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Associations Between HDL Particles and Ischemic Events by Vascular Domain, Gender, and Ethnicity: A Pooled Cohort Analysis

Running Title: *Singh et al.; HDL Particles and Events by Vascular Domain*

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Abstract

Background: High density lipoprotein cholesterol concentration (HDL-C) is an established atheroprotective marker, in particular for coronary artery disease; however, HDL particle concentration (HDL-P) may better predict risk. The associations of HDL-C and HDL-P with ischemic stroke and with myocardial infarction (MI) among women and Blacks has not been well studied. We hypothesized that HDL-P would be consistently associated with MI and stroke among women and Blacks compared with HDL-C.

Methods: We analyzed individual level participant data in a pooled cohort of four large population studies without baseline atherosclerotic cardiovascular disease (ASCVD) – the Dallas Heart Study (DHS) (n=2,535), Atherosclerotic Risk in Communities (ARIC) Study (n=1,595), Multi Ethnic Study of Atherosclerosis (MESA) (n=6,632) and Prevention of Renal and Vascular Endstage Disease (PREVEND) (n=5,022). HDL markers were analyzed in adjusted Cox proportional hazard models for MI and ischemic stroke.

Results: In the overall population (n=15,784), HDL-P was inversely associated with the combined outcome of MI and ischemic stroke, adjusted for cardiometabolic risk factors, [hazard ratio (HR) for Q4 vs Q1 0.64, 95% confidence interval [CI] 0.52 to 0.78] as was HDL-C (HR for Q4 vs Q1: 0.76, 95% CI 0.61 to 0.94). Adjustment for HDL-C did not attenuate the inverse relationship between HDL-P and ASCVD, while adjustment for HDL-P attenuated all associations between HDL-C and events. HDL-P was inversely associated with the individual endpoints of MI and ischemic stroke in the overall population, including in women. HDL-P was inversely associated with MI among White participants but not among Black participants (HR Q4 vs Q1 for White 0.49, 95%CI 0.35-0.69; for Black 1.22, 95%CI 0.76-1.98; $p_{interaction} = 0.001$). Similarly, HDL-C was inversely associated with MI among White participants (HR Q4 vs Q1 0.53, 95%CI 0.36-0.78) but had a weak direct association with MI among Black participants (HR Q4 vs Q1 1.75, 95%CI 1.08-2.83; $p_{interaction} < 0.0001$).

Conclusions: In comparison to HDL-C, HDL-P was consistently associated with MI and ischemic stroke in the overall population. Differential associations of both HDL-C and HDL-P for MI by Black ethnicity suggest that ASCVD risk may differ by vascular domain and ethnicity. Future studies should examine individual outcomes separately.

Key Words: HDL; lipids; cholesterol; biomarker; risk; myocardial infarction; stroke; race/ethnicity

Non-standard Abbreviations and Acronyms

HDL-C; HDL-P; ASCVD; DHS; ARIC; MESA; PREVEND; HR; CI; MI; CHD; BIOLINCC; NMR; MRI; LP3; EDTA; Q1; Q4; LDL-C; BMI; hs-CRP; SD; LDL-P; CETP; REGARDS

Clinical Perspective

What is new?

- HDL-P is inversely associated with the specific endpoint of ischemic stroke overall and among women, whereas HDL-C is not associated with ischemic stroke.
- Neither HDL-P nor HDL-C are associated with myocardial infarction in Blacks.

What are the clinical implications?

- HDL-P but not HDL-C may be a useful risk marker for ischemic stroke.
- HDL-P may be a useful risk marker for both myocardial infarction and ischemic stroke among women.
- There is likely minimal utility of HDL markers for risk prediction of myocardial infarction in the Black population.

Introduction

High density lipoprotein cholesterol concentration (HDL-C) is associated with atherosclerotic cardiovascular disease (ASCVD) and remains part of the ASCVD Pooled Cohort Equations as well as the European SCORE risk charts.[1, 2] However, recent epidemiologic studies have suggested that HDL particle concentration (HDL-P) may better associate with ASCVD outcomes, even among those on statin therapy.[3] This is underscored by observations showing that drugs that most potently raise HDL-C, such as niacin and cholesteryl transfer protein inhibitors, do not have consistent effects on HDL-P levels and have not consistently improved ASCVD outcomes.[4-8] However, there remain several relevant gaps in the role of HDL-P and its association with ASCVD, especially in distinct vascular territories and among women and Black populations.[9-12]

Most of the studies investigating HDL-P have been performed in single cohort studies assessing solely coronary heart disease (CHD) or composite outcomes inclusive of different vascular beds.[3, 13-16] Recent investigations of HDL parameters suggest preserved association of HDL-P with CHD but a lack of association with ischemic cerebrovascular disease.[10, 16-19] Thus, whether HDL-P is a robust marker for ischemic stroke remains unknown, especially since strokes typically comprise relatively few events in any single population-based cohort and not uncommonly include ischemic and non-ischemic etiologies as a combined endpoint.

Furthermore, whether HDL-P associates with CHD or ischemic stroke among women or Blacks is not well studied. Among cohorts that include women or Black participants, the numbers of events represented by these groups remains small, limiting the ability to fully assess these relationships.[17, 20-22] In a prior study, we observed a potential interaction by race on the

association between HDL-C but not HDL-P on a composite ASCVD outcome but were limited in exploring interactions for CHD and stroke separately.[20]

Lastly, some reports have suggested that indexing HDL-C to HDL-P or HDL size to HDL-P may capture HDL functionality, with increased cholesterol/size to particle ratio reflecting potential HDL dysfunction.[23] Increasing cholesterol content or size per HDL particle may represent HDL particles that are overloaded with cholesterol or larger and potentially dysfunctional and less able to participate in reverse cholesterol transport. Whether these ratios add additional information with respect to risk prediction of incident cardiovascular events remains unknown.

We sought to investigate specific associations between the markers HDL-P, HDL-C, HDL-C/HDL-P, and HDL size/HDL-P and the outcomes of myocardial infarction and stroke as well as overall ASCVD. We further assessed whether gender or Black ethnicity modified these associations. To overcome the limitations of prior studies, we conducted an individual participant pooled cohort analysis from four separate cohorts: the Dallas Heart Study (DHS), the Multi-Ethnic Study of Atherosclerosis (MESA), the Atherosclerosis Risk in Communities Study (ARIC), and the Prevention of Renal and Vascular End-stage Disease (PREVEND).

Methods

Anonymized data and materials for MESA and ARIC have been made publicly available at BIOLINCC and can be accessed at https://biolincc.nhlbi.nih.gov/home/. Data for PREVEND is available upon request at https://www.maelstrom-research.org/mica/individual-study/prevend and for DHS at https://www.utsouthwestern.edu/research/translational-medicine/doing-research/dallas-heart/.

