ORIGINAL RESEARCH

High-Density Lipoprotein Subclasses and Noncardiovascular, Noncancer Chronic Inflammatory-Related Events Versus Cardiovascular Events: The Multi-Ethnic Study of Atherosclerosis

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Background—High-density lipoprotein (HDL) particles have properties beyond reverse cholesterol transport. We hypothesized that their protection extends to inflammation-related disease. The predictive value of HDL particle subclasses and inflammatory markers was studied for noncardiovascular, noncancer chronic inflammation–related death and hospitalization, and for incident cardiovascular disease (CVD).

Methods and Results—A multiethnic, multicenter, prospective observational study was conducted in 6475 men and women (aged 45 to 84 years) free of known CVD at baseline with median follow-up of 10.1 years. Fasting venous samples were analyzed for baseline lipid profile and lipoprotein particles. We focused on the HDL family of variables (small-, medium-, and large-diameter HDL particles and HDL cholesterol). Analyses identified the sum of small- plus medium-diameter HDL particles as important. Small- plus medium-diameter HDL particles were inversely associated with diagnostic code–based noncardiovascular, noncancer chronic inflammation–related death and hospitalization (n=1054) independent of covariates: relative risk per SD 0.85 (95% CI: 0.79 to 0.91, $P<0.0001$). Small- plus medium-diameter HDL particles were also associated with adjudicated fatal and nonfatal coronary heart disease events (n=423): relative risk per SD 0.88 (95% CI 0.77 to 0.98, $P=0.02$).

Conclusions—Small- plus medium-diameter HDL particles are an independent predictor for noncardiovascular, noncancer chronic inflammation–related death and hospitalization and for coronary heart disease events in subjects initially free of overt CVD. These findings support the hypothesis that smaller HDL particles of diameter <9.4 nm have anti-inflammatory properties in the general population. **(*J Am Heart Assoc.* 2015;4:e002295 doi: 10.1161/JAHA.115.002295)**

Key Words: cardiovascular events • high-density lipoprotein • lipoprotein particles • Multi-Ethnic Study of Atherosclerosis • noncardiovascular, noncancer chronic inflammation–related death and hospitalization

The inverse association between high-density lipoprotein (HDL) cholesterol (HDL-C) and coronary heart disease (CHD) was described several decades ago.[1] The most plausible mechanism of the antiatherosclerotic effect of HDL-C was considered to be reverse cholesterol transport.[2] With the development of medical therapy to increase HDL-C levels, randomized clinical trials were performed but with unexpectedly adverse[3] or null[4] outcomes, including increases in both cardiovascular disease (CVD) and non-CVD events. HDL particle concentrations, which have been shown to predict CVD events,[5] encompass a heterogeneous family of different sized particles that differ in lipid and protein content, with protective and nonprotective components not reflected by HDL-C.[6] HDL particle subclasses may therefore be relevant

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Accompanying Tables S1 through S5 are available at http://jaha.ahajournals.org/content/4/9/e002295/suppl/DC1

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to understanding the potential role(s) played by HDL in both CVD and non-CVD.

Inflammation is a common mechanism in underlying infection and chronic respiratory, gastrointestinal, and autoimmune diseases, as well as CVD.[7] There is now evidence that HDL particles inhibit vascular inflammation,[2] acting oppositely to interleukin-6 (IL-6).[8] HDL particles also have antioxidant[9] and antithrombotic properties[10] and suppress the production and mobilization of monocytes and neutrophils from bone marrow.[11] Lipoprotein particles generally play an important part in host defense against infection.[12] In HIV-positive patients, low total, large-diameter, and smaller-diameter HDL particles were predictive for CVD events and smaller-diameter HDL particles had strong relations with non-CVD death.[8,13]

During the past 2 decades, risk factor associations with conditions other than CVD have been found in several epidemiological studies.[14–20] Most of these study cohorts were free of overt CVD at the beginning of the study and showed similar numbers of deaths attributed to CVD, cancer, and non-CVD other than cancer. In our experience, most non-CVD, noncancer deaths have an important inflammatory component, either chronically or secondary to severe infection. However, there has been little study of the relation of HDL to non-CVD.

Here, we focused on variables in the HDL family, namely HDL particles of small (HS-P), medium (HM-P), and large (HL-P) diameter, plus HDL-C, while adjusting for other blood lipid variables and 6 lipoprotein particle subclass variables in the low-density lipoprotein (LDL) family. Both in vitro and in vivo findings prompted the hypothesis that smaller HDL particles are inversely related to non-CVD, noncancer chronic inflammation–related disease (ChrIRD) death and hospitalization, as well as to CVD. ChrIRD events encompass clinically serious events including many non-CVD, noncancer diagnoses.

