ORIGINAL RESEARCH

Concentration of Smaller High-Density Lipoprotein Particle (HDL-P) Is Inversely Correlated With Carotid Intima Media Thickening After Confounder Adjustment: The Multi Ethnic Study of Atherosclerosis (MESA)

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***Background*—**Recent studies have failed to establish a causal relationship between high-density lipoprotein cholesterol levels (HDL-C) and cardiovascular disease (CVD), shifting focus to other HDL measures. We previously reported that smaller/denser HDL levels are protective against cerebrovascular disease. This study sought to determine which of small+medium HDL particle concentration (HDL-P) or large HDL-P was more strongly associated with carotid intima-media thickening (cIMT) in an ethnically diverse cohort.

***Methods and Results*—**In cross-sectional analyses of participants from the Multi Ethnic Study of Atherosclerosis (MESA), we evaluated the associations of nuclear magnetic resonance spectroscopy–measured small+medium versus large HDL-P with cIMT measured in the common and internal carotid arteries, through linear regression. After adjustment for CVD confounders, low-density lipoprotein cholesterol (LDL-C), HDL-C, and small+medium HDL-P remained significantly and inversely associated with common (coefficient=−1.46 μm; P=0.00037; n=6512) and internal cIMT (coefficient=−3.82 μm; P=0.0051; n=6418) after Bonferroni correction for 4 independent tests (threshold for significance=0.0125; α=0.05/4). Large HDL-P was significantly and inversely associated with both cIMT outcomes before HDL-C adjustment; however, after adjustment for HDL-C, the association of large HDL-P with both common (coefficient=1.55 μm; P=0.30; n=6512) and internal cIMT (coefficient=4.84 μm; P=0.33; n=6418) was attenuated. In a separate sample of 126 men, small/medium HDL-P was more strongly correlated with paraoxonase 1 activity (r_p=0.32; P=0.00023) as compared to both total HDL-P (r_p=0.27; P=0.0024) and large HDL-P (r_p=0.02; P=0.41) measures.

***Conclusions*—**Small+medium HDL-P is significantly and inversely correlated with cIMT measurements. Correlation of small+medium HDL-P with cardioprotective paraoxonase 1 activity may reflect a functional aspect of HDL responsible for this finding. **(*J Am Heart Assoc*. 2016;5:e002977 doi: 10.1161/JAHA.115.002977)**

Key Words: antioxidant • carotid intima media thickening • cerebrovascular disease • high-density lipoprotein cholesterol • high-density lipoprotein particle concentration • paraoxonase 1

Several large, randomized, clinical trials[1–3] and Mendelian randomization studies[4] have failed to show a causal role for high-density lipoprotein cholesterol (HDL-C) levels in cardioprotection, decreasing interest in HDL as a therapeutic target. However, recent work from the Multi Ethnic Study of Atherosclerosis (MESA) found that total HDL particle concentration (HDL-P) measured by nuclear magnetic resonance (NMR) spectroscopy is a significant predictor of incident cardiovascular events and carotid intima-media thickening (cIMT), even when adjusting for the effects of HDL-C and

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other cardiovascular disease (CVD) confounders.[5] Other recent work in MESA has found that the combination of small and medium HDL-P concentration was an independent predictor for noncardiovascular inflammation-related death/hospitalization and, separately, incident coronary heart disease events.[6] These studies suggested that unmeasured aspects of HDL not reflected by HDL-C (such as antioxidant and anti-inflammatory properties[6]) might be responsible for the long-held and consistent cardioprotective associations of HDL.[7]

The HDL particle is a heterogeneous molecule with over 70 associated proteins.[8] Broadly, HDL can be distinguished into two subfractions by density: HDL2 and HDL3. Unlike HDL3, HDL2 is larger, less dense, and strongly associated with apolipoprotein A1 (apoA1). HDL2 carries the majority of cholesterol reflected in HDL-C measurements.[9] On the other hand, HDL3 carries enzymes that prevent oxidative stress[10] and receives cholesterol from reverse cholesterol transport through the ATP-binding cassette transporter A1 (ABCA1) mediators.[11] We have previously demonstrated that HDL3 cholesterol (HDL3-C) is well approximated by the sum of small and medium HDL-P concentration, whereas HDL2 cholesterol (HDL2-C) correlates strongly with large HDL-P concentration.[12,13]

Previous work by Kontush and Chapman has established the hypothesis that smaller and denser HDL-P are the source of overall HDL cardioprotection.[14–16] Several functions have been proposed for this observation of small and dense HDL's cardioprotection: its ability to accept cholesterol from foam cells by ABCA1,[17] antioxidant activity,[10] antiapoptotic actions,[18,19] and its anti-inflammatory properties,[20] among other hypotheses previously reviewed in detail.[15,21]

To investigate whether one of these unmeasured functional aspects of HDL associated with HDL2 or HDL3 were responsible for the cardioprotective effects of HDL, we previously performed analyses to determine whether HDL-C, HDL2-C, HDL3-C, or apoA1 was the best predictor of carotid artery disease status.[22] In that work, the smaller HDL3-C subfraction cholesterol was found to be the best predictor of reduced odds of carotid artery disease, demonstrating the utility of the HDL3-C measure versus HDL-C.[22] Follow-up work in a subset of this carotid artery disease case-control study found that differences in small and medium HDL-P concentration were the primary determinants of HDL-P concentration differences between cases and controls.[13] In a separate study of ≈1000 stroke-free subjects, HDL3-C was negatively associated with carotid plaque area, whereas HDL2-C was positively associated.[23] These results support the aforementioned hypothesis that cholesterol-poor HDL subspecies, which are under-represented in measures of HDL-C, may be important protective factors against cerebrovascular disease.[14–16]

Given these findings, we sought to validate the association of HDL3-C with cIMT in an independent cohort. Because HDL3-C is closely approximated by the sum of small and medium HDL-P concentration (small+medium HDL-P), our primary goal in the present study was to determine whether small+medium HDL-P concentration was associated with decreased common and internal cIMT in a cross-sectional analysis of MESA participants. We note that the combination of small+medium HDL-P has been used in several other studies as a measure of small, dense, and potentially cardioprotective HDL.[6,24] We simultaneously sought to evaluate whether large HDL-P concentration was similarly cardioprotective in cross-sectional analyses after covariate adjustment. Finally, as a follow-up analysis, we evaluated the correlation between the HDL-P subspecies as measured by ion mobility and paraoxonase 1 (PON1) activity, which has previously been reported as protective against carotid artery disease[25–28] in the Seattle-based CLEAR study. Notably, ion mobility was developed subsequent to NMR spectroscopy measurement of HDL-P and provides similar, though not identical, estimates.[13] The MESA cohort did not have these PON1 activity data available.

Methods

Ethics Statement

Informed, written consent was obtained from each participant of MESA at the time of recruitment between July 2000 and August 2002. Institutional review boards at each participating center of MESA approved of the study. The University of Washington Institutional Review Board approved analyses presented in this work.

MESA Cohort Sample

MESA is a multicenter, longitudinal cohort study of the prevalence and progression of subclinical atherosclerotic disease phenotypes. Between July 2000 and August 2002, MESA recruited 6814 participants between 45 and 84 years of age from 6 field sites: Baltimore City and Baltimore County, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County, California; northern Manhattan and the Bronx, New York; and St Paul, Minnesota. Participants were of African-American, Hispanic, white, and Chinese-American self-reported ancestry. Baseline exclusion criteria for recruitment were: self-reported CVD history, pregnancy, cancer, cognitive impairment, or weight >136 kg. At the time of recruitment, each participant underwent examination, including blood draw, anthropometric measurements, and collection of questionnaire data.

