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ORIGINAL RESEARCH

Novel Size-Based High-Density Lipoprotein Subspecies and Incident Vascular Events

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BACKGROUND: High-density lipoprotein (HDL) particle concentration likely outperforms HDL cholesterol in predicting atherosclerotic cardiovascular events. Whether size-based HDL subspecies explain the atheroprotective associations of HDL particle concentration remains unknown. Our objective was to assess whether levels of specific size-based HDL subspecies associate with atherosclerotic cardiovascular disease in a multiethnic pooled cohort and improve risk prediction beyond traditional atherosclerotic cardiovascular disease risk factors.

METHODS AND RESULTS: Seven HDL size-based subspecies were quantified by nuclear magnetic resonance (LP4 algorithm; H1=smallest; H7=largest) among participants without prior atherosclerotic cardiovascular disease in ARIC (Atherosclerosis Risk in Communities), MESA (Multi-Ethnic Study of Atherosclerosis), PREVEND (Prevention of Renal and Vascular Endstage Disease), and DHS (Dallas Heart Study) cohorts (n=15 371 people). Multivariable Cox proportional hazards models were used to evaluate the association between HDL subspecies and incident myocardial infarction (MI) or ischemic stroke at follow-up (average 8–10 years) adjusting for HDL cholesterol and risk factors. Improvement in risk prediction was assessed via discrimination and reclassification analysis. Within the pooled cohort (median age 57 years; female 54%; Black 22%) higher H1 (small) and H4 (medium) concentrations were inversely associated with incident MI (hazard ratio [HR]/SD, H1 0.88 [95% CI, 0.81–0.94]; H4 0.89 [95% CI, 0.82–0.97]). H4 but not H1 improved risk prediction indices for incident MI. Increasing H2 and H4 were inversely associated with improved risk prediction indices for composite end point of stroke, MI, and cardiovascular death (HR/SD, H2 0.94 [95% CI, 0.88–0.99]; H4 0.91 [95% CI, 0.85–0.98]). Levels of the large subspecies (H6 and H7) were not associated with any vascular end point.

CONCLUSIONS: Two of 7 HDL size-based subspecies modestly improved risk prediction for MI and composite vascular end points in a large multiethnic pooled cohort. These findings support assessment of precise HDL subspecies for future studies regarding clinical utility.

Key Words: HDL ■ HDL size ■ HDL-C ■ MI ■ multiethnic ■ stroke

High-density lipoprotein (HDL) cholesterol concentration (HDL-C) remains a cornerstone marker of atherosclerotic cardiovascular disease (ASCVD) risk[1]; however, the evidence supporting its utility is primarily derived from older cohorts.[2–4] As contemporary studies expand the diversity of participants to be more reflective of the US population, there is accumulating evidence demonstrating that HDL-C's associations are nonlinear and blunted with respect to myocardial infarction (MI), stroke, and all-cause mortality.[5–8] This

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CLINICAL PERSPECTIVE

What Is New?

- We demonstrate for the first time that specific size-based high-density lipoprotein subspecies improve risk prediction of incident myocardial infarction beyond high-density lipoprotein cholesterol levels and overall particle numbers.

What Are the Clinical Implications?

- The clinical implications of our study are that focusing on measurement of specific high-density lipoprotein species rather than total high-density lipoprotein cholesterol may enhance risk prediction of atherosclerotic cardiovascular disease and uncover potential new therapeutic targets.

Nonstandard Abbreviations and Acronyms

HDL-P	high-density lipoprotein particle concentration
NMR	nuclear magnetic resonance
NRI	net reclassification improvement

is underscored by failure of novel drugs that robustly increase HDL-C to affect incidence of MI and ischemic stroke,[9] further supporting the disconnection between HDL-C and ASCVD.

HDL does not exist as uniform particles but as a mixed collection of structurally and functionally unique subspecies that are not represented by HDL-C per se.[10–12] Properties reflective of the unique physiochemistry of HDL, such as HDL particle concentration (HDL-P), have more consistent relationships with ASCVD event risk than HDL-C, even in select populations such as those on statin therapy, with type 1 diabetes, and children.[5,7,13,14] HDL-P, much like HDL-C, represents a composite of many unique HDL subspecies, with significant heterogeneity in size and relative abundance. Creating more homogeneity by partitioning particles by size and measuring the particle concentrations of these size-based HDL subspecies may add further precision. Whether assessment of these specific size-based HDL subspecies improves ASCVD risk prediction remains unknown.

HDL composition can be analyzed practically in a clinical setting by a variety of methods.[15] Nuclear magnetic resonance (NMR) is one technique that has been well studied in large populations;[16–19] it measures HDL-C to the same precision as tradition biochemical assays[20] and has added advantage of measuring particle heterogeneity, namely HLD-P and HDL-particle size. These size-based HDL subspecies correlate with ASCVD in some but not all studies, leaving unclear associations between HDL particle size and ASCVD.[21–24] This inconsistency may be partly due to the limitations of inadequate resolution of previous methods that were unable to separate the heterogeneous conglomerate of HDL particles into a sufficient number of discrete homogeneous subspecies. However, advances in NMR algorithms can now determine 7 different size-based HDL subspecies,[25] which offers an opportunity to explore the associations between precise HDL subspecies and ASCVD risk.

Some limitations of prior studies include use of mostly single cohorts with limited numbers of events. Further, lack of ethnic diversity and inadequate representation of women in some previous studies limit generalizability of those findings. Therefore, we pooled individual-level data from the ARIC (Atherosclerosis Risk in Communities), MESA (Multi-Ethnic Study of Atherosclerosis), PREVEND (Prevention of Renal and Vascular Endstage Disease), and DHS (Dallas Heart Study), which all used the same NMR platform to measure HDL size-based (H1–H7) subspecies. The objective of the current study is to use a large diverse pooled cohort to investigate the associations between the novel 7 size-base HDL subspecies and ASCVD.

