ORIGINAL RESEARCH

High-Density Lipoprotein Subspecies Defined by Apolipoprotein C-III and Subclinical Atherosclerosis Measures: MESA (The Multi-Ethnic Study of Atherosclerosis)

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Background—Apolipoprotein C-III (apoC-III), a small proinflammatory protein present on 6% to 7% of high-density lipoprotein (HDL) particles, defines a subspecies of HDL adversely associated with coronary heart disease in primarily white cohorts. In a multi-ethnic population free of clinical cardiovascular disease, we evaluated the relationship between apoC-III–defined HDL subspecies and subclinical markers of atherosclerotic pathology.

Methods and Results—We investigated cross-sectional associations between apolipoprotein A-I concentrations of apoC-III–defined HDL subspecies, measured via ELISA and imaging measures of subclinical atherosclerosis, among 4659 participants in the MESA (The Multi-Ethnic Study of Atherosclerosis) at baseline (2000–2002). HDL particles containing and lacking apoC-III were divergently associated with coronary artery calcification in women (*P*-heterogeneity=0.002) but not in men (*P*-heterogeneity=0.31) and with carotid plaque score (*P*-heterogeneity=0.02) and intima-media thickness (*P*-heterogeneity=0.06) in the overall study population. HDL lacking apoC-III was inversely associated with all outcome measures (coronary artery calcification, women: odds ratio per SD=0.81 [95% confidence interval [CI], 0.73–0.90]; carotid plaque, overall: odds ratio per SD=0.92 [95% CI, 0.84–1.00]; intima-media thickness, overall: mean difference per SD=−14.0 μm [95% CI, −21.1 to −6.7 μm]), whereas HDL containing apoC-III was positively associated (coronary artery calcification, women: odds ratio=1.10 [95% CI, 0.99–1.22]; plaque, overall: odds ratio=1.10 [95% CI, 1.01–1.19]) or unassociated. Neither total HDL nor HDL subspecies was associated with changes in subclinical atherosclerosis measures up to 10 years later.

Conclusions—The presence of apoC-III defined a subspecies of HDL not inversely associated with baseline measures of subclinical atherosclerosis, supporting a role of apoC-III in the pathophysiology of cardiovascular disease. **(*J Am Heart Assoc*. 2018;7: e007824. DOI: 10.1161/JAHA.117.007824.)**

Key Words: apolipoprotein • atherosclerosis • coronary artery calcium • high-density lipoprotein • plaque

Observational evidence supports a strong inverse association between high-density lipoprotein (HDL) cholesterol concentrations and risk of cardiovascular disease (CVD).[1,2] However, the inconclusive evidence from large-scale clinical trials for a cardioprotective effect of HDL cholesterol-raising agents[3–6] has cast doubt on the causal relationship between HDL cholesterol and CVD and has suggested that measures of HDL beyond its cholesterol concentration may better capture its functional properties.

Emerging evidence suggests that the heterogeneous protein composition of HDL may dictate its metabolic and functional properties and define distinct HDL subspecies with respect to CVD risk. In our recent work, HDL containing the atherogenic protein apolipoprotein C-III (apoC-III) was

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Accompanying Tables S1 through S6 are available at http://jaha.ahajournals.org/content/7/6/e007824/DC1/embed/inline-supplementary-material-1.pdf

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Clinical Perspective

What Is New?

- In this multi-ethnic US population, high-density lipoprotein (HDL; assessed via concentrations of apolipoprotein A-I) only displayed a protective association with measures of subclinical atherosclerosis when it lacked the proinflammatory protein apolipoprotein C-III (apoC-III).
- In cross-sectional analyses, HDL lacking apoC-III was inversely associated with coronary artery calcification (women only), carotid plaque, and carotid intima-media thickness. HDL containing apoC-III was positively associated with coronary artery calcification and plaque and unassociated with intima-media thickness.
- These findings support our previously reported divergent associations of apoC-III–defined HDL subspecies with incident coronary heart disease in primarily white populations.

What Are the Clinical Implications?

- Although the subdivision of HDL into fractions with and without apoC-III is far from clinical application, our results support the potential use of apoC-III–defined HDL subspecies for cardiovascular disease risk reclassification.

positively associated with incident coronary heart disease (CHD), whereas HDL lacking apoC-III was inversely associated.[7] ApoC-III, although present on a minority of lipoproteins, may have a critical influence on pathophysiologic processes contributing to the development of CVD. ApoC-III delays the clearance of apolipoprotein B–containing lipoproteins (low-density lipoprotein [LDL] and very-LDL) from circulation by blocking their interaction with hepatic receptors[8] and at high levels may impair their lipolysis.[9–11] Although the role of apoC-III on HDL is less well understood, apoC-III might similarly impede HDL clearance and may also affect the anti-inflammatory properties of HDL, particularly its ability to inhibit monocyte adhesion to endothelial cells.[12]

Subclinical atherosclerosis, the main underlying precursor of clinical CVD, constitutes a decades-long continuum that begins as early as adolescence with the development of fatty streaks in the arterial walls that subsequently develop into fibrous lipid-rich plaques, which can ultimately become calcified and/or rupture.[13] Because atherosclerosis is characterized by both disturbances in lipoprotein metabolism and upregulation of proinflammatory mechanisms,[14] studying the role of apoC-III and apoC-III–based HDL subspecies in the development and progression of atherosclerosis might provide important insight into the etiology of CVD. Although genetic studies support the involvement of apoC-III in atherosclerosis,[15–17] evidence from epidemiologic studies is sparse,[18] and no prior studies have examined whether apoC-III might modulate associations between HDL and subclinical atherosclerosis. Furthermore, because prior analyses of apoC-III–based HDL subspecies and CHD have been conducted in predominantly white populations,[7] it is unclear whether the role of these subspecies in atherosclerotic development might differ by race/ethnicity.

In the current study, we investigated the relationship between apoC-III and HDL subspecies defined by the presence or absence of apoC-III in relation to subclinical atherosclerosis in a multi-ethnic population. Using coronary artery calcification (CAC), carotid intima-media thickness (IMT), and carotid plaque scores as noninvasive measures of subclinical atherosclerosis, we assessed associations both at baseline and with change in outcome measures over a 10-year follow-up period.

Methods

The data, analytic methods, and study materials will be made available to other researchers for purposes of reproducing the results only with proper institutional review board approvals and strict adherence to cohort-specific regulations. Requests for data can be directed to R.L.M.

Study Population

MESA (The Multi-Ethnic Study of Atherosclerosis) is a multicenter prospective cohort study that began in 2000 to 2002 with the enrollment of 6814 men and women of white (38%), black (28%), Hispanic (22%), and Chinese American (12%) descent between the ages of 45 to 84 years without a history of clinical CVD.[19] Participants attended 4 follow-up visits through 2012 (2002–2003 [examination 2], 2004–2005 [examination 3], 2005–2007 [examination 4], and 2010–2012 [examination 5]). Informed consent was obtained from all study participants, and the institutional review board at each study site approved the study protocol.

Apolipoprotein Measurements

Apolipoproteins were measured in baseline plasma samples from 5796 MESA participants (1000 participants were randomly excluded by the MESA Steering Committee to preserve sample volume, and an additional 18 participants had insufficient sample volume for analyses). After exclusion of participants with undetectable (N=11) or implausibly low (N=29) values of any apolipoprotein exposure or with incomplete baseline covariate information (N=66), 5690 participants had complete apolipoprotein measures.

Samples were removed from −80°C storage and thawed at room temperature. Whole plasma was fractionated into lipoproteins containing apoC-III and those deficient of apoC-III by immunoaffinity separation on a 96-well microplate (Greiner

Bio-One MICROLON 600; VWR catalog no. 82050-734) coated with rabbit anti-human apoC-III antibody (Academy Biomedical, catalog no. 33A-R1b; 10 μg/mL in 1× PBS). After overnight incubation at 4°C, the unbound fraction depleted of apoC-III–containing lipoproteins was collected for analysis. After washing with 1× PBS, ELISA diluent (1× PBS/2% BSA/0.05% Tween 20) was added to each well to dissociate the components of the bound apoC-III–containing lipoproteins from the plate during a 2-hour incubation at 37°C and was collected for analysis.