Lipid Measurements

In MESA, all lipid and covariate data were collected at physical examination during study enrollment. Blood was drawn after a 12-h fast, and samples were stored at −70°C with EDTA. Plasma HDL-P concentration was measured at LipoScience, Inc. (Raleigh, NC) using NMR spectroscopy and the LipoProfile-3 algorithm.[29,30] All other lipids and fasting glucose were measured at a separate central laboratory (Collaborative Studies Clinical Laboratory at Fairview University Medical Center, Minneapolis, MN). HDL-C was measured after precipitation of non-HDL cholesterol through exposure to magnesium/dextran, using the cholesterol oxidase method (Roche Diagnostics, Indianapolis, IN). LDL-C was calculated using the Friedwahl equation.[31] The sum of small and medium HDL-P measures (small+medium HDL-P) was used as a proxy variable for HDL3-C,[12,13] because we previously have found HDL3-C to be protective against carotid artery disease.[22]

Covariates

Demographic information such as body mass index (BMI), blood pressure, smoking history, and medication use were all collected at the baseline MESA physical examination. Smoking was defined as never, former (smoked ≥100 cigarettes in a lifetime), or current. Pack-years of smoking were also calculated from this survey information. Fasting glucose was measured after an overnight fast. Diabetes status was defined as a fasting glucose ≥126 mg/dL or use of an antidiabetic medication. Systolic blood pressure (SBP) was measured at rest from the brachial artery with the participant in a sitting position. Antihypertensive medication (HTN Rx) and lipid-lowering medication use (LLM Rx) were determined by self-report at the baseline MESA examination.

cIMT Measurement

High-resolution B-mode ultrasound was used to measure carotid atherosclerosis as previously described.[32] Total cIMT in micrometers (μm) was calculated separately from maximal carotid artery thickening at 4 sites (right and left; near and far walls) for the common and internal carotid arteries, respectively.[33]

Carotid Lesion Epidemiology And Risk Study Sample

The Carotid Lesion Epidemiology And Risk (CLEAR) study is a Seattle-based prevalent carotid artery disease case-control study, comprised primarily of veterans, with controls distribution matched by sex and age at diagnosis of carotid artery disease cases. Exclusion criteria for the CLEAR study included familial hypercholesterolemia, total fasting cholesterol >400 mg/dL, hypocoagulable state or use of hypocoagulant medication, postorgan transplant, or inability to consent. Diabetes status was defined as hemoglobin A1c ≥6.5% or use of oral hypoglycemic therapy or insulin. Medication use was determined by self-report and then matched to pharmacy records. We previously have reported a carotid artery disease–protective effect of small+medium HDL-P concentration and HDL3 in CLEAR.[13,22] The analyzed subset of CLEAR described here is composed of 126 men without CVD not on statin pharmacotherapy.

Standard methods were used in the CLEAR study to determine plasma levels of total cholesterol, triglycerides, very low-density lipoprotein cholesterol, and HDL-C using an Abbott Spectrum analyzer (Abbott Laboratories, Abbott Park, IL). All samples were analyzed at a central laboratory, the Northwest Lipid Research Laboratory, and used fasting plasma stored with lithium heparin. In the CLEAR study, PON1 enzyme activity was measured by rate of enzymatic degradation of phenylacetate (AREase) by a continuous spectrophotometric assay with lithium heparin plasma as previously described.[25–27] PON1 AREase activity had an approximate normal distribution. HDL-P was measured using calibrated ion mobility analysis, as previously described.[13] This ion mobility method of determining HDL-P is expected to deliver closely correlated, but distinct measurements of HDL-P as compared to NMR,[13] which was used in MESA.

Statistical Analysis

All analyses and graphics were performed in R software (http://r-project.org). For our primary analyses, the outcomes of total common and internal cIMT were separately analyzed for association with either the sum of small+medium HDL-P or large HDL-P concentrations. We used a linear regression model adjusting for the confounders of age, sex, race (coded as a dummy variable with whites, the largest subgroup, as the reference group and blacks, Hispanics, and Asians as the comparison groups), BMI, cigarette smoking status (former or current, with the never group as reference), pack-years of smoking, SBP, antihypertensive medication use, fasting glucose, diabetes status, LLM Rx use, LDL-C, and HDL-C. A Bonferroni-corrected threshold of significance of 0.0125 (α=0.05/4) was applied because of the 4 tests in our primary hypothesis: common cIMT with small+medium HDL-P and large HDL-P and internal cIMT with small+medium HDL-P and large HDL-P.

To aid in interpretation, we present results in a staged regression model, adjusting first for demographic (age, sex, and race), then adding cardiovascular risk factors (BMI, cigarette smoking status, pack-years of smoking, SBP, HTN Rx, fasting glucose, diabetes status, and LLM Rx),

subsequently adding LDL-C, and finally adding HDL-C to the linear regression model. We note that staged regression models were not used in the primary analyses, but are presented in table form to illustrate the effect of HDL-C on the association of small+medium HDL-P versus large HDL-P on the cIMT outcomes.

To illustrate multivariable regression results, we separately plotted covariate-adjusted mean internal and common cIMT for quartiles of small+medium HDL-P and large HDL-P, respectively. Two lines are presented in each plot: one for covariate-adjusted internal or common cIMT with all covariates except HDL-C in the model and a second line with all covariates in the final regression model.

Sensitivity analyses were performed by race group and sex for the association of small+medium HDL-P with both common and internal cIMT. Significant differences in association of small+medium HDL-P with either outcome were followed-up with addition of a formal interaction term to the regression model (eg, sex×small+medium HDL-P). Presence of effect modification was concluded if the interaction term was significantly different than zero ($P \leq 0.05$).

Results

MESA participants analyzed in this work were multiethnic men and women without baseline CVD with a mean age of 62.2 years (see Table 1). Men composed 47.2% of the study population. Self-reported whites were the largest ethnic group, representing 38.5% of the participants, followed by blacks (27.8%), Hispanics (21.9%), and Asians (11.8%). Common and internal artery cIMT had means of 870.51 and 1071.52 μm, respectively. Baseline clinical and demographic characteristics of the MESA participants are summarized in Table 1. Correlations of demographic, lipid, and clinical variables with small, medium, large, and small+medium HDL-P have been reported recently by Duprez et al. in a largely overlapping data set from MESA.[6]

After full adjustment (including LDL-C and HDL-C), small+medium HDL-P concentration was significantly and inversely associated common cIMT at a Bonferroni-corrected threshold of α=0.0125 (P=0.00037; see Table 2), with a final covariate-adjusted beta coefficient of −1.46 μm. In contrast, after full adjustment (including LDL-C and HDL-C), large HDL-P concentration was not significantly associated with common cIMT (P=0.30).

A similar pattern was observed for associations with internal cIMT. After full adjustment, small+medium HDL-P concentration was significantly and inversely associated at a Bonferroni-corrected threshold of α=0.0125 with internal cIMT (P=0.0051; beta coefficient=−3.82 μm; see Table 3). In contrast, after full adjustment, large HDL-P concentration was not significantly associated with internal cIMT (P=0.33). Full regression coefficients for all covariates are presented in Table 4 for small+medium HDL-P and both common and internal cIMT, and separately in Table 5 for large HDL-P with both common and internal IMT.

Notably, in post-hoc analyses, large HDL-P was strongly and inversely associated with common and internal cIMT after adjustment for demographic covariates, CVD risk factors, and LDL-C. However, addition of HDL-C caused both of these associations to attenuate and become nonsignificant (see Tables 2 and 3). Moreover, in final regression models including HDL-C, the direction of effect for large HDL-P indicated that increasing levels of large HDL-P was associated

Table 1. Baseline Multi Ethnic Study of Atherosclerosis (MESA) Cohort Demographic and Clinical Characteristics

	N	Mean (±SD) or N (%)
Age, y	6814	62.2 (±10.2)
Race	6814	
White, %		2622 (38.5)
Asian, %		804 (11.8)
Black, %		1892 (27.8)
Hispanic, %		1496 (21.9)
Men, %	6814	3213 (47.2)
Body mass index, kg/m^2	6814	28.3 (±5.48)
Smoking status	6792	
Never, %		3418 (50.3)
Former, %		2487 (33.6)
Current, %		887 (13.1)
Cigarette pack-years	6720	11.3 (±20.9)
Systolic blood pressure, mm Hg	6811	126.6 (±21.5)
Antihypertensive Rx, %	6811	2536 (37.2)
Fasting glucose, mg/dL	6789	104.5 (±30.9)
Diabetes status, %	6814	859 (12.6)
Lipid-lowering Rx, %	6811	1100 (16.2)
LDL-C, mg/dL	6701	117.2 (±31.5)
HDL-C, mg/dL	6701	50.9 (±14.8)
Triglycerides, mg/dL	6786	135.1 (±68.9)
Large HDL-P, μmol/L	6786	6.02 (±3.46)
Small+medium HDL-P, μmol/L	6786	28.01 (±5.48)
Small HDL-P, μmol/L	6786	13.27 (±6.84)
Medium HDL-P, μmol/L	6786	14.74 (±5.73)
Common cIMT, μm	6726	870.51 (±193.43)
Internal cIMT, μm	6629	1071.52 (±603.44)

cIMT indicates carotid intima-media thickening; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; LDL-C, low-density lipoprotein cholesterol; Rx, medication.