METHODS

ARIC and MESA cohort data are available publicly at BIOLINCC (https://biolincc.nhlbi.nih.gov/home/). Data for PREVEND and DHS are available, respectively, at https://umcgresearch.org/w/prevend and https://www.utsouthwestern.edu/research/translational-medicine/doing-research/dallas-heart/. Approval of local medical ethics committees was obtained for all cohorts, and all participants provided written informed consent. The studies were carried out in accordance with the Helsinki declaration.

ARIC, MESA, PREVEND, and DHS are all prospective, community-based cohort studies. We pooled individual participant-level data into a single harmonized cohort, excluding participants with known cardiovascular disease. Each study had similar protocols for obtaining lipid data.[26] Fasting venous blood samples were collected and stored at −80 °C. Standard methods were used to measure total cholesterol, HDL-C, and triglycerides, and calculate non-HDL-C. Low-density lipoprotein cholesterol concentration levels were calculated with the Friedewald equation.[27] The 7 novel size-based subspecies concentrations were quantified in serum or EDTA plasma specimens by NMR LipoProfile (LipoScience [now Labcorp], Morrisville,

NC) testing using a 400-MHz NMR Profiler or Vantera Clinical Analyzer, a fully automated NMR platform, and the LP4 deconvolution algorithm.[25] H1 is the smallest subgroup and H7 the largest: H1 (7.4–7.7 nm), H2 (7.8–8.6 nm), H3 (8.7–9.4 nm), H4 (9.5–10.2 nm), H5 (10.3–10.7 nm), H6 (10.8–11.9 nm), and H7 (12.0–13.0 nm). Details on how NMR is able to accurately quantify lipoprotein particle of different sizes can be found in Jeyarajah et al.[20]

ARIC is a prospective cohort examining cardiovascular disease incidence in Black and White adults 45 to 60 years of age from Forsyth County, North Carolina; Jackson, Mississippi; suburbs of Minneapolis, Minnesota; and Washington County, Maryland.[28] The carotid magnetic resonance imaging substudy (N=1686) performed additional lipoprotein analysis with NMR only; these participants and their corresponding lipid profiles were used for the pooled cohort. MESA is a multiethnic cohort (N=6814) of adults 45 to 84 years old from 6 centers in the United States serially monitored for development of clinically significant ASCVD.[29] PREVEND's cohort (N=6214) is based in Groningen, the Netherlands, initially investigating the association of microalbuminuria and cardiovascular disease in participants 28 to 75 years of age.[30] DHS (N=2782) intentionally recruited ~50% Black Dallas County residents to create a more ethnically diverse cohort (ages 30–65).

All cohorts had self-reported history of sex, ethnicity, and smoking status. Diabetes was defined uniformly across all cohorts as fasting glucose ≥126 mg/dL or 7 mmol/L or use of glucose-lowering medications. Hypertension was defined as average systolic blood pressure ≥140 mm Hg and average diastolic blood pressure ≥90 mm Hg or use of blood pressure-lowering medication.

Methods of obtaining clinical events were individualized to each cohort. ARIC combined death certificate information, hospital discharge information, follow-up phone calls, and independent adjudicators to report events outlined on their website (https://sites.cscc.unc.edu/aric/surveillance-manuals). MESA called participants every 9 to 12 months, with committees adjudicating events. PREVEND used information from the national registry of hospital discharge diagnoses and the Dutch Central Bureau for Statistics. DHS methods of adjudication have been previously described.[31] Each cohort had approximately similar mean lengths of follow-up, with a range of 8 to 12 years.

Co-primary outcomes were defined as (1) fatal and nonfatal MI and (2) fatal and nonfatal ischemic stroke events. All definite or suspected embolic or hemorrhagic stroke events were excluded from primary outcomes. Secondary outcomes were (1) combined end points of nonfatal and fatal MI and ischemic stroke and (2) composite cardiovascular end point defined as cardiovascular death, MI, and all strokes.

Statistical Analysis

All 4 cohorts were harmonized into a single population and subsequently analyzed using individual participant level data. Spearman correlations were used to determine correlations across all HDL markers. Main exposures were reported as medians with interquartile intervals. Cox proportional hazards models for time to first events were used to determine hazard ratios (HR) for HDL subspecies as both continuous markers (per 1 SD) and as quartiles (quartiles 2, 3, and 4 versus 1). Markers were log-transformed when required to maintain linearity. For all models, stratified baseline hazards were used, allowing for a different baseline hazard function for each cohort. Robust SEs were used to account for possible correlation within cohorts. Proportional hazards assumptions were satisfied by assessing Schoenfeld residuals. A log-likelihood chi-square test was used to assess the *P* trend.

Models were adjusted for the traditional risk factors age, sex, race or ethnicity, hypertension, diabetes, smoking, lipid medications, low-density lipoprotein cholesterol, and triglycerides as well as body mass index, waist circumference (centimeters), and high-sensitivity C-reactive protein. Fully adjusted models for the main results included all 7 HDL subspecies. Serial adjustments were made for HDL-C. HDL-P was not included in these models because all 7 HDL size species sum to total HDL-P.

Risk prediction performance was analyzed using metrics of discrimination,[32] C-statistic and integrated discrimination improvement, and reclassification,[33] net reclassification improvement (NRI). Positive, nonzero numbers for these value suggest experimental model is superior in risk prediction compared with base model. Base models[34] included age, sex, race or ethnicity, hypertension, diabetes, smoking, lipid medications, low-density lipoprotein cholesterol, and triglycerides as well as body mass index, waist circumference (centimeters), and high-sensitivity C-reactive protein. The additional contributions of HDL-C, total HDL-P, and the 7 HDL subspecies were tested in separate models. Lastly, the 7 HDL subspecies were each compared with models that included the aforementioned risk factors as well as HDL-C. All analyses were conducted using SAS 9.3 (SAS Institute, Raleigh Durham, Cary, NC).

