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Apolipoprotein B discordance with low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol in relation to coronary artery calcification in the Multi-Ethnic Study of Atherosclerosis (MESA)

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Abstract

Background—Discordant levels of apolipoprotein B (apo B) relative to low-density lipoprotein cholesterol (LDL-C) or non-high-density lipoprotein cholesterol (non-HDL-C) may be associated with subclinical atherosclerotic cardiovascular disease (ASCVD).

Objective—The present study investigated whether discordance between apo B and LDL-C or non-HDL-C levels was associated with subclinical ASCVD measured by coronary artery calcium (CAC).

Methods—This study was conducted in a subpopulation of the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, aged 45 to 84 years, free of ASCVD, and not taking lipid-lowering medications at baseline (2000-2002) (prevalence analytic N=4,623; incidence analytic N=2,216; progression analytic N=3,947). Apo B discordance relative to LDL-C and non-HDL-C was defined using residuals and percentile rankings (>5/10/15 percentile). Associations with

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AUTHOR CONTRIBUTION STATEMENT

Jing Cao, Sarah O. Nomura, Brian T. Steffen, Weihua Guan, and Michael Y. Tsai contributed to study design, data analysis and manuscript writing. Alan T. Remaley, Amy B. Karger, Pamela Ouyang, and Erin D. Michos contributed to manuscript writing.

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prevalent and incident CAC (CAC>0 vs. CAC=0) were assessed using prevalence ratio/relative risk regression and CAC progression (absolute increase/year) using multinomial logistic regression.

Results—Higher apo B levels were associated with CAC prevalence, incidence and progression. Apo B discordance relative to LDL-C or non-HDL-C was inconsistently associated with CAC prevalence and progression. Discordantly high apo B relative to LDL-C and non-HDL-C was associated with CAC progression. Associations for apo B discordance with non-HDL-C remained after further adjustment for metabolic syndrome components.

Conclusion—Apo B was associated with CAC among adults 45 years not taking statins, but provided only modest additional predictive value of apo B for CAC prevalence, incidence or progression beyond LDL-C or non-HDL-C. Apo B discordance may still be important for ASCVD risk assessment and further research is needed to confirm findings.

Keywords

apolipoprotein B; low-density lipoprotein cholesterol; non-high-density lipoprotein cholesterol; coronary artery calcium; discordance

INTRODUCTION

Cholesterol attributable to atherogenic lipoproteins, including low-density lipoprotein cholesterol (LDL-C) and non-high-density lipoprotein cholesterol (non-HDL-C) [total cholesterol minus high-density lipoprotein cholesterol (HDL-C)], are often utilized for assessing atherosclerotic cardiovascular disease (ASCVD) risk [1]. However, recent evidence suggests the total number of lipoprotein particles and particle size may be more relevant [2–6], and neither LDL-C nor non-HDL-C address particle number or size as cholesterol levels in particles varies [7, 8]. Direct measurement of particles would be ideal, but is expensive and remains challenging to implement clinically. Alternatively, apolipoprotein B (apo B) may be a better, or a useful additional, marker for ASCVD risk assessment since it is present on atherogenic lipoproteins, and thus, is more reflective of particle numbers [7–15]. Importantly, apo B is highly correlated with LDL-C and non-HDL-C; therefore, the clinical utility of measuring apo B, in addition to cholesterol, is controversial. Most recently, the 2018 American College of Cardiology (ACC)/American Heart Association/Multisociety Guidelines for the management of blood cholesterol has included apo B as a risk assessment enhancer that may be of value under certain circumstances [1].

Among individuals with more small, dense LDL-P or large, lipid-rich LDL-P, measured apo B levels would be expected to be higher/lower, respectively, in comparison to LDL-C or non-HDL-C (i.e. discordant). Evaluating apo B discordance in relation to LDL-C and non-HDL-C allows for assessment of the potential additional benefit of measuring apo B by addressing dilution of associations that occur with highly correlated biomarkers [10, 11, 16]. Additionally, discordance is calculated at the individual level making it possible to directly evaluate associations between discordant compared to concordant measurements and outcomes [17]. Several previous studies have reported higher ASCVD risk among

individuals with discordantly high apo B concentrations relative to LDL-C or non-HDL-C [7, 10, 12, 15] and lower risk among those with discordantly low apo B when compared to individuals with concordant measurements [7, 10, 15]. These findings suggest the additional measurement of apo B may be beneficial, particularly for improving ASCVD risk classification in discordant individuals.

Coronary artery calcification (CAC) is a measure of subclinical atherosclerotic disease that strongly correlates with ASCVD risk [18]. To our knowledge, only one previous study, the Coronary Artery Risk Development in Young Adults (CARDIA) study, has evaluated apo B discordance with LDL-C and non-HDL-C in relation to atherosclerotic disease measured by CAC. This study reported apo B, and discordance between apo B and LDL-C and non-HDL-C, measured at ages 18-34, better predicted risk of CAC twenty-five years later [8]. It is beneficial to ascertain whether discordance is present and associated with intermediate risk factors (e.g. CAC), as this would indicate whether discordance is an early sign of the disease process and relevant to evaluate in early assessment and monitoring of ASCVD risk. While the results from CARDIA provide important insights, the ages at measurement of the lipid-related biomarkers in CARDIA are generally younger than the ages at which these measurements are routinely assessed in the general population [19]. The objective of the present study was to evaluate whether similar associations are observed in an older, racial/ethnically diverse population, by investigating the discordance between apo B and LDL-C or non-HDL-C measurements and its association with CAC prevalence and CAC incidence or progression among participants not taking lipid-lowering medication at baseline in the Multi-Ethnic Study of Atherosclerosis (MESA).