Table 2. Staged Linear Regression Model Association of Small+Medium HDL-P and Large HDL-P Concentration With Carotid Intima-Media Thickening (μm) in the Common Carotid Arteries (n=6512)

	Small+Medium HDL-P, μmol/L		Large HDL-P, μmol/L	
	Coefficient (μm) ±SE	*P* Value	Coefficient (μm) ±SE	*P* Value
Small+medium HDL-P or large HDL-P, unadjusted	−1.63±0.43	0.00024	−4.15±0.69	<0.0001
+Demographic characteristics*	−0.95±0.41	0.019	−5.45±0.67	<0.0001
+Above and CVD risk factors†	−1.94±0.40	<0.0001	−2.95±0.69	<0.0001
+Above and LDL-C	−1.76±0.39	<0.0001	−2.28±0.69	0.0011
+Above and HDL-C	−1.46±0.41	0.00037	1.55±1.50	0.30

Final regression model coefficients/*P* values for association with internal cIMT are available in Tables 4 (small+medium HDL-P) and 5 (large HDL-P). cIMT indicates carotid intima-media thickening; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; LDL-C, low-density lipoprotein cholesterol.
*Demographic characteristics are defined as age, sex, and race (Asian, black, or Hispanic, with white as the reference group).
†Cardiovascular disease (CVD) risk factors defined as body mass index, cigarette smoking status (former or current, with never group as reference), pack-years of smoking, systolic blood pressure, antihypertensive medication, fasting glucose, diabetes status, and lipid-lowering medication use.

with increased common (beta coefficient=1.55 μm; *P*=0.30) and internal (beta coefficient=4.84 μm; *P*=0.31) cIMT. In contrast, the inverse association of small+medium HDL-P with both common and internal cIMT remained robust even after the addition of HDL-C to the model (see Tables 2 and 3). Graphical representations of the effect of HDL-C on regression model prediction of common and internal cIMT are presented in Figures 1 and 2, respectively, for both small+medium and large HDL-P.

To examine the possibility of either small or medium HDL-P driving the association of small+medium HDL-P and both cIMT measures, a separate multivariable linear regression model was fit (see footnote of Table 4). Both small HDL-P (beta coefficient=−1.35 μm; *P*=0.0052) and medium HDL-P (beta coefficient=−1.52 μm, *P*=0.00054) were separately and significantly associated with decreased common cIMT. Small HDL-P was significantly associated with decreased internal cIMT (beta coefficient=−4.44 μm; *P*=0.0059). Medium HDL-P was also associated with internal cIMT (beta coefficient=−3.44 μm; *P*=0.019).

Sensitivity analyses were performed for the association of small+medium HDL-P with common cIMT (Table 6) and internal cIMT (Table 7) across racial groups. Notably, after full adjustment, small+medium HDL-P concentration was consistently associated with decreased common and internal cIMT for whites (n=2506; beta coefficient=−1.15 μm and *P*=0.079 with common cIMT and n=2470; beta coefficient=−5.59 μm and *P*=0.013 with internal cIMT) and blacks (n=1808; beta coefficient=−2.15 μm and *P*=0.0058 with common cIMT and n=1782; beta coefficient=−4.11 μm and *P*=0.10 with internal cIMT; see Tables 6 and 7). In Hispanics, small+medium HDL-P concentration was associated with decreased common cIMT (n=1430; beta coefficient=−1.84 μm and *P*=0.045; see Table 6), but was not associated with internal cIMT (n=1409; beta coefficient=−0.81 μm and *P*=0.79; see Table 7). Finally, after full adjustment, small+medium HDL-P was not associated

Table 3. Staged Linear Regression Model Association of Small+Medium HDL-P and Large HDL-P Concentration With Carotid Intima-Media Thickening (μm) in the Internal Carotid Arteries (n=6418)

	Small+Medium HDL-P, μmol/L		Large HDL-P, μmol/L	
	Coefficient (μm) ±SE	*P* Value	Coefficient (μm) ±SE	*P* Value
Small+medium HDL-P or large HDL-P, unadjusted	−2.88±1.38	0.037	−10.91±2.18	<0.0001
+Demographic characteristics*	−2.15±1.34	0.11	−12.17±2.22	<0.0001
+Above and CVD risk factors†	−4.91±1.33	0.00036	−6.36±2.28	0.0054
+Above and LDL-C	−4.45±1.33	0.00081	−4.62±2.31	0.045
+Above and HDL-C	−3.82±1.36	0.0051	4.84±4.99	0.33

Final regression model coefficients/*P* values for association with internal cIMT are available in Tables 4 (small+medium HDL-P) and 5 (large HDL-P). cIMT indicates carotid intima-media thickening; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; LDL-C, low-density lipoprotein cholesterol.
*Demographic characteristics are defined as age, sex, and race (Asian, black, or Hispanic, with white as the reference group).
†Cardiovascular disease (CVD) risk factors defined as body mass index, cigarette smoking status (former or current, with never group as reference), pack-years of smoking, systolic blood pressure, antihypertensive medication, fasting glucose, diabetes status, and lipid-lowering medication use.

Table 4. Full Multivariable Regression Model Coefficients for Association of Small+Medium HDL-P and Common cIMT and Internal cIMT

	Common cIMT, μm (N=6512)		Internal cIMT, μm (N=6418)	
Covariates	Coefficient (μm) ±SE	*P* Value	Coefficient (μm) ±SE	*P* Value
Age, y	7.11±0.23	<0.0001	15.79±0.76	<0.0001
Black, %	20.48±5.44	0.00017	−60.70±18.11	0.00081
Hispanic, %	−13.39±5.71	0.019	−73.96±18.98	<0.0001
Asian, %	−25.12±7.13	0.00043	−205.88±23.72	<0.0001
Male sex, %	33.25±4.67	<0.0001	80.29±15.55	<0.0001
BMI, kg/m^2	3.05±0.44	<0.0001	1.73±1.47	0.24
Former smoker, %	11.35±5.24	0.030	47.69±17.44	0.0063
Current smoker, %	5.23±7.37	0.48	96.79±24.51	<0.0001
Pack-years	0.46±0.12	0.00013	2.49±0.40	<0.0001
SBP, mm Hg	1.52±0.11	<0.0001	2.71±0.36	<0.0001
HTN Rx, %	4.06±4.77	0.39	70.25±15.86	<0.0001
Fasting glucose, mg/dL	0.17±0.094	0.079	0.50±0.31	0.11
Diabetes status, %	20.47±8.70	0.019	84.21±28.96	0.0037
LDL-C, mg/dL	0.42±0.067	<0.0001	1.08±0.22	<0.0001
HDL-C, mg/dL	−0.54±0.16	0.00086	−1.12±0.54	0.039
LLM Rx, %	20.28±5.87	0.00055	135.40±19.52	<0.0001
Small+medium HDL-P, μmol/L	−1.46±0.41*	0.00037*	−3.82±1.36†	0.0051†

BMI indicates body mass index; cIMT, carotid intima-media thickening; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; HTN Rx, hypertension medication treatment; LDL-C, low-density lipoprotein cholesterol; LLM Rx, lipid-lowering medication treatment; SBP, systolic blood pressure.

*When modeling small and medium HDL-P separately (rather than the sum of small+medium HDL-P) with common cIMT, small HDL-P has a beta coefficient of −1.35 and *P*=0.0052 whereas medium HDL-P has a beta coefficient of −1.52 and a *P*=0.00054.