RESULTS

The pooled cohort consisted of 15 371 participants free of known underlying ASCVD (Table 1) at baseline. The median age was 56 years; 54% were female and 22% were Black. Median HDL-C was 48 mg/dL (Table 1). During 11.3 years of median follow-up time (total follow-up time 204 887 years), 721 fatal/nonfatal MI, 467 fatal/

Table 1. Baseline Characteristics of Pooled and Constituent Cohorts

	Overall (n=15371)	ARIC (n=1595)	MESA (n=6632)	PREVEND (N=5022)	DHS (n=2535)
Age, y	56.8 (13.1)	70.9 (5.6)	62.2 (10.2)	53.1 (11.9)	43.7 (9.87)
Female sex, n (%)	8850 (54.2)	888 (55.7)	3506 (52.9)	2730 (54.4)	1413 (55.7)
Black, n (%)	3520 (22.3)	412 (25.8)	1831 (27.6)	44 (0.9)	1212 (47.8)
Systolic blood pressure, mmHg	126 (19)	125 (14)	127 (21)	126 (19)	124 (18)
Low-density lipoprotein cholesterol, mg/dL	115 (32)	118 (34)	117 (32)	115 (29)	107 (35)
Total cholesterol, mg/dL	125 (94)	197 (40)	194 (35)	212 (40)	181 (39)
Body mass index, kg/m^2	28 (6.0)	28.9 (5.3)	28.3 (5.4)	26.6 (4.4)	29.6 (7.0)
Fasting glucose, mg/dL	95 (27)	107 (24)	97 (30)	90 (21)	101 (41)
Diabetes, n (%)	1808 (10)	332 (19.9)	851 (12.6)	352 (5.6)	273 (9.8)
Waist circumference, cm	96 (14)	98.9 (12.7)	98.1 (14.4)	91.7 (12.7)	98.9 (16.6)
Smoking, n (%)	3505 (20)	151 (9)	878 (13)	1727 (28)	749 (27)
HDL-cholesterol, mg/dL	48 (40–57)	48 (40–58)	48 (40–59)	47 (40–56)	48 (40–57)
HDL-particle concentration µmol/L	32.5 (28.8–36.8)	34.9 (31.2–39.3)	33.4 (29.3–38)	31.2 (27.8)	32.8 (28.9–37.1)
HDL size, nm	9.1 (8.8–9.5)	9.1 (8.7–9.5)	9.2 (8.9–9.6)	9.1 (8.7–9.6)	9.0 (8.7–9.3)
H1 µmol/L, median (IQR)	4.20 (2.70–5.50)	1.91 (1.06–3.03)	5.13 (4.20–6.06)	3.43 (2.24–4.64)	4.50 (2.70–5.0)
H2 µmol/L, median (IQR)	10.37 (8.50–12.40)	13.08 (10.86–15.17)	9.45 (7.63–11.44)	10.59 (9.03–12.22)	10.50 (8.50–12.60)
H3 µmol/L, median (IQR)	2.37 (0.90–4.08)	2.76 (1.40–4.31)	1.58 (0.43–3.50)	3.18 (1.97–4.45)	1.30 (0.20–3.33)
H4 µmol/L, median (IQR)	1.38 (0.78–2.09)	1.25 (0.72–1.87)	1.09 (0.56–1.73)	1.71 (1.12–2.46)	1.40 (0.070–2.10)
H5 µmol/L, median (IQR)	0.85 (0.35–1.63)	0.94 (0.54–1.58)	1.56 (0.98–2.48)	0.30 (0.05–0.62)	0.90 (0.50–0.80)
H6 µmol/L, median (IQR)	0.42 (0.13–1.01)	0.57 (0.26–1.15)	0.27 (0.04–0.69)	0.63 (0.26–1.38)	0.40 (0.10–0.80)
H7 µmol/L, median (IQR)	0.32 (0.13–0.59)	0.16 (0.06–0.32)	0.42 (0.24–0.67)	0.33 (0.13–0.63)	0.20 (0.10–0.40)

Baseline properties of individual cohorts. ARIC indicates Atherosclerosis Risk in Communities; DHS, Dallas Heart Study; HDL-C, high-density lipoprotein concentration; IQR, interquartile range; MESA, Multi-Ethnic Study of Atherosclerosis; and PREVEND, Prevention of Renal and Vascular Endstage Disease. Median concentration for HDL subspecies with interquartile range for pooled cohort.

nonfatal ischemic stroke, and 1781 events for composite end point occurred (cardiovascular death, MI, and all stroke types).

HDL-C was moderately correlated with HDL-P (r=0.72; Table S1). The HDL subspecies with the strongest correlations with HDL-C included the 2 largest, H6 (r=0.55) and H7 (r=0.60), as well as H3 (r=0.47). Besides an isolated association between H6 (large) and H3 (medium; R=0.58) (P<0.01 for each), the 7 HDL subspecies did not have strong associations among each other (Table S1).

In risk factor adjusted models (without HDL-C) including all 7 HDL subspecies, H1, H2, and H4 were inversely associated with incident MI. For all 3 significant HDL subspecies, there was 11% to 14% lower incidence of MI per 1 SD higher value at baseline in our pooled cohort (HR per SD, H1 0.86 [95% CI, 0.80–0.93]; H2 0.89 [95% CI, 0.83–0.97]; H4 0.86 [95% CI, 0.79–0.93]) (Figure 1). Subsequent quartile analysis demonstrated 27% to 32% lower incidence of MI comparing top versus bottom quartiles (HR Q4 versus Q1, H1 0.73 [95% CI, 0.58–0.91]; H2 0.69 [95% CI, 0.54–0.89]; H4 0.68 [95% CI, 0.54–0.86]; Figure 2).

With respect to the second co-primary end point of ischemic stroke, H2 and H4 were inversely associated in models adjusted for risk factors (without HDL-C) and all 7 HDL subspecies (HR per SD higher value, H2 0.88 [95% CI, 0.80–0.96]; H4 0.87 [95% CI, 0.78–0.97]) (Figure 3). Quartile analyses revealed 25% to 32% lower incidence in ischemic stroke in the top versus bottom quartile (HR Q4 versus Q1, H2 0.69 [95% CI, 0.52–0.92]; H4 0.75 [95% CI, 0.56–1.02]) (Figure 4).

