High-Density Lipoprotein and Long-Term Incidence and Progression of Aortic Valve Calcification: The Multi-Ethnic Study of Atherosclerosis

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High-Density Lipoprotein and Long-term Incidence and Progression of Aortic Valve Calcification: The Multi-Ethnic Study of Atherosclerosis

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Disclosures

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Supplemental Material

Figures S1–S3

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Abstract

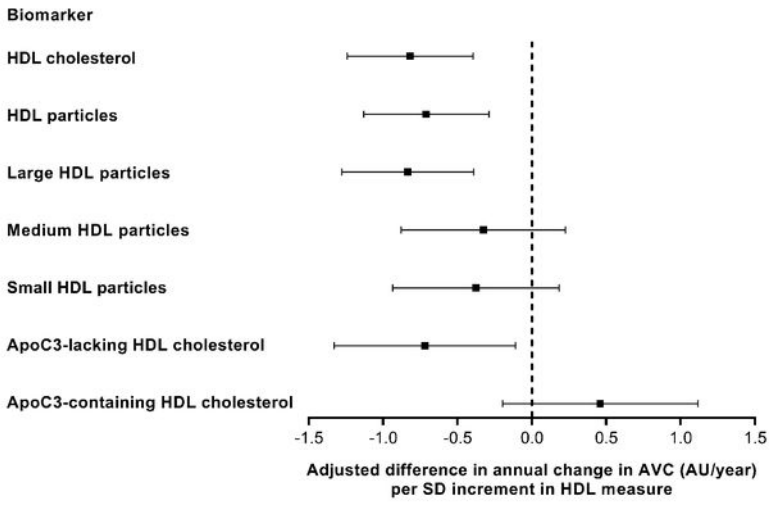
Background—Aortic valve calcification (AVC) shares pathologic features with atherosclerosis. Lipoprotein components have been detected in aortic valve tissue, including high density lipoprotein (HDL). HDL measures have inverse associations with cardiovascular disease, but relationships with long-term AVC progression are unclear. We investigated associations of HDL cholesterol, particles, apoC3-defined HDL subtypes, and, secondarily, cholesteryl ester transfer protein (CETP) mass and activity, with long-term incidence and progression of AVC.

Methods—We used linear mixed-effects models to evaluate the associations of baseline HDL indices with AVC. AVC was quantified by Agatston scoring of up to 3 serial computed tomography (CT) scans over a median of 8.9 (maximum 11.2) years of follow-up in the Multi-Ethnic Study of Atherosclerosis (n=6814).

Results—After adjustment, higher concentrations of HDL-C, HDL-P, large HDL-P, and apoC3-lacking HDL-C were significantly associated with lower incidence/progression of AVC, as was higher CETP mass. Neither small nor medium HDL-P, apoC3-containing HDL-C, or CETP activity were significantly associated with AVC incidence/progression in the main analyses. When included together, a significant association was observed only for HDL-C, but not for HDL-P. In exploratory analyses, inverse associations for HDL-C, HDL-P, large HDL-P, and apocC3-lacking HDL with AVC incidence/progression were more pronounced for adults ≥65 years, men, and White participants. ApoC3-containing HDL-C only showed a positive association with AVC in these subgroups.

Conclusions—In a multi-ethnic population, HDL-C, HDL-P, large HDL-P, and apoC3-lacking HDL-C were inversely associated with long-term incidence and progression of AVC. Further investigation of HDL composition and mechanisms could be useful in understanding pathways that slow AVC.

Graphical Abstract



Keywords

valve; lipoprotein; cholesterol; aortic stenosis; Lipids and Cholesterol; Clinical Studies; Biomarkers

Introduction

The proportion of adults 65 and older is expected to rise in North America to approximately 25% by 2060, bringing an accompanying surge in aging-related cardiovascular disease (CVD).[1] A hallmark of pathologic cardiovascular aging is calcification, a process that particularly affects the aortic valve.[2] Aortic valve calcification (AVC) progresses over decades and may culminate in markedly restricted leaflet opening and severe aortic stenosis (AS). Among U.S. adults ≥65 years, severe AS is present in 3.7–4.7%.[2,3] Once symptomatic, median survival for this condition is 2 years unless the aortic valve is replaced through surgical or endovascular procedures.[4] As yet, there are no therapies of proven efficacy for AVC, underscoring the need to better understand the determinants and mechanisms responsible for the disorder.

AVC and atherosclerosis share common pathophysiologic features.[5] Histologic studies have documented subendothelial lipid deposits, superimposed by immune cells and adjacent microcalcifications, in aortic valve tissues in early stages of disease.[5] Circulating lipids, a prominent risk factor for coronary heart disease, have also been linked to AVC.[6] This includes elevated levels of low density lipoprotein cholesterol (LDL-C) and lipoprotein (Lp)(a), both of which have been implicated in Mendelian randomization studies as potentially causal for AVC.[7,8] Randomized trials of statins, however, failed to show a reduction in AS progression with LDL lowering.[9] But these trials focused largely on patients with moderate or greater AS, a stage at which hemodynamic stress, and not lipids, may be the dominant driver of progression.[10] Low levels of high density lipoprotein cholesterol (HDL-C) have likewise been associated with AVC, but Mendelian randomization analysis did not support a causal basis.[8,11] Given the complex biology of HDL particles (-P), however, this does not exclude the possibility that a protective effect could relate to a different measure, such as HDL-P concentration, particle size, associated proteins or lipids, or its functionality as a cholesterol acceptor.