†When modeling small and medium HDL-P separately (rather than the sum of small+medium HDL-P) with internal cIMT, small HDL-P has a beta coefficient of −4.44 and *P*=0.0059 whereas medium HDL-P has a beta coefficient of −3.44 and a *P*=0.019.

with either cIMT outcome in Asians (n=768; beta coefficient=−0.23 μm and *P*=0.85 with common cIMT and n=757; beta coefficient=−2.25 μm and *P*=0.51 with internal cIMT; see Tables 6 and 7). Statistical interaction tests of race group and small+medium HDL-P were conducted on full regression models predicting both common and internal cIMT. No effect modification by race group of the association of HDL-P was found either common (*P*=0.17) and internal cIMT (*P*=0.94) outcomes.

Separate sensitivity analyses were performed for the association of small+medium HDL-P with common cIMT and internal cIMT across sex (see Table 8). After full adjustment, small+medium HDL-P concentration was consistently associated with decreased common cIMT in men (n=3072; beta coefficient=−2.91 μm and *P*<0.0001), but not in women (n=3440; beta coefficient=−0.70 μm and *P*=0.15). In contrast, after full adjustment, the opposite pattern was observed with internal cIMT, with women having a significant association of small+medium HDL-P (n=3381; beta coefficient=−3.89 μm and *P*=0.018), but not men (n=3037; beta coefficient=−3.46 μm, and *P*=0.15; results summarized in Table 8). Statistical interaction tests of gender (with females as the reference) and small+medium HDL-P were conducted on full regression models predicting both common and internal cIMT. The male sex-by-small+medium HDL-P interaction for a full linear regression model of internal cIMT was not significant (*P*=0.69); however, the interaction for common cIMT was significant (*P*=0.0024).

To determine whether PON1 could potentially mediate the inverse association of small+medium HDL-P with common and internal cIMT, we performed a follow-up analysis in the CLEAR study where PON1 measures were available. For this analysis, 126 men without CVD and not taking statins were evaluated for HDL-P concentrations and PON1 AREase activity. Pearson's pair-wise correlation coefficients were then calculated for each subgroup of HDL-P and PON1 AREase activity (see Figure 3). PON1 AREase activity was most strongly correlated with small+medium HDL-P (r_p=0.32; *P*=0.00023), followed by total HDL-P (r_p=0.27; *P*=0.0024). PON1 AREase activity was not correlated with large HDL-P (r_p=0.02; *P*=0.41). Total HDL-P was strongly correlated with small+medium HDL-P (r_p=0.83; *P*<0.0001) followed by large HDL-P (r_p=0.61; *P*<0.0001).

Table 5. Full Multivariable Regression Model Coefficients for Association of Large HDL-P and Common cIMT and Internal cIMT

	Common cIMT, μm (N=6512)		Internal cIMT, μm (N=6418)	
Covariates	Coefficient (μm) ±SE	*P* Value	Coefficient (μm) ±SE	*P* Value
Age, y	7.12±0.23	<0.0001	15.80±0.76	<0.0001
Black, %	23.32±5.39	<0.0001	−53.58±17.94	0.0028
Hispanic, %	−12.59±5.72	0.028	−72.13±19.03	0.00015
Asian, %	−24.79±7.16	0.00054	−205.34±23.81	<0.0001
Male sex, %	36.47±4.466	<0.0001	89.04±15.49	<0.0001
BMI, kg/m^2	2.97±0.44	<0.0001	1.53±1.47	0.29
Former smoker, %	11.01±5.25	0.036	46.87±17.45	0.0073
Current smoker, %	5.43±7.37	0.46	97.39±24.52	<0.0001
Pack-years	0.46±0.12	0.00013	2.49±0.40	<0.0001
SBP, mm Hg	1.49±0.11	<0.0001	2.62±0.36	<0.0001
HTN Rx, %	3.77±4.77	0.43	69.53±15.87	<0.0001
Fasting glucose, mg/dL	0.15±0.094	0.11	0.47±0.31	0.14
Diabetes status, %	21.56±8.71	0.013	87.07±28.95	0.0026
LDL-C, mg/dL	0.45±0.068	<0.0001	1.16±0.23	<0.0001
HDL-C, mg/dL	−0.99±0.34	0.0040	−2.45±1.15	0.032
LLM Rx, %	18.50±5.85	0.0016	130.95±19.47	<0.0001
Large HDL-P, μmol/L	1.55±1.50	0.30	4.84±4.99	0.33

BMI indicates body mass index; cIMT, carotid intima-media thickening; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; HTN Rx, hypertension medication treatment; LDL-C, low-density lipoprotein cholesterol; LLM Rx, lipid-lowering medication treatment; SBP, systolic blood pressure.

Discussion

Functional aspects of HDL not reflected by HDL-C measurements[14–16] may be responsible for the repeated observation that HDL is protective against CVD.[12,34–36] In the current work, we present further evidence that small, dense HDL-P—which is more closely correlated with cardioprotective PON1 activity[25,37]—is inversely associated with cIMT. Specifically, we have used linear regression in a large cross-sectional analysis of ≈6500 MESA participants to demonstrate that cholesterol-poor small+medium HDL-P concentration is significantly and inversely associated with common and internal cIMT after adjustment for HDL-C and other cardiovascular confounders, whereas large HDL-P was not. Amount of cIMT is positively associated with incident coronary heart disease[38,39]; thus, the finding of decreased common and internal

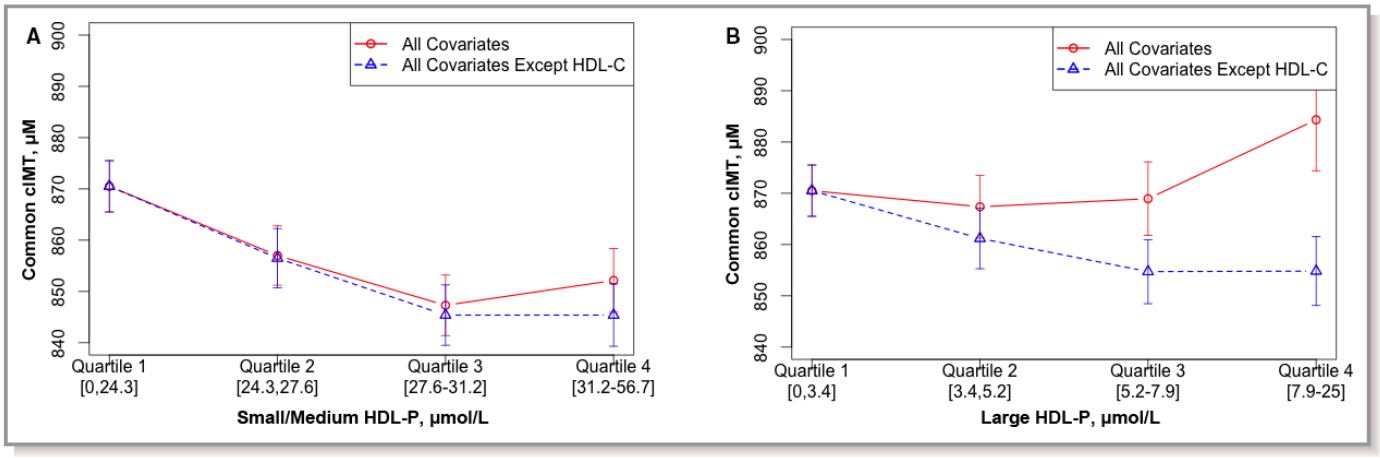

Figure 1. Mean covariate-adjusted common cIMT (μm) for small+medium high-density lipoprotein particle concentration (HDL-P) (A) and large HDL-P (B) before and after adjustment for HDL-C. See Tables 4 and 5 for list of covariates. cIMT indicates carotid intima-media thickening; HDL-C, high-density lipoprotein cholesterol.