The concentrations of apolipoproteins were measured by sandwich ELISA using polyclonal antibodies (Academy Biomedical Company, Houston TX): apoC-III in whole plasma (coating antibody catalog no. 33A-R1b at 10 μg/mL in 1× PBS, detection antibody catalog no. 33H-G2b at 1 μg/mL in 1× PBS); and apolipoprotein A-I (apoA-I) in HDL containing apoC-III and in HDL lacking apoC-III (coating antibody catalog no. 11A-G2b at 5 μg/mL in 1× PBS, detection antibody catalog no. 11H-G1b at 1 μg/mL in 1× PBS). For all ELISAs, the coating antibody was incubated for 1 hour at 37°C. Plates were washed 3 times with washing buffer (0.1% Tween 20 in 1× PBS), then blocked (Pierce, casein in 1× PBS at 1% w/v; VWR catalog no. PI37528) with 1-hour incubation at 37°C, followed by washing 3 times with washing buffer. Three extensively characterized plasma pools whose apoA-I concentrations in whole plasma and the apoC-III plasma fractions had been calibrated using commercially available standards and immunoaffinity column chromatography were used, 1 for the calibration curve and the other 2 as known-concentration controls to assess batch validity and between-batch variance. The calibration curve was prepared in dilutions starting at 10 000× and serially 2× further to 640 000× in 1× PBS containing 0.5% BSA, creating a reliable second-degree polynomial curve fit. The calibration curve, known controls, and unknown samples were diluted in 1× PBS containing 0.5% BSA and incubated overnight at 4°C or 1 hour at 37°C. After incubation, plates were washed 3 times with wash buffer, appropriate detection antibody was added, as described, plates were incubated 1 hour at 37°C, and then washed 3 times with wash buffer. Avidin peroxidase was then added (Sigma Aldrich, catalog no. A7419-2ML; 0.01 μg/mL in 1× PBS), and plates were incubated for 1 hour at 37°C, then washed 3 times with wash buffer. Finally, o-phenylenediamine (Sigma Aldrich, catalog no. P9187-50SET) was added to all plates to develop color for 1 hour and 20 minutes at room temperature and the absorbance was read at 450 nm.

Each sample was measured in duplicate. All laboratory personnel were blinded to the case status. Using this method, the within-run average coefficients of variation were 5% for apoA-I in HDL lacking apoC-III, 8% for apoA-I in HDL containing apoC-III, and 4% for total apoC-III. The detection ranges are (in g/L): apoA-I in HDL containing apoC-III, 0.01 to 0.4; apoA-I in HDL lacking apoC-III, 0.1 to 3.5; and apoC-III, 0.005 to 0.5.

To account for moderate variation in apolipoprotein levels by batch, values of each apolipoprotein exposure were recalibrated to represent the average distribution across batches.[20] Log-transformed apolipoprotein variables were regressed on batch and age as well as other variables associated with apolipoprotein levels (sex, race, study site, smoking status, alcohol intake, education, and body mass index [BMI]) using linear regression models. We used the back-transformed apolipoprotein measures in our analyses.

CAC Assessment

CAC was measured on all participants at baseline, as described previously.[21,22] Participants received a second CAC scan by random assignment at either examination 2 or examination 3, and additional CAC measurements were obtained in a subset of participants at examination 5.

We excluded all scans performed subsequent to cardiac revascularization procedures during follow-up (N=175), resulting in 5690 examination 1 scans, 2448 examination 2 scans, 2275 examination 3 scans, and 2521 examination 5 scans available for our analyses among participants with apolipoprotein measurements.

CAC was measured at examinations 1 to 3 using either a cardiac-gated electron-beam computed tomography scanner (Chicago, IL; Los Angeles, CA; and New York, NY, field centers) or multidetector computed tomography system (Baltimore, MD; Forsyth County, NC; and St Paul, MN field centers), with phantom adjustment used to calibrate scans across sites and technicians.[21] All study centers used the multidetector system at examination 5. Two scans were obtained for each participant and read centrally at the Harbor-University of California, Los Angeles Research and Education Institute. Scans were scored using the Agatson method,[23] with undetectable CAC set to 0. The mean score from the 2 scans was used in analyses.

Carotid IMT and Plaque Assessment

Ultrasound imaging for the measurement of carotid IMT and carotid plaque was performed at baseline and examination 5 via high-resolution B-mode ultrasonography (Logic 700; General Electric Co, UK). Examination 1 ultrasounds from participants in the MESA Air (The Multi-Ethnic Study of Atherosclerosis and Air Pollution) substudy were reread concurrently with examination 5 ultrasounds at the University of Wisconsin Ultrasound Reading Center in the subset of participants with measures at both examinations. Available examination 1 ultrasounds from MESA Air participants without examination 5 ultrasounds were reread in 2015. Reread examination 1 IMT and plaque measurements were used in our analyses to ensure consistency with examination 5 measures; they also were more reproducible[24,25] and plaque

assessments were more concordant with clinical consensus recommendations[26,27] than original measurements. Details of ultrasound IMT and plaque rereadings have been described previously.[28,29] The small number of participants with ultrasound data at only examination 5 (N=5) were excluded from IMT and plaque analyses.

Common carotid artery IMT measurements were available for 3805 participants in the apolipoprotein data set at baseline and 2678 participants at examination 5. The mean of measurements from the left and right far walls of the distal (final 10 mm) common carotid artery was used in analyses.

Carotid plaque presence was defined as a discrete focal wall thickening ≥1.5 mm or focal wall thickening ≥50% of the surrounding IMT.[26] A carotid plaque score was used in our main analyses and calculated as the total number of plaques in the internal, bifurcation, and common carotid artery segments (range, 0–12).[29] Carotid plaque scores were available for 4319 participants with apolipoprotein measurements at baseline and 2753 participants at examination 5.

Statistical Analyses

Baseline participant characteristics and distributions of exposure and outcome variables were examined as means (SDs) or medians (interquartile ranges) for continuous variables or as proportions for categorical variables.

We first examined cross-sectional associations between apolipoprotein exposures and subclinical atherosclerosis measures at baseline. Apolipoproteins were modeled across quintiles and per SD on the basis of distributions in the overall study population, and tests of trend were performed across quintile medians. Carotid IMT, which was approximately normally distributed, was analyzed continuously via linear regression. To accommodate the large proportion of 0 CAC and plaque scores and the highly right-skewed score distributions, we used ordinal logistic regression as our primary analysis method for both outcomes. The following clinically defined CAC severity categories were used in ordinal logistic regression analyses: 0, 1 to 10, 11 to 100, 101 to 400, and >400.[30] Because clinical cut points have not been established for the carotid plaque score, distribution-driven cut points (0, 1, 2–3, and ≥4 plaques) were selected for plaque analyses. Similar results in sensitivity analyses using alternative cut points for CAC and carotid plaque scores indicated that the proportional odds assumption was met for both end points.

Associations between apolipoproteins and change in subclinical atherosclerosis over follow-up were investigated by modeling each outcome measure at examination 5 (an average of 9.6 years after baseline) with adjustment for the corresponding baseline measure and baseline covariates. In sensitivity analyses, we examined associations for change in CAC between baseline and the time of the second scan at examination 2 or 3 (an average of 2.4 years after baseline). Modeling approaches were similar to those used for cross-sectional analyses (ordinal regression for CAC and plaque scores and linear regression for IMT). As an additional sensitivity analysis, we used logistic regression to investigate associations with the development of new plaque and CAC through the end of follow-up among participants without detectable measures at baseline.