Greater HDL-P levels could better capture the favorable properties of HDL, as has been documented in relation to ischemic stroke and CHD.[12–14] Furthermore, HDL-P size appears also to be of relevance to HDL functionality. Larger HDL-P size, which in part, relates inversely to cholesteryl ester transfer protein (CETP) activity, has shown a stronger inverse relationship with longevity and CVD, although conflicting reports have documented this for smaller HDL-P instead.[15–17] Such differences could relate to distinct lipid or protein composition of HDL subclasses that may modulate their protective cardiovascular properties.[18] One protein in particular, apolipoprotein (apo) C3, has been shown to modify the associations of HDL with CVD events.[19] Specifically, HDL lacking apoC3 was inversely associated with CVD, while HDL containing apoC3 was positively associated. Thus, higher apoC3 content could be a defining feature of dysfunctional HDL.[19–21]

Studying HDL particle subtypes may therefore contribute to understanding which proteins or cholesterol removal (efflux) pathways could be associated with AVC. We undertook this study in MESA, investigating baseline measurement of HDL particles and serial computed tomography (CT) measurements of AVC, with nearly a decade of CT follow-up for many participants. We hypothesized that (i) higher HDL-C and HDL-P concentration, (ii) larger HDL particle size, and (iii) higher levels of apoC3-lacking HDL-C(i.e., more functional HDL) would be associated with lower incidence or progression of AVC. We also explored whether lower CETP mass and activity would be associated with less AVC progression.

Materials and Methods

Study Population and Cardiac Computed tomography (CT)

Data requests by qualified researchers may be directed to MESA at https://www.mesa-nhlbi.org. The authors will share their analysis upon request. In brief, MESA enrolled 6814 participants of self-reported White, Black, Hispanic/Latino, and Chinese race-ethnicity, ages 45–84 years, at 6 US centers to ascertain subclinical risk factors for atherosclerosis.[22] Participants signed informed consent to enroll in MESA and the study was approved by the institutional review boards of participating institutions.

Serial CT scans were for AVC by Agatston scoring in a semi-automated fashion, as previously described.[23] Scores were measured in duplicate and averaged with high agreement (kappa >0.9) within and between readers. AVC was measured at baseline (n=6814, 2000–2002), then at Exams 2 (2002–2004) or 3 (2004–2006), with the cohort evenly divided in random fashion between the two Exams (total n=5886), and at Exam 5 (2010–2012, n=3,304). In the baseline examination, electron beam (EB) CT and multi-detector (MD) CT varied by site; subsequently, all participants underwent MDCT. Prevalent AVC was defined as a score>0 at baseline, incident AVC as a score>0 at follow-up with absent baseline calcification, and progression as a positive difference between scans. The study population comprised all participants with HDL measures of interest at the baseline examination who also completed a cardiac CT.

Measurement of HDL-P, ApoC3-containing HDL, HDL-C and CETP

Blood was sampled after 12 h of fasting at the baseline study visit and EDTA plasma stored at −70°C. HDL-P concentration, small (SHDL-P, 7.3–8.2 nm), medium (MHDL-P, 8.3–9.4 nm) and large (LHDL-P, 9.5–14 nm) HDL particle subfractions, mean HDL-P size (ZHDL-P), and LDL-P were measured at LipoScience, Inc. (Raleigh, NC) by nuclear magnetic resonance (NMR) spectroscopy and the LipoProfile-3 algorithm, as previously described.[24,25]

ApoC3-containing HDL-C was measured using a modified sandwich ELISA assay at the Harvard T.H. Chan School of Public Health (Boston, MA) from baseline samples after excluding 1000 random samples that had undergone extensive testing and were depleted in volume, as previously reported.[19] All lipoproteins containing apoC3 were bound in the first step and then apoAI was measured in the bound and unbound fractions to quantify apoC3-

containing and depleted HDL. Outliers (n=45) with implausible values were excluded for a final n=5657.

HDL-C and CETP measures were performed at Fairview University Medical Center (Minneapolis, MN), as detailed elsewhere.[26] Briefly, plasma was precipitated with magnesium chloride and dextran sulfate, by the cholesterol oxidase method (Chol R1, Roche Diagnostics Corporation, Indianapolis, IN), and analyzed for HDL-C on a Roche/Hitachi 911 Automatic Analyzer (Roche Diagnostics Corporation). Serum CETP mass was measured with the CETP ELISA (Wako Chemicals USA, Richmond VA). Plasma CETP activity was measured with the CETP Activity Kit (Roar Biomedical, Inc., New York, NY).

Demographic and Clinical Covariates

Sex, race-ethnicity, cigarette smoking, alcohol consumption, and educational attainment were obtained from self-report. Heavy alcohol use was defined as >14 drinks/week for men and >7 drinks/week for women. Body mass index (BMI) was calculated as weight (kg)/height squared (m^2). Systolic blood pressure (SBP) was measured with a Dinamap model Pro 100 automated oscillometric sphygmomanometer on seated participants three times and the last two measurements were averaged. Antihypertensive therapy was defined as being on at least 1 pharmacologic agent to reduce blood pressure. Diabetes mellitus was defined as fasting glucose ≥126 mg/dL or use of a pharmacologic agent (oral or insulin) to lower blood glucose. Time since menopause and estrogen use were documented at the baseline visit.

LDL-C was calculated from the Friedewald equation for samples having triglycerides (TG) <400 mg/dL. TG were measured with a glycerol blanked enzymatic method (Trig/GB, Roche Diagnostics Corporation). Statin use was defined as being on a 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitor. Lp(a) mass concentration was measured by Health Diagnostics Laboratory (Richmond, Virginia) with a latex-enhanced turbidimetric immunoassay (Denka Seiken, Tokyo, Japan).[27] Estimated glomerular filtration rate (eGFR) was calculated from serum creatinine using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) 2009 equation. GlycA is a composite measure of glycosylated acute-phase inflammatory proteins, which has been shown to interact with HDL-P, comprising; α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin, and transferrin clustered as a single peak on NMR LipoProfile-3.[28] Intra-assay and inter-assay coefficients of variability for GlycA in MESA were 1.9% and 2.6%, respectively.[29]