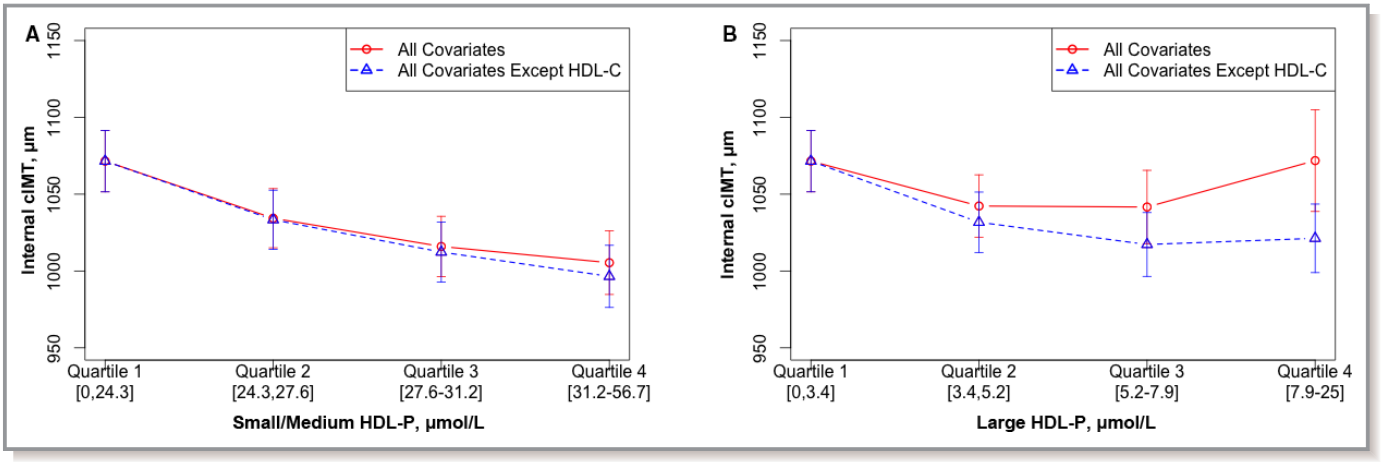

Figure 2. Mean covariate-adjusted internal cIMT (µm) for small+medium high-density lipoprotein particle concentration (HDL-P) (A) and large HDL-P (B) before and after adjustment for HDL-C. See Tables 4 and 5 for list of covariates. cIMT indicates carotid intima-media thickening; HDL-C, high-density lipoprotein cholesterol.

cIMT with increasing small+medium HDL-P is suggestive of possible protection from atherosclerotic end-organ damage. Finally, using 126 CLEAR participants, we report that PON1 AREase activity is more strongly correlated with small+medium HDL-P (r_p=0.32; P=0.00023) than large HDL-P concentration (r_p=0.02; P=0.41).

Large HDL-P is the cholesterol-rich subfraction of HDL.[9,40] In these data, large HDL-P is significantly associated with decreased common and internal cIMT after adjustment for a range of covariates. However, the association of large HDL-P with both common and internal cIMT is attenuated by addition of HDL-C to the multivariable regression model. In contrast, the association of small+medium HDL-P was robust to post-hoc step-wise addition of HDL-C to the regression model. These results suggest that HDL-C is responsible for the inverse correlation between large HDL-P and both common and internal cIMT, while also demonstrating possible antiatherogenic properties of small+medium HDL-P that are not fully reflected by HDL-C measurements alone, as first hypothesized by Kontush and Chapman.[14–16]

Table 6. Sensitivity Analysis by Race of Association of Small+Medium HDL-P With Common cIMT (µm)

	White (N=2506)		Black (N=1808)		Hispanic (N=1430)		Asian (N=768)	
Covariates	Coefficient±SE	*P* Value	Coefficient±SE	*P* Value	Coefficient±SE	*P* Value	Coefficient±SE	*P* Value
Age, y	8.04±0.36	<0.0001	6.79±0.46	<0.0001	6.96±0.50	<0.0001	4.77±0.62	<0.0001
Male sex, %	25.65±7.57	0.00072	47.53±9.28	<0.0001	37.46±10.22	0.00026	15.01±13.44	0.26
BMI, kg/m^2	3.46±0.73	<0.0001	2.44±0.78	0.0018	3.21±0.95	0.00075	5.06±1.85	0.0063
Former smoker, %	15.34±7.98	0.055	8.70±10.47	0.41	10.53±11.36	0.35	8.52±18.71	0.65
Current smoker, %	14.75±12.48	0.24	−14.73±13.38	0.27	23.56±14.94	0.11	20.68±27.52	0.45
Pack-years	0.38±0.16	0.019	0.55±0.26	0.033	0.29±0.32	0.36	0.93±0.49	0.061
SBP, mm Hg	1.71±0.18	<0.0001	1.23±0.20	<0.0001	1.53±0.23	<0.0001	1.74±0.29	<0.0001
HTN Rx, %	7.42±7.58	0.33	−3.96±9.05	0.66	17.17±10.58	0.10	−9.33±13.91	0.50
Fasting glucose, mg/dL	0.22±0.21	0.31	0.18±0.17	0.31	0.051±0.16	0.74	0.36±0.27	0.19
Diabetes status, %	28.26±18.48	0.13	6.65±14.78	0.65	32.14±16.83	0.056	26.46±23.31	0.26
LDL-C, mg/dL	0.33±0.11	0.0039	0.39±0.13	0.0017	0.63±0.14	<0.0001	0.41±0.19	0.037
HDL-C, mg/dL	−0.58±0.26	0.023	−0.64±0.31	0.037	−0.25±0.39	0.52	−0.43±0.49	0.39
LLM Rx, %	11.54±8.96	0.19	46.81±11.43	<0.0001	−8.29±13.73	0.55	34.09±17.19	0.048
Small+Med HDL-P, µmol/L	−1.15±0.65	0.079	−2.15±0.78	0.0058	−1.84±0.92	0.045	−0.23±1.19	0.85

Linear regression analyses of small+medium HDL-P and all other covariates in Table 4, association with common cIMT in the Multi-Ethnic Study of Atherosclerosis (MESA) study, stratified by race. BMI indicates body mass index; cIMT, carotid intima-media thickening; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; HTN Rx, hypertension medication treatment; LDL-C, low-density lipoprotein cholesterol; LLM Rx, lipid-lowering medication treatment; SBP, systolic blood pressure.

Table 7. Sensitivity Analysis by Race of Association of Small+Medium HDL-P With Internal cIMT (μm)

	White (N=2470)		Black (N=1782)		Hispanic (N=1409)		Asian (N=757)	
Covariates	Coefficient±SE	*P* Value	Coefficient±SE	*P* Value	Coefficient±SE	*P* Value	Coefficient±SE	*P* Value
Age, y	18.48±1.24	<0.0001	15.47±1.53	<0.0001	17.12±1.65	<0.0001	6.69±1.77	0.00017
Male sex, %	66.34±25.96	0.011	52.03±31.13	0.095	150.34±33.61	<0.0001	62.58±38.44	0.10
BMI, kg/m^2	2.73±2.49	0.27	1.11±2.62	0.67	2.08±3.13	0.51	−4.80±5.29	0.36
Former smoker, %	21.49±27.38	0.43	99.98±35.10	0.0044	58.23±37.35	0.12	2.27±53.52	0.97
Current smoker, %	91.29±42.76	0.033	107.16±44.85	0.017	167.97±49.13	0.00065	−22.49±78.73	0.78
Pack-years	3.29±0.56	<0.0001	0.97±0.86	0.26	1.49±1.04	0.15	3.26±1.41	0.021
SBP, mm Hg	2.89±0.62	<0.0001	3.81±0.68	<0.0001	1.99±0.75	0.0077	1.56±0.84	0.062
HTN Rx, %	147.90±25.97	<0.0001	4.48±30.35	0.88	54.97±34.79	0.11	19.39±39.80	0.64
Fasting glucose, mg/dL	0.41±0.73	0.57	0.042±0.58	0.94	0.079±0.52	0.88	1.77±0.77	0.023
Diabetes status, %	21.47±63.34	0.73	68.53±49.56	0.17	200.96±55.36	0.00029	98.56±66.69	0.14
LDL-C, mg/dL	1.39±0.39	0.00039	0.37±0.43	0.39	1.73±0.45	0.00012	0.73±0.56	0.19
HDL-C, mg/dL	−1.18±0.88	0.18	−1.44±1.03	0.16	−0.13±1.28	0.92	−2.01±1.43	0.16
LLM Rx, %	118.81±30.70	0.00011	204.28±38.33	<0.0001	50.03±45.16	0.27	201.14±49.19	<0.0001
Small+med HDL-P, μmol/L	−5.59±2.24	0.013	−4.11±2.61	0.10	−0.81±3.02	0.79	−2.25±3.42	0.51

Linear regression analyses of small+medium HDL-P and all other covariates in Table 4, association with internal cIMT in the Multi-Ethnic Study of Atherosclerosis (MESA) study, stratified by race. BMI indicates body mass index; cIMT, carotid intima-media thickening; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; HTN Rx, hypertension medication treatment; LDL-C, low-density lipoprotein cholesterol; LLM Rx, lipid-lowering medication treatment; SBP, systolic blood pressure.

