table 1

Baseline demographic characteristics of Multi-Ethnic Study of Atherosclerosis participants at Exams 1 and 2.

	All	Women	Men	<i>p value</i>
Exam 1 (n=6730)				
Age, mean (SD)	62 (10)	62 (10)	62 (10)	0.98
Systolic blood pressure (mm Hg), mean (SD)	126 (21)	127 (23)	126 (19)	0.016
BMI (kg/m ²), mean (SD)	28 (5)	29 (6)	28 (4)	<0.001
Hypertension medication, n (%)	2485 (37)	1368 (38)	1117 (35)	0.015
Lipid lowering medication, n (%)	1076 (16)	573 (16)	503 (16)	0.92
Education, n (%)				<0.001
< High school	1212 (18)	701 (20)	511 (16)	
High school completed	1222 (18)	737 (21)	485 (15)	
> High school	4273 (64)	2126 (60)	2147 (68)	
Smoking, n (%)				<0.001
Never	3386 (50)	2110 (59)	1276 (41)	
Former	2448 (36)	1037 (29)	1411 (45)	
Current	874 (13)	417 (12)	457 (15)	
Race /ethnicity, n (%)				0.094
White	2589 (38)	1353 (38)	1236 (39)	
Chinese	792 (12)	411 (11)	381 (12)	
Black	1874 (28)	1042 (29)	832 (26)	
Hispanic	1475 (22)	770 (22)	705 (22)	
Exam 2 (n=6019)				
Age	64 (10)	64 (10)	64 (10)	0.6
Systolic blood pressure, (mm Hg), mean (SD)	124 (21)	125 (23)	124 (19)	0.08
BMI (kg/m ²), mean (SD)	28 (5)	29 (6)	28 (4)	<0.001
Hypertension medication, n (%)	2391 (41)	1287 (43)	1104 (40)	0.064
Lipid lowering medication, n (%)	1264 (22)	639 (21)	625 (23)	0.14
Education, n (%)				<0.001
< High school	1003 (17)	574 (18)	429 (15)	
High school completed	1084 (18)	649 (21)	435 (15)	
> High school	3917 (65)	1924 (61)	1993 (70)	
Smoking, n (%)				<0.001
Never	2762 (46)	1784 (57)	978 (35)	
Former	2542 (43)	1038 (33)	1504 (53)	
Current	671 (11)	323 (10)	348 (12)	
Race/ethnicity, n (%)				0.12
White	2387 (40)	1222 (39)	1165 (41)	
Chinese	702 (12)	358 (11)	344 (12)	
Black	1628 (27)	893 (28)	735 (26)	
Hispanic	1302 (22)	681 (22)	621 (22)	

Values are n(%) for categorical variables

* Two-sample t-test or chi-square test as appropriate comparing females and males

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HDL cholesterol (mg/dL) and lipoprotein concentrations (nmol/L) of Multi-Ethnic Study of Atherosclerosis participants at Exam 1. HDL cholesterol at Exam 2.

Table 2

	All				Women				Men				P*
	n	Mean (SD)	median	range	n	Mean (SD)	median	range	n	Mean (SD)	median	range	
Exam 1													
HDL-C (mg/dL)	6651	51 (14)	48	15 – 100	3512	56 (14)	54	20 – 100	3139	45 (11)	43	15 – 99	<0.001
HDL-P (mol/L)	6702	34 (7)	33	14 – 63	3559	37 (7)	36	16 – 63	3143	31 (5)	31	14 – 60	<0.001
Small HDL (mol/L)	6702	15 (6)	15	0 – 45	3559	14 (6)	14	0 – 45	3143	15 (5)	15	0 – 36	<0.001
Medium HDL (mol/L)	6702	13 (7)	12	0–54	3559	15 (7)	14	0–54	3143	11 (5)	11	0 – 45	<0.001
Large HDL (mol/L)	6702	6.0 (3.5)	5.2	0 – 25	3559	7.3 (3.6)	6.7	0 – 25	3143	4.6 (2.7)	3.8	0.7 – 23	<0.001
Exam 2													
HDL-C (mg/dL)	6056	51 (14)	49	21 – 100	3174	56 (14)	54	24 – 100	2882	46 (12)	44	21 – 98	<0.001

* Two-sample t-test

Table 3
Endothelial activation marker concentrations (ng/mL) across subsets of MESA participants.