For this individual participant pooled cohort analysis, four cohorts were included that comprised participants without clinically manifest or self-reported atherosclerotic disease at baseline and that had available HDL data measured by nuclear magnetic resonance (NMR) spectroscopy using the same analytic platform (*NMR LipoProfile*® test; LipoScience (now LabCorp), Raleigh, NC, USA). The Dallas Heart Study (DHS) is a multiethnic population cohort of Dallas County residents with deliberate oversampling of Black participants.[24] From 2000 to 2002, 2782 participants completed detailed in-home surveys, laboratory testing and imaging studies. MESA (Multi-Ethnic Study of Atherosclerosis) is a large, ethnically diverse cohort of 6814 participants aged 45 to 84 years old recruited from six sites in the United States (US) between 2000-2002.[25] Data from the MESA study was obtained via the NHLBI BIOLINCC repository. ARIC (Atherosclerosis Risk in Communities) is a population-based cohort to study cardiovascular disease incidence in Black and White adults ages 45 to 64 years from four US communities.[26] The ARIC Carotid MRI (magnetic resonance imaging) sub-study recruited approximately 2000 participants with previous carotid ultrasound testing to undergo additional imaging with carotid MRI as well as advanced lipoprotein analysis with NMR. Prevention of Renal and Vascular Endstage Disease (PREVEND) is a prospective cohort based in the city of Groningen, The Netherlands, designed to assess the association of urinary albumin excretion with renal and cardiovascular disease.[27] Between 1997 and 1998, participants aged 28 to 75 years were invited to participate with 8592 subjects (6000 subjects with urinary albumin excretion >10 mg/L and 2592 without) completing the screening program and outpatient visit. For the present analysis, data was used from participants who completed the second screening and had available outcome data, leaving a cohort of 6241 participants with complete information for the present

analysis.[28] For each cohort, the study was approved by an institutional review committee and the subjects gave informed consent.

Ethnicity, gender, smoking status, and previous history of ASCVD were self-reported in each cohort. Hypertension was defined uniformly across cohorts as average systolic blood pressure >/= 140 mmHg and average diastolic blood pressure >/= 90 mmHg or use of anti-hypertensive medication. Diabetes was also defined uniformly across cohorts as fasting glucose >/=126 mg/dL or 7 mmol/L or use of diabetic medications.

For all cohorts, venous blood was collected in the fasting state. Total cholesterol, triglycerides, and HDL-C were measured enzymatically using standard methods and expressed in mg/dL or mmol/L. Low density lipoprotein (LDL-C) levels were calculated using the Friedewald equation. Non-HDL-C was calculated as the difference between total cholesterol and HDL-C. Body mass index was calculated as weight divided by height squared. HDL particle concentration (HDL-P) and particle size (HDL-size) were measured on serum or EDTA plasma specimens by *NMR LipoProfile*® testing using a 400 MHz NMR Profiler or Vantera automated analyzer employing the LipoProfile-3 (LP3) deconvolution algorithm in order to obtain uniformity across all cohorts in the measurement of the exposure variables. Spearman's rank correlation coefficients between HDL-C measured enzymatically and HDL-C derived by NMR LP3 deconvolution algorithm were 0.92 for ARIC, 0.87 for DHS, 0.96 for MESA and 0.95 for PREVEND (Figure I in the Supplement).

Clinical events were ascertained in each individual cohort. Methods of adjudication of events in DHS have been described previously.[24] ARIC utilized a combination of follow up phone calls and assessment of hospital discharge information and death certificate information as well as independent adjudicators as described on their website

(https://sites.cscc.unc.edu/aric/surveillance-manuals). In MESA, events were identified through follow up phone calls to participants every 9 to 12 months with adjudication committees determining cardiovascular events. Information about cardiovascular endpoints was obtained from the Dutch Central Bureau for Statistics and the national registry of hospital discharge diagnoses in PREVEND.[29] The length of mean follow up for each cohort was similar, with a range of 8 to 12 years.

The two primary outcomes of interest were defined as 1) first fatal and non-fatal MI and 2) fatal and non-fatal ischemic stroke events. For inclusion of ischemic stroke, we excluded all definite or probable hemorrhagic and embolic stroke events in the cohorts. We defined two additional outcomes – 1) first fatal and non-fatal MI and ischemic strokes combined; 2) a composite outcome including first fatal and non-fatal MI and ischemic strokes as well as coronary and peripheral revascularization procedures.

Statistical Analysis

Variables from all cohorts were harmonized and synthesized into one large cohort which was then analyzed in one step by using individual patient level data. Baseline HDL-C, HDL-P and HDL particle size were expressed as medians with interquartile intervals. We tested linearity in Cox models via a supremum test with 1000 bootstrap replications and found that the majority of HDL parameters were either not normally distributed or had non-linear associations with outcomes other than associations with ischemic stroke. Cox proportional hazards models were used to determine hazard ratios (HR) per increasing race- and gender-specific quartiles of HDL-C, HDL-P, HDL-size, HDL-C/HDL-P and HDL-size/HDL-P for time to first events. Hazard ratios were reported for quartile 4 (Q4) using quartile 1 (Q1) as a reference (quartiles for HDL-C and HDL-P in Table I in the Supplement). For all of the Cox models, we used stratified baseline

hazards, allowing a different baseline hazard function for each study. We also used robust standard errors to account for the possible correlation of the same patients within the same cohort. Proportional hazards assumptions were satisfied by checking Schoenfeld residuals. Restricted cubic splines were generated with 5 knots at the 5th, 25th, 50th, 75th, and 95th percentiles.

Models were adjusted for cohort and traditional risk factors such as age, hypertension, diabetes, smoking, lipid medications, LDL-C, triglycerides as well as body mass index (BMI), waist circumference (cm) and high sensitivity C-reactive protein (hs-CRP). In addition, for the HDL-C models, adjustments were made for all these covariates as well as HDL-P. Similarly, independent associations of HDL-P were assessed with adjustments for the same covariates as well as HDL-C. Data for both models prior to and after adjustment are reported. No additional adjustment was made in the quartile analysis for race/gender since the quartiles generated were race/gender specific; whereas race/gender were included in continuous spline analyses. Interaction testing was performed by gender and ethnicity (Black vs White) followed by stratified models, with P for interaction ≤ 0.05 considered a significant interaction. Otherwise, two-sided p values <0.05 were considered as indicating statistical significance. No adjustments were made for multiple testing. All analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, North Carolina).

Results

The overall pooled cohort comprised 15,784 participants without baseline atherosclerotic disease. The median age was 56 years, 46% were male and 22% were Black. Baseline characteristics of the participants by cohort are displayed in Table 1. The median HDL-C was 48

mg/dL, median HDL-P was 32.5 µmol/L and median HDL-size was 9.1 nm (overall and cohort HDL characteristics displayed in Table 2). Over the mean follow-up period of 8 to 12 years across cohorts, there were 515 fatal/non-fatal MI events, 321 fatal/non-fatal ischemic stroke events and 1,242 overall ASCVD events (Table 3). The pooled cohort consisted of 8,550 women and 3,520 Black participants with variation in the overall number and proportion across cohorts. The number of events by ethnicity, gender and cohort are summarized in Table 3.