Reverse cholesterol transport (RCT), whereby cholesterol is removed from peripheral cells and atherosclerotic plaque,[41,42] represents another possible function of HDL that may underlie its cardioprotective effects. A recent study has demonstrated the importance of RCT through direct measurement of macrophage cholesterol efflux capacity in a longitudinal

Table 8. Sensitivity Analysis by Sex of Association of Small+Medium HDL-P With Common cIMT and Internal cIMT

	Common cIMT, μm (N=6512)				Internal cIMT, μm (N=6418)			
	Male (N=3072)		Female (N=3440)		Male (N=3037)		Female (N=3381)	
Covariates	Coefficient±SE	*P* Value	Coefficient±SE	*P* Value	Coefficient±SE	*P* Value	Coefficient±SE	*P* Value
Age, y	7.53±0.3−5	<0.0001	6.80±0.30	<0.0001	16.49±1.15	<0.0001	15.26±1.02	<0.0001
Black, %	28.59±8.22	0.00051	17.03±7.24	0.019	−78.07±27.33	0.0043	−42.64±24.26	0.079
Hispanic, %	−12.53±8.50	0.14	−13.67±7.69	0.076	−43.39±28.26	0.12	−100.04±25.75	0.00011
Asian, %	−20.03±10.67	0.061	−24.94±9.70	0.010	−211.51±35.46	<0.0001	−194.93±32.49	<0.0001
BMI, kg/m^2	5.84±0.81	<0.0001	1.90±0.52	0.00024	3.77±2.69	0.16	0.66±1.73	0.70
Former smoker, %	12.98±7.67	0.091	10.37±7.34	0.16	12.29±25.52	0.63	86.79±24.56	0.00042
Current smoker, %	13.97±10.86	0.20	−2.42±10.16	0.81	82.72±36.11	0.022	113.18±34.03	0.00089
Pack-years	0.42±0.16	0.0073	0.45±0.19	0.022	2.98±0.52	<0.0001	1.52±0.66	0.021
SBP, mm Hg	1.55±0.18	<0.0001	1.52±0.13	<0.0001	3.21±0.59	<0.0001	2.48±0.45	<0.0001
HTN Rx, %	7.71±7.23	0.29	0.85±6.28	0.89	95.93±24.04	<0.0001	49.24±21.04	0.019
Fasting glucose, mg/dL	0.010±0.13	0.94	0.39±0.14	0.0054	0.45±0.42	0.29	0.55±0.47	0.24
Diabetes status, %	41.37±12.49	0.00094	−7.06±12.17	0.56	87.69±41.52	0.035	76.32±40.76	0.061
LDL-C, mg/dL	0.61± 0.10	<0.0001	0.30±0.088	0.00062	1.33±0.35	0.00012	0.91±0.29	0.0019
HDL-C, mg/dL	−0.41±0.29	0.16	−0.61±0.19	0.0019	−0.73±0.98	0.45	−1.54±0.65	0.018
LLM Rx, %	24.91±8.88	0.0050	18.19±7.78	0.020	106.47±29.51	0.00031	163.67±26.07	<0.0001
Small+med HDL-P, μmol/L	−2.91±0.72	<0.0001	−0.70±0.49	0.15	−3.46±2.40	0.15	−3.89±1.64	0.018

Linear regression analyses of small+medium HDL-P and all other covariates in Table 4, association with common and internal cIMT in the Multi-Ethnic Study of Atherosclerosis (MESA) study, stratified by sex. BMI indicates body mass index; cIMT, carotid intima-media thickening; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; HTN Rx, hypertension medication treatment; LDL-C, low-density lipoprotein cholesterol; LLM Rx, lipid-lowering medication treatment; SBP, systolic blood pressure.

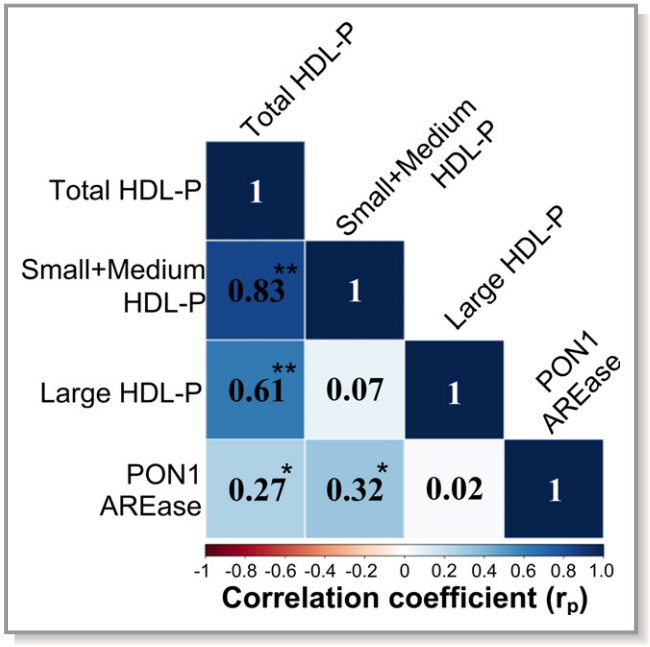

Figure 3. Paraoxonase-1 (PON1) arylesterase hydrolysis activity (AREase) is associated with small and medium high-density lipoprotein particle concentration (HDL-P) concentration (r_p=0.32; P=0.00023), but not large HDL-P concentration (r_p=0.02; P=0.41) in the carotid lesion epidemiology and risk (CLEAR) study (n=126). Cell values reported are Pearson's pair-wise correlation coefficients. *0.05≤P≤0.0001; **P<0.0001.

study.[43] In this study, 2924 adults without baseline CVD were followed for a median of 9.4 years, and the highest quartile of cholesterol efflux had a 67% reduction in CVD incidence as compared to the lowest quartile.[43] Notably, cholesterol efflux has an inverse relationship with HDL particle size and density, with the smallest HDL particles having the highest efficiency of cholesterol efflux from macrophage foam cells.[17]

The antioxidant properties of HDL are another function of HDL that may be responsible for its cardioprotection. Past work found that, similar to cholesterol efflux,[17] antioxidant properties of HDL were enriched in the smallest and densest HDL particles.[10] PON1 is a glycoprotein enzyme produced in the liver with extremely broad substrate specificity (for further in-depth discussion of PON1, please refer to a recent review[44]) that is strongly associated with the smaller/denser HDL particles.[10] Rare mutations in the PON1 gene have previously been reported to be predictive of ischemic stroke.[37] Additionally, PON1 has been shown to prevent in vitro oxidation of LDL[45–47] and HDL.[48] Thus, in this context, our findings that small+medium HDL-P is strongly associated with decreased common and internal cIMT may, at least in part, reflect the increased cardioprotective enzyme activity of PON1 in these particles[10] as compared to large HDL-P.

Antiapoptotic and anti-inflammatory properties of small, dense HDL may also play a role in its protective effects against CVD.[14–16] Recent work in MESA found that small+-medium HDL-P was inversely associated with noncardiovascular inflammation-related death or hospitalization, suggesting that HDL plays an important role in the pathogenesis of numerous inflammation-related diseases.[6] In this regard, the HDL content of sphingosine-1-phosphate (S1P) and other lipids carried on HDL are important considerations as possible molecular mediators of this anti-inflammatory effect. S1P is enriched in small and dense HDL[18] and is inversely correlated with endothelial cell apoptosis.[18,19] In addition, S1P has been shown to inhibit neutrophil migration and leukocyte adhesion to sites of cellular injury.[49] S1P and other HDL-related lipids were not measured in MESA, and their effects on cIMT independent of small+medium HDL-P could not be determined in the current work.

Numerous studies have found that potentially cardioprotective properties increase in potency with decreasing size and density of HDL (ie, antioxidation,[10] anti-inflammatory effects,[6] cholesterol efflux,[17] and S1P content[18]). To test the possibility that the small+medium HDL-P association with both common and internal cIMT was driven by small HDL-P, we performed a post-hoc analysis including all HDL-P measures (see Table 6). We found that both small and medium HDL-P were independently and significantly associated with common and internal cIMT. Further investigation in other longitudinal studies with HDL-P measures is required to determine whether either of small or medium HDL-P is more strongly associated with decreased cardiovascular disease incidence.