Statistical Analysis

Participant characteristics were described by sex. Continuous measures were compared using the student t test if normally distributed or Mann Whitney U test otherwise. Pairwise correlations of lipid measures were assessed by Pearson correlation coefficients. We determined the annual rate of AVC change over time in participants without and with calcification at baseline using linear mixed-effects models (LMM). This approach is equivalent to examining combined incidence and progression of AVC. Although AVC was largely zero-weighted and skewed, we analyzed this variable in its original scale given that its distribution becomes asymptotically normal when the sample size is large, as done previously in MESA.[30–32] Briefly, the model included AVC at available time points as

the dependent variable, a random intercept per participant to account for correlation in the longitudinal data, and main effects for time from baseline and the independent variable, as well as their interaction. The parameter of interest was the interaction term which quantifies the difference in AVC change per year (i.e., the change in slopes) associated with one standard deviation (SD) increment in continuous exposure level. The model's inclusion of baseline AVC provided adjustment for the starting level of calcification. The independent variables included baseline concentrations of HDL-C, HDL-P, apoC3-containing and apoC3-lacking HDL, small, medium and large HDL-P, with mean HDL-P (ZHDL-P) concentration as a secondary measure, and, in an exploratory analysis, CETP activity and CETP mass. HDL-C, HDL-P, ZHDL-P and CETP measures were considered individually, whereas HDL subclasses (SHDL-P, MHDL-P, and LHDL-P) and subspecies (apoC3-containing and -lacking HDL concentrations) were each entered together in the same model. The difference in regression coefficients for apoC3-containing and -lacking HDL was assessed with the Wald test.

We first performed unadjusted analyses (Model 0). We next adjusted for age, sex, race-ethnicity, scanner type and site (Model 1). We then further adjusted for Model 1 variables and education, BMI, smoking, SBP, antihypertensive therapy, diabetes, LDL-P, TG, statin use, and eGFR (Model 2, main model). In sensitivity analyses, we next assessed the impact of additional adjustment for GlycA[33,34]; for Lp(a) in the subset with available measurement; for heavy alcohol use; and for estrogen replacement therapy or time since menopause in women.[35]

We tested for three-way interactions of exposure measures and time by age ≥65 or <65 years, sex, race-ethnicity, as well as by GlycA, based on previous evidence indicating variable affects with these factors, by including cross-product terms in Model 2. To preserve maximum power for assessment of our seven primary exposures, we did not undertake adjustment for multiple testing, but defined statistical significance as a two-tailed $p<0.05$. Three-way interaction testing was considered exploratory, and a Chi-squared test was used to evaluate model fit. Analyses were performed in R (Vienna, Austria). Forest plots were generated with GraphPad Prism 9.1.0 (La Jolla, CA).

Results

Baseline Characteristics

The numbers of participants having baseline laboratory and CT measures at each examination in MESA are indicated in Figure 1. Our study sample comprised 6784 participants with available AVC and HDL parameter measures at baseline. Sex-stratified characteristics are shown in Table 1. Women had lower prevalent diabetes and smoking than men, but women had higher SBP and antihypertensive use, higher BMI, lower eGFR, and less education. HDL-C and HDL-P concentrations, as well as mean HDL-P size, medium and large HDL-P concentrations, and Lp(a) were higher in women. ApoC3-containing and -lacking HDL, CETP mass and activity, as well as GlycA concentrations were higher in women. Small HDL particles and TG were lower in women. Pairwise correlations of lipids/lipoproteins and CETP measures are shown in Figure 2.

Women less often had any AVC, and when present, had a lower extent of calcification than men. Mean AVC at baseline was 121.1±264.0 (range 1.90–2453.3) AU for women and 248.7±657.2 (range 1.90–7672.3) AU for men. Participants were followed for a median of 8.9 (maximum of 11.2) years. Among the 5875 participants free of AVC at baseline, 433 participants developed new AVC during follow-up with mean longitudinal change of 13.8±40.2 AU/year. Among the 909 with positive AVC at baseline, 744 had follow-up AVC measured, whose mean longitudinal change was 18.2±110.5 AU/year. Of those with prevalent AVC, 274 participants exhibited a decrease in calcification of 31.3±95.4 AU/year. Associations with HDL indices were evaluated in the overall cohort, including those with and without baseline AVC, in whom mean change was 2.3±34.5 AU/year.

Exposure Measures and Rates of AVC Increase

Higher levels of HDL-C, HDL-P, apoC3-lacking HDL, LHDL-P and ZHDL-P were significantly associated with lower rates of AVC incidence and slower progression in unadjusted, minimally adjusted and fully adjusted models (Fig. 3A,B). No significant associations with incidence/progression of AVC were observed for apoC3-containing HDL, MHDL-P or SHDL-P. Additional adjustment for GlycA had no meaningful impact on the risk estimates (Supplemental Fig. 1). Results were similar for SHDL-P, MHDL-P, or LHDL-P when each was included individually in the sequential models. For the analysis of apoC3-lacking and apoC3-containing HDL, the change in AVC per year was significantly slower in those with higher level of the exposure (p=0.028). Examination of apoC3-containing and apoC3-lacking HDL-C separately in sequential models showed no significant associations with AVC incidence/progression. When HDL-C and HDL-P were entered together in Model 2, only HDL-C remained significantly associated with AVC incidence/progression (HDL-C: β per SD= −0.629 [95% CI=−1.221, −0.036] AU/year, p=0.038; HDL-P: β per SD =−0.270 [95% CI=0.862, 0.323] AU/year, p=0.372). Higher CETP mass was also significantly associated with lower incidence/progression of AVC without meaningful attenuation of the regression coefficients at higher levels of adjustment (β per SD=−1.144 [95% CI=−0.376, −1.911] AU/year, p=0.003, in the main model). This was not the case, however, for CETP activity, for which a similar, non-significant association was observed at all levels of adjustment (β per SD=−0.673 [95% CI=0.126, −1.492] AU/year, p=0.098, in the main model.)

In sensitivity analyses (Supplemental Fig. 1), we adjusted for Lp(a) in the subset of the cohort having this measurement (n=4661), which resulted in no meaningful change in the associations studied. Additional adjustment in Model 2 for heavy alcohol use or estrogen replacement therapy likewise had no material impact on the results. Adjustment for time since menopause in Model 2 in the subset of post-menopausal women had no meaningful influence on the risk estimates (Supplemental Fig. 2).