To assess the robustness of ordinal regression CAC and plaque score results, we additionally performed tobit regression, which is uniquely suited for estimating linear associations in the presence of clustered right- or left-censored values.[31] Because tobit regression requires noncensored values to conform to linear regression assumptions, we used the cube root of (CAC+1) and (plaque+1) to normalize the right-skewed CAC and plaque score distributions, as previously recommended by Han and Kronmal.[32] β Coefficients from tobit models were back-transformed to allow associations to be presented as ratios. Because tobit regression results for CAC and carotid plaque were generally in close agreement with those from ordinal regression models (with a similar direction and significance for nearly all estimates in both cross-sectional and longitudinal analyses using both approaches), ordinal regression was used for our main analyses. (Comparisons of ordinal and tobit results for cross-sectional analyses are presented in Table S1.) To address the potential for survival bias in analyses restricted to the subset of participants with examination 5 measurements, we compared baseline characteristics in the subsets with and without outcome measures at examination 5.

Baseline lipid-lowering medication users were excluded from final models, resulting in a final analytic sample size of 4659 (2240 men, 2419 women). Analyses of subclinical atherosclerosis change were adjusted for any lipid-lowering medication use through the examination at which the outcome measure was assessed (examination 5 in main analyses of all outcome measures and examination 2/3 in sensitivity analyses of CAC). Baseline estimates with and without exclusions for lipid medication use are presented in Table S2.

Because the associations of total apoC-III and total HDL with baseline CAC were significantly modified by sex (P for interaction=0.0001 for total apoC-III and P for interaction=0.006 for total HDL), we included an interaction term between their main effects and sex and present sex-specific estimates derived from this model for the baseline CAC associations. We additionally assessed effect modification by age (<65 versus ≥65 years), race/ethnicity (black, Hispanic, and Chinese American versus white), and BMI (normal weight [18.5–24.9 kg/m^2], overweight [25.0–29.9 kg/m^2], and obese [≥30 kg/m^2]).[33]

Basic models were adjusted for age (years), race (white, black, Hispanic, and Chinese American), sex, and study site

(Forsyth County, NC; Northern Manhattan and the Bronx, NY; Baltimore City and Baltimore County, MD; St Paul, MN; Chicago, IL; and Los Angeles County, CA). We then additionally adjusted for other important predictors of subclinical atherosclerosis in multivariable models: BMI (weight [kg]/height [m]^2; continuous), smoking (never, former, or current smoker), alcohol consumption (never, former, or current drinker), systolic blood pressure (mm Hg; continuous), diabetes mellitus (fasting blood glucose ≥7.0 mmol/L or use of insulin or oral hypoglycemic medications),[34] income (< $25 000, $25 000–$49 999, $50 000–$74 999, or ≥$75 000 per year), and current antihypertensive medication use. HDL particles containing and lacking apoC-III were simultaneously included in HDL subspecies analyses because the 2 subspecies sum to total HDL. We assessed the heterogeneity between associations for the 2 HDL subspecies with risk of subclinical atherosclerosis measures in models with both subfractions included as linear terms. We then tested the linear hypothesis that the regression coefficients for the 2 subfractions were equal (using a 1 *df* Wald test).

Because covariate adjustment influenced some estimates (in particular, associations for total apoC-III were slightly attenuated), full multivariable models were used in final analyses. In additional models, we further adjusted for LDL cholesterol and log-transformed triglycerides as well as log-transformed inflammatory markers (C-reactive protein and interleukin-6).

All analyses were performed in SAS, with $P<0.05$ indicating statistical significance.

Results

Baseline Characteristics

Both men and women were, on average, 63 years old at baseline. Women had higher levels of all apolipoprotein exposures, whereas men had a higher burden of subclinical atherosclerosis, as determined by all 3 outcome measures (Table 1). Prevalence of lipid-lowering medication use was ≈18% in both sexes at baseline and increased to 40% in men and 38% in women by examination 5.

Baseline Associations Between Apolipoproteins and Subclinical Atherosclerosis Measures

Total apoC-III

In multivariable models, higher total apoC-III levels were associated with greater baseline CAC in men (odds ratio [OR] per SD=1.21; 95% confidence interval [CI], 1.12–1.32; *P*-trend <0.0001), whereas no association was present in women (Table 2). At baseline, total apoC-III was positively associated with carotid plaque (OR per SD=1.08; 95% CI, 1.01–1.16; *P*-trend=0.01) and with IMT across quintiles in the total study population (mean difference, quantile 5 versus quintile 1=18.3 μm; 95% CI, −0.2 to 36.8 μm; *P*-trend=0.03), but associations for continuous apoC-III were not statistically significant.

Total HDL and apoC-III–defined HDL subspecies

In men, both total HDL and HDL lacking apoC-III were unassociated with CAC (Figure—Panel A and Table S3). In women, total HDL was associated with reduced CAC (OR per SD=0.87; 95% CI, 0.79–0.94), with a slightly stronger inverse association for HDL not containing apoC-III (OR per SD=0.81; 95% CI, 0.73–0.90) (Figure—Panel B and Table S4). A borderline positive association with CAC was observed for HDL containing apoC-III among both men (OR per SD=1.09; 95% CI, 0.97–1.21) and women (OR per SD=1.10; 95% CI, 0.99–1.22), although no association was observed across quintiles in either sex (*P*-trend, men=0.20; *P*-trend, women=0.83). Continuously modeled linear associations of the 2 HDL subspecies were significantly different in women (*P*-heterogeneity=0.002) but not in men (*P*-heterogeneity=0.31).

Associations with carotid plaque were more strongly inverse for HDL lacking apoC-III (OR per SD=0.92; 95% CI, 0.84–1.00) than for total HDL (OR per SD=0.97; 95% CI, 0.91–1.04), whereas associations with IMT were similarly inverse for the 2 apolipoprotein exposures (total HDL: mean difference=−14.9 μm [95% CI, −20.5 to −9.2 μm]; HDL lacking apoC-III: mean difference=−14.0 μm [95% CI, −21.2 to −6.7 μm]) (Figure—Panels C and D and Tables S4 and S5).

HDL particles containing apoC-III displayed a slight positive association with carotid plaque (OR per SD=1.10; 95% CI, 1.01–1.19) that was significantly different from the inverse association for HDL particles not containing apoC-III (*P*-heterogeneity=0.02). HDL containing apoC-III was unassociated with IMT, but the difference in associations for the 2 HDL subspecies was borderline significant (*P*-heterogeneity=0.06).

The positive associations for total apoC-III with CAC in men and with carotid plaque in the combined population of men and women were attenuated with adjustment for triglycerides and LDL cholesterol (Table S2). Triglyceride and LDL cholesterol adjustment had minimal influence on most other estimates, and adjustment for inflammatory markers (C-reactive protein and interleukin-6) also did not materially change any estimates.

Baseline Apolipoproteins and Change in Subclinical Atherosclerosis

None of the apolipoprotein exposures was significantly associated with change in any of the subclinical atherosclerosis measures between examinations 1 and 5 (Table 3) or with change in CAC in sensitivity analyses using the additional follow-up measures available through examinations 2 or 3 (Table S6). Consistent with these findings, no associations

Table 1. Baseline (2000–2002) Characteristics of Participants With Apolipoprotein and Subclinical Atherosclerosis Measures in MESA