Sensitivity analyses of the relationship of small+medium HDL-P with common and internal cIMT measures across racial groups have limited power. Notably, the direction of the relationship of the HDL measures with both common and internal cIMT is negative across all racial groups and both sex groups, if not always statistically significant. However, we note that Asians and women have a near-zero association of small+medium HDL and common CIMT. In addition, Hispanics had an attenuated beta coefficient and nonsignificant association between small+medium HDL-P and internal cIMT. Though a formal interaction test was not significant for effect modification by race group (including Asians) of small+medium HDL-P association with either common or internal cIMT, we did note a significant interaction between male sex and small+medium HDL-P for the association with common cIMT. Notably, work by Mackey et al., analyzing 5597 overlapping participants from this current work, did not find evidence of effect modification by sex or race for total HDL-P or HDL-C and cIMT measures.[5] Further work is required to elucidate whether these findings represent true population differences in associations of small+medium HDL-P concentration, or whether they result from random population variance.

Limitations of this study should be considered. First, this study is cross-sectional and observational. Second, these

results imply that unmeasured functional aspects of small+medium HDL-P are at least partially responsible for the HDL-C independent and inverse association of small+medium HDL-P and carotid atherosclerosis. However, with the current data, it is not currently possible to distinguish whether the effects are attributed to PON1 or other antioxidant protein activity, RCT, S1P, or other molecule-associated anti-inflammatory and antiapoptotic properties, or possibly other HDL-related functions.[15] Similarly, PON1 enzyme activity requires calcium ions for proper function. Therefore, plasma stored with EDTA or other calcium-chelating agents cannot be measured for their PON1 activity; consequently, PON1 activity could not be measured in the MESA cohort. Finally, several variables with importance to CVD in general and HDL in particular (eg, S1P) were not available and thus could not be included in analyses. Strengths of this study include its multiethnic and sex-balanced design, which allow for greater sex- and genetic ancestry–specific generalizations regarding the associations of HDL-P subclasses with cIMT outcomes.

In summary, we have performed analyses on participants in the large, well-characterized MESA cohort and report that small+medium HDL-P is strongly and inversely associated with cIMT in both the common and internal carotid arteries. Large HDL-P is not associated with cIMT in either carotid artery branch after adjustment for HDL-C, suggesting that cholesterol carried by HDL is likely not important in prevention of cardiovascular and cerebrovascular disease. Finally, we report, from separate data in the CLEAR study, that cardioprotective PON1 AREase activity is most strongly correlated with small+medium HDL-P, as compared to both total HDL-P and large HDL-P measures. Future work should evaluate the specific functional aspects of HDL, including PON1 activity, to identify biomarkers for clinical intervention.

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Disclosures

None.

References

1. AIM-HIGH Investigators, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. *N Engl J Med*. 2011;365:2255–2267.
2. Schwartz GG, Olsson AG, Abt M, Ballantyne CM, Barter PJ, Brumm J, Chaitman BR, Holme IM, Kallend D, Leiter LA, Leitersdorf E, McMurray JJV, Mundl H, Nicholls SJ, Shah PK, Tardif J-C, Wright RS. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med*. 2012;367:2089–2099.
3. HPS2-THRIVE Collaborative Group, Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, Wallendszus K, Craig M, Jiang L, Collins R, Armitage J. Effects of extended-release niacin with laropiprant in high-risk patients. *N Engl J Med*. 2014;371:203–212.
4. Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, Hindy G, Hólm H, Ding EL, Johnson T, Schunkert H, Samani NJ, Clarke R, Hopewell JC, Thompson JF, Li M, Thorleifsson G, Newton-Cheh C, Musunuru K, Pirruccello JP, Saleheen D, Chen L, Stewart AF, Schillert A, Thorsteinsdottir U, Thorgeirsson G, Anand S, Engert JC, Morgan T, Spertus J, Stoll M, Berger K, Martinelli N, Girelli D, McKeown PP, Patterson CC, Epstein SE, Devaney J, Burnett MS, Mooser V, Ripatti S, Surakka I, Nieminen MS, Sinisalo J, Lokki M-L, Perola M, Havulinna A, de Faire U, Gigante B, Ingelsson E, Zeller T, Wild P, de Bakker PI, Klungel OH, Maitland-van der Zee AH, Peters BJM, de Boer A, Grobbee DE, Kamphuisen PW, Deneer VH, Elbers CC, Onland-Moret NC, Hofker MH, Wijmenga C, Verschuren WM, Boer JM, van der Schouw YT, Rasheed A, Frossard P, Demissie S, Willer C, Do R, Ordovas JM, Abecasis GR, Boehnke M, Mohlke KL, Daly MJ, Guiducci C, Burtt NP, Surti A, Gonzalez E, Purcell S, Gabriel S, Marrugat J, Peden J, Erdmann J, Diemert P, Willenborg C, König IR, Fischer M, Hengstenberg C, Ziegler A, Buysschaert I, Lambrechts D, Van de Werf F, Fox KA, El Mokhtari NE, Rubin D, Schrezenmeir J, Schreiber S, Schäfer A, Danesh J, Blankenberg S, Roberts R, McPherson R, Watkins H, Hall AS, Overvad K, Rimm E, Boerwinkle E, Tybjaerg-Hansen A, Cupples LA, Reilly MP, Melander O, Mannucci PM, Ardissino D, Siscovick D, Elosua R, Stefansson K, O'Donnell CJ, Salomaa V, Rader DJ, Peltonen L, Schwartz SM, Altshuler D, Kathiresan S. Plasma HDL cholesterol and risk of myocardial infarction: a Mendelian randomisation study. *Lancet*. 2012;380:572–580.
5. Mackey RH, Greenland P, Goff DC, Lloyd-Jones D, Sibley CT, Mora S. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events. *J Am Coll Cardiol*. 2012;60:508–516.
6. Duprez DA, Otvos J, Tracy RP, Feingold KR, Greenland P, Gross MD, Lima JAC, Mackey RH, Neaton JD, Sanchez OA, Jacobs DR. High density lipoprotein subclasses and noncardiovascular, noncancer chronic inflammatory related events versus cardiovascular events: the Multi Ethnic Study of Atherosclerosis. *J Am Heart Assoc*. 2015;4:e002295 doi: 10.1161/JAHA.115.002295.
7. Castelli WP. Cardiovascular disease and multifactorial risk: challenge of the 1980s. *Am Heart J*. 1983;106:1191–1200.
8. Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, Byun J, Vuletic S, Kassim S, Singh P, Chea H, Knopp RH, Brunzell J, Geary R, Chait A, Zhao X-Q, Elkon K, Marcovina S, Ridker P, Oram JF, Heinecke JW. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. *J Clin Invest*. 2007;117:746–756.
9. Asztalos BF, Tani M, Schaefer EJ. Metabolic and functional relevance of HDL subspecies. *Curr Opin Lipidol*. 2011;22:176–185.
10. Kontush A, Chantepie S, Chapman MJ. Small, dense HDL particles exert potent protection of atherogenic LDL against oxidative stress. *Arterioscler Thromb Vasc Biol*. 2003;23:1881–1888.
11. de la Llera-Moya de M, Drazul-Schrader D, Asztalos BF, Cuchel M, Rader DJ, Rothblat GH. The ability to promote efflux via ABCA1 determines the capacity of serum specimens with similar high-density lipoprotein cholesterol to remove cholesterol from macrophages. *Arterioscler Thromb Vasc Biol*. 2010;30:796–801.
12. Kim DS, Hutchins PM, Jarvik GP. The HDL particle: frontiers for new discovery in cardioprotection. *Clin Lab Int*. 2015;38:16–19.
13. Hutchins PM, Ronsein GE, Monette JS, Pamir N, Wimberger J, He Y, Anantharamaiah GM, Kim DS, Ranchalis JE, Jarvik GP, Vaisar T, Heinecke JW. Quantification of HDL particle concentration by calibrated ion mobility analysis. *Clin Chem*. 2014;60:1393–1401.
14. Kontush A, Chapman MJ. Antiatherogenic small, dense HDL—guardian angel of the arterial wall? *Nat Clin Pract Cardiovasc Med*. 2006;3:144–153.
15. Kontush A, Chapman MJ. Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. *Pharmacol Rev*. 2006;58:342–374.