Continuous analysis for the secondary end point of combined MI and ischemic stroke demonstrated inverse associations with H1, H2, H4, and H5 (HR per SD higher value, H1 0.90 [95% CI, 0.85–0.99]; H2 0.89 [95% CI, 0.84–0.95]; H4 0.86 [95% CI, 0.81–0.92]; H5 0.90 [95% CI, 0.83–0.98]). H1, H2, and H4 remained associated in quartile analyses (HR Q4 versus Q1, H1 0.78 [95% CI, 0.64–0.97]; H2 0.69 [95% CI, 0.57–0.83]; H4 0.70 [95% CI, 0.57–0.84]);

With respect to the secondary composite end point of cardiovascular death, MI, and all strokes, H2, H4, and H5 were inversely associated (HR per SD higher value, H2 0.90 [95% CI, 0.85–0.95], H4 0.88 [95% CI, 0.83–0.94], H5 0.92 [95% CI, 0.85–0.99]). Only H2 and H4 demonstrated significance in Q4 versus Q1 quartile analysis (HR Q4 versus Q1, H2 0.74 [95% CI, 0.63–0.86]; H4 0.75 [95% CI, 0.63–0.89]).

In risk factor adjusted models including all 7 HDL subspecies, after serially adjusting for HDL-C for the

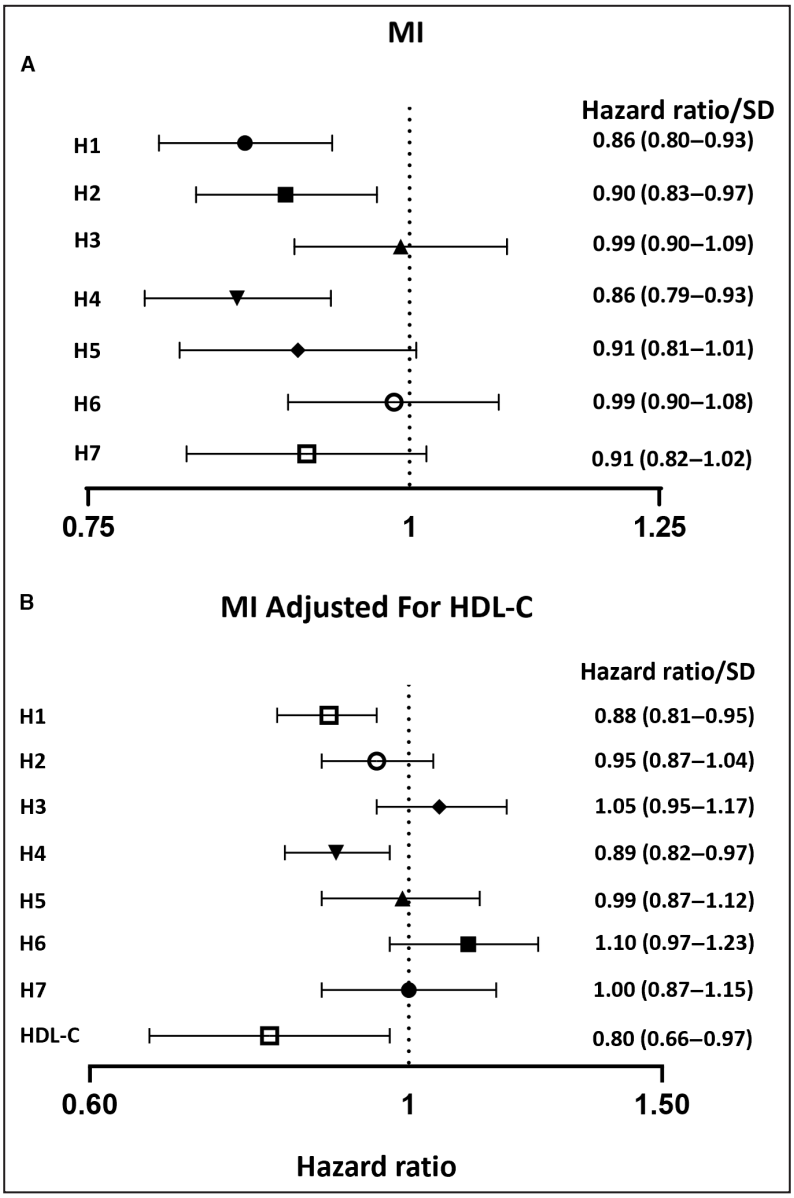

Figure 1. Size-based HDL subspecies associations with incident myocardial infarction per 1 SD increment.
H1 (7.4–7.7 nm), H2 (7.8–8.6 nm), H3 (8.7–9.4 nm), H4 (9.5–10.2 nm), H5 (10.3–10.7 nm), H6 (10.8–11.9 nm), H7 (12.0–13.0 nm). **A,** Cox proportional hazard models of size-based HDL subspecies and incident myocardial infarction. Models include adjustment for age, race or ethnicity, sex, diabetes, hypertension, smoking, low-density lipoprotein cholesterol, triglycerides, lipid-lowering medications, body mass index, waist circumference, and high-sensitivity C-reactive protein and all 7 HDL subspecies. **B**, Adjusted for HDL-C in addition to previous risk factors. HDL-C indicates high-density lipoprotein cholesterol; and MI, myocardial infarction.

primary end points, only H1 and H4 retained significant associations with MI (HR per SD higher value, H1 0.88 [95% CI, 0.81–0.95], H4 0.89 [95% CI, 0.82–0.97]), and relationships between all HDL subspecies and ischemic stroke were attenuated. For MI, HR trends across increasing quartiles were significant for H1 (P_{trend}=0.004), H2 (P_{trend}=0.03), and H4 (P_{trend}=0.001).