	All				Women				Men				P*
	n	Mean (SD)	median	range	n	Mean (SD)	median	range	n	Mean (SD)	median	range	
VCAM-1	2386	730 (204)	698	252 – 1603	1262	724 (200)	687	299 – 1558	1124	737 (208)	708	252 – 1603	0.12
ICAM-1	2373	252 (86)	234	44 – 697	1257	254 (87)	235	53 – 683	1116	250 (85)	231	44 – 697	0.27
E-selectin	978	54 (23)	52	5–140	561	51 (23)	49	5 – 140	417	57 (23)	55	10 – 139	<0.001
L-selectin	2397	888 (193)	874	281 – 1686	1268	929 (193)	921	418 – 1686	1129	843 (182)	820	281 – 1668	<0.001
MMP-1	2378	5.4 (3.7)	4.4	0.09 – 21.6	1254	5.6 (3.8)	4.7	0.09 – 21.1	1124	5.1 (3.6)	4.2	0.24 – 21.6	0.002
residual of ln P-selectin	6017	0 (0.3)	0.01	–0.99 – 0.94	3154	–0.04 (0.31)	–0.03	–0.99 – 0.92	2863	0.05 (0.31)	0.06	–0.98 – 0.94	<0.001

Two-sample t-test

Table 4

Associations of HDL-C and HDL-P (per SD) with circulating levels of select endothelial activation markers in a subcohort of MESA participants.

	All		Women		Men	
	HDL-C	HDL-P	HDL-C	HDL-P	HDL-C	HDL-P
ln sVCAM-1	0.98 (0.97–0.99) p=0.002	0.97 (0.95–0.98) p<0.001	0.99 (0.97–1.00) p=0.07	0.97 (0.95–0.98) p<0.001	0.98 (0.96–1.00) p=0.02	0.97 (0.95–0.99) p=0.004
ln sICAM-1	0.94 (0.93–0.95) p<0.001	0.96 (0.95–0.97) p<0.001	0.94 (0.92–0.96) p<0.001	0.97 (0.95–0.99) p=0.004	0.95 (0.92–0.97) p<0.001	0.94 (0.92–0.97) p<0.001
ln sE-selectin	0.98 (0.95–1.02) p=0.4	0.96 (0.92–0.99) p=0.01	0.98 (0.94–1.02) p=0.3	0.95 (0.90–0.99) p=0.02	1.01 (0.95–1.07) p=0.8	1.00 (0.94–1.05) p=0.9
ln sL-selectin	0.99 (0.98–1.00) p=0.04	0.98 (0.97–0.99) p=0.001	1.00 (0.98–1.01) p=0.5	0.98 (0.97–1.00) p=0.02	0.98 (0.96–1.00) p=0.02	0.98 (0.96–1.00) p=0.03
ln MMP-1	1.00 (0.97–1.04) p=0.9	1.04 (1.00–1.08) p=0.05	0.98 (0.94–1.03) p=0.5	1.06 (1.01–1.19) p=0.03	1.01 (0.95–1.07) p=0.8	1.00 (0.94–1.06) p=0.9
Residual of sP-selectin	0.98 (0.97–0.99) p<0.001	1.00 (0.99–1.01) p=0.5	0.98 (0.96–0.99) p<0.001	1.00 (0.99–1.01) p=0.9	0.97 (0.96–0.99) p=0.001	0.99 (0.97–1.01) p=0.2

For the regression analyses log adhesion proteins are the outcome and HDL measures are the independent variables. HDL measures are scaled by dividing the HDL measure by the overall standard deviation (SD) for each HDL measure. For HDL-C at exam 1 and exam 2 the SD is 14; the SD for HDL-P is 7. The effect size and 95% confidence intervals are calculated by exponentiating the parameter estimates from the regression models. Age, sex, race/ethnicity, education level, systolic blood pressure, hypertension medication use, BMI, smoking status, lipid lowering medication use, serum creatinine, diabetes, LDL-C, and coronary artery calcium were included as covariates.