16. Camont L, Chapman MJ, Kontush A. Biological activities of HDL subpopulations and their relevance to cardiovascular disease. *Trends Mol Med*. 2011;17:594–603.
17. Du X-M, Kim M-J, Hou L, Le Goff W, Chapman MJ, Van Eck M, Curtiss LK, Burnett JR, Cartland SP, Quinn CM, Kockx M, Kontush A, Rye K-A, Kritharides L, Jessup W. HDL particle size is a critical determinant of ABCA1-mediated macrophage cellular cholesterol export. *Circ Res*. 2015;116:1133–1142.
18. Kontush A, Therond P, Zerrad A, Couturier M, Negre-Salvayre A, de Souza JA, Chantepie S, Chapman MJ. Preferential sphingosine-1-phosphate enrichment and sphingomyelin depletion are key features of small dense HDL3 particles: relevance to antiapoptotic and antioxidative activities. *Arterioscler Thromb Vasc Biol*. 2007;27:1843–1849.
19. Kimura T, Sato K, Malchinkhuu E, Tomura H, Tamama K, Kuwabara A, Murakami M, Okajima F. High-density lipoprotein stimulates endothelial cell migration and survival through sphingosine 1-phosphate and its receptors. *Arterioscler Thromb Vasc Biol*. 2003;23:1283–1288.
20. Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. *Arterioscler Thromb Vasc Biol*. 1995;15:1987–1994.
21. Rosenson RS, Brewer HB, Ansell B, Barter P, Chapman MJ, Heinecke JW, Kontush A, Tall AR, Webb NR. Translation of high-density lipoprotein function into clinical practice: current prospects and future challenges. *Circulation*. 2013;128:1256–1267.
22. Kim DS, Burt AA, Rosenthal EA, Ranchalis JE, Eintracht JF, Hatsukami TS, Furlong CE, Marcovina S, Albers JJ, Jarvik GP. HDL-3 is a superior predictor of carotid artery disease in a case-control cohort of 1725 participants. *J Am Heart Assoc*. 2014;3:e000902. doi: 10.1161/JAHA.114.000902.
23. Tiozzo E, Gardener H, Hudson BI, Dong C, Della-Morte D, Crisby M, Goldberg RB, Elkind MSV, Cheung YK, Wright CB, Sacco RL, Rundek T. High-density lipoprotein subfractions and carotid plaque: the Northern Manhattan Study. *Atherosclerosis*. 2014;237:163–168.
24. McGarrah RW, Craig DM, Haynes C, Dowdy ZE, Shah SH, Kraus WE. High-density lipoprotein subclass measurements improve mortality risk prediction, discrimination and reclassification in a cardiac catheterization cohort. *Atherosclerosis*. 2016;246:229–235.
25. Kim DS, Burt AA, Ranchalis JE, Richter RJ, Marshall JK, Eintracht JF, Rosenthal EA, Furlong CE, Jarvik GP. Additional common polymorphisms in the PON gene cluster predict PON1 activity but not vascular disease. *J Lipids*. 2012;2012:476316.
26. Jarvik GP, Rozek LS, Brophy VH, Hatsukami TS, Richter RJ, Schellenberg GD, Furlong CE. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is PON1192 or PON155 genotype. *Arterioscler Thromb Vasc Biol*. 2000;20:2441–2447.
27. Jarvik GP, Hatsukami TS, Carlson C, Richter RJ, Jampsa R, Brophy VH, Margolin S, Rieder M, Nickerson D, Schellenberg GD. Paraoxonase activity, but not haplotype utilizing the linkage disequilibrium structure, predicts vascular disease. *Arterioscler Thromb Vasc Biol*. 2003;23:1465–1471.
28. Kim DS, Burt AA, Ranchalis JE, Vuletic S, Vaisar T, Li W-F, Rosenthal EA, Dong W, Eintracht JF, Motulsky AG, Brunzell JD, Albers JJ, Furlong CE, Jarvik GP. PLTP activity inversely correlates with CAAD: effects of PON1 enzyme activity and genetic variants on PLTP activity. *J Lipid Res*. 2015;56:1351–1362.
29. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med*. 2006;26:847–870.
30. Otvos JD. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. *Clin Lab*. 2002;48:171–180.
31. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18:499–502.
32. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med*. 1999;340:14–22.
33. Mora S, Szklo M, Otvos JD, Greenland P, Psaty BM, Goff DCJ, O'Leary DH, Saad MF, Tsai MY, Sharrett AR. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*. 2007;192:211–217.
34. Javaheri A, Rader DJ. Apolipoprotein A-I and cholesterol efflux: the good, the bad, and the modified. *Circ Res*. 2014;114:1681–1683.
35. deGoma EM, deGoma RL, Rader DJ. Beyond high-density lipoprotein cholesterol levels. *J Am Coll Cardiol*. 2008;51:2199–2211.
36. Rader DJ, Tall AR. The not-so-simple HDL story: is it time to revise the HDL cholesterol hypothesis? *Nat Med*. 2012;18:1344–1346.
37. Kim DS, Crosslin DR, Auer PL, Suzuki SM, Marsillach J, Burt AA, Gordon AS, Meschia JF, Nalls MA, Worrall BB, Longstreth WT, Gottesman RF, Furlong CE, Peters U, Rich SS, Nickerson DA, Jarvik GP; on behalf of the NHLBI Exome Sequencing Project. Rare coding variation in paraoxonase-1 is associated with ischemic stroke in the NHLBI Exome Sequencing Project. *J Lipid Res*. 2014;55:1173–1178.
38. Ebrahim S, Papacosta O, Whincup P, Wannamethee G, Walker M, Nicolaides AN, Dhanjil S, Griffin M, Belcaro G, Rumley A, Lowe GDO. Carotid plaque, intima media thickness, cardiovascular risk factors, and prevalent cardiovascular disease in men and women: the British Regional Heart Study. *Stroke*. 1999;30:841–850.
39. Nambi V, Chambless L, Folsom AR, He M, Hu Y, Mosley T, Volcik K, Boerwinkle E, Ballantyne CM. Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk. *J Am Coll Cardiol*. 2010;55:1600–1607.
40. Asztalos BF, Roheim PS, Milani RL, Lefevre M, McNamara JR, Horvath KV, Schaefer EJ. Distribution of ApoA-I-containing HDL subpopulations in patients with coronary heart disease. *Arterioscler Thromb Vasc Biol*. 2000;20:2670–2676.
41. Wolfbauer G, Albers JJ, Oram JF. Phospholipid transfer protein enhances removal of cellular cholesterol and phospholipids by high-density lipoprotein apolipoproteins. *Biochim Biophys Acta*. 1999;1439:65–76.
42. Chirackal Manavalan AP, Kober A, Metso J, Lang I, Becker T, Hasslitzer K, Zandl M, Fanaee-Danesh E, Pippal JB, Sachdev V, Kratky D, Stefulj J, Jauhiainen M, Panzenboeck U. Phospholipid transfer protein is expressed in cerebrovascular endothelial cells and involved in high density lipoprotein biogenesis and remodeling at the blood-brain barrier. *J Biol Chem*. 2014;289:4683–4698.
43. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, Shaul PW. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med*. 2014;371:2383–2393.
44. Kim DS, Marsillach J, Furlong CE, Jarvik GP. Pharmacogenetics of paraoxonase activity: elucidating the role of high-density lipoprotein in disease. *Pharmacogenomics*. 2013;14:1495–1515.
45. Mackness MI, Arrol S, Durrington PN. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. *FEBS Lett*. 1991;286:152–154.
46. Mackness MI, Arrol S, Abbott C, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. *Atherosclerosis*. 1993;104:129–135.
47. Watson AD, Berliner JA, Hama SY, La Du BN, Faull KF, Fogelman AM, Navab M. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J Clin Invest*. 1995;96:2882–2891.
48. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *J Clin Invest*. 1998;101:1581–1590.
49. Theilmeier G. High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury in vivo via the S1P3 lysophospholipid receptor. *Circulation*. 2006;114:1403–1409.