With respect to risk prediction performance beyond traditional risk factors, for the primary end point of MI, addition of HDL-C did not improve C-statistic or NRI but did improve the integrated discrimination improvement (Table 2). Addition of HDL-P did not improve the C-statistic but did improve both the NRI and integrated discrimination improvement. Analyzing each

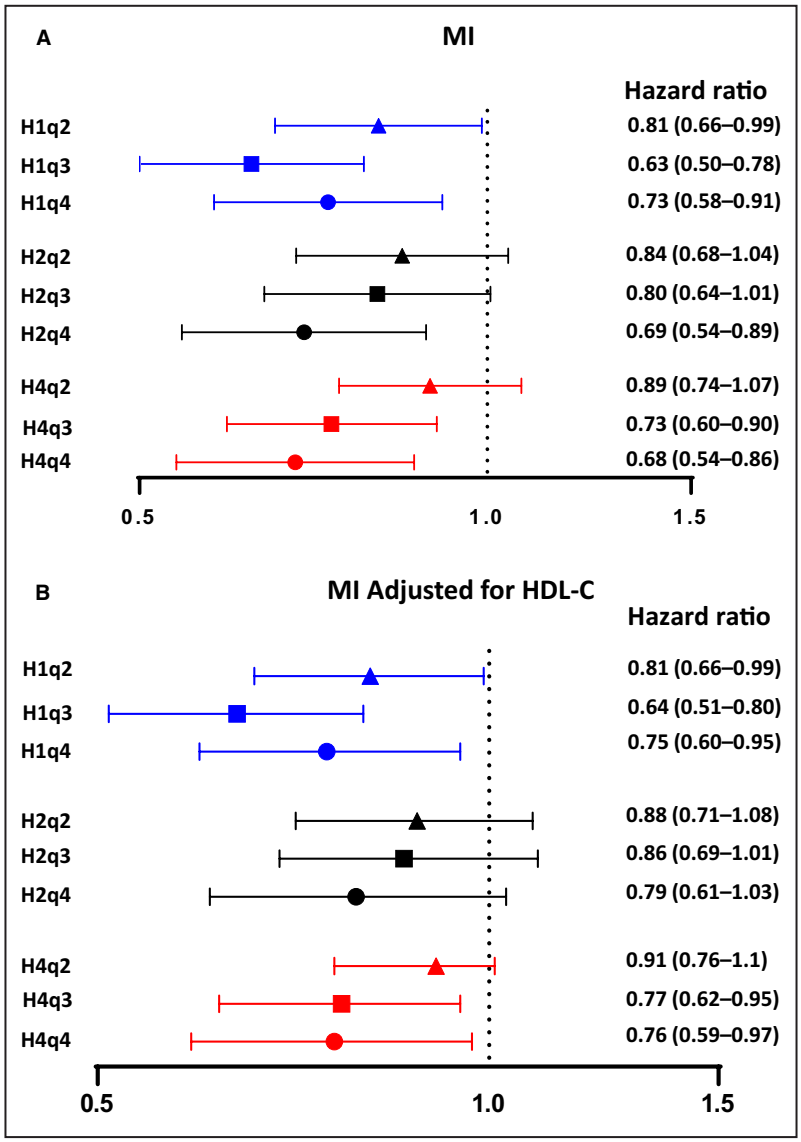

Figure 2. Associations between quartiles of high-density lipoprotein particles H1 (7.4–7.7 nm), H2 (7.8–8.6 nm), and H4 (9.5–1.2 nm) and incident myocardial infarction.

A, Cox proportional hazard models of quartile 2 (Q2), quartile 3 (Q3), and quartile 4 (Q4) vs quartile 1 for H1, H2, H4, and incident myocardial infarction. Models include adjustment for age, race or ethnicity, sex, diabetes, hypertension, smoking, low-density lipoprotein cholesterol, triglycerides, lipid-lowering medications, body mass index, waist circumference, and high-sensitivity C-reactive protein and all 7 HDL subspecies. **B**, Adjusted for HDL-C in addition to previous risk factors. HDL-C indicates high density lipoprotein cholesterol; MI, myocardial infarction.

subspecies separately with regard to MI, H4 was the only subspecies that improved the C-statistic, NRI, and integrated discrimination improvement when added to risk factor adjusted models with and without HDL-C.

For risk prediction performance for the combined end point of cardiovascular death, MI, and all strokes, compared with risk factor adjusted models including HDL-C, H2 and H4 improved both the C-statistic and NRI.

DISCUSSION

In our large, multiethnic pooled cohort, novel size-based HDL subspecies were associated with incident ASCVD events. Of 7 HDL subspecies, higher levels of H2 (small) and H4 (medium) were consistently associated with reduced risk of MI, ischemic stroke, and the composite end point of cardiovascular death, MI, and all stroke. In addition to H2 and H4, higher levels of