Variables	Men (N=2728)	Women (N=2962)
Age, mean (SD), y	62.6 (10.2)	62.7 (10.3)
Postmenopausal (women only), N (%)	…	2462 (83.2)
Race/ethnicity, N (%)		
White	1024 (37.5)	1092 (36.9)
Chinese American	346 (12.7)	352 (11.9)
Black	751 (27.5)	877 (29.6)
Hispanic	607 (22.3)	641 (21.6)
CAC score, median (IQR)	19.4 (0–197)	0 (0–35.8)
Carotid plaque score, median (IQR)*	1 (0–2)	0 (0–2)
Carotid IMT mean (SD), μm*	806 (205)	758 (179)
Diabetes mellitus, N (%)	390 (14.3)	338 (11.4)
Hypertension, N (%)	1196 (43.8)	1395 (47.1)
Use of blood pressure–lowering drugs, N (%)	1005 (36.8)	1139 (38.5)
Use of lipid-lowering drugs, N (%)	488 (17.9)	543 (18.3)
Systolic blood pressure, mean (SD), mm Hg	126 (18.8)	128 (23.3)
Waist circumference, mean (SD), cm	99.2 (12.2)	97.2 (16.0)
Body mass index, mean (SD), kg/m^2	27.8 (4.5)	28.7 (6.2)
Physical activity, median (IQR), MET-h/wk†	63.5 (31.8–117.8)	51.5 (25.0–90.0)
Current smoker, N (%)	390 (14.3)	327 (11.0)
Current alcohol consumption ≥1 drink/wk, N (%)	1277 (46.8)	763 (25.8)
Income >$75 000/y, N (%)	730 (26.8)	491 (16.6)
HDL-C, median (IQR), mmol/L	1.1 (1.0–1.3)	1.4 (1.2–1.7)
Total apoA-I in HDL, median (IQR), g/L	1.2 (1.0–1.3)	1.4 (1.2–1.6)
ApoA-I in HDL containing apoC-III, median (IQR), g/L	0.07 (0.06–0.09)	0.09 (0.07–0.11)
ApoA-I in HDL not containing apoC-III, median (IQR), g/L	1.1 (0.9–1.3)	1.3 (1.1–1.5)
Proportion of HDL that contains apoC-III, median (IQR), %‡	6.1 (5.3–7.1)	6.5 (5.6–7.4)
Total apoC-III, median (IQR), g/L	0.08 (0.06–0.10)	0.09 (0.07–0.12)
Triglycerides, median (IQR), mmol/L	1.3 (0.9–1.9)	1.2 (0.9–1.8)
Total cholesterol, median (IQR), mmol/L	4.8 (4.3–5.4)	5.1 (4.6–5.7)

To convert apoA-I and apoC-III to mg/dL, divide by 0.01; HDL-C and total cholesterol to mg/dL, divide by 0.0259; triglycerides to mg/dL, divide by 0.0113; ApoA-I indicates apolipoprotein A-I; apoC-III, apolipoprotein C-III; CAC, coronary artery calcification; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; IMT, intima-media thickness; IQR, interquartile range; MESA, The Multi-Ethnic Study of Atherosclerosis; and MET, metabolic equivalent.

*IMT available in 1835 men and 1970 women, and plaque score available in 2072 men and 2247 women.

†Physical activity includes moderate and vigorous activity. Participants reporting >18 h/d of total physical activity excluded (men: N=2407; women: N=2514, after exclusions).

‡HDL assessed via concentrations of apoA-I.

were detected between any of the apolipoprotein exposures and the development of new CAC or plaque in logistic regression analyses (Table 4).

Effect Modification

Although several significant interactions were found between apolipoprotein measures and potential effect modifiers at baseline (positive associations for total apoC-III with IMT in whites but not blacks [*P*-interaction=0.03], stronger inverse associations for total HDL [*P*-interaction=0.04] and HDL lacking apoC-III [*P*-interaction=0.03] with carotid plaque among those with lower BMI), none of the potential effect modifiers (age, race/ethnicity, and BMI) was associated with any of the apolipoprotein exposures across >1 outcome measure. Consistent with our overall findings, associations with changes in subclinical atherosclerosis measures were null in all subgroup analyses.

Table 2. Baseline (2000–2002) Associations Between Total ApoC-III Levels and Subclinical Atherosclerosis Measures in MESA

Variables	N	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	Per SD	*P* Value for Trend
ApoC-III quintile median (range) or SD, g/L		0.05 (0.01–0.06)	0.07 (0.06–0.08)	0.09 (0.08–0.09)	0.10 (0.09–0.12)	0.14 (0.12–0.53)	0.04	
Coronary artery calcification, OR (95% CI)*								
Men	2240	1.0 (Reference)	1.09 (0.87 to 1.37)	1.04 (0.82 to 1.32)	1.36 (1.06 to 1.74)	1.63 (1.25 to 2.12)	1.21 (1.12 to 1.32)	<0.0001
Women	2419	1.0 (Reference)	1.14 (0.84 to 1.54)	0.91 (0.68 to 1.23)	1.03 (0.77 to 1.37)	0.98 (0.72 to 1.32)	0.95 (0.87 to 1.05)	0.69
Carotid plaque, OR (95% CI)	3546	1.0 (Reference)	1.10 (0.89 to 1.35)	1.05 (0.86 to 1.30)	1.24 (1.01 to 1.53)	1.31 (1.06 to 1.63)	1.08 (1.01 to 1.16)	0.01
Carotid intima-media thickness, mean difference (95% CI)	3140	1.0 (Reference)	6.3 (−10.9 to 23.6)	8.6 (−8.8 to 26.0)	9.0 (−2.1 to 33.1)	18.3 (−0.2 to 36.8)	1.9 (−4.1 to 7.8)	0.03

ORs obtained from ordinal regression models for coronary artery calcification and plaque analyses. Lipid-lowering medication users excluded. Models adjusted for age, sex, race/ethnicity, study site, body mass index, smoking, alcohol, systolic blood pressure, diabetes mellitus, income, and antihypertensive medication use. ApoC-III indicates apolipoprotein C-III; CI, confidence interval; MESA, The Multi-Ethnic Study of Atherosclerosis; and OR, odds ratio.
*P for interaction with sex=0.0001.

Discussion

Previously, we reported in individuals of primarily European descent that HDL subspecies, defined by the presence or absence of apoC-III, are differentially associated with the risk of CVD, with a strong inverse association for HDL lacking apoC-III and a direct association for HDL containing apoC-III.[7] The current study in a multi-ethnic population of men and women extends these findings to the subclinical spectrum of CVD. In cross-sectional analyses, higher baseline levels of total apoC-III were positively associated with CAC in men and with carotid plaque and IMT in combined analyses of men and women. Supporting our prior findings for CVD, HDL not containing apoC-III was inversely associated with all measures of subclinical atherosclerosis, whereas HDL containing apoC-III was positively associated with CAC and plaque and unassociated with IMT. Despite these divergent associations for the 2 HDL subspecies in cross-sectional analyses, no associations were found between apolipoprotein exposures and change in subclinical atherosclerosis measures over the 10-year follow-up period.

Genetic studies support a role of apoC-III in the atherosclerotic process. Three *APOC3* gene loss-of-function variants associated with a reduced risk of CVD in large Mendelian randomization studies[35,36] were subsequently found to also be associated with lower levels of CAC in the multi-ethnic BioImage Study,[16] although no associations were observed with carotid plaque or IMT. One of these genetic variants was also associated with CAC in an Amish population.[15] In addition, sequence variation in the *A-I/C-III/A-IV* gene cluster was associated with carotid IMT in a small sample from the ARIC (Atherosclerosis Risk in Communities) Study.[17] Although total circulating apoC-III has been associated with higher CVD incidence or mortality in several observational studies,[37] data on associations with subclinical atherosclerosis are limited, with 1 cross-sectional analysis reporting a positive association between plasma apoC-III and CAC among 1422 participants with type 2 diabetes mellitus (tobit regression ratio per SD higher apoC-III, 1.78; 95% CI, 1.27–2.50).[18]

ApoC-III has multiple effects on lipid metabolism that may directly contribute to atherosclerosis and diminish the antiatherogenic properties of HDL. ApoC-III delays the clearance of triglyceride-rich lipoproteins from circulation by blocking their interaction with hepatic receptors,[8] and preliminary data from our laboratory suggest that apoC-III may similarly inhibit HDL clearance. ApoC-III may also enhance the production of new triglyceride-rich lipoproteins[38,39] and at high concentrations inhibit their lipolysis.[9–11] The attenuation of total apoC-III estimates with adjustment for triglycerides and LDL cholesterol, similar to that seen in triglyceride-adjusted analyses of apoC-III and CAC