HDL-P

In the pooled cohort, HDL-P was inversely associated with MI+stroke as well as the individual endpoints of MI (HR for Q4 vs Q1: 0.63, 95% confidence interval [CI] 0.49- 0.81) and ischemic stroke (HR for Q4 vs Q1: 0.66, 95% CI 0.48-0.93) in a model adjusted for established cardiovascular risk factors (Figure 1). The outcome of ischemic stroke met the linearity assumption and to maximize the power of our analysis, we also examined the relationship between HDL-P and ischemic stroke using continuous hazard ratios. Per one standard deviation (SD) increase in HDL-P, there was a significant reduction in ischemic stroke risk (HR per 1 SD increase: 0.84, 95% CI 0.73-0.96). After additional adjustment for HDL-C, HDL-P remained inversely associated with all outcomes of interest with the exception that the association between HDL-P and ischemic stroke was no longer significant in both the continuous and quartile analysis (Figure 1).

Gender did not modify the association between HDL-P and MI + stroke ($p_{interaction} = 0.1$). (Figure 2). The inverse associations between HDL-P and combined MI+stroke (HR for Q4 vs Q1: 0.50, 95% CI 0.36- 0.69), MI (HR for Q4 vs Q1: 0.51, 95% CI 0.34-0.78) and ischemic stroke (HR for Q4 vs Q1: 0.54, 95% CI 0.33-0.88) were also observed in the women in our

pooled cohort. After adjustment for HDL-C, the association between HDL-P and composite outcomes remained statistically significant in women (data not shown).

Black ethnicity modified the association between HDL-P and MI + stroke ($p_{interaction}$ = 0.03; Figure 3). This was driven by the MI endpoint such that HDL-P was inversely associated with MI among White participants (HR Q4 vs Q1: 0.49, 95% CI 0.35-0.69) but not among Black participants (HR Q4 vs Q1: 1.22, 95% CI 0.76-1.98; Figure 4). Adjustment for HDL-C attenuated the relationship with MI in Whites somewhat but did not attenuate the effect modification by ethnicity ($p_{interaction}$ = 0.001). Interaction testing by cohort did not modify these results.

HDL-C

In the overall pooled cohort, HDL-C was inversely associated with MI + stroke (HR for Q4 vs Q1: 0.76, 95 CI 0.61-0.94) in a model adjusted for the same cardiovascular risk factors as above (Figure 4). The associations between HDL-C and the individual endpoints of MI (HR for Q4 vs Q1: 0.79, 95% CI 0.61 -1.02) and ischemic stroke (HR for Q4 vs Q1: 0.77, 95% CI 0.54-1.10) were not statistically significant. When analyzing ischemic stroke as a continuous variable to maximize power, there was a significant reduction in ischemic stroke risk per one standard deviation increase in HDL-C (HR per 1 SD increase: 0.85, 95% CI 0.75-0.97). However, after additional adjustment for HDL-P, there was no remaining association between HDL-C and combined MI and stroke (HR for Q4 vs Q1: 0.99, 95% CI 0.76- 1.29), or individual MI and ischemic stroke (Figure 4).

Gender did not modify these associations, with no significant interaction for combined or individual endpoints. The inverse association between HDL-C and combined MI+stroke (HR for Q4 vs Q1: 0.61, 95% CI 0.42-0.90) as well as MI (HR for Q4 vs Q1:0.59, 95% CI 0.35-0.97)

were preserved in women (Figure 2). HDL-C was not associated with ischemic stroke in women (HR Q4 vs Q1: 0.75, 95% CI 0.44-1.31). After adjustment for HDL-P, all associations between HDL-C and outcomes in women were no longer statistically significant (data not shown).

Similar to the results for HDL-P, Black ethnicity modified the associations between HDL-C and events, driven in particular by MI (Figure 3). HDL-C was inversely associated with the combined hard endpoint of MI and ischemic stroke as well as the composite endpoint among White participants but had no association in Black participants ($p_{interaction}$ 0.02). Whereas HDL-C was inversely associated with MI among White participants (HR Q4 vs Q1: 0.53, 95%CI 0.36-0.78), this was not observed among Black participants (HR Q4 vs Q1: 1.75, 95%CI 1.08-2.83; $p_{interaction} < 0.0001$) (Figure 3). No relationship was evident between HDL-C and ischemic stroke among either Black or White participants (Figure 3). Using HDL-C values obtained from the LP3 algorithm did not change our results (data not shown). Interaction testing by cohort revealed a modification of the results by inclusion of participants from PREVEND cohort ($p_{interaction}$ 0.001).

Quartiles defining values of HDL-C and HDL-P by ethnicity and gender are displayed in Tables II and III in the Supplement.

Effect Modification by Ethnicity for MI

Black ethnicity modified the association with MI events for both HDL-C and HDL-P in our pooled cohort. To examine this further, we stratified our results by ethnicity in individual cohorts (Figure 5). Given the small sample size of Black participants in PREVEND (2 MI events out of a total of 44 Black participants), hazard ratios were reported for White but not for Black participants in this cohort. The relationship between MI and each HDL parameter in each individual cohort paralleled the different results by ethnicity observed in our pooled cohort

(Figure 5). Spline curves demonstrating the differences in the curves between HDL-C and HDL-P with MI by Black and White participants are shown in Figure 6. Adjustment of HDL-C for HDL-P did not attenuate the effect modification by ethnicity for MI or combined endpoints ($p_{interaction}$ for MI < 0.0001).

Additional HDL Parameters

Associations between outcomes and ratios of HDL cholesterol concentration and size indexed to particle number were also explored. The HDL-C/HDL-P ratio was not associated with either individual events or overall ASCVD in adjusted models (Table IV in the Supplement). In contrast, increasing HDL-size/HDL-P was associated with both individual and composite outcomes (HR for composite Q4 vs Q1: 1.21, 95% CI 1.13-1.29) even after adjustment for risk factors (Table V in the Supplement). However, the point estimates and confidence intervals for the inverse ratio of HDL-P (1/HDL-P) were similar to those of HDL size/HDL-P (Table V in the Supplement). The results were similar for the subgroups of ethnicity and gender (data not shown).

HDL-size alone was not significantly associated with ASCVD after adjustment for cardiovascular risk factors (HR for composite outcome Q4 vs Q1: 0.91, 95% CI 0.77-1.09) as shown in Table IV in the Supplement. These results were unchanged when stratified by ethnicity or gender (data not shown).