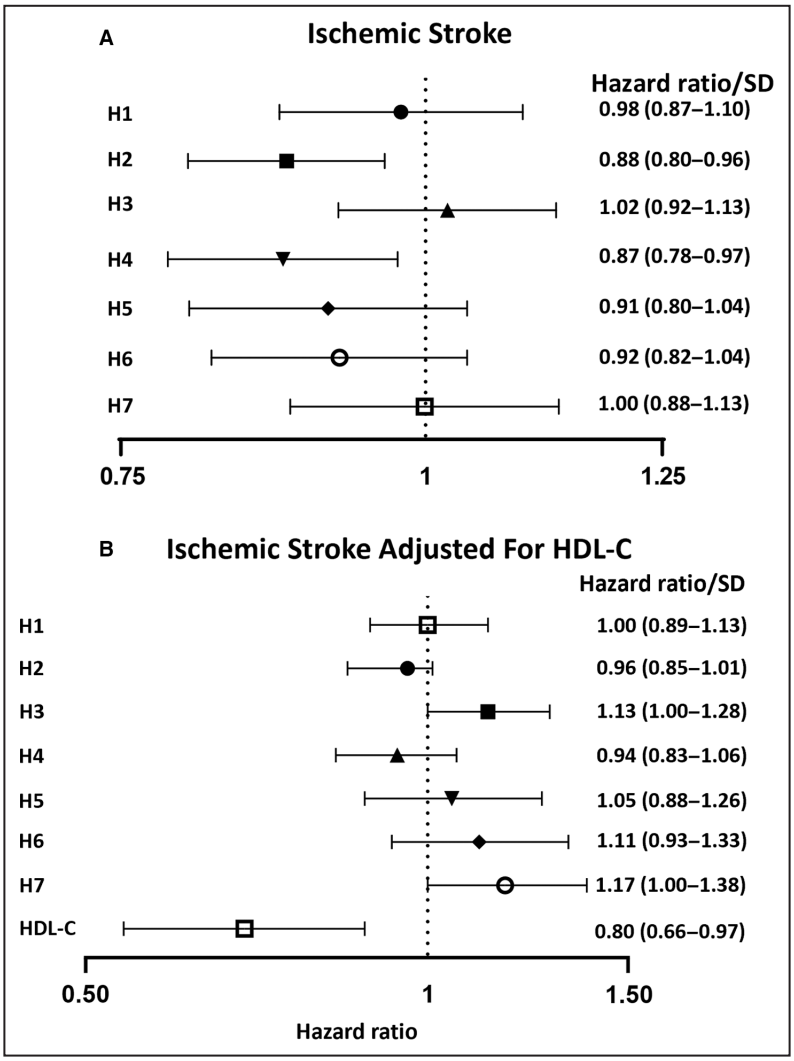

Figure 3. Size-based HDL subspecies associations with incident ischemic stroke per 1 SD increment.
H1 (7.4–7.7 nm), H2 (7.8–8.6 nm), H3 (8.7–9.4 nm), H4 (9.5–10.2 nm), H5 (10.3–10.7 nm), H6 (10.8–11.9 nm), H7 (12.0–13.0 nm). **A,** Cox proportional hazard models of size-based HDL subspecies and incident ischemic stroke. Models include adjustment for age, race or ethnicity, sex, diabetes, hypertension, smoking, low-density lipoprotein cholesterol, triglycerides, lipid-lowering medications, body mass index, waist circumference, and high-sensitivity C-reactive protein and all 7 HDL subspecies. **B**, Adjusted for HDL-C in addition to previous risk factors. HDL-C indicates high-density lipoprotein cholesterol; and MI, myocardial infarction.

H1 (smallest size) were also associated with reduced risk of incident MI. Intriguingly, none of the large subspecies (H6–H7) were associated with any specific or composite end point despite prior associations with favorable cardiometabolic phenotypes.[35,36] These findings expand on prior studies that had demonstrated associations between total HDL particle concentration (HDL-P) and MI by now revealing specific size-based HDL subspecies that may contribute to that protective relationship in a large, diverse pooled cohort across 4 distinct studies (Figure 5).

Although HDL has purported atheroprotective effects, whether markers of HDL metabolism have clinical utility in risk prediction remains unknown. The overall cholesterol content of HDL (HDL-C) has been the principal measurement over the decades but with inconsistent results in terms of CVD risk prediction. Recent studies reveal that HDL exists as a heterogenous set of subspecies with diversity in structure and function[37–39]; using the simplistic HDL-C in current models of ASCVD does not consider this physicochemical complexity but only its cholesterol cargo. It is likely that specific

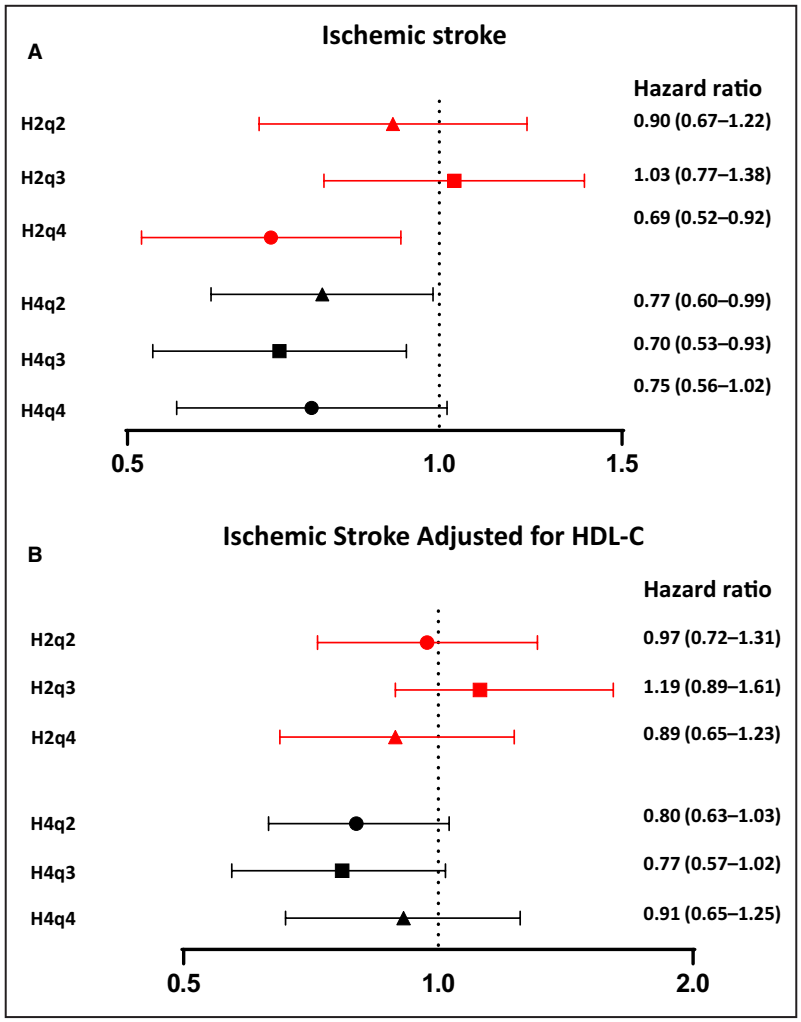

Figure 4. Associations between quartiles of high-density lipoprotein particles H2 (7.8–8.6 nm) and H4 (9.5–1.2 nm) and incident ischemic stroke. H1 (7.4–7.7 nm), H2 (7.8–8.6 nm), H3 (8.7–9.4 nm), H4 (9.5–10.2 nm), H5 (10.3–10.7 nm), H6 (10.8–11.9 nm), H7 (12.0–13.0 nm). **A,** Cox proportional hazard models of size-based HDL subspecies and incident ischemic stroke. Models include adjustment for age, race or ethnicity, sex, diabetes, hypertension, smoking, low-density lipoprotein cholesterol, triglycerides, lipid-lowering medications, body mass index, waist circumference, and high-sensitivity C-reactive protein and all 7 HDL subspecies. **B**, Adjusted for HDL-C in addition to previous risk factors. HDL-C, high-density lipoprotein cholesterol; and MI, myocardial infarction.