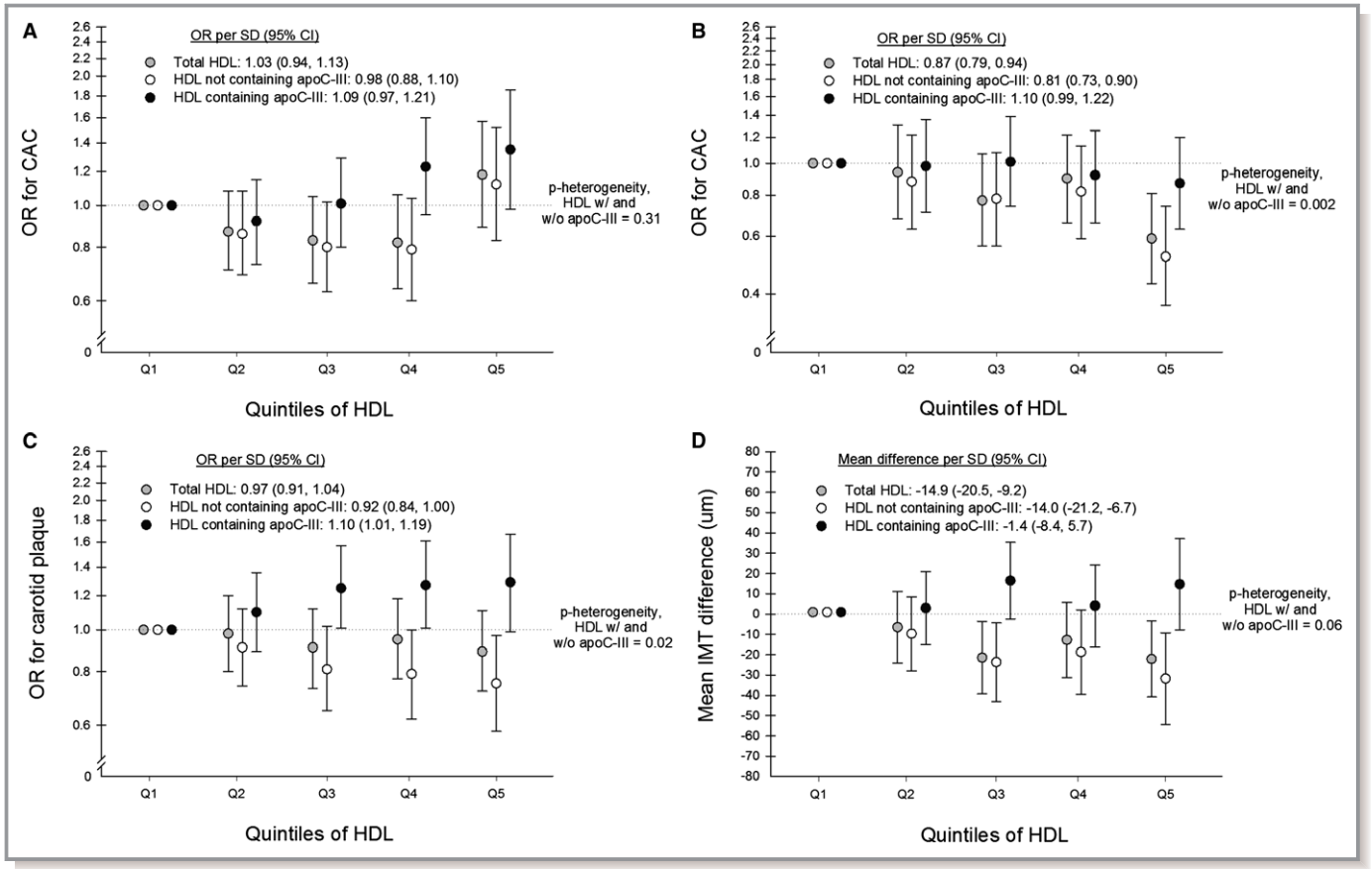

Figure. A through D, Baseline multivariable associations for total high-density lipoprotein (HDL) and apolipoprotein C-III (apoC-III)–based HDL subspecies with coronary artery calcification (CAC) in men (A) and women (B) and with carotid plaque (C) and carotid intima-media thickness (IMT; D) (men and women combined). Lipid-lowering medication users excluded. Models adjusted for age, sex, race/ethnicity, study site, body mass index, smoking, alcohol, systolic blood pressure, diabetes mellitus, income, and antihypertensive medication use. HDL assessed via concentrations of apolipoprotein A-I (apoA-I). Total apoC-III, SD=0.04 g/L; total apoA-I in HDL, SD=0.37 g/L; apoA-I in HDL lacking apoC-III, SD=0.35 g/L; apoA-I in HDL containing apoC-III, SD=0.03 g/L. CI indicates confidence interval and OR, odds ratio.

by Qamar et al,[18] suggests that apoC-III may influence the atherosclerotic process, in part through the promotion of dyslipidemia. Supporting this mechanism, *APOC3* genetic variants predictive of reduced CAC have been associated with lower circulating triglyceride levels.[15]

Beyond its influence on lipid metabolism, apoC-III may also be involved in atherosclerotic development through the promotion of inflammation. ApoC-III stimulates the production of inflammatory cytokines,[12,40,41] and only HDL lacking apoC-III impairs monocyte adhesion to endothelial cells,[12] an early step in the atherosclerotic process.[42] Although the minimal change in estimates with adjustment for C-reactive protein and interleukin-6 in our study argues against an inflammatory link between apoC-III and atherosclerosis, the availability of additional inflammatory biomarkers in future studies might allow this hypothesis to be explored more fully.

Because CAC, plaque, and IMT likely represent distinct aspects of the atherosclerotic process and were only moderately correlated in our study population (ρ=0.32–0.41 for baseline measures), some variation in associations across outcome measures was expected. Intima-media thickening is typically the earliest detectable atherosclerotic change,[43] whereas calcification occurs more commonly in advanced lesions.[44] Correspondingly, CAC has been most strongly associated with incident CHD in MESA, whereas associations were more modest for carotid plaque and only marginally significant for carotid IMT.[24] Nonetheless, the relative consistency of associations of apolipoprotein measures with these diverse measures of atherosclerosis tends to support the robustness of our findings.

Further investigation is needed to better understand the observed differences by sex for baseline CAC associations. Given that we observed similar associations for men and women for carotid plaque and IMT, as well as for incident CHD in a prior study,[7] chance is certainly a possible explanation. The stronger associations for total apoC-III with CAC in men and more divergent associations between the 2 HDL subspecies in women may largely reflect the considerable

Table 3. Associations Between Baseline Apolipoprotein Exposures and Change in Subclinical Atherosclerosis Measures in MESA (Examinations 1–5)

Variable	SD, g/L	Coronary Artery Calcification OR per SD (95% CI) (N=2070)	Carotid Plaque OR per SD (95% CI) (N=1957)	Carotid IMT Mean Difference per SD (95% CI), μm (N=1686)
Total apoC-III	0.04	0.94 (0.84 to 1.04)	1.07 (0.96 to 1.19)	2.1 (−4.1 to 8.2)
Total apoA-I in HDL	0.37	0.95 (0.87 to 1.05)	0.97 (0.88 to 1.07)	−0.2 (−5.8 to 5.5)
ApoA-I in HDL lacking apoC-III	0.35	1.00 (0.89 to 1.12)	0.96 (0.85 to 1.07)	0.2 (−6.8 to 7.2)
ApoA-I in HDL containing apoC-III	0.03	0.93 (0.83 to 1.05)	1.03 (0.92 to 1.16)	−0.5 (−7.2 to 6.3)

Data are given as OR or mean difference (95% CI). Baseline lipid-lowering medication users excluded. Models adjusted for age, sex, race/ethnicity, study site, body mass index, smoking, alcohol, systolic blood pressure, diabetes mellitus, income, antihypertensive medication use, and any lipid-lowering medication use through examination 5. *P*-heterogeneity, apoA-I in HDL containing and lacking apoC-III: coronary artery calcification, *P*-heterogeneity=0.51; carotid plaque, *P*-heterogeneity=0.47; IMT, *P*-heterogeneity=0.92. ApoA-I indicates apolipoprotein A-I; apoC-III, apolipoprotein C-III; CI, confidence interval; HDL, high-density lipoprotein; IMT, intima-media thickness; MESA, The Multi-Ethnic Study of Atherosclerosis; and OR, odds ratio.