Table 5

Associations of nuclear magnetic resonance spectroscopy-derived HDL-P subclasses (per SD) and select endothelial activation markers in a subcohort of MESA participants.

	Small HDL-P	Medium HDL-P	Large HDL-P
ln sVCAM-1 (N=2015)	1.00 (0.99–1.01) p=0.87	0.97 (0.96–0.98) p<0.001	1.01 (0.99–1.02) p=0.36
ln sICAM-1 (N=2004)	1.01 (0.99–1.02) p=0.34	0.97 (0.96–0.99) p<0.001	0.97 (0.95–0.98) p<0.001
ln sE-selectin (N=951)	1.00 (0.96–1.03) p=0.79	0.97 (0.94–1.00) p=0.03	1.01 (0.97–1.04) p=0.62
ln sL-selectin (N=2022)	0.99 (0.98–1.00) p=0.25	0.99 (0.98–1.00) p=0.03	1.00 (0.99–1.01) p=0.65
ln MMP-1 (N=2009)	1.00 (0.97–1.04) p=0.08	1.02 (0.99–1.06) p=0.17	1.01 (0.97–1.05) p=0.56
residual of sP-selectin (N=5092)	1.01 (1.00–1.02) p=0.12	1.00 (0.99–1.01) p=0.90	0.98 (0.97–0.99) p<0.001

For regression analyses, log adhesion proteins are the outcome and HDL measures are the independent variables. HDL measures are scaled by dividing the HDL measure by the overall standard deviation (SD) for each HDL measure. For small HDL-P the SD is 6; for medium HDL-P the SD is 7; and for large HDL-P the SD is 3.5. The effect size and 95% confidence intervals are calculated by exponentiating the parameter estimates from the regression models. Regression models were adjusted for age, sex, race/ethnicity, education level, systolic blood pressure, hypertension medication use, BMI, smoking status, lipid lowering medication use, serum creatinine, diabetes, LDL-C, and coronary artery calcium.

Table 6

Mean levels (SD) of endothelial activation markers among individuals stratified by HDL cutoff values: HDL-C above and below 40 mg/dL cutoff; HDL-P above and below the 25th percentile*.

	HDL-C					HDL-P				
	<40 mg/dL		40mg/dL			<25 th %ile		25 th %ile		
	n	Mean (SD)	n	Mean (SD)	p-value	n	Mean (SD)	n	Mean (SD)	p-value
sVCAM-1, ng/mL (SD)	473	752 (220)	1898	724 (199)	0.01	579	747 (227)	1799	725 (196)	0.004
sICAM-1, ng/mL (SD)	469	276 (100)	1889	247 (82)	<0.001	576	264 (95)	1789	249 (83)	<0.001
sE-selectin, ng/mL (SD)	199	60 (24)	773	52 (23)	0.39	241	59 (24)	736	52 (23)	0.39
sL-selectin, ng/mL (SD)	478	877 (192)	1093	891 (193)	0.08	584	876 (191)	1805	893 (193)	0.51
Ln MMP-1, ng/mL (SD)	478	5.4 (3.8)	1884	5.4 (3.7)	0.79	583	5.2 (3.6)	1787	5.5 (3.7)	0.31
Residual of sP-selectin, ng/mL (SD)	1198	0.08 (0.30)	4768	-0.02 (0.31)	<0.001	1437	0.04 (0.32)	4563	-0.01 (0.31)	0.24

* 25th percentile of HDL-P equated to 29.3 nmol/L and was calculated using the entire MESA cohort in participants with non-missing data (N=6730).

Regression models were used to assess the association of HDL groups with adhesion proteins. Adhesion proteins were the outcome and HDL measures were the independent variables. Regression models were adjusted for age, sex, race/ethnicity, education level, systolic blood pressure, hypertension medication use, BMI, smoking status, lipid lowering medication use, serum creatinine, diabetes, LDL-C, and coronary artery calcium.