subspecies of HDL drive associations with ASCVD, and ability to measure these subspecies could lead to more consistent associations and potential therapeutic targets.[26] Advanced methods have evolved to precisely measure these subspecies and have been scaled for clinical application. Size-based characterization of HDL subspecies is an attractive approach. Density-based methods were methodologically robust but laborious and provided inconsistent associations with CVD end points across population-based cohorts and clinical trials.[40–42] Ion mobility[43] and NMR are high-throughput technologies used both in research and clinically that provide refined size-based assessment of HDL subspecies. NMR-based lipoprotein particle measurement has been used in multiple cohorts with CVD end points and now provides improved resolution, with the ability to assess 7 size-based HDL subspecies, a significant advance beyond the conventional 2 to 5 size-based subspecies approach with prior methods. Recently, using the same NMR methodology as this study, Sokooti et al found some of the 7 size-based HDL subspecies associated with incident type 2 diabetes both in the general population[11] and in kidney transplant recipients.[44] Here we showed that applying this more precise technique to our pooled cohort revealed that particles that were

Table 2. Risk Prediction Performance of HDL Subspecies for Myocardial Infarction

	C-statistic (95% CI)	Delta_C-statistic (95% CI)	NRI (95% CI); *P* value	IDI (95% CI); *P* value
Base model	0.77 (0.75–0.78)	…	…	…
Base model+HDL-P	0.77 (0.75–0.78)	0.0004 (−0.0008–0.002)	0.12 (0.03–0.20); *P*=0.007	0.002 (0.0008–0.004); *P*=0.0007
Base model+HDL-C	0.77 (0.75–0.78)	0.0007 (−0.002–0.003)	0.05 (−0.04–0.15); *P*=0.26	0.002 (0.0006–0.003); *P*=0.003
Base model+H1	0.77 (0.75–0.78)	0.0002 (−0.001–0.001)	0.05 (−0.05–0.15); *P*=0.31	−0.00009 (−0.0007–0.0005); *P*=0.77
Base model+H2	0.77 (0.75–0.78)	0.002 (−0.0001–0.004)	0.03 (−0.08–0.15); *P*=0.58	0.00003 (−0.0006–0.0007); *P*=0.93
Base model+H4	0.77 (0.75–0.78)	0.004 (0.0001–0.01)	0.16 (0.08–0.24); *P*=0.0001	0.002 (0.0009–0.004); *P*=0.001
Base model+H1+HDL-C	0.77 (0.75–0.78)	0.0008 (−0.002–0.003)	0.06 (−0.05–0.16); *P*=0.29	0.00001 (−0.0007–0.0008); *P*=0.98
Base model+H2+HDL-C	0.77 (0.75–0.79)	0.002 (−0.001–0.005)	0.01 (−0.09–0.11); *P*=0.82	−0.0001 (−0.0008–0.0006); *P*=0.78
Base model+H4+HDL-C	0.77 (0.75–0.79)	0.004 (0.0002–0.01)	0.15 (0.07–0.21); *P*=0.0001	0.002 (0.0003–0.003); *P*=0.01

C-statistic, delta-C-statistic, NRI, and (IDI) values comparing the base model of risk prediction to base model+biomarker (either HDL-C, HDL-P) or H1, H2, and H4 for myocardial infarction. Nonzero NRI and IDI values indicate an increase in performance of reclassification. HDL-C indicates high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; IDI, integrated discrimination index; and NRI, net reclassification index.

once previously grouped together have heterogeneous associations with ASCVD (Figure 5).

Despite the fact that atherosclerotic processes mediate both coronary heart disease and ischemic stroke, differential associations with HDL-C have been noted.[45–47] HDL-C has inverse associations with coronary heart disease, whereas the associations with stroke are less clear.[46] HDL-C has been reported to have inverse,[46,48] positive,[46,48] and null associations[49] with incident stroke, suggesting heterogenous associations across different vascular beds. Shortcomings of prior studies investigating size-based HDL particles and ASCVD either have low number of events[42] or use surrogate markers of MI.[50,51] Our pooled cohort design provided sufficient numbers of MI and ischemic strokes to assess associations with each vascular end point separately. We found that H1 and H4 were consistently inversely associated with MI even when