Table 4. Logistic Regression ORs and 95% CIs for Associations of Apolipoprotein Levels With CAC and Carotid Plaque in MESA, 2000 to 2012

	CAC (N=2464)		Carotid Plaque (N=1829)	
Variable	Quintile 5 vs 1	Per SD	Quintile 5 vs 1	Per SD
Total apoC-III	0.92 (0.68–1.24)	0.99 (0.90–1.08)	1.34 (0.94–1.92)	1.03 (0.92–1.16)
Total apoA-I in HDL	0.78 (0.58–1.06)	0.97 (0.88–1.06)	1.00 (0.70–1.44)	1.05 (0.93–1.17)
ApoA-I in HDL not containing apoC-III	0.95 (0.66–1.36)	1.01 (0.90–1.13)	1.00 (0.65–1.55)	1.08 (0.94–1.24)
ApoA-I in HDL containing apoC-III	0.77 (0.53–1.11)	0.93 (0.82–1.05)	1.04 (0.68–1.58)	0.95 (0.82–1.10)

Baseline lipid-lowering medication users excluded. Multivariable models adjusted for age, sex, race/ethnicity, study site, body mass index, smoking, alcohol, systolic blood pressure, diabetes mellitus, income, and antihypertensive medication use. Total apoC-III, SD=0.04 g/L; total apoA-I in HDL, SD=0.37 g/L; apoA-I in HDL not containing apoC-III, SD=0.35 g/L; apoA-I in HDL containing apoC-III, SD=0.03 g/L. *P*-heterogeneity, apoA-I in HDL containing and lacking apoC-III: CAC, *P*-heterogeneity=0.42; plaque, *P*-heterogeneity=0.32. ApoA-I indicates apolipoprotein A-I; apoC-III, apolipoprotein C-III; CAC, coronary artery calcification; CI, confidence interval; HDL, high-density lipoprotein; MESA, The Multi-Ethnic Study of Atherosclerosis; and OR, odds ratio.

differences in exposure and outcome distributions by sex; all apolipoprotein levels were higher in women, whereas CAC scores were substantially higher in men. Sex differences in lipid metabolism[45] or in the determinants or pathophysiological characteristics of atherosclerosis[46] may also contribute to the discrepant findings for men and women in baseline CAC analyses.

Given that atherosclerosis is a decades-long process, it is not entirely surprising that we observed associations with subclinical atherosclerosis at baseline but not with progression over follow-up. Apolipoprotein measures at baseline presumably represent long-term levels and, therefore, baseline associations for apoC-III and HDL subspecies likely capture the influence of apolipoproteins on atherosclerotic development over the course of many years (mean age=63 years at baseline). Therefore, even with 10 years of follow-up, the magnitude of additional change in our outcome measures may have been insufficient to detect associations with further subclinical atherosclerosis progression, at least in a sample this size. Because progression analyses required participants to survive until examination 5, the differential survival of participants with examination 5 measurements was also a concern, although the similar distribution of exposure and outcome variables among those with and without examination 5 measurements partly mitigates our concern about potential survival bias (Table 5).

Strengths of our study include the relatively large number of participants, which allowed us to assess effect modification by sex, race/ethnicity, and other CVD risk factors, and our decade-long follow-up period. Our use of a novel modified sandwich ELISA to measure apolipoprotein concentrations allowed for the efficient and highly reproducible measurement of apolipoprotein exposures. In addition, the availability of repeated measures of CAC, IMT, and carotid plaque scores permitted the comprehensive evaluation of apolipoprotein associations with subclinical atherosclerosis. We also used a rigorous methodological approach, which demonstrated the robustness of our findings to both ordinal and tobit regression.

Our study is not without limitations. The measurement of apolipoproteins at one point in time may not have fully captured long-term apolipoprotein levels and did not allow us

Table 5. Baseline (2000–2002) Characteristics of Participants With Apolipoprotein and Subclinical Atherosclerosis Measures MESA Subsets With and Without CAC Measurements at Examination 5*

Variables	Men Without CAC Scans at Examination 5 (N=1532)	Men With CAC Scans at Examination 5 (N=1196)	Women Without CAC Scans at Examination 5 (N=1637)	Women With CAC Scans at Examination 5 (N=1325)
Age, mean (SD), y	64.3 (10.5)	60.4 (9.4)	64.4 (10.6)	60.6 (9.4)
White race, N (%)	559 (36.5)	465 (38.9)	617 (37.7)	475 (35.9)
CAC score, median (IQR)	38.0 (0–275)	5.1 (0–104)	0 (0–62.3)	0 (0–11.7)
IMT, mean (SD), μm†	838 (215)	779 (192)	789 (189)	734 (167)
Carotid plaque score, median (IQR)†	1 (0–3)	0 (0–2)	1 (0–2)	0 (0–2)
Diabetes mellitus, N (%)	259 (16.9)	131 (11.0)	214 (13.1)	124 (9.4)
Hypertension, N (%)	719 (46.9)	477 (39.9)	818 (50.0)	577 (43.6)
Use of blood pressure–lowering drugs, N (%)	596 (38.9)	409 (34.2)	670 (40.9)	469 (35.4)
Use of lipid-lowering drugs, N (%)	262 (17.1)	226 (18.9)	318 (19.4)	225 (17.0)
Systolic blood pressure, mean (SD), mm Hg	128 (20.2)	124 (18.2)	130 (24.0)	125 (22.0)
Body mass index, mean (SD), kg/m^2	27.7 (4.6)	27.9 (4.2)	28.8 (6.4)	28.7 (5.9)
Current smoker, N (%)	240 (15.7)	150 (12.5)	185 (11.3)	142 (10.7)
Current alcohol consumption ≥1 drink/wk, N (%)	697 (45.5)	580 (48.5)	400 (24.4)	363 (27.4)
Income >$75 000/y, N (%)	349 (22.8)	381 (31.9)	251 (15.3)	240 (18.1)
ApoA-I, median (IQR), g/L	1.2 (1.0–1.3)	1.2 (1.0–1.3)	1.4 (1.2–1.6)	1.4 (1.2–1.6)
ApoA-I in HDL with apoC-III, median (IQR), g/L	0.07 (0.06–0.09)	0.07 (0.06–0.09)	0.09 (0.07–0.11)	0.09 (0.07–0.11)

ApoA-I indicates apolipoprotein A-I; apoC-III, apolipoprotein C-III; CAC, coronary artery calcification; HDL, high-density lipoprotein; IMT, intima-media thickness; IQR, interquartile range; and MESA, The Multi-Ethnic Study of Atherosclerosis.

*Examination 5 CAC subset is shown for comparison purposes; differences were comparable for examination 1 vs examination 5 plaque and IMT subsets.

†IMT available in 836 men and 858 women, and plaque score available in 922 men and 976 women, in subset without examination 5 measures; IMT available in 999 men and 1112 women, and plaque score available in 1150 men and 1271 women, in subset with examination 5 measures.

to evaluate associations for changes in apolipoprotein levels. Although apolipoprotein exposures and subclinical atherosclerosis outcome measures were assessed with a high degree of precision, potential measurement error may have biased associations towards the null. In addition, the smaller number of individuals with IMT and plaque measurements limited our ability to detect associations and evaluate effect modification in this subset.

In conclusion, apoC-III was positively associated with measures of subclinical atherosclerosis and diminished or reversed the inverse associations for HDL in cross-sectional analyses, whereas no associations were found with changes in subclinical atherosclerosis over the 10-year follow-up period. Although these results are consistent with a prolonged effect of apoC-III and apoC-III–defined subspecies on the decades-long clinical course of atherosclerosis and support our prior associations for incident CHD, additional research is needed to determine the potential utility of apoC-III–defined HDL subspecies for CVD risk reclassification. Future studies are also needed to further explore potential differences in associations by sex and to provide insight into the biologic pathways through which apoC-III and apoC-III–defined subspecies might influence the development of atherosclerosis.

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Disclosures

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SUPPLEMENTAL MATERIAL

Table S1. Comparison of ordinal regression odds ratios (ORs) and tobit regression ratios for baseline (2000 – 2002) per SD associations of apolipoprotein levels with coronary artery calcification and carotid plaque score in the Multi-Ethnic Study of Atherosclerosis.