Effect Modification

Three-way interactions for HDL-C, HDL-P, LHDL-P, and apoC3-containing HDL-Cand apoC3-lacking HDL-C with time and age, sex, and race-ethnicity are presented in the Supplemental Fig. 3. There was evidence of significant interaction for all HDL measures by these demographic factors, such that higher HDL-C, HDL-P, and LHDL-P were particularly

associated with lower AVC incidence and slower progression in older adults, men, White participants, and, in the case of HDL-P, Hispanic/Latino participants. ApoC3-containing HDL-C was associated with greater and apoC3-lacking HDL-C associated with lower AVC incidence and slower progression in men and White participants. In additional exploratory analyses, there was no interaction between HDL measures and GlycA (all p>0.121).

Discussion

We investigated the relationships of HDL-C, HDL-P, HDL size and apoC3-defined particle subtypes, along with CETP, with AVC over long-term follow-up in a large multi-ethnic cohort. We found that higher HDL-C and HDL-P concentration, large HDL particle size, and apoC3-lacking HDL-C were significantly associated with lower incidence and progression of AVC after adjustment for cardiovascular risk factors, but no association was observed for small or medium HDL particles or for apoC3-containing HDL. Secondary analyses revealed that higher CETP mass, but not activity, was associated with lower AVC incidence and slower long-term progression. The findings were not altered after additional adjustment for the novel marker of inflammation, GlycA, nor by further adjustment for Lp(a). Exploratory testing for interaction showed stronger inverse associations for HDL-C, HDL-P, LHDL-P and apoC3-lacking HDL-C with incidence/progression of AVC among older, male, and White participants, and among those subgroups, a positive association of apoC3-containing HDL-C with this outcome.

Proteomic analysis of human AS tissue specimens has demonstrated enrichment in proteins from oxidative, complement, innate immunity, anti-calcific and anti-thrombotic pathways.[36–38] Mass spectrometry has also identified proteins carried by a spectrum of HDL sizes to be localized to AS tissue, including apoAI, apoAII, apoAIV, apoM, apoJ, apoC3 and paroxonase-1, among others.[36–38] This suggests that HDL particles, either directly through cholesterol efflux or indirectly through associated proteins or lipids, have a yet unidentified, but potentially important, counterregulatory role in AVC.

At the population level, studies evaluating the relationship between HDL and AVC have thus far limited their focus to HDL-C concentration, documenting a similar inverse relationship as reported earlier for atherosclerotic CVD. The Framingham Heart Study found that higher long-term average level of HDL-C was associated with lower prevalence of CT-determined AVC.[7] Prior work from MESA documented that lower HDL-C was associated with incident CT-determined AVC, but not AVC progression, at mean follow-up of 2.4 years.[39] These associations for HDL-C were not reproduced in relation to new-onset aortic stenosis in the Malmo Diet and Cancer Study, but the point estimate, which lacked precision, was nonetheless consistent with an inverse relationship.[8]

The present investigation newly extends previous epidemiologic findings on HDL-C and calcific aortic valve disease to both incidence and progression of AVC over long-term follow-up assessed by linear mixed-effects models. To our knowledge, the current study is also the first to investigate the prospective association of HDL-C alongside those of HDL-P, HDL-P size, and apoC3-defined HDL subclasses with AVC.

Reverse cholesterol transport by HDL particles to the liver is regarded as a key protective mechanism against peripheral cholesterol accumulation, but its relationship to AVC is less well defined than for atherosclerosis.[40,41] Cholesterol efflux from peripheral cells involves several mechanisms, including aqueous diffusion, protein-mediated transfer via the ATP-Binding Cassette (ABC) A1 transporter to lipid-lacking apoAI, and ABCG1- or Scavenger Receptor (SR) B1-mediated transfer to lipid-rich spherical HDL. Associations with specific HDL subclasses may be informative as to mechanism of efflux, with protection against disease relating to small HDL particles pointing to an ABCA1-predominant effect, while protection relating to medium and large HDL particles pointing to aqueous diffusion, ABCG1 or SR-B1 pathways.[42] Treatment with apoAI Milano and apoAI mimetic peptide, potentially through interaction with ABCA1, decreased AVC in rabbit models of disease, suggesting a role for efflux in protection against AVC.[43,44] A case-control study evaluating HDL size and cholesterol efflux capacity via ABCA1 and SR-B1 pathways from serum of individuals with severe AS versus age and sex-matched controls was null, but the sample size was small.[45]

We found that HDL-C and HDL-P exhibited comparable associations with incidence and progression of AVC. When HDL-C and HDL-P were adjusted for each other, however, only HDL-C remained significantly associated with the outcome. This finding contrasts with previous analyses from MESA showing that on mutual adjustment HDL-P, but not HDL-C, level was inversely associated with carotid intima-media thickness and incident CVD.[13] The superiority of HDL-P over HDL-C for CVD risk prediction has been supported by various other studies[12], although the Women's Health Study found the association to be stronger for HDL-C than HDL-P.[46] In our cohort, however, HDL-C was more strongly correlated with large HDL particles than was HDL-P, as expected. Hence, the inverse association of HDL-C with the rate of AVC change, independent of HDL-P, could reflect its close association with LHDL-P, where the association with AVC appears specifically to reside.

Indeed, we observed that higher LHDL-P concentration was associated with lower long-term incidence/progression of AVC with or without adjustment for medium and small HDL-P levels. This finding is consistent with the more favorable association of larger HDL-P for other outcomes.[14,15] Yet, multiple studies have also found that higher levels of smaller HDL-P are associated with lower risk of CHD.[16,17] Such discrepancies may relate to the balance between enhanced larger HDL-P interaction with ABCG1 and SR-B1 to effect reverse cholesterol transport, and the greater anti-inflammatory and anti-oxidative properties documented for smaller HDL-P, among other differences. The inverse association for LHDL-P, but not MHDL-P or SHDL-P, with AVC incidence and progression documented here occurred despite the reported preferential cargo of certain favorable proteins such as paroxonase-1, apo-M and apo-J1 in smaller HDL-P (HDL_3), and the higher content of the adverse protein apoC3 in larger HDL-P (HDL_2).[47] Yet, in our cohort, LHDL-P showed identical correlations with apoC3-lacking and apoC3-containing HDL. Our data cannot address the mechanistic basis for the preferential association of LHDL-P with rate of AVC change. The extent to which differential protein or lipid content of smaller and larger particles explain these findings justify further in vitro study.