Discussion

In this pooled cohort analysis of individual participants free of CVD across four cohorts, increasing HDL-P inversely correlated with both MI and ischemic stroke while the relationship of HDL-C with these endpoints was more modest and not statistically significant. In contrast,

increasing HDL-C was only associated with reduced ASCVD risk among White participants. The association of both HDL-C and HDL-P with the individual endpoint of MI was significantly modified by ethnicity, with no association between either HDL marker and MI in the Black population. With a relatively large number of ischemic stroke events in this combined cohort analysis, we were able to demonstrate an inverse association between both HDL-C as well as HDL-P and stroke. HDL-P attenuated all associations between HDL-C and events whereas HDL-C had negligible effects on associations between HDL-P and events in the overall population.

Although traditional analyses have focused on the cholesterol content of lipoprotein particles (LDL-C and HDL-C), recent studies have elucidated the concept that lipoprotein particle concentration may have a stronger association with ASCVD risk compared to cholesterol content. In the case of LDL-C, when concentrations are in agreement (concordant) with LDL particle concentration (LDL-P), there is a reliable, graded relationship with ASCVD risk and response to therapy. However, discordances between LDL-C and LDL-P can occur within the milieu of marked dyslipidemia and insulin resistance as well as with certain lipid-modifying therapies such as cholesterylester transfer protein (CETP) inhibitors.[30, 31] In these situations, LDL-P typically is linked more strongly to risk and better reflects treatment efficacy.[32] Thus, the hypothesis that HDL particle concentration may also provide better risk prediction compared to HDL-C is justified, despite the fact that HDL-C remains a key and easily measured lipid marker in guideline-recommended risk score algorithms.[31] HDL-C is also required to calculate non-HDL cholesterol, which captures cholesterol in all apoB-containing lipoproteins and is proven to predict risk ASCVD risk in all age categories of men and women.[33] However, in predominantly Caucasian cohorts, the inverse association between Apo A-I with coronary events remained

significant while HDL-C had no association with coronary events after adjustment for Apo A-I.[34] Furthermore, the most potent HDL-C-raising therapies such as niacin and CETP inhibitors have not improved ASCVD outcomes.[8, 35-37]

In this regard, our pooled cohort analysis confirms that HDL-P more consistently associates with ASCVD as compared to HDL-C and essentially attenuates all associations between HDL-C and individual and combined ASCVD outcomes. We aimed to extend these observations to events by specific vascular domains, namely MI and ischemic stroke, and to events in specific populations that have been underrepresented in most longitudinal cohort studies of HDL markers, namely women and Blacks. Our strategy to use a pooled cohort study design specifically addressed the key limitation of prior single-cohort studies: limited numbers of events and subsequent reduced statistical power in investigating these relationships.

Analysis of MI and ischemic stroke endpoints in this pooled cohort analysis revealed complex interactions for both HDL-C and HDL-P. Among women, HDL-C was inversely associated with MI but the association with ischemic stroke was not significant. These inconsistent relationships by gender and vascular domain have not been reported thus far for HDL-C and highlight its further limitations as an overall ASCVD risk marker. In contrast, HDL-P was consistently associated with both MI and ischemic stroke among women. Most previous analysis in single cohorts such as MESA and ARIC revealed inconsistent associations between HDL-C and HDL-P with stroke or examined subclinical endpoints of cerebrovascular disease. [12, 18, 19, 38, 39] Our current pooled cohort analysis includes the largest number of ischemic strokes in a multi-ethnic cohort analyzed for HDL parameters and strongly suggests that HDL-P is inversely related to ischemic stroke risk. We demonstrate that HDL-P was inversely associated with ischemic stroke not just in our overall cohort, but also in women. This is contrasted with a lack

of association in MESA with total strokes (N=176 total and 150 ischemic strokes), likely due to limited power and a lack of association with ischemic strokes in the Heart Protection Study, which was high risk and predominantly European.[16, 39] Neither explored the impact of gender on these associations. Therefore, our cohort is one of the first studies to demonstrate inverse associations between HDL-P and hard cerebrovascular events in women. Furthermore, the lack of association between HDL-C and ischemic stroke overall and in women in our large multi-ethnic pooled cohort contrasts with prior reports with fewer events and less ethnic diversity.[40, 41] This suggests the need to examine HDL-P as a risk marker for ischemic stroke overall and global ASCVD among women in further studies. Although not assessed in this analysis, cholesterol efflux, a primary anti-atherosclerotic function of HDL, inversely associated with incident coronary heart disease in both the MESA and PREVEND cohorts, however it did not associate with carotid plaque progression or with incident ischemic stroke in the MESA cohort.[42, 43] Thus, parameters reflecting different aspects of HDL metabolism, from cholesterol content to particle concentration to function, appear to contain heterogeneous information regarding atherosclerotic risk. Of all these measures, HDL-P most consistently associates with risk for both MI and ischemic stroke in the overall population.

The most striking and unexpected finding was an effect modification by Black race/ethnicity for both HDL-C and HDL-P and risk of MI. Among White participants, HDL-C and HDL-P were inversely associated with incident MI. Initial epidemiologic studies, which were done primarily in predominantly White cohorts, consistently show this association with HDL-C, leading to its inclusion as a major risk biomarker for heart disease. It is also consistent with more contemporary studies in exclusively White or predominantly White cohorts such as EPIC-Norfolk and PREVEND.[44, 45] In contrast, among Black participants in our pooled cohort,

HDL-C and HDL-P did not have an inverse association with MI. This is suggested in the Pooled Cohort Equation, where the beta coefficients for HDL-C and overall ASCVD risk are much weaker in Black (-0.307) versus White men (-13.578), although they do not capture differences in vascular domains of coronary versus cerebrovascular disease. Prior studies from multi-ethnic cohorts such as MESA did not reveal significant effect modifications of HDL-C by race/ethnicity for combined ASCVD endpoints but similarly did not parse out MI separate from stroke or other ASCVD endpoints and were likely not powered to test for interactions by race/ethnicity.[15] A prior study in the DHS suggested effect modification by Black race/ethnicity for composite ASCVD but did not parse out myocardial infarction vs. ischemic stroke due to small numbers of events.[20] However, our results parallel the findings from a meta-analysis of the Jackson Heart Study with 4114 Black participants and the Framingham Offspring Cohort, which was predominantly White. While other risk factors like age, diabetes, BMI and triglycerides were significantly different among Black participants with and without coronary heart disease, HDL-C was not significantly different. HDL-C was not associated with coronary heart disease among the Black participants in adjusted models in this study, similar to our findings.[46] Our pooled cohort had a higher number of MI events (n=166) among a similar number of Black participants compared to the Jackson Heart Study. Increasing HDL-C was not associated with fewer coronary events among the Black population in the ARIC-Carotid MRI sub study we examined, which could explain the difference in association when compared to prior analyses of ARIC, which served as one of the cohorts for validation of Framingham coronary heart disease prediction. However, even with exclusion of participants from ARIC-Carotid MRI, there was no inverse association between HDL-C and MI in the Black population among the remaining cohorts, challenging traditional notions of HDL-C as a biomarker of inverse risk in this ethnic group. A

recently published analysis from the REasons for Geographical And Racial Differences in Stroke (REGARDS) cohort identified an HDL paradox with lower risk of coronary heart disease at an HDL-C range of 30 to <40 mg/dL among the Black population, consistent with our findings that higher HDL-C did not translate to lower MI risk.[47] Intriguingly, in our pooled cohort analyses, another novel observation was the lack of association between HDL-P and MI in Black participants, suggesting that both HDL cholesterol and particle concentration have distinct associations with MI among Blacks as compared to Whites.