	Total apoC-III	**Total HDL**	**HDL not containing apoC-III**	**HDL containing apoC-III**
Standard deviation, mg/dL	4.0	36.9	34.7	3.4
CAC, Males (N = 2240)				
Ordinal regression OR (95% CI)	1.21 (1.12, 1.32)	1.03 (0.94, 1.13)	0.98 (0.88, 1.10)	1.09 (0.97, 1.21)
Tobit regression ratio (95% CI)	1.54 (1.27, 1.85)	1.08 (0.87, 1.34)	0.94 (0.73, 1.21)	1.26 (0.98, 1.62)
CAC, Females (N = 2419)				
Ordinal regression OR (95% CI)	0.95 (0.87, 1.05)	0.87 (0.79, 0.94)	0.87 (0.79, 0.94)	1.10 (0.99, 1.22)
Tobit regression ratio (95% CI)	0.88 (0.71, 1.10)	0.75 (0.61, 0.91)	0.65 (0.51, 0.84)	1.21 (0.96, 1.53)
Carotid plaque score (N = 3546)				
Ordinal regression OR (95% CI)	1.08 (1.01, 1.16)	0.97 (0.91, 1.04)	0.92 (0.84, 1.00)	1.10 (1.01, 1.19)
Tobit regression ratio (95% CI)	1.02 (1.00, 1.03)	1.00 (0.99, 1.01)	0.99 (0.97, 1.01)	1.02 (1.00, 1.03)

Baseline lipid-lowering medication users excluded.
Multivariable models adjusted for age, sex, race/ethnicity, study site, BMI, smoking, alcohol, systolic blood pressure, diabetes, income, anti-hypertensive medication use.

p-heterogeneity, HDL containing and not containing apoC-III, CAC (males) = 0.31 for ordinal regression, 0.19 for tobit regression
p-heterogeneity, HDL containing and not containing apoC-III, CAC (females) = 0.002 for ordinal regression, 0.005 for tobit regression
p-heterogeneity, HDL containing and not containing apoC-III, plaque = 0.02 for ordinal regression, 0.09 for tobit regression

Table S2. Associations between baseline (2000 – 2002) apolipoprotein levels in standard deviation increments and subclinical atherosclerosis in the Multi-Ethnic Study of Atherosclerosis, model comparisons

	Total apoC-III	Total HDL	HDL not containing apoC-III	HDL containing apoC-III	P-heterogeneity, HDL containing and not containing apoC-III
Standard deviation, mg/dL	4.0	36.9	34.7	3.4	
CAC, Males (OR and 95% CI)					
Model 1: Basic model	1.26 (1.16, 1.37)	1.00 (0.91, 1.10)	0.96 (0.86, 1.07)	1.08 (0.97, 1.21)	0.23
Model 2: Multivariable model	1.21 (1.12, 1.32)	1.03 (0.94, 1.13)	0.98 (0.88, 1.10)	1.09 (0.97, 1.21)	0.31
Model 3: Multivariable model + lipid medication users	1.19 (1.11, 1.28)	1.04 (0.96, 1.14)	0.98 (0.88, 1.08)	1.12 (1.01, 1.24)	0.14
Model 4: Multivariable model + triglycerides and LDL-C	1.14 (1.02, 1.27)	1.04 (0.95, 1.14)	1.01 (0.90, 1.13)	1.05 (0.93, 1.18)	0.73
Model 5: Multivariable model + CRP and IL-6	1.23 (1.13, 1.34)	1.04 (0.94, 1.14)	0.99 (0.89, 1.11)	1.08 (0.97, 1.21)	0.38
CAC, Females (OR and 95% CI)					
Model 1: Basic model	0.99 (0.90, 1.09)	0.83 (0.77, 0.91)	0.79 (0.71, 0.89)	1.07 (0.97, 1.19)	0.002
Model 2: Multivariable model	0.95 (0.87, 1.05)	0.87 (0.79, 0.94)	0.87 (0.79, 0.94)	1.10 (0.99, 1.22)	0.002
Model 3: Multivariable model + lipid medication users	1.03 (0.95, 1.11)	0.89 (0.82, 0.95)	0.84 (0.77, 0.93)	1.07 (0.98, 1.17)	0.004
Model 4: Multivariable model + triglycerides and LDL-C	0.92 (0.82, 1.03)	0.88 (0.81, 0.96)	0.83 (0.74, 0.92)	1.10 (0.99, 1.22)	0.004
Model 5: Multivariable model + CRP and IL-6	0.97 (0.88, 1.07)	0.87 (0.80, 0.95)	0.81 (0.73, 0.91)	1.11 (1.00, 1.23)	0.002
Carotid plaque score* (Mean difference and 95% CI)					
Model 1: Basic model	1.12 (1.05, 1.20)	0.96 (0.90, 1.02)	0.91 (0.83, 0.98)	1.09 (1.00, 1.18)	0.01
Model 2: Multivariable model	1.08 (1.01, 1.16)	0.97 (0.91, 1.04)	0.92 (0.84, 1.00)	1.10 (1.01, 1.19)	0.02
Model 3: Multivariable model + lipid medication users	1.11 (1.04, 1.17)	0.98 (0.92, 1.04)	0.93 (0.86, 1.00)	1.09 (1.01, 1.17)	0.01
Model 4: Multivariable model + triglycerides and LDL-C	1.05 (0.95, 1.15)	0.99 (0.93, 1.07)	0.94 (0.86, 1.03)	1.09 (1.00, 1.18)	0.06
Model 5: Multivariable model + CRP and IL-6	1.09 (1.02, 1.17)	0.98 (0.91, 1.05)	0.92 (0.84, 1.00)	1.10 (1.01, 1.20)	0.02
IMT* (Mean difference and 95% CI)					
Model 1: Basic model	6.3 (0.2, 12.4)	-11.8 (-17.7, -5.8)	-12.3 (-19.7, -4.8)	0.7 (-6.5, 8.0)	0.05
Model 2: Multivariable model	1.9 (-4.1, 7.8)	-14.9 (-20.5, -9.2)	-14.0 (-21.2, -6.7)	-1.4 (-8.4, 5.7)	0.06
Model 3: Multivariable model + lipid medication users	3.9 (-1.5, 9.3)	-5.6 (-11.2, 0.0)	-6.8 (-13.6, 0.0)	1.9 (-4.7, 8.5)	0.15
Model 4: Multivariable model + triglycerides and LDL-C	-6.4 (-14.2, 1.4)	-6.9 (-12.9, -1.0)	-7.6 (-14.9, -0.2)	0.9 (-6.2, 8.1)	0.20
Model 5: Multivariable model + CRP and IL-6	0.6 (-5.4, 6.6)	-8.6 (-14.6, -2.5)	-10.0 (-17.4, -2.6)	2.1 (-5.1, 9.2)	0.07

* Associations for IMT and carotid plaque in the combined population of men and women.

Lipid-lowering medication users excluded from all models except Model 3.

Multivariable models adjusted for age, sex, race/ethnicity, study site, BMI, smoking, alcohol, systolic blood pressure, diabetes, income, anti-hypertensive medication use.