Materials and Methods

Study Sample

The Multi-Ethnic Study of Atherosclerosis (MESA) was initiated to investigate the prevalence, correlates, and progression of subclinical CVD in people initially free of overt clinical CVD, although they may have had non-CVD conditions before baseline.[21] Between 2000 and 2002, 6814 men and women of white, black, Hispanic, or Chinese race/ethnicity, aged 45 to 84 years, were recruited from 6 US communities. Participants missing any lipoprotein particle variable or any covariates or without any follow-up were excluded, leaving a total of 6475. The institutional review boards at all participating centers approved the study, and all participants provided signed informed consent.

Laboratory Analysis

Lipids, lipoprotein particles, and other laboratory assays

Blood was drawn after a 12-hour fast, and EDTA-anticoagulated plasma samples were stored at −70°C. Lipids were measured at the Collaborative Studies Clinical Laboratory (Fairview-University Medical Center, Minneapolis, MN) within 2 weeks of sample collection, by using Centers for Disease Control and Prevention/National Heart, Lung, and Blood Institute standards. HDL-C concentration was measured by using the cholesterol oxidase method (Roche Diagnostics) after precipitation of non–HDL-C with magnesium/dextran (CV 2.9%). LDL-C concentration was calculated by using the Friedewald equation.

Lipoprotein particle subclass concentrations were measured with proton NMR spectroscopy at LipoScience, Inc (now part of LabCorp).[22] Amplitudes of the spectroscopically distinct lipid methyl group signals emitted by the different lipoprotein subclasses, derived through deconvolution of the plasma methyl signal envelope by using the LipoProfile-3 algorithm, were used to calculate lipoprotein subclass particle concentrations. Estimated diameter ranges of the subclasses are given in Table 1. Interassay CVs, determined from 80 replicate analyses of 8 plasma pools over 20 days, was 9%, 14%, and 6% for HL-P, HM-P, and HS-P, respectively.

Inflammatory markers

High-sensitivity C-reative protein (hs-CRP) was measured by using the BNII nephelometer (Dade Behring Inc). Intra-assay and interassay analytic CVs ranged from 2.3% to 4.4% and from 2.1% to 5.7%, respectively. IL-6 was measured by using an ultrasensitive ELISA (Quantikine HS Human IL-6 Immunoassay, R&D Systems). The laboratory analytic CV for this assay was 6.3%. D-dimer was measured by using an immunoturbidimetric assay on a Sta-R analyzer (Liatest D-DI; Diagnostica Stago). The lower limit of detection was 0.01 μg/mL.

ChrIRD death and hospitalization events and fatal and nonfatal CVD

Deaths and hospitalizations were identified by direct participant contact at 9-month intervals. Deaths of participants who had emigrated or who dropped out of MESA were identified through the National Death Index.

ChrIRD events were based on adjudication of the *International Classification of Diseases* (ICD) diagnoses in death (ICD-10) and hospital records (ICD-9), with exclusion of CVD, cancer, injury, acute organ failure, psychoses, substance abuse, and some metabolic disorders such as diabetes mellitus, similar to previous studies.[14–20] Conceptually, we considered non-CVD, noncancer ChrIRD events to reflect a chronic inflammatory, oxidative stress, or infectious

Table 1. Subject Characteristics at MESA Baseline (2000–2002) and CVD and Non-CVD ChrIRD Events During MESA Follow-up

	No.	Mean or %	SD
Covariates			
Age, y	6475	62.1	10.3
Race, %			
White	2508	38.7	
Chinese	787	12.2	
Black	1753	27.1	
Hispanic	1427	22.0	
Female, %	3425	52.9	
Body mass index, kg/m^2	6475	28.3	5.4
Systolic blood pressure, mm Hg	6475	126.5	21.5
Diastolic blood pressure, mm Hg	6475	71.9	10.3
Blood pressure–lowering medication, %	2128	32.9	
Cholesterol-lowering medication, %	1048	16.2	
Smoking			
Current, %	834	12.9	
Former, %	2367	36.6	
Diabetes, %	808	12.5	
Biomarkers			
Interleukin-6, pg/mL	6475	1.55	1.22
High-sensitivity C-reactive protein, mg/L	6475	3.59	5.20
D-dimer, μg/mL	6475	0.36	0.77
Lipids			
Total cholesterol, mg/dL	6475	194.2	35.4
LDL cholesterol, mg/dL	6397	117.3	31.4
HDL cholesterol, mg/dL	6475	51.0	14.8
Triglycerides, mg/dL	6475	130.5	78.1
Lipoprotein particles			
HDL particle family of variables			
HS-P 7.3 to 8.2 nm, μmol/L	6475	14.8	5.7
HM-P 8.2 to 9.4 nm, μmol/L	6475	13.3	6.9
HL-P 9.4 to 14 nm, μmol/L	6475	6.0	3.5
HS-P+HM-P (HMS-P) 7.3 to 9.4 nm, μmol/L	6475	28.1	5.5
LDL particle family of variables			
Large LDL 20.5 to 23 nm, nmol/L	6475	587.6	259.3
Small LDL 18 to 20.5 nm, nmol/L	6475	535.0	382.0
IDL 23 to 29 nm, nmol/L	6475	125.9	98.5
Small VLDL 29 to 35 nm, nmol/L	6475	34.0	20.0
Medium VLDL 35 to 60 nm, nmol/L	6475	28.8	21.8
Large VLDL >60 nm, nmol/L	6475	5.0	6.5