There may be some possible explanations for ethnic differences in HDL biology. In general, Blacks have higher HDL-C and lower triglyceride levels compared to Whites, but these characteristics do not necessarily translate into a lower risk of coronary heart disease.[48-51] Based on our analysis of participants by race/ethnicity in individual cohorts, the surprising observation that higher HDL-C may even be directly associated with MI among Blacks may be partly explained by differences not only in HDL subclass composition, but also different relationships between HDL_2-C and HDL_3-C levels and the risk of coronary disease in White and Black populations.[46] Studies examining HDL functionality have found that HDL in Black populations had lower anti-oxidant and anti-inflammatory activity compared to White populations, which may be one explanation of this paradoxical result. While known genetic polymorphisms in hepatic lipase activity may partly explain the higher HDL levels observed in Blacks, there is also data to suggest that these higher HDL levels may not be anti-atherogenic. Blacks also have higher lipoprotein (a) levels compared to Whites, but the direct associations with ischemic/thrombotic events are similar.[52]

Lastly, with respect to ischemic stroke, though there was no effect modification by ethnicity, HDL-C was not associated with ischemic stroke among White or Black participants.

By way of comparison, Black race/ethnicity modified the inverse associations between HDL-P and MI but not between HDL-P and ischemic stroke. Overall HDL-P is a more consistent risk marker compared to HDL-C, with the exception that Black race/ethnicity seems to modify risk associations between HDL-related markers and MI.

We also explored the concept that cholesterol overloaded HDL may be dysfunctional and impart increased risk. Prior studies have suggested that varying metrics of overloaded HDL, such as HDL-size or increased HDL-C to HDL-P ratios may be cross-sectionally associated with increased atherosclerotic disease.[23, 34] In our study, while HDL-C indexed to HDL-P was not linked to any outcomes, HDL size/HDL-P did not impart any additional information beyond HDL-P alone. Theoretically, the cholesterol overloaded HDL particle may be less efficient at cholesterol uptake and reverse cholesterol transport, but simple ratios of overall HDL concentration and size to particle number may be too crude to reflect this dynamic process.

Our analysis had several limitations. Although the diverse ethnic and geographic makeup of our pooled cohort improves overall generalizability, the significant heterogeneity of the populations recruited in the individual cohorts could have biased our results. Geographical or environmental factors that were not adjusted for could have impacted our analysis, especially with respect to the differences in outcomes by race that have not been reported in previous epidemiologic studies. The PREVEND cohort was enriched with participants with albuminuria, which is a known risk factor for increased metabolic abnormalities and cardiovascular morbidity and mortality, which we attempted to account for by adjustment for the cohort in our analyses.[29, 53-55] Although there is a more consistent association between HDL-P and ASCVD events, our study does not address whether HDL-P would improve clinical risk stratification for ASCVD over HDL-C as it stands in current risk prediction models. We did not see effect modification by

gender in the overall population, but whether there is a difference between genders within the racial subgroups was not addressed by our analysis. Given overall healthy baseline cohorts and our goal to examine outcomes for MI and ischemic stroke, we may not have sufficient power to examine these differences. All four cohorts in our study used the identical proprietary NMR algorithm to measure HDL-P which is critical since there is significant variation between the absolute measurements of HDL-P derived by different methodologies.[56] It is unknown whether measurement of HDL-P by alternative methods such as calibrated ion mobility would have altered our primary findings, although it is important to note that even with different methodologies, the inverse association between HDL-P and atherosclerotic disease has been consistently present.[57-59]

In conclusion, our study suggests that HDL-C may not be as consistent a marker for ASCVD as previously thought, especially for ischemic stroke. Our large pooled cohort demonstrated that HDL-P is more consistent than HDL-C in associating with MI and ischemic stroke in the general population and in women. An important exception was that neither HDL-C nor HDL-P was associated with MI in the Black population, suggesting that ethnicity differentially impacts the association between HDL parameters and atherosclerotic disease in different vascular beds. Future refinements of risk prediction algorithms should more precisely parse out ischemic endpoints by race/ethnicity if HDL-C is to remain as a risk factor in these equations for the Black population. An important next step is examining whether HDL particle composition imparts additional risk prediction information.

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Supplemental Materials

Supplementary Figure I

Supplementary Tables I-V

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Table 1. Baseline characteristics of participants in individual cohorts.

	Overall (n=15784)	DHS (n=2535)	MESA (n=6632)	ARIC (n=1595)	PREVEND (n=5022)
Age (yrs)	56.8 (13.1)	43.7 (9.87)	62.2 (10.2)	70.9 (5.6)	53.1 (11.9)
Gender (female)	8550, 54.2%	1413, 55.7%	3506, 52.9%	888, 55.7%	2730,54.4%
Black	3520, 22.3%	1212, 47.8%	1831, 27.6%	412, 25.8%	44, 0.9%
SBP (mmHg)	126 (19)	124 (18)	127 (21)	125 (14)	126 (19)
LDLc (mg/dL)	115 (32)	107 (35)	117 (32)	118 (34)	115 (29)
Total cholesterol (mg/dL)	125 (94)	181 (39)	194 (35)	197 (40)	212 (40)
BMI (kg/m2)	28 (6.0)	29.6 (7.0)	28.3 (5.4)	28.9 (5.3)	26.6 (4.4)
Fasting glucose (mg/dL)	95 (27)	101 (41)	97 (30)	107 (24)	90 (21)
Diabetes	1808, 10%	273, 9.8%	851, 12.6%	332, 19.9%	352, 5.6%
Waist circumference (cm)	96 (14)	98.9 (16.6)	98.1 (14.4)	98.9 (12.7)	91.7 (12.7)
Smoking	3505, 20%	749, 27%	878, 13%	151, 9%	1727, 28%

Table 2. HDL characteristics of overall and individual cohorts.