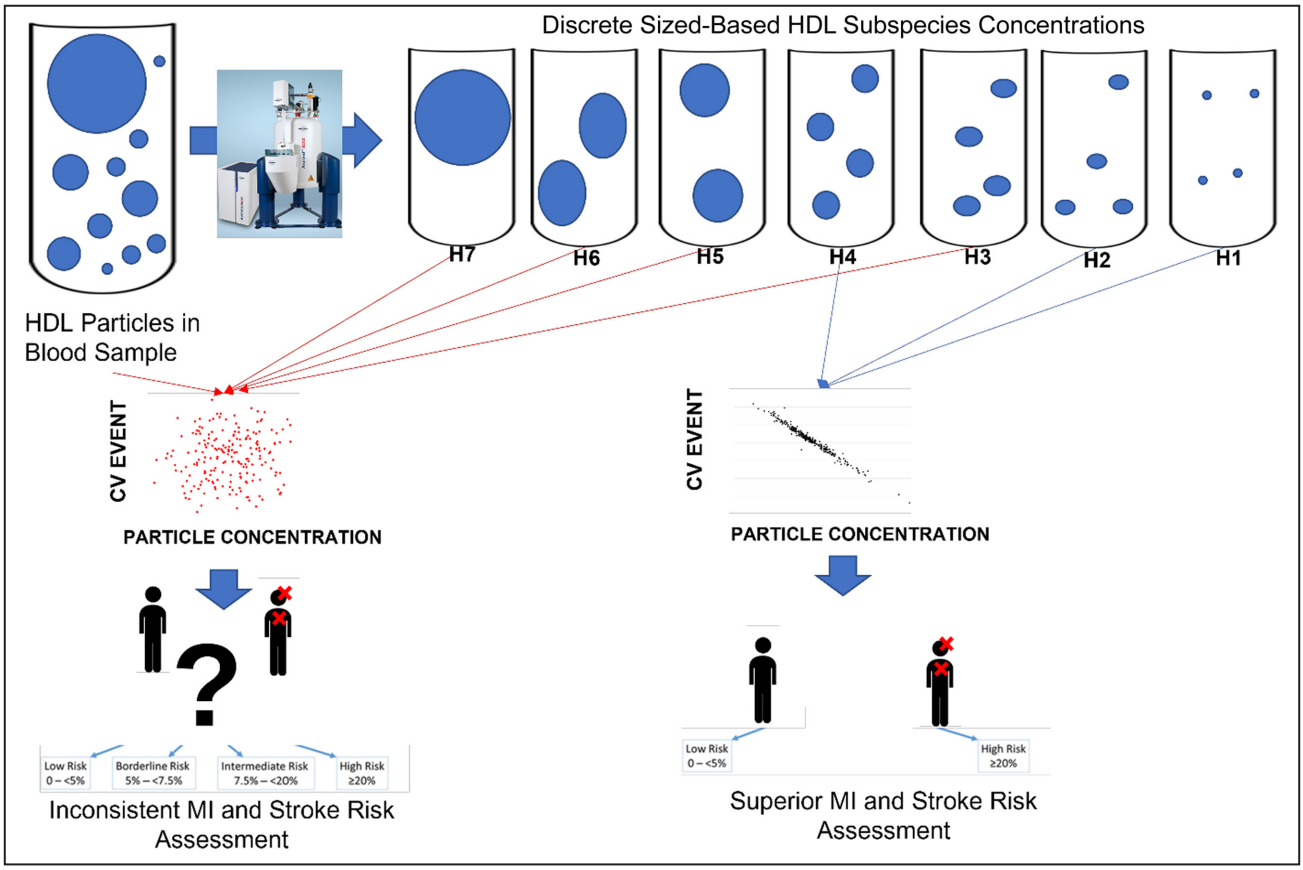

Figure 5. Size-based HDL subspecies that associate with vascular events enhance risk assessment.
CV indicates cardiovascular; HDL-C, high-density lipoprotein cholesterol; and MI, myocardial infarction.

adjusted for HDL-C. H1 is the smallest sized HDL particle detected by NMR and, although controversial, may be the most readily available to accept cholesterol from peripheral tissues such as the arterial wall via the ABCA1 cell membrane transporter. Of note, H1 does not represent pre-beta HDL particles, the smallest most lipid-poor cholesterol acceptors, which are not detected by NMR. H4 is a medium-sized HDL particle but not the most abundant HDL species and not highly correlated with any other HDL parameter. It represents the center point of HDL size subspecies and, thus, may facilitate reverse cholesterol transport to both small and medium-sized particles via ABCA1 and ABCG1 transporters. H2 (small HDL) is the most abundant HDL subspecies and was inversely associated with MI and ischemic stroke but was attenuated when adjusted for HDL-C despite minimal correlations with HDL-C. It is unclear why H2 and H3 did not have significant associations with MI despite significant associations with adjacent size-based particles (H1 and H4). H2 was associated with the composite cardiovascular end point even with adjustment for HDL-C. Lastly, the largest HDL subspecies (H7 and H6) did not associate with MI or stroke in risk factor adjusted models even before adjustment for HDL-C, suggesting that the largest size-based HDL subspecies has no added benefit in ASCVD risk prediction beyond traditional risk factors. Overall, our current findings suggest that specific size-based HDL species independently associate with MI and likely contribute to the overall association of HDL-P and ASCVD events.

Whether HDL markers improve actual ASCVD risk prediction to justify clinical utility in contemporary diverse cohorts remains unclear. Our study shows that adding total HDL-P to traditional risk factors improves reclassification for MI, suggesting modest clinical utility. Inclusion of specific size-based HDL subspecies improved metrics of both discrimination and reclassification beyond traditional risk factors and HDL-C. These findings support potential clinical utility, but the overall magnitude of improvement was modest. Future studies are needed to confirm these findings across different populations and determine if the improvement in risk prediction in specific populations is sufficient for clinical relevance. Whether H1, H2, and H4 associate with subclinical atherosclerosis or non-ASCVD is unknown but would further enhance understanding of the role of HDL metabolism in cardiometabolic disease.

Limitations

Although the pooled cohort design reflected strengths in number of events and diversity with respect to sex, race, and geography, there remained some limitations. These include the observational nature of the study, which precluded assessment of causality and lack of other measures of HDL composition and function. Only variables available in all 4 cohorts and able to be harmonized were included. Although cohorts differed in demographics and risk factor prevalence, cohort status was accounted for in the multivariable analyses. The size-based subspecies in this study are derived from NMR spectroscopy, which uses an analytic approach to partition discrete size-based lipoprotein particles.

CONCLUSIONS

The large numbers of events across 4 cohorts with varying diversity, age, and risk was anticipated to provide more robust estimates concerning the association of HDL subspecies with incident ASCVD. H1 and H4 were found to be inversely associated with MI and modestly improved risk prediction of MI beyond risk factors including HDL-C; None of the 7 subspecies were independently associated with ischemic stroke. Although H2 and H4 improved risk prediction of composite atherosclerotic CV end points beyond risk factors and HDL-C, the size of this difference was small. These findings support future studies of the role of specific HDL subspecies in cardiometabolic health.

ARTICLE INFORMATION

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Supplemental Material

Table S1

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