Continued

Table 1. Continued

	No.	Mean or %	SD
Events			
CVD events, fatal or nonfatal, %	756	11.7	
ChrIRD, death or hospitalization, %	1054	16.3	
Both entities: CVD and ChrIRD, %	345	5.3	

MESA indicates Multi-Ethnic Study of Atherosclerosis; CVD, cardiovascular disease; ChrIRD, noncardiovascular, noncancer chronic inflammation–related hospitalization and death; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HS-P, small-diameter HDL particles; HM-P, medium-diameter HDL particles; HL-P, large-diameter HDL particles; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

component as a predominant pathophysiology. Diagnostic codes were considered from among the following chronic and severe pathologic conditions: infectious diseases; endocrine, nutritional, and metabolic diseases; nervous, respiratory and digestive system diseases; skin diseases; musculoskeletal and connective tissue disorders; genitourinary diseases; and blood disorders. Most event records had multiple ICD codes.

Adjudication of the ChrIRD group of disorders was done as follows: 2 physicians independently identified the ICD codes reflecting non-CVD and noncancer disorders that had an important inflammatory component. These physicians had no information about the participant aside from the single ICD code. There was substantial agreement in this aspect, and, finally, a minimal set of events were discussed to obtain full agreement. It was decided to separate minor conditions from serious conditions. Serious conditions were defined as those that, in the judgment of both physicians, were likely to result in intensive hospital care or death. As a result, the list was altered. Nine codes were proposed as serious inflammation-related conditions, among which 7 were not previously noted among the 22 originally proposed by the first physician. With these refined definitions in hand, further coding resulted in near-unanimous agreement between the 2 physicians. We classified codes one at a time; consequently, a person could be placed in both CVD and non-CVD, noncancer ChrIRD. Restricting analysis to a hierarchical definition did not alter any conclusions. A complete list of ICD codes and their frequencies of occurrence is given in Tables S1 and S2.

A physician committee adjudicated CVD events based on medical records. CVD was defined as myocardial infarction, angina, stroke, transient ischemic attack, heart failure, peripheral arterial disease, and CVD death, as previously described.

Statistical Analysis

Mean and SD or counts and percentages were calculated for description. We defined HDL-related variables to include HS-P,

HM-P, HL-P, and HDL-C. Based on analyses described next, we added a fifth HDL-related variable, namely the sum of HM-P plus HS-P (HMS-P). These 5 variables constitute information about the HDL family. The remaining blood lipid variables were fasting triglycerides and total cholesterol (used instead of LDL-C so as to not lose 78 subjects with triglyceride levels ≥400 mg/dL). The remaining lipoprotein particle subclass variables were small LDL, large LDL, intermediate-density lipoprotein (IDL), small very low-density lipoprotein (VLDL), medium VLDL, and large VLDL. These 8 variables constitute information about the LDL family. We computed Pearson correlation (partial age, race/ethnicity, sex, except that correlations with age were not adjusted) of the 5 HDL-related variables with (1) each other and the remaining 8 lipid and lipoprotein particle variables in the LDL family and (2) a set of covariates measured at baseline, including age, race/ethnicity, sex, height (cm), heart rate (beats/min), body mass index (kg/m^2), systolic and diastolic blood pressure (mm Hg), blood pressure–lowering medication use (yes/no), cholesterol-lowering medication use (yes/no), smoking status (never, former, current), diabetes (yes/no), IL-6 (pg/mL), hsCRP (mg/L), and D-dimer (μg/mL).

We performed Poisson regression (incidence density of the earliest qualifying event over a median period of 10.1 years) predicting the following 5 clinical outcome variables from HDL-related variables: ChrIRD, incident fatal and nonfatal CVD, fatal and nonfatal CHD, fatal and nonfatal stroke, and fatal and nonfatal heart failure. We also studied subsets of ChrIRD. Given the high correlations among lipid and lipoprotein particle variables, including across the HDL and LDL families, isolation of regression effects to a single one of the HDL-related variables is complex. Within the HDL family, there were 2 highly correlated pairs: HS-P and HM-P (r=−0.62) and HL-P and HDL-C (r=0.89).