	Overall	DHS	MESA	ARIC	PREVEND
HDL-C (mg/dL)	48 (40-57)	48 (40-57)	48 (40-59)	48 (40-58)	47 (40-56)
HDL-P (μmol/L)	32.5 (28.8-36.8)	32.8 (28.9-37.1)	33.4 (29.3-38)	34.9 (31.2-39.3)	31.2 (27.8-34.5)
HDL size (nm)	9.1 (8.8-9.5)	9.0 (8.7-9.3)	9.2 (8.9-9.6)	9.1 (8.7-9.5)	9.1 (8.7-9.6)
HDL-C/HDL-P (10 mg/μmol)	1.47 (1.31-1.66)	1.45 (1.26-1.70)	1.45 (1.30-1.64)	1.35 (1.21-1.56)	1.52 (1.37-1.69)
HDL size/HDL-P (nm/μmol/L)	0.28 (0.25-0.31)	0.27 (0.24-0.31)	0.28 (0.25-0.31)	0.26 (0.24-0.29)	0.29 (0.27-0.32)

Median values are reported with interquartile interval in parenthesis.
ARIC=Atherosclerotic Risk in Communities Study; DHS=Dallas Heart Study; MESA=Multi Ethnic Study of Atherosclerosis; PREVEND=Prevention of Renal and Vascular Endstage Disease; HDL=high density lipoprotein; HDL-C=high density lipoprotein concentration; HDL-P=high density lipoprotein particle concentration.

Table 3. Number of first events for each primary and composite outcome stratified by ethnicity, gender and cohort.

	MI	Ischemic Stroke	MI + Stroke	Composite
Men (n=7234)	340	173	491	786
Women (n=8550)	175	148	314	456
Black (n=3520)	149	100	238	347
White (n=9371)	280	178	441	713
ARIC (n=1595)	126	94	207	217
DHS (n=2535)	89	46	127	185
MESA (n=6632)	218	118	328	536
PREVEND (n=5022)	82	63	143	304
Total (n=15784)	515	321	805	1242

MI=myocardial infarction; ARIC=Atherosclerotic Risk in Communities Study; DHS=Dallas Heart Study; MESA=Multi Ethnic Study of Atherosclerosis; PREVEND=Prevention of Renal and Vascular Endstage Disease.

Figure Legends

Figure 1. Association of HDL-P with individual and composite ASCVD outcomes before and after adjustment for HDL-C.

Cox proportional hazards models of sex/ethnicity-adjusted quartile 4 vs quartile 1 of HDL-P for stroke, MI and composite ASCVD outcomes before and after adjustment for HDL-C. Both models include adjustment for risk factors and cohort. Risk factors adjusted for: age, diabetes, hypertension, smoking, LDL-C, triglycerides, lipid lowering medications, BMI, waist circumference and hs-CRP.

Figure 2. Association of HDL-C with individual and composite ASCVD outcomes before and after adjustment for HDL-P.

Cox proportional hazards models of sex/ethnicity-adjusted quartile 4 (Q4) vs quartile 1 (Q1) of HDL-C for stroke, MI and composite ASCVD outcomes before and after adjustment for HDL-P. Both models include adjustment for risk factors and cohort. Risk factors adjusted for: age, diabetes, hypertension, smoking, LDL-C, triglycerides, lipid lowering medications, BMI, waist circumference and hs-CRP.

Figure 3. Association of HDL-C and HDL-P with outcomes stratified by gender.

Cox proportional hazards models of sex/ethnicity-adjusted quartile 4 (Q4) vs quartile 1 (Q1) of HDL-C and HDL-P for stroke, MI and composite ASCVD outcomes in men and women. Both models include adjustment for risk factors (age, diabetes, hypertension, smoking, LDL-C, triglycerides, lipid lowering medications, BMI, waist circumference and hs-CRP) and cohort.

Figure 4. Association of HDL-C and HDL-P with outcomes stratified by ethnicity.

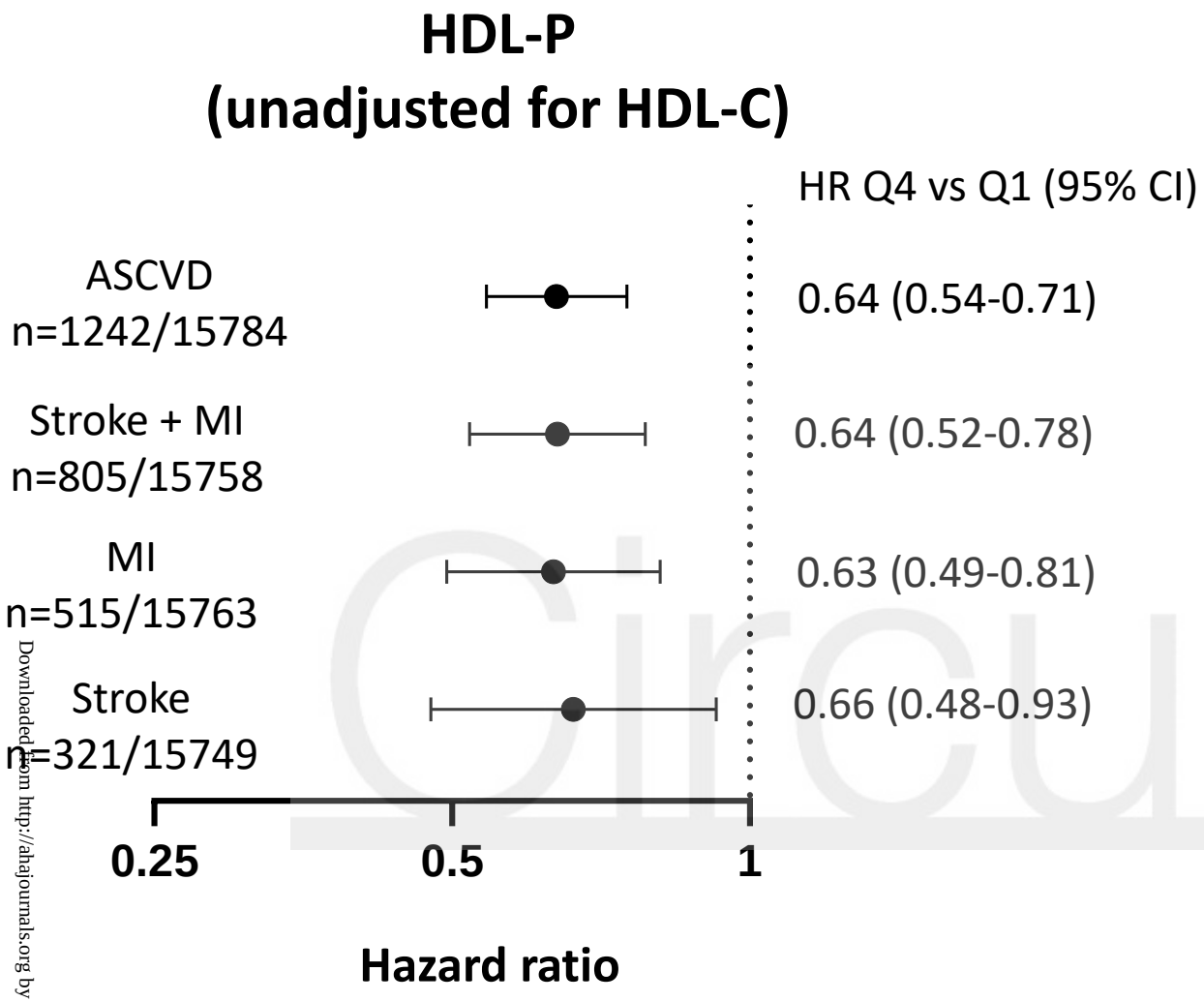
Cox proportional hazards models of sex/ethnicity-adjusted quartile 4 (Q4) vs quartile 1 (Q1) of HDL-C and HDL-P for stroke, MI and composite ASCVD outcomes stratified by Black vs White participants. Both models include adjustment for risk factors (age, diabetes, hypertension, smoking, LDL-C, triglycerides, lipid lowering medications, BMI, waist circumference and hs-CRP) and cohort. In this model, no additional adjustment for HDL-P or HDL-C was made.