Our finding that apoC3-lacking HDL, but not apoC3-containing HDL, was inversely associated with AVC progression on mutual adjustment is consistent with previous work suggesting that apoC3 may be a marker of HDL dysfunction. A pooled analysis of 4 cohorts, including MESA, showed that HDL lacking apoC3 was associated with a lower risk of incident CHD, while HDL containing apoC3 was instead associated with a higher risk.[19] There are several mechanisms by which HDL-associated apoC3 may impair the particle's functionality, including abolition of its anti-inflammatory properties, reversal of its anti-apoptotic effects, and diminution of its cholesterol efflux capacity.[19,20,48] Although the present analyses did not demonstrate a significant positive association for apoC3-containing HDL-Cand AVC progression overall, exploratory testing of effect modification by demographic factors did show stronger positive associations of apoC3-containing HDL, which were significant in men and White participants. ApoC3, which is present in a fraction of HDL-P as well as other lipoproteins, has been detected in AS tissue.[36] More recently, apoC3 was shown to induce valve interstitial cell calcification through mitochondrial dysfunction and a related inflammatory mechanism.[49] While apoC3 is preferentially carried by large HDL-Ps, which showed a protective relationship with AVC incidence/progression, the observation that apoC3-containing and apoC3-lacking HDL-C exhibited identical positive correlations with LHDL-P in our cohort helps to reconcile these findings.[47] Regardless, the present results on apoC3 composition support the importance of the HDL protein cargo to the particles' properties, not only in relation to coronary artery calcification, but also in relation to AVC.

In secondary analyses, higher CETP mass, but not activity, was associated with lower AVC progression. CETP mass was moderately-to-strongly correlated with its activity, but showed no meaningful correlations with HDL measures, and only mild correlations with LDL-C and TG. The inverse association with AVC seen for CETP mass is contrary to expectation, given that genetic variants associated with decreased CETP levels are associated with higher HDL-C, lower LDL-C, and reduced atherosclerotic CVD.[15,26,50] Stimulation of CETP production by hepatic oxysterol content, which is lowered by statins, would favor a positive association of circulating CETP with AVC progression, while CETP levels can be suppressed lipopolysaccharide-mediated inflammation, which would contribute to the inverse association observed for CETP mass.[51] The latter association, however, persisted after adjustment for LDL-P, statins, and GlycA. The basis for this result is therefore unclear and requires independent replication.

The finding of effect modification for our main exposure measures, such that their relationships were stronger among older adults, men, and White participants, is of particular interest. These groups are generally known to have higher AVC progression, and thus, it might have been easier to detect an inverse association of AVC with HDL measures than with other groups who started at lower levels and therefore had less progression. Association of HDL-C with CVD risk in women has been recently challenged with evolving data supporting a complex relationship that is impacted by timing of menopause.[35] Similar to the current findings of a stronger negative association of large HDL-P with AVC at older ages, we previously reported that greater concentration of large HDL-P was significantly associated with lower carotid intima-media thickness mainly after longer time since menopause, suggesting a potential adverse change in the quality of these particles

close to menopause, which seems to be at least partly reversed later in life.[35] Inflammation alters HDL from anti-atherogenic to pro-atherogenic.[33] We did not find evidence that GlycA modified the associations of HDL measures with incidence or progression of AVC, although we did not adjust for other markers of inflammation.

In our study, the magnitude of the associations detected was modest. For example, in the sex-stratified analysis, each SD increment in LHDL-P was associated with a 1.60 AU/year lower rate of AVC progression in men. In comparison, each 1-year decrease in age among men was associated with a 3.5 AU/year lower rate of AVC progression. However, HDL parameters were measured once at baseline and the aggregate impact of repeated measures would be expected to be higher, especially in those burdened with greater cardiovascular risk. Furthermore, identifying the specific protein and lipid components of HDL that most account for this protective relationship could translate to a stronger magnitude of effect, better identifying targets for intervention.

The strengths of this study include longitudinal investigation of a well-characterized cohort with detailed lipid and covariate information and serial CT measures of AVC over long-term follow-up. There are several limitations, however. Given the nature of our healthy population, the mean AVC Agatston score was low, such that only a small minority had calcification even in the mild-moderate range. Therefore, our findings apply to early, subclinical stages of calcification, and not necessarily to more advanced or clinical disease. The present analyses did not correct for multiple testing in order to preserve power to detect meaningful associations. Available literature on biological mechanisms and associations with atherosclerotic CVD and other outcomes provides helpful context. But given the possibility of an inflated false-positive rate, the present associations await confirmation in future studies. Exploratory testing for interaction was prespecified and results consistent with known differences in AVC by age, sex, and race/ethnicity, but the findings will require replication. Further studies are required to determine if the cholesterol/phospholipid carrying ability of HDL, or an unmeasured protein component is accountable for the inverse association of HDL-C, HDL-P, and larger HDL particles with AVC.

Conclusions

In conclusion, our observation of an inverse association for HDL-C, HDL-P, LHDL-P, and apoC3-lacking HDL-C with long-term AVC incidence and progression, coupled with studies indicating HDL-associated proteins present in AS tissue, suggests a protective role either related to HDL's function in reverse cholesterol transport, protein cargo, or its other pleiotropic effects. We found no benefit, but also no harm, from higher total HDL-P at a given level of HDL-C, or higher small or medium HDL-P at a given level of HDL-P.