In the first analytic phase, we compared whole regression models by using differences in −2 log likelihood (Δ−2LL). For a given outcome variable and a fixed sample (here, n=6475), a better-fitting model has lower Δ−2LL. Δ−2LL changes when the number of predictor variables increases, in which case interpretation used the negative of the P=0.05 cut point for a χ^2 variable, with degrees of freedom equal to the number of additional variables: −3.84, −5.99, and −9.49 for 1, 2, and 4 additional variables, respectively. Our rule for interpretation comparing 2 models that have the same number of predictor variables was that Δ−2LL of <−3.84 was a statistically significant difference.

This procedure does not make statements specific to single variables but is helpful for understanding highly correlated predictors by comparing 2 whole regression models that differ by >1 variable. We investigated the HDL-related variables singly and in highly correlated pairs. Given generally small correlations of the HDL-related variables with the set of covariates, the first set of whole regression comparisons was with base model 1, which included the set of covariates but no lipid or lipoprotein variables. The second set of comparisons was with base model 2, which added lipid and lipoprotein variables in the LDL family to the set of covariates. Additional comparisons were described in the text.

In the second analytic phase, we identified a composite variable that captured available information derived from the pair HS-P and HM-P in a single dimension and computed relative risks across that variable. We linearly transformed the pair of variables into uncorrelated parts, thereby retaining all information. Specifically, we regressed HM-P on HS-P to estimate the residual of HM-P given HS-P, which by definition is uncorrelated with HS-P. The algebraic form of this variable is HM-P+0.76 HS-P−24.5; substantively, it quantitates how much more or less HM-P concentration was than would be expected given the concentration of HS-P. In regressions predicting outcomes with this residual and HS-P, HS-P was never a significant predictor (data not shown). Because this residual differs little from HMS-P, we focused on HMS-P as the single informative variable, reported for 3 models: one model adjusting only for age, race/ethnicity, and sex; a second model adding the rest of the set of covariates; and a third model adding the lipid and lipoprotein LDL family variables. To study goodness of fit of the linearity assumption, we presented outcome incidence densities in the third model by quartiles of HMS-P.

Improvement in Prediction Probability

The regression coefficient of a significant predictor may borrow from changes to the coefficients of other variables in the model without affecting the overall predictive performance of the whole model. We therefore studied whether there was improvement in prediction based on the whole model when HMS-P was added to the model including all other variables. The procedure used is called improvement in prediction probability (IPP) and is conceptually similar to study of net reclassification improvement.[23]

Details of the IPP Procedure

In IPP, by using Poisson regression, we computed the probability of observing an event under the base model including all other variables and then under an alternative model that included HMS-P. The reclassification probability is the expected event probability under the alternative model minus the expected event probability under the base model. We then computed observed cumulative event rates for those with positive and negative reclassification probability as one measure of the success of reclassification. As a second measure of whether reclassification improved prediction in a graded fashion, we regressed observed event rates in Poisson

Table 2. Variables in the HDL Particle Family: Mean and SE Values According to Sex and Race/Ethnicity, N=6475

	No.	HS-P	HM-P	HL-P	HDL-C	HM-P+HS-P (HMS-P)
Sex						
Female	3425	14.2±0.11	15.1±0.13	7.3±0.06	56.3±0.26	29.3±0.10
Male	3050	15.4±0.09	11.3±0.10	4.6±0.05	45.1±0.21	26.7±0.08
Race/ethnicity						
White	2508	14.0±0.12	15.0±0.15	6.1±0.07	52.4±0.31	29.0±0.11
Chinese	787	16.8±0.20	11.0±0.21	6.1±0.11	49.7±0.45	27.7±0.16
Black	1753	15.3±0.13	11.8±0.14	6.4±0.09	52.4±0.36	27.0±0.12
Hispanic	1427	14.5±0.14	13.4±0.17	5.4±0.08	47.7±0.35	27.9±0.13

HDL indicates high-density lipoprotein; HS-P, small-diameter HDL particles; HM-P, medium-diameter HDL particles; HL-P, large HDL particles; HDL-C, HDL cholesterol.

regression on continuous reclassification probability, adjusting for base model probability.

We considered $P<0.05$ to be noteworthy in general screening of findings. Analyses were performed by using PC-SAS version 9.3 (SAS institute).

Results

Table 1 summarizes the participant characteristics. Total HDL particle concentration was 34.1 μmol/L; smaller HDL particles (HS-P and HM-P) constituted the majority of total HDL-P. Of the 34.1 μmol/L, mean HS-P represented 43%; mean HM-P, 39%; and mean HL-P, 18%. HSM-P constituted a mean of 28.1±5.3 μmol/L. During the median of 10.1-year follow-up, there were 1054 ChrIRD hospitalization and death events and 756 fatal and nonfatal CVD events. Given that 345 people qualified for both types of events, 67% (709/1054) of ChrIRD deaths and hospitalizations had no concurrent adjudicated CVD diagnosis, while 54% (411/756) of CVD cases had no concurrent ChrIRD diagnosis.