Table S3. Ordinal regression odds ratios and 95% confidence intervals for baseline (2000 – 2002) associations between apolipoproteins and coronary artery calcification among men (N = 2240) and women (N = 2419) in the Multi-Ethnic Study of Atherosclerosis

	Q1	Q2	Q3	Q4	Q5	Per SD	P-trend
Total apoC-III							
Quintile median (range) or SD, mg/dL	5.35 (1.26 – 6.46)	7.31 (6.46 – 8.01)	8.70 (8.01 – 9.45)	10.40 (9.46 – 11.77)	14.09 (11.77 – 53.10)	4.0	
Multivariable model, men	*1.0 (ref)*	1.09 (0.87, 1.37)	1.04 (0.82, 1.32)	1.36 (1.06, 1.74)	1.63 (1.25, 2.12)	1.21 (1.12, 1.32)	<0.0001
Multivariable model, women	*1.0 (ref)*	1.14 (0.84, 1.54)	0.91 (0.68, 1.23)	1.03 (0.77, 1.37)	0.98 (0.72, 1.32)	0.95 (0.87, 1.05)	0.69
Total HDL							
Quintile median (range) or SD, mg/dL	92.0 (27.3 – 102.9)	111.4 (102.9 – 119.0)	125.8 (119.0 – 133.5)	143.0 (133.5 – 155.4)	176.4 (155.4 – 376.8)	36.9	
Multivariable model, men	*1.0 (ref)*	0.87 (0.71, 1.08)	0.83 (0.66, 1.05)	0.82 (0.64, 1.06)	1.18 (0.89, 1.57)	1.03 (0.94, 1.13)	0.70
Multivariable model, women	*1.0 (ref)*	0.94 (0.68, 1.31)	0.77 (0.56, 1.07)	0.90 (0.66, 1.22)	0.59 (0.43, 0.81)	0.87 (0.79, 0.94)	0.0002
HDL not containing apoC-III							
Quintile median (range) or SD, mg/dL	86.1 (20.4 – 96.4)	104.4 (96.4 – 111.5)	117.5 (111.5 – 124.8)	133.2 (124.8 – 145.2)	165.0 (145.3 – 352.2)	34.7	
Multivariable model, men	*1.0 (ref)*	0.86 (0.69, 1.08)	0.80 (0.63, 1.02)	0.79 (0.60, 1.04)	1.12 (0.83, 1.52)	0.98 (0.88, 1.10)	0.77
Multivariable model, women	*1.0 (ref)*	0.88 (0.63, 1.22)	0.78 (0.56, 1.08)	0.82 (0.59, 1.13)	0.52 (0.37, 0.74)	0.81 (0.73, 0.90)	0.001
HDL containing apoC-III							
Quintile median (range) or SD, mg/dL	5.03 (1.12 – 5.94)	6.62 (5.94 – 7.31)	7.94 (7.31 – 8.62)	9.44 (8.62 – 10.65)	12.41 (10.65 – 39.31)	3.4	
Multivariable model, men	*1.0 (ref)*	0.92 (0.73, 1.15)	1.01 (0.80, 1.29)	1.23 (0.95, 1.60)	1.35 (0.98, 1.86)	1.09 (0.97, 1.21)	0.20
Multivariable model, women	*1.0 (ref)*	0.98 (0.71, 1.36)	1.01 (0.74, 1.39)	0.92 (0.66, 1.26)	0.87 (0.63, 1.20)	1.10 (0.99, 1.22)	0.83

Lipid-lowering medication users excluded (N for males and females reflects numbers after exclusions).
Multivariable models adjusted for age, sex, race/ethnicity, study site, scanner model, BMI, smoking, alcohol, systolic blood pressure, diabetes, income, anti-hypertensive medication use.
p-heterogeneity, HDL containing and not containing apoC-III: p-het (males) = 0.31; p-het (females) = 0.002

Table S4. Ordinal regression odds ratios (ORs) and 95% confidence intervals for baseline (2000 – 2002) cross-sectional associations between apolipoprotein levels and carotid plaque score in the Multi-Ethnic Study of Atherosclerosis (N = 3546)

	Q1	Q2	Q3	Q4	Q5	Per SD	P-trend
Total apoC-III							
Multivariable model	*1.0 (ref)*	1.10 (0.89, 1.35)	1.05 (0.86, 1.30)	1.24 (1.01, 1.53)	1.31 (1.06, 1.63)	1.08 (1.01, 1.16)	0.01
Total HDL							
Multivariable model	*1.0 (ref)*	0.98 (0.80, 1.20)	0.91 (0.73, 1.12)	0.95 (0.77, 1.18)	0.89 (0.72, 1.11)	0.97 (0.91, 1.04)	0.30
HDL not containing apoC-III							
Multivariable model	*1.0 (ref)*	0.91 (0.74, 1.12)	0.81 (0.65, 1.02)	0.79 (0.62, 1.00)	0.75 (0.58, 0.97)	0.92 (0.84, 1.00)	0.03
HDL containing apoC-III							
Multivariable model	*1.0 (ref)*	1.10 (0.89, 1.36)	1.25 (1.01, 1.57)	1.27 (1.01, 1.61)	1.29 (0.99, 1.67)	1.10 (1.01, 1.19)	0.06

Lipid-lowering medication users excluded (Total N reflects numbers after exclusions).
Multivariable models adjusted for age, sex, race/ethnicity, study site, BMI, smoking, alcohol, systolic blood pressure, diabetes, income, anti-hypertensive medication use.
Standard deviations and quintile medians and ranges given in Table S1.
p-heterogeneity, HDL containing and not containing apoC-III = 0.02

Table S5. Mean difference (um) and 95% confidence intervals for baseline (2000 – 2002) cross-sectional associations between apolipoprotein levels and common carotid intima-media thickness in the Multi-Ethnic Study of Atherosclerosis (N = 3140)

	Q1	Q2	Q3	Q4	Q5	Per SD	P-trend
Total apoC-III							
Multivariable model	*1.0 (ref)*	6.3 (-10.9, 23.6)	8.6 (-8.8, 26.0)	9.0 (-2.1, 33.1)	18.3 (-0.2, 36.8)	1.9 (-4.1, 7.8)	0.03
Total HDL							
Multivariable model	*1.0 (ref)*	-6.5 (-24.0, 11.1)	-21.4 (-39.2, -3.5)	-12.8 (-31.2, 5.7)	-22.1 (-40.8, -3.3)	-14.9 (-20.5, -9.2)	0.02
HDL not containing apoC-III							
Multivariable model	*1.0 (ref)*	-9.7 (-27.9, 8.4)	-23.6 (-43.0, -4.3)	-18.7 (-39.5, 2.2)	-31.8 (-54.4, -9.2)	-14.0 (-21.2, -6.7)	0.01
HDL containing apoC-III							
Multivariable model	*1.0 (ref)*	3.0 (-15.0, 20.9)	16.5 (-2.4, 35.4)	4.1 (-16.2, 24.3)	14.7 (-7.8, 37.2)	-1.4 (-8.4, 5.7)	0.23

Lipid-lowering medication users excluded (Total N reflects numbers after exclusions).
Multivariable models adjusted for age, sex, race/ethnicity, study site, BMI, smoking, alcohol, systolic blood pressure, diabetes, income, anti-hypertensive medication use.
Standard deviations and quintile medians and ranges given in Table S1.
p-heterogeneity, HDL containing and not containing apoC-III = 0.06

Table S6. Change in coronary artery calcification according to apolipoprotein levels (per SD), Exam 1 to 2/3 and Exam 1 to 5.

	SD, mg/dL	CAC, Exam 1 to 2/3* OR per SD (95% CI) (N = 3861)	CAC, Exam 1 to 5 OR per SD (95% CI) (N=2070)
Total apoC-III			
OR (95% CI)	4.0	1.07 (0.98, 1.17)	0.94 (0.84, 1.04)
Total HDL			
OR (95% CI)	36.9	1.05 (0.96, 1.15)	0.95 (0.87, 1.05)
HDL not containing apoC-III			
OR (95% CI)	34.7	1.06 (0.95, 1.18)	1.00 (0.89, 1.12)
HDL containing apoC-III			
OR (95% CI)	3.4	0.99 (0.89, 1.10)	0.93 (0.83, 1.05)

*Participants received a second CT at either Exam 2 (N=1998) or Exam 3 (N=1863), and therefore participants with Exam 2 and 3 scans were combined.
Baseline lipid-lowering medication users excluded. Multivariable model adjusted for age, sex, race/ethnicity, study site, BMI, smoking, alcohol, systolic blood pressure, diabetes, income, anti-hypertensive medication use, lipid-lowering medication use at follow-up exams.

p-heterogeneity, HDL containing and not containing apoC-III:
CAC, Exam 1 to 2/3 p-het = 0.45
CAC, Exam 1 to 5 p-het = 0.51