Evidence of stronger inverse associations for these measures with incidence and progression of AVC in older participants, men and Whites, and the positive association observed for apoC3-containing HDL, may reflect the higher susceptibility for AVC documented in these groups. Further investigation of large HDL and apoC3-lacking HDL-C particles, their role in cholesterol efflux, lipid composition, or protein cargo, and possible modulation or

counterbalance of LDL-C and/or Lp(a), could be useful in developing treatments to slow or stop progression of AVC in those at elevated risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AVC	aortic valve calcification
AS	aortic stenosis
HDL	high density lipoprotein
-C	HDL cholesterol
-P	particle
large	LHDL-P
small	SHDL-P
medium	MHDL-P
mean HDL size	ZHDL-P
apo	apolipoprotein
CETP	cholesteryl ester transfer protein
CT	computed tomography
MESA	Multi-Ethnic Study of Atherosclerosis
LDL-C	low density lipoprotein cholesterol
Lp (a)	lipoprotein

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Highlights

- In a multi-ethnic population-based US cohort, higher levels of HDL-C, HDL-P, large HDL-P, and HDL lacking apoC3 were associated with lower incidence/progression of AVC over long-term follow-up.
- In exploratory analyses, these associations were stronger in older, male, and White participants. A higher level of HDL containing apoC3 was associated with higher AVC progression in these subgroups, as well.
- Further investigation of HDL composition and its protective mechanisms could be useful in developing treatments to delay progression of AVC.

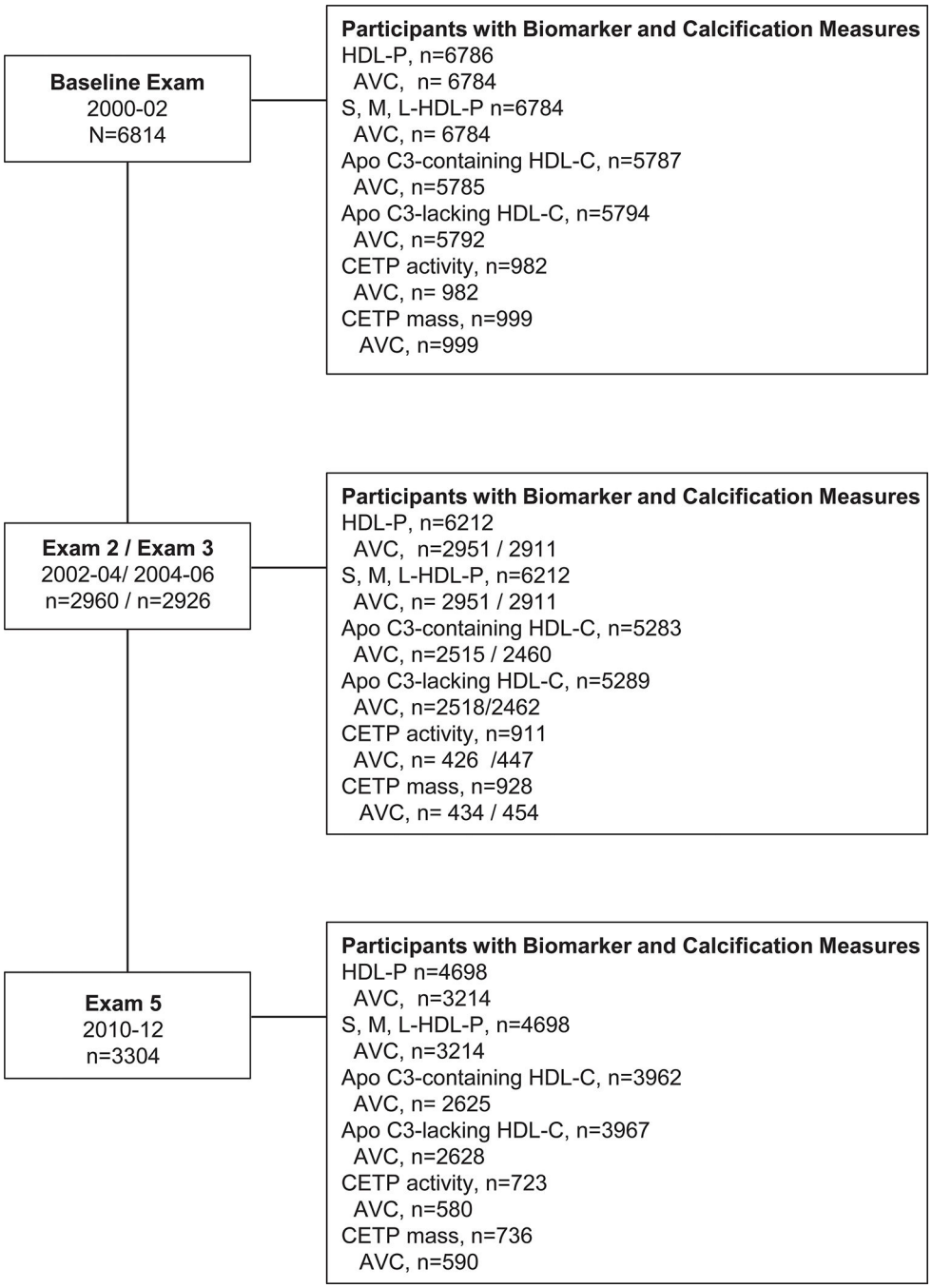


Fig. 1.

The number of participants with lipid and aortic valve calcification (AVC) measures at each examination in the Multi-Ethnic Study of Atherosclerosis. apo=apolipoprotein, -C=cholesterol, CETP=cholesteryl ester transfer protein, HDL=high density lipoprotein, L=large, M=medium, -P=particle concentration, S=small.