In Table 2, mean±SE values of the 5 HDL-related variables (HS-P, HM-P, HL-P, HDL-C, and HMS-P) were higher in women than in men, with the exception of HS-P. Whites had the highest HM-P and HMS-P, while Chinese and blacks had the lowest levels of these variables. HS-P was highest in Chinese and blacks.

Table 3 shows correlations of the 5 HDL-related variables with each other and with other lipid and lipoprotein entities as well as the set of covariates. There was little correlation of the HDL-related variables with total or LDL cholesterol; correlations were stronger with triglycerides. In contrast, there was close to complete overlap between HL-P and HDL-C ($r=0.89$). Among lipoprotein particle variables, HS-P and HM-P were highly correlated ($r=-0.62$) and HL-P had strong and opposite correlations with large LDL particles ($r=0.5$) and small LDL particles ($r=-0.67$). Other correlations among lipoprotein particle size variables are substantial (ie, between 0.2 and 0.4). HM-P correlated highly with HMS-P ($r=0.55$). A noteworthy pattern of decreasing correlation is seen across HS-P, HM-P, and HL-P—for example, with small LDL particles, where $r=0.37$, -0.32, and -0.67, respectively, with similar patterns for triglycerides and all VLDL particle variables. In contrast, the corresponding correlations were increasing for HDL-C and large LDL particles. Correlations with nonlipid covariates were much smaller, with the exception of body mass index. The pattern of decreasing correlation across HS-P, HM-P, and HL-P was reiterated for several variables, including body mass index, use of blood pressure–lowering medication, diabetes, and IL-6. HMS-P had a correlation of 0.13 with heart rate.

Nonlipid, nonlipoprotein predictors of ChrIRD include age, Hispanic versus Chinese race/ethnicity, male sex, heart rate, systolic blood pressure, antihypertensive medication use, diabetes, IL-6, and D-dimer (Table S3).

Associations of Sets of HDL-Related Variables With Outcome Events

Adjusting for the set of covariates but in the absence of lipid and lipoprotein variables in the LDL family (base model 1, Table S4), the most negative $\Delta-2LL$ values occur in conjunction with adding the pair HS-P and HM-P for predicting ChrIRD, but contributions to $\Delta-2LL$ are similar from the pair HL-P and HDL-C or from the pair HS-P and HM-P for predicting CVD and CHD. Prediction of stroke arises predominantly from the pair HL-P and HDL-C. When the LDL family of variables was added to the model to create base model 2 (Table S5), the greatest improvement in prediction was for ChrIRD by using the pair HS-P and HM-P. HM-P alone was a less significant predictor of ChrIRD and the pair HS-P and HM-P was a marginally significant predictor of CVD. The pair HL-P

Table 3. Pearson Partial Correlations* Between Variables in the HDL Particle Family and Lipids, Lipoprotein Particles, and Other Covariates, N=6475

	HS-P	HM-P	HL-P	HDL-C	HM-P+HS-P (HMS-P)
Lipids and lipoproteins					
Total cholesterol	0.02	0.05	0.07	0.16	0.08
LDL cholesterol	0.01	−0.07	—0.14	−0.07	−0.08
HDL cholesterol	−0.28	0.4	0.89	1	0.19
Triglycerides	0.29	−0.1	−0.34	−0.41	0.19
HS-P 7.3 to 8.2 nm	1	−0.62	−0.29	−0.28	0.31
HM-P 8.2 to 9.4 nm	−0.62	1	0.21	0.4	0.55
HL-P 9.4 to 14 nm	−0.29	0.21	1	0.89	−0.05
Large LDL 20.5 to 23 nm	−0.3	0.18	0.5	0.5	−0.10
Small LDL 18 to 20.5 nm	0.37	−0.32	−0.67	−0.62	0.01
IDL 23 to 29 nm	−0.05	0.13	−0.11	−0.07	0.10
Small VLDL 29 to 35 nm	0.25	−0.04	−0.29	−0.33	−0.01
Medium VLDL 35 to 60 nm	0.28	−0.2	−0.38	−0.41	0.05
Large VLDL >60 nm	0.09	−0.09	−0.19	−0.19	0.23
Covariates					
Age	0.12	−0.07	0.11	0.08	0.04
Height	0.01	−0.04	−0.02	−0.03	−0.04
Heart rate	0.1	0.02	−0.11	−0.07	0.13
Systolic blood pressure	0.06	0.03	−0.04	−0.04	0.09
Diastolic blood pressure	0.04	0.03	−0.03	−0.01	0.08
Antihypertensive medication	0.1	−0.03	−0.09	−0.09	0.06
Hypertension	0.09	0	−0.08	−0.07	0.09
Body mass index	0.18	−0.12	−0.29	−0.28	0.05
Former smoker	0.02	0.02	0.02	0.04	0.05
Current smoker	−0.04	0.01	−0.05	−0.05	−0.03
Diabetes	0.13	−0.1	−0.12	−0.12	0.02
Interleukin-6	−0.03	−0.05	−0.13	−0.15	−0.08
High-sensitivity C-reactive protein	−0.01	0.01	−0.08	−0.08	0.01
D-dimer	−0.05	−0.02	0	−0.02	−0.08