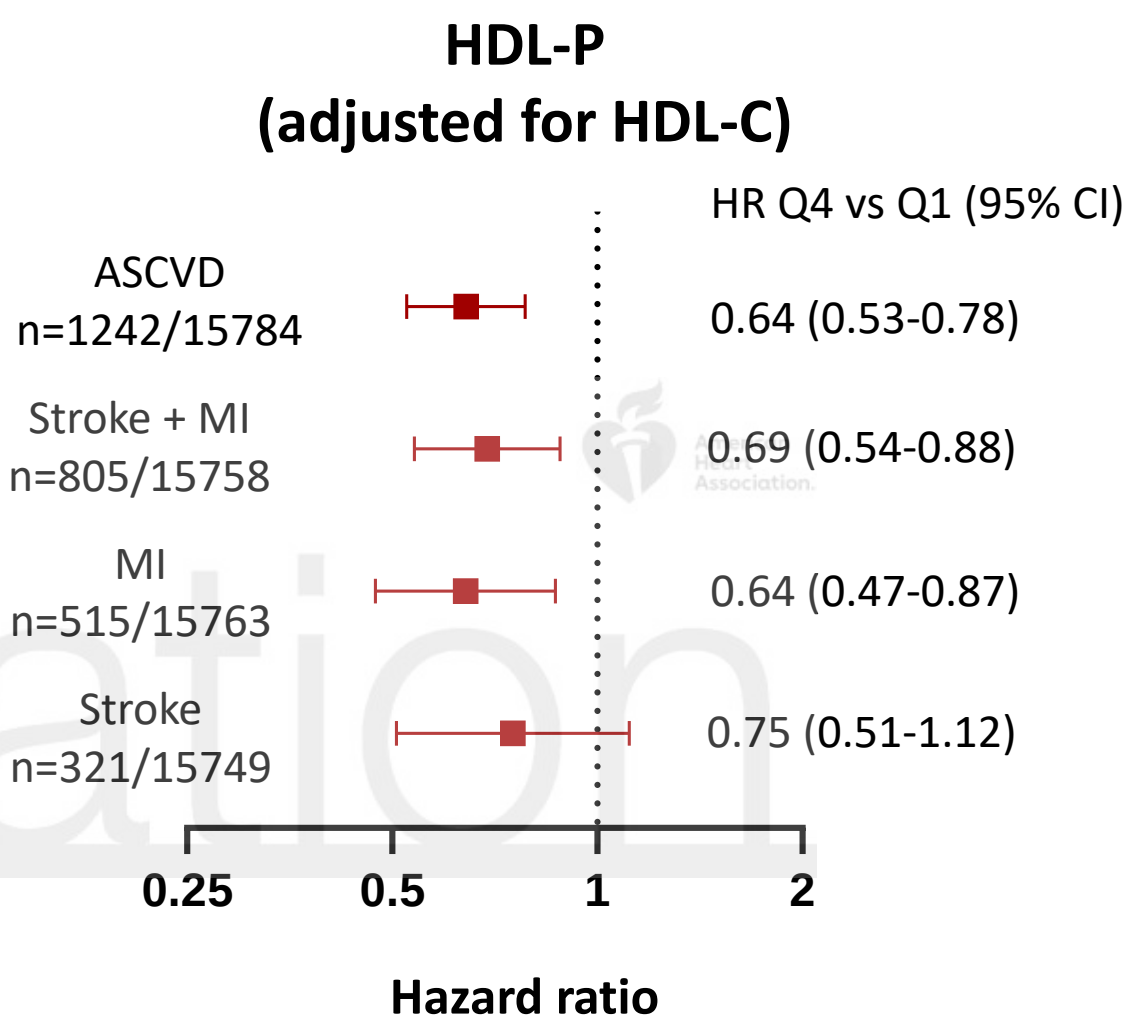
Figure 5. Association of HDL-C and HDL-P with MI stratified by race and cohort.