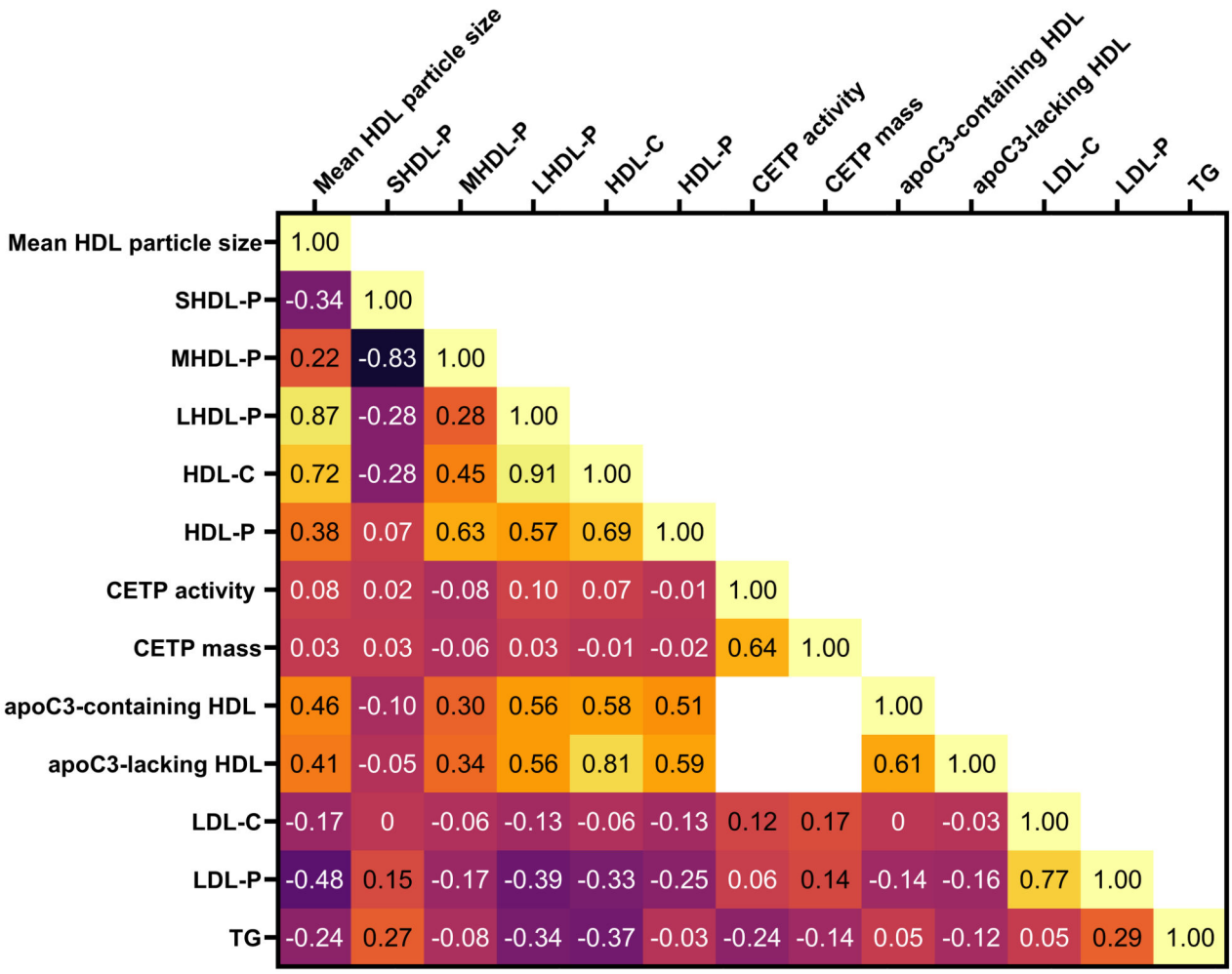
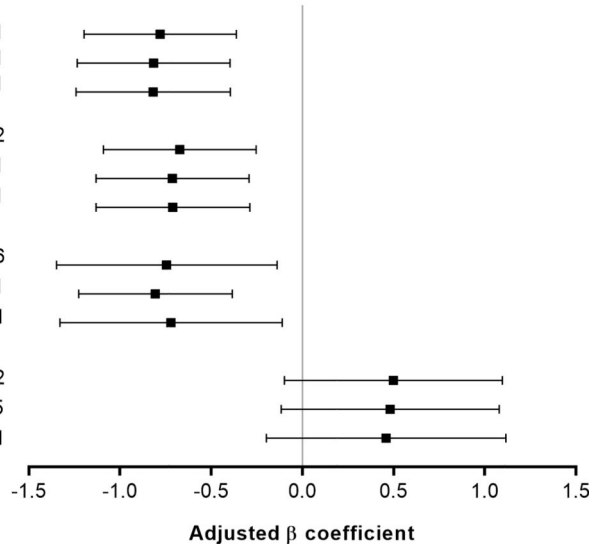


Fig. 2.

Pearson correlations between laboratory measures. apo=apolipoprotein, -C=cholesterol, CETP=cholesteryl ester transfer protein, HDL=high density lipoprotein, L=large, LDL=low density lipoprotein, M=medium, P=particle concentration, S=small, TG=triglyceride. There was no overlap of data for ApoC3 and CETP measures.

Biomarker	Model	n	β	95% CI (AU/year)	P value
HDL-C	0	6784	-0.779	(-1.197, -0.362)	<0.001
	1	6784	-0.816	(-1.235, -0.397)	<0.001
	2	6744	-0.818	(-1.241, -0.396)	<0.001
HDL-P	0	6784	-0.672	(-1.091, -0.253)	0.002
	1	6784	-0.713	(-1.132, -0.293)	<0.001
	2	6741	-0.711	(-1.132, -0.289)	<0.001
ApoC3-lacking	0	5784	-0.744	(-1.348, -0.139)	0.016
HDL-C	1	5784	-0.806	(-1.227, -0.385)	<0.001
	2	5738	-0.720	(-1.330, -0.111)	0.021
ApoC3-containing	0	5784	0.499	(-0.099, 1.097)	0.102
HDL-C	1	5784	0.481	(-0.116, 1.079)	0.115
	2	5738	0.458	(-0.198, 1.115)	0.171