HDL indicates high-density lipoprotein; HS-P, small-diameter HDL particles; HM-P, medium-diameter HDL particles; HL-P, large HDL particles; HDL-C, HDL cholesterol; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

*All correlation coefficients are adjusted for age, race/ethnicity, and sex, except that age is unadjusted. $r<0.03$ corresponds to $P<0.05$ given the large sample size.

and HDL-C did not add to prediction in any model that included the LDL family.

Associations of Medium Plus Small HDL Particle Concentration With Outcome Events

Table 4 gives the findings for prediction of the 5 outcome variables for HMS-P. In all models (except the least complex model for any CVD), HMS-P was a significant predictor of ChrIRD, CVD, and CHD but did not predict either stroke or heart failure. Incidence densities per 100 participants followed for 10.1 years are shown in Table 5 for each outcome variable across quartiles of HMS-P, including adjustment for the LDL family. Incidence densities in the most adjusted model monotonically decreased with increasing HMS-P for ChrIRD (15.7 for quartile 1 to 11.2 for quartile 4). Decreases in incidence density were also apparent for CVD and CHD outcomes. Subsets of ChrIRD were similarly predicted by HMS-P. In the model that included demographics, CVD risk factors, and the LDL-related variables, relative

Table 4. Prediction of Outcome Events (N=6475 at Risk) in Poisson Regression For Medium- Plus Small-Diameter HDL Particle Concentration During Median Follow-up 10.1 Years

	ChrIRD (n=1054)	Any CVD (n=756)	CHD (n=423)	Stroke (n=172)	HF (n=222)
Minimal model (age, race/ethnicity, sex adjusted)	0.86 (0.81 to 0.92), <0.0001	0.95 (0.88 to 1.02), 0.17	0.91 (0.82 to 1.01), 0.09	0.99 (0.85 to 1.16), 0.91	0.96 (0.83 to 1.11), 0.59
Risk factor model (minimal model+other covariates*)	0.86 (0.80 to 0.92), <0.0001	0.91 (0.85 to 0.99), 0.03	0.88 (0.79 to 0.98), 0.02	0.97 (0.83 to 1.14), 0.71	0.93 (0.81 to 1.08), 0.34
Risk factor and LDL family model (risk factor model+LDL family)	0.85 (0.79 to 0.91), <0.0001	0.90 (0.83 to 0.98), 0.01	0.88 (0.78 to 0.98), 0.02	0.97 (0.82 to 1.15), 0.76	0.92 (0.79 to 1.06), 0.25

Relative risk (95% CI), *P* trend. HDL indicates high-density lipoprotein; ChrIRD, noncancer chronic inflammation–related hospitalization and death; CVD, cardiovascular disease; CHD, coronary heart disease; HF, heart failure; LDL, low-density lipoprotein.
*Height, heart rate, body mass index, systolic and diastolic blood pressure, blood pressure– and cholesterol-lowering medication use, smoking status, diabetes, interleukin-6, high-sensitivity C-reactive protein, and D-dimer.

risks were all inverse. Specifically, for ChrIRD deaths (n=287), relative risk per 1 SD of HMS-P was 0.81 (95% CI 0.71 to 0.93), *P* for trend 0.003. Corresponding relative risks, CIs, and *P* values were for CHrIRD hospitalization (n=928 includes some deaths), 0.86 (0.79 to 0.92), <0.0001; for infectious disease death or hospitalization (n=464), 0.79 (0.71 to 0.87), <0.0001; for septicemia (n=168), 0.91 (0.75 to 1.09), 0.31; for acute renal failure death or hospitalization (n=261), 0.8 (0.7 to 0.92), 0.0023; and for syncope or collapse hospitalization (n=166), 0.89 (0.75 to 1.05), 0.16. Most other subsets of ChrIRD were also inversely related to HMS-P (data not shown). We note that because of multiple cause coding, each of these conditions may be accompanied by other conditions.

Improvement in Prediction Probability

Table 6 shows significantly higher event rates for predicting ChrIRD in those reclassified up versus down (17.6% versus 15.0%). It also shows that reclassification was graded: significant regression of ChrIRD events on the level of reclassification probability indicates that stronger reclassification, the higher is the reclassification probability. For CHD, event rates for those reclassified up versus down were 7.1% versus 6.0% (*P*=0.07). The regression coefficient for CHD events on continuous reclassification probability was not significant.