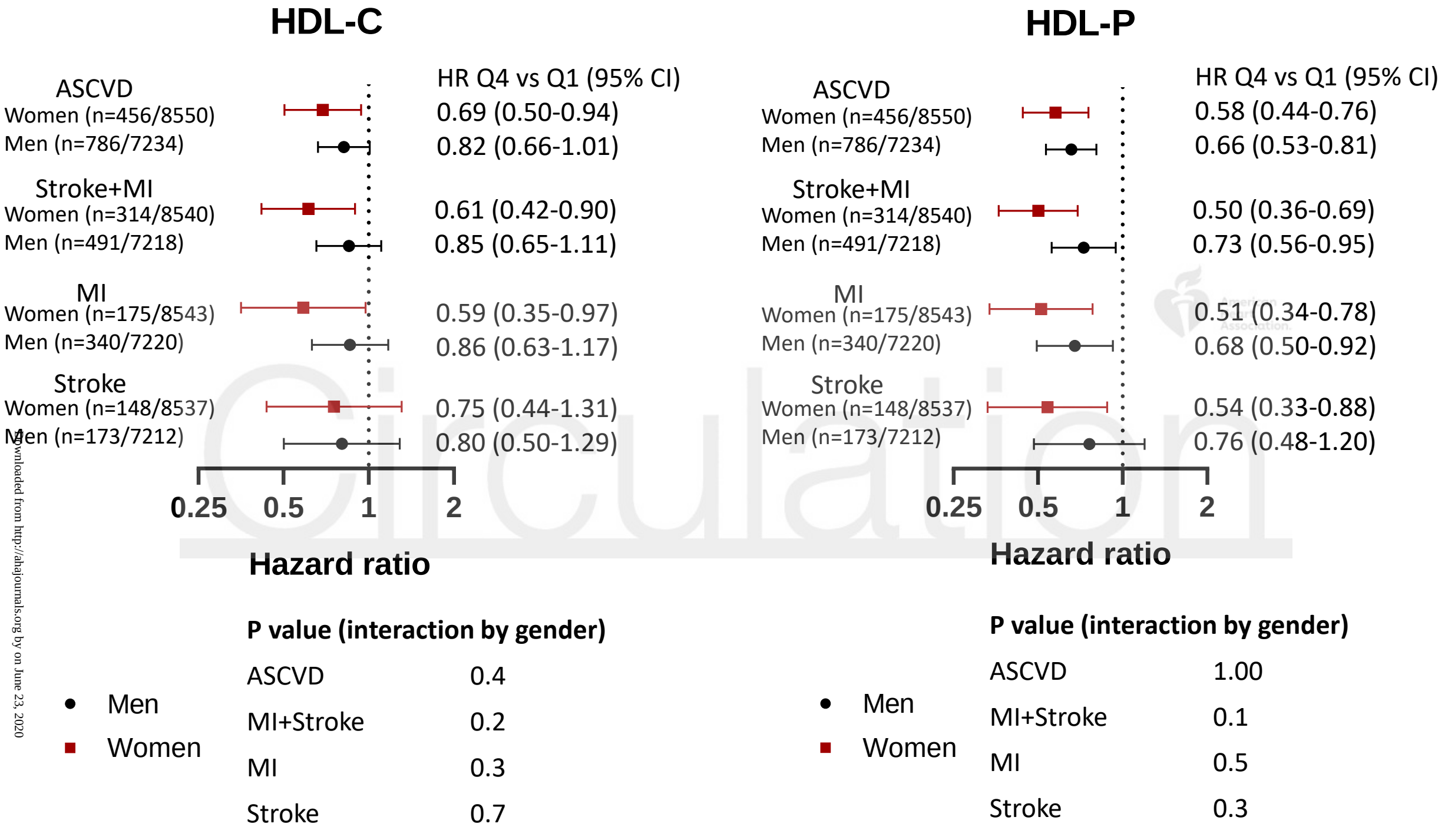
Cox proportional hazards models of sex/ethnicity-adjusted quartile 4 (Q4) vs quartile 1 (Q1) of HDL-C and HDL-P for fatal/non-fatal MI outcomes stratified by race and cohort. The number of Black participants in each cohort is specified in the figure legend. This model is adjusted for risk factors (age, diabetes, hypertension, smoking, LDL-C, triglycerides, lipid lowering medications, BMI, waist circumference and hs-CRP). No additional adjustment for HDL-P or HDL-C was made in this model.

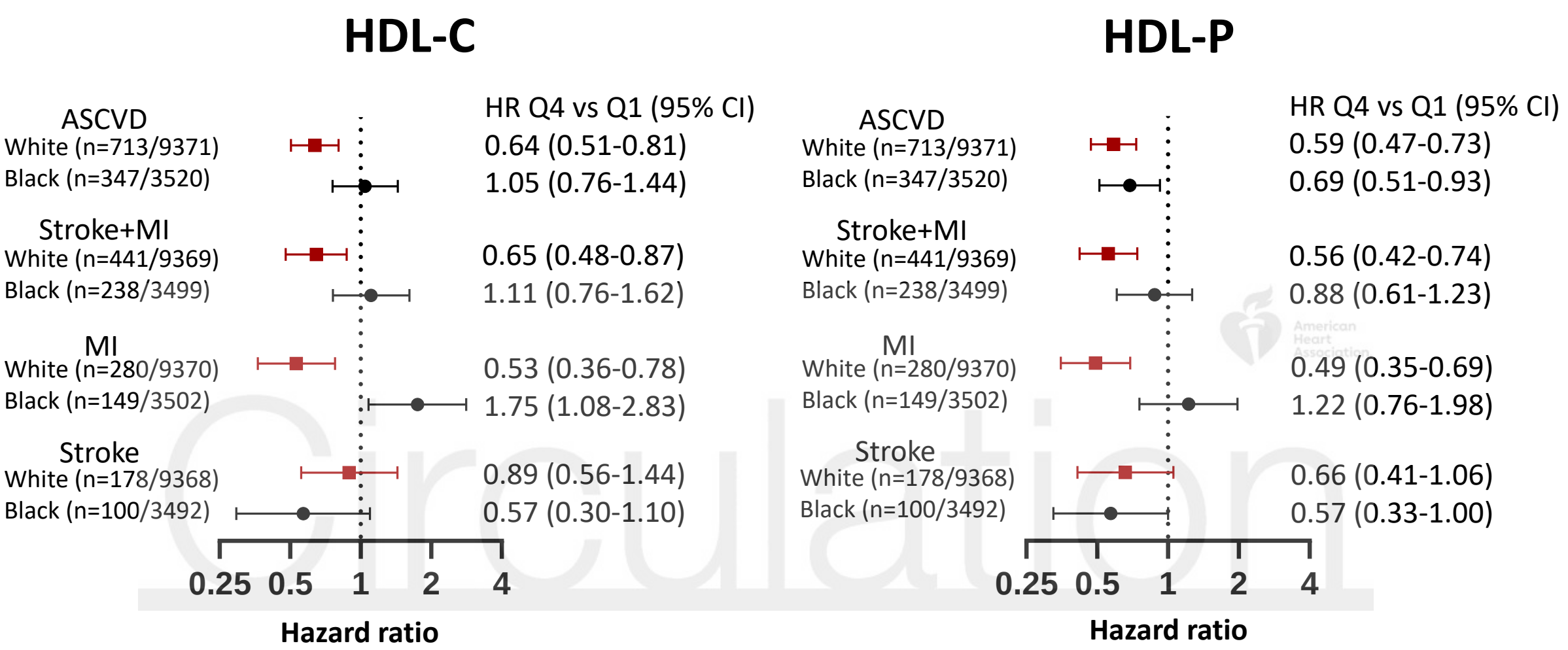
Figure 6. Spline curves demonstrating the relationship between HDL-C and HDL-P with MI by Black vs White participants.

Spline curves of adjusted hazard ratios for the association between HDL-C and HDL-P with MI in Black and White populations in our pooled cohort. This model is adjusted for risk factors (age, sex, race/ethnicity, diabetes, hypertension, smoking, LDL-C, triglycerides, lipid lowering medications, BMI, waist circumference and hs-CRP). No additional adjustment for HDL-P or HDL-C was made in this model. Shaded area around the spline curves represents 95% CI.









P value (interaction by race)

ASCVD	0.1
MI+Stroke	0.02
MI	<0.0001
Stroke	0.3

- Black
- White

P value (interaction by race)

ASCVD	0.4
MI+Stroke	0.03
MI	0.001
Stroke	0.8