Biomarker	Model	n	β	95% CI (AU/year)	P value
Mean HDL size	0	6784	-0.686	(-1.109, -0.262)	0.002
	1	6784	-0.684	(-1.111, -0.257)	0.002
	2	6744	-0.680	(-1.110, -0.251)	0.002
LHDL-P	0	6784	-0.811	(-1.249, -0.374)	<0.001
	1	6784	-0.835	(-1.270, -0.394)	<0.001
	2	6744	-0.835	(-1.278, -0.392)	<0.001
MHDL-P	0	6784	-0.291	(-0.837, 0.256)	0.297
	1	6784	-0.329	(-0.878, 0.221)	0.241
	2	6744	-0.326	(-0.879, 0.226)	0.247
SHDL-P	0	6784	-0.326	(-0.880, 0.228)	0.249
	1	6784	-0.376	(-0.932, 0.181)	0.186
	2	6744	-0.377	(-0.936, 0.183)	0.187

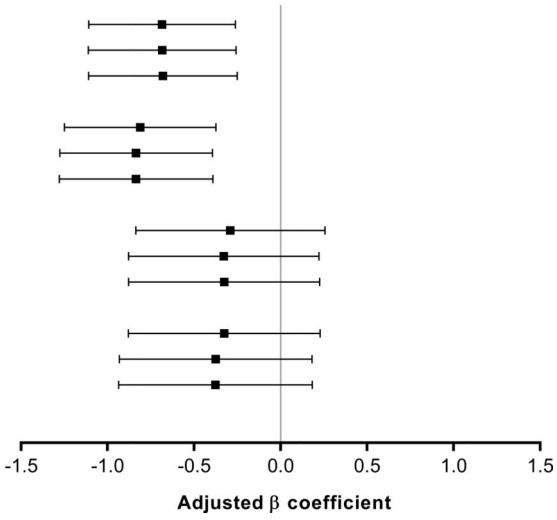


Fig. 3.

Associations of high-density lipoprotein (HDL) measures with incidence and progression of aortic valve calcification (AVC). Coefficients are per SD increment in HDL measures. Panels provide results for different HDL measures as follows: **A**, HDL cholesterol (HDL-C), HDL particle (HDL-P) concentration (each entered individually in the models), and apoC3-lacking or -containing HDL (entered jointly in the models); **B**, Mean HDL particle size (entered individually) and concentrations of large (LHDL-P), medium (MHDL-P) and small (SHDL-P) particles (entered jointly). Model (M) 0: unadjusted; Model 1: adjusted for age, sex, race/ethnicity, scanner type and site; Model 2: adjusted for Model 1 covariates and education, body mass index, systolic blood pressure, antihypertensive therapy, diabetes, smoking, low density lipoprotein particles, triglycerides, statin use, and

estimated glomerular filtration rate. Standard deviations: LHDL-P 3.46 mg/dL, MHDL-P 6.84 mg/dL, SHDL-P 5.73 mg/dL.

Table 1.

Baseline demographics, clinical characteristics, and laboratory measures in women and men enrolled in the Multi-Ethnic Study of Atherosclerosis who completed computed tomography at baseline.

Characteristic	Women N=3583	Men N=3201	P value
Age, years	62 ± 10	62 ± 10	0.831
Race/Ethnicity, n (%)			0.063
White	1355 (37.8)	1257 (39.3)	
Chinese	413 (11.5)	390 (12.2)	
Black	1042 (29.1)	836 (26.1)	
Hispanic	773 (21.6)	718 (22.4)	
BMI, kg/m^2	28.8 ± 6.2	27.9 ± 4.4	<0.001
Education, n (%)			<0.001
<High School	702 (19.7)	51 (16.2)	
High School	738 (20.7)	491 (15.4)	
Some college	1059 (29.7)	866 (27.1)	
College Grad	561 (15.7)	607 (19.0)	
Grad School	511 (14.3)	709 (22.2)	
Smoking, n (%)			<0.001
Never	2114 (59.2)	1295 (40.6)	
Former	1041 (29.2)	1434 (44.9)	
Current	416 (11.6)	462 (14.5)	
Heavy alcohol use, n (%)	151 (4.2)	193 (6.0)	
SBP, mm Hg	127±23	126 ±19	0.036
Antihypertensive medication, n (%)	1376 (38.4)	1146 (35.8)	0.023
Diabetes, n (%)	408 (11.4)	445 (13.9)	0.002
Time since menopause, years	16±11	NA	NA
Estrogen supplementation, n (%)	908 (28.1)	NA	NA
eGFR, ml/min/1.73 m^2	79.9±17.5	82.6±19.4	<0.001
LDL-C, mg/dL	118±32	117±31	0.185
HDL-C, mg/dL	56±15	45±12	<0.001
Triglycerides, mg/dL	127±74	134±82	0.001
Statin, n (%)	536 (15.0)	471 (14.7)	0.837
Lipoprotein (a), nmol/L	32±34	27±29	<0.001
apoC3-containing HDL, mg/dL	9±4	7±3	<0.001
apoC3-lacking HDL, mg/dL	132±37	111±30	<0.001
HDL-P, μmol/L	37±7	31±5	<0.001
SHDL-P, μmol/L	14±6	15±5	<0.001
MHDL-P, μmol/L	15±7	11±5	<0.001
LHDL-P, μmol/L	7±4	5±3	<0.001
ZHDL size, nm	9.4±0.4	9.1±0.4	<0.001

Characteristic	Women N=3583	Men N=3201	P value
Cholesteryl ester transfer protein activity, nmol/mL/hour	41.9±12.4	37.9±12.5	<0.001
Cholesteryl ester transfer protein mass, μg/mL	1.8±0.5	1.7±0.5	<0.001
GlycA, μmol/L	395.1±62.4	366.6±58.5	<0.001
Any AVC, n (%)	364 (10.1)	549 (17.1)	<0.001

Values are given as mean ± standard deviation or median (interquartile range).

Apo=apolipoprotein, BMI= body mass index, C=cholesterol, GlycA=glycoprotein N-acetyl methyl groups, EBCT=electron beam computerized tomography, eGFR=estimated glomerular filtration rate, HDL=high density lipoprotein, L=large particle size, LDL=low density lipoprotein, M=medium particle size, NA=not applicable, P=particle, S=small particle size, SBP=systolic blood pressure, Z=mean particle size