Sensitivity Analyses

There were no associations of HMS-P with total cancer hospitalization or death (n=553). When we omitted anyone whose ChrIRD or CVD event or censoring date was within 2 years of baseline, we were left with 6288 people in the analysis with 569 CVD events and 6317 people in the analysis with 896 ChrIRD. Findings were similar to those in Tables 4 (*P*<0.0001 for ChrIRD and *P*=0.04 for CVD).

Discussion

Our major finding was that the concentration of the smaller HDL particles, namely HMS-P, was significantly predictive for non-CVD, non-cancer ChrIRD and less strongly for fatal and nonfatal CVD in the MESA cohort initially free of overt CVD during a median follow-up of 10.1 years, even after accounting for other risk factors and variables in the HDL and LDL families. The highly correlated pair HL-P and HDL-C was significantly related to CVD without adjustment for variables

Table 5. Prediction of Outcome Events (N=6475 at Risk) in Poisson Regression For Medium- Plus Small-Diameter HDL Particle Concentration During Median Follow-up 10.1 Years

	ChrIRD	CVD	CHD	Stroke	HF
Quartile 1 (3.7 to 24.3 μmol/L)	15.7 (13.9 to 17.8)	9.3 (7.9 to 11)	5.3 (4.2 to 6.6)	1.6 (1.1 to 2.4)	2.2 (1.6 to 3)
Quartile 2 (24.4 to 27.6 μmol/L)	14.9 (13.3 to 16.8)	9.3 (8.0 to 10.8)	5.4 (4.4 to 6.6)	1.9 (1.4 to 2.6)	2.1 (1.6 to 2.9)
Quartile 3 (27.7 to 31.2 μmol/L)	11.9 (10.5 to 13.5)	8.6 (7.4 to 10.0)	4.9 (4.0 to 6.0)	2.0 (1.5 to 2.8)	1.8 (1.3 to 2.5)
Quartile 4 (31.3 to 56.7 μmol/L)	11.2 (9.7 to 13.0)	7.9 (6.6 to 9.4)	4.1 (3.2 to 5.3)	1.4 (1.0 to 2.1)	2.3 (1.6 to 3.2)

Incidence density per 100 persons followed for 10.1 years (95% CI), by quartiles of medium- plus small- HDL particle concentration in the risk factor and LDL family model. HDL indicates high-density lipoprotein; ChrIRD, noncancer chronic inflammation–related hospitalization and death; CVD, cardiovascular disease; CHD, coronary heart disease; HF, heart failure; LDL, low-density lipoprotein.

Table 6. Improvement in Prediction Probability for Model 3, Without (Base Model) and With Medium- Plus Small-Diameter HDL Particle Concentration (Alternative Model)

	Reclassification Down: % Events (n/N)	Reclassification Up: % Events (n/N)	Relative Risk	*P* Categorical Reclassification*	*P* Continuously Graded Reclassification†
ChrIRD	15.0 (486/3242)	17.6 (568/3233)	1.17	0.005	<0.0001
CVD	11.3 (355/3145)	12.0 (401/3330)	1.06	0.34	0.13
CHD	6.0 (190/3187)	7.1 (233/3288)	1.18	0.07	0.25
Stroke	2.9 (88/3028)	2.4 (84/3447)	0.83	0.24	0.19
HF	3.3 (104/3078)	3.5 (118/3397)	1.06	0.83	0.22

HDL indicates high-density lipoprotein; ChrIRD, noncancer chronic inflammation–related hospitalization and death; CVD, cardiovascular disease; CHD, coronary heart disease; HF, heart failure.

**P* based on 2-sample z test for proportions comparing cumulative event rates for those reclassified up versus those reclassified down.

†*P* for graded reclassification (Poisson regression of outcome on reclassification probability adjusted for base probability).

in the LDL family but lost significance when the LDL family was entered into the model. Sorting out the associations of HL-P and HDL-C versus elements of the LDL family is beyond the scope of study of the HDL family.

A previous MESA publication concerning 5597 people not taking lipid-lowering medication by Mackey et al described the association of HDL-C and total HDL-P with CHD (n=227) during 6 years of follow-up.[5] HDL-C was not significantly related to CHD after adjustment for total HDL-P, while the association of total HDL-P with CHD persisted with adjustment for HDL-C, total LDL particles, LDL-C, and triglycerides. Findings were similar for the combined end point of CHD, stroke, and other atherosclerotic death. The present report clarifies the findings of Mackey et al[5] by emphasizing the importance of the smaller HDL particle subclasses and showing that the prediction of CHD and CVD due to HL-P is statistically bound with the LDL family.

A unique aspect of our approach is that we entered variables in the HDL family in pairs, then identified a function of HS-P and HM-P, the predictive pair, which singly predicted ChrIRD and, to a lesser extent, CHD. Other studies have examined the variables in the HDL family one at a time; this approach invites confusion in interpretation due to strong intercorrelations among HDL particle subclasses, LDL particle subclasses, and blood lipid concentrations. Akinkuolie et al[24] studied CHD in the Women's Health Study and used a 5–HDL-P subclass system. Their best predictor of CHD, with adjustment for other lipoprotein variables, was their large HDL particle variable, which has substantial overlap in particle diameter with our HM-P. Among our single variables, HM-P was the most predictive of the HDL subclasses but was not as good a predictor as HMS-P. Several other studies of single HDL particle subclasses adjusted for other lipoprotein variables also found that smaller HDL particles predicted CHD.[25–27] No other study has examined HDL particles in relation to ChrIRD.

The classic view is that HDL-C is "good cholesterol." This view is limited and recent advances show a great deal of complexity among HDL particles, in particular relating to a wide variety of proteins that are carried on HDL particles.[28] Our findings provide evidence consistent with HDL particles representing a spectrum of physicochemical and functional properties. HDL particles are thought to have antithrombotic activity in human platelets, anti-inflammatory activity in blood, and antiapoptotic activity in endothelial cells is engendered mainly by small dense HDL particles.[9] HDL particles undergo constant, dynamic remodeling as they transport cholesterol and other lipids between circulating cells, lipoproteins, tissues, and organs. Circulating HDL particles are heterogeneous, differing in size, charge, and protein and lipid composition.[28,29] The most prevalent HDL proteins are apolipoprotein (apo)A-I (≈70%) and apoA-II (≈20%), which can be considered to form a skeleton for HDL.[9] Other proteins include several forms of apoC (mainly apoC-III, ≈2% to 4%), apoD, apoE (<2%), and many other proteins in smaller concentrations and specific to different HDL subfractions. It is likely that many of these low concentration proteins are gained and lost as HDL particles are continuously converted in the plasma, including changes in diameter and shape (spherical versus discoidal). HDL particles seem to interact with most, if not all, enzymes and lipid transfer proteins involved in lipoprotein metabolism. HDL constituent turnover rates range from a few hours to several days.[29] We postulate that this process of HDL particle conversion is important in the different functional properties among the HDL particle subclasses. Although based on a single assessment of HDL particle subclasses, we speculate that the predictive power of HMS-P might indicate that it is beneficial in this dynamic flux to have more smaller HDL particles. Our finding that HMS-P predicts non-CVD, noncancer ChrIRD even better than it predicts CHD incidence bolsters the argument that HDL particles are involved in anti-inflammatory activity.

Our study has some strengths and weaknesses. Strengths include our large multiethnic study cohort with incident follow-up events. There were a large number of CVD and non-CVD, noncancer ChrIRD events. Diagnoses were based on multiple ICD codes and reviewed by 2 blinded physicians. A strength is that we included nonfatal non-CVD, noncancer ChrIRD events. While others have studied fatal non-CVD, noncancer chronic inflammatory conditions,[19,20] our study of nonfatal events is novel.

The adjudication of the non-CVD chronic inflammatory end points of death or hospitalization events is less precise than the adjudication of the CVD end points. The non-CVD events are based solely on ICD codes. A focus on each of the many included diseases would not be feasible, and, in fact, such a requirement would mean that no study would be able to group conditions as we did. On the other hand, ICD codes for the extremely serious conditions including in the non-CVD outcome are not assigned lightly by hospitals or in making death certificates. The biological background of the selected conditions is well known to have a commonality of low-grade or more-intense inflammation. Our clinical impression is that the ICD coding of these conditions is sufficiently precise to support our definition. Another limitation is that we did not know whether non-CVD, noncancer ChrIRD events were incident, although the more severe, life-threatening aspects of these inflammatory processes are unlikely to preexist, because many people with such conditions do die. In this context, it is interesting that exclusion of the first 2 years of early ChrIRD events did not alter conclusions, which would suggest that ongoing low-level inflammatory processes did not create reverse causality. We only had lipoprotein particle subclass measures and inflammatory markers at baseline; thus, we were not able to study their changes over time and the correlations of their changes with changes in lipids. We focused only on the HDL family and have noted that findings for HL-P and HDL-C were indeterminate because we restricted to disentangling only the HDL family. Because we have reported an observational study, residual confounding is possible. Our use of the term "predict" implies that baseline measures predict future disease, but we can only speculate that those associations are causal.

We found that smaller HDL particles were inversely related to future ChrIRD, a novel composite of serious noncardiovascular, noncancer inflammation-related diseases. Smaller HDL particles also predicted incident CHD. While epidemiologic studies do not provide mechanisms underlying these associations, our findings support the concept of multiple biological functions of HDL particles. Our findings emphasize the need for further study of the key biological activities of HDL subpopulations, as well as clinically relevant, anti-inflammatory, and atheroprotective HDL components.

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