MATERIALS AND METHODS

STUDY POPULATION

The study population was derived from the prospective MESA cohort, details of which have been described previously [20]. In brief, between July 2000 and August 2002, 6,814 male and female White, Black, Hispanic, or Chinese American participants aged 45 to 84 and free of clinically apparent cardiovascular disease were recruited at six centers in the United States (Baltimore, MD; Chicago, IL; Forsyth County, ND; Los Angeles County, CA; New York, NY; St. Paul, MN). The data used for the present analysis were collected at the baseline exams (2000-2002) with the exception of CAC incidence and progression data, which includes participants with at least one CAC measurement during follow-up though exam 5. Informed consent was obtained from all study participants and the study was approved by the institutional review boards of all MESA field centers.

The present analysis included all MESA participants with baseline data for the exposures of interest (apo B, LDL-C and non-HDL-C levels), the primary outcome (CAC) and all covariates. Apo B was not measured in participants who were taking lipid-lowering medication at baseline or those in the 'MESA 1000', and therefore, were excluded from the analysis (N=2,125). Among participants with no measurement of apo B, mean values for total cholesterol, HDL-C and LDL-C were lower than those included in the analysis, while non-HDL-C levels were similar. Additionally, those lacking apo B measurements were more likely to be diabetic (excluded with diabetes=17.2%, included with diabetes=11.0%) and to

have prevalent CAC>0 at baseline (CAC>0 among excluded=55.3%, included=47.3%). These differences were attributable to the exclusion of statin users, as the 'MESA 1000' participants were similar to those included in the present analysis. After excluding additional participants with missing data (N=56) the final analytic cohort for the prevalent CAC analysis included 4,623 participants. Participants without at least one follow-up CAC measurements through exam 5 (N=521) were excluded from CAC incidence and progression analyses. Additionally, participants with negative CAC progression were excluded (N=155) as there is currently no approach for reversing CAC and there was no way to determine the reasons for a negative measurement (e.g. weight change, measurement error, etc.) (prospective analytic N=3,947).

DATA COLLECTION

Exposure Measurement—Fasting (12-hour) blood samples were collected at baseline study visits and stored at -70°C . Lipids were measured on EDTA plasma at a central laboratory within approximately two weeks of collection. Total cholesterol was measured via cholesterol oxidase methods (coefficient of variation: 1.6%) and triglycerides (coefficient of variation: 4.0%) were measured using triglyceride GB on a Roche COBAS FARA centrifugal analyzer (Roche Diagnostics, Indianapolis, IN). High-density lipoprotein cholesterol (HDL-C) was quantified by cholesterol oxidase methods following precipitation of non-HDL-C by magnesium/dextran (Roche Diagnostics, Indianapolis, IN) (coefficient of variation: 2.9%). Non-HDL-C was tabulated by subtracting measured HDL-C from total cholesterol. LDL-C was calculated using the Friedewald equation [21]. Apo-B concentrations were quantified at Health Diagnostics Laboratory Inc. (Richmond, VA) using the Tina-quant Apolipoprotein B ver.2 immunoassay on a Roche Modular P analyzer (Roche Diagnostics, Indianapolis, IN) (coefficient of variation <5%).

Outcome Assessment—A detailed report on CAC measurement methods was previously published [22]. CAC measurements were assessed in participants at exam 1 using either an electron beam or multidetector computed tomography scanner. Follow-up CAC measurements were conducted among participants at either exam 2 (N=1,782) or 3 (N=1,723), with additional measurements in a random subset at exam 4 (N=726) and among those included in an ancillary study (MESA AIR) at exam 5 (N=2,013). At baseline exams, radiographic phantoms containing standardized and known concentrations of calcium were placed beneath the thorax of each participant and scanned twice by certified technologists. All scans were read at a single center by a radiologist or cardiologist. Agatston scores were calculated for each scan and the mean of the two scans were used for this analysis. Intra- and inter-observer agreement for baseline measurements were high with kappa statistics of 0.90 and 0.93, respectively [22]. For the present analysis, baseline CAC was defined as present (CAC Agatston score >0) or absent (CAC Agatston score=0). In the prospective analysis, CAC incidence was defined as an Agatston score >0 during follow-up among participants with CAC=0 at baseline. CAC progression was any increase in CAC from baseline through last CAC measurement for each participant.

Covariates—Demographic, personal and medical history and lifestyle behaviors were collected by interview or questionnaire at the baseline exam. Anthropometric measures,

blood pressure and fasting glucose were measured by study staff. Body mass index (BMI) was calculated from measured height and weight as kg/m^2 and a BMI $\geq 30 \text{ kg/m}^2$ was considered obese. Hypertension was defined as taking anti-hypertensive medication or a systolic blood pressure $\geq 140 \text{ mmHG}$. Participants were considered diabetic if they were taking diabetes medication or had a fasting glucose $>125 \text{ mg/dL}$. Participants were classified as having metabolic syndrome if they met three of the five following criteria: (1) waist circumference $\geq 89 \text{ cm}$ for women or $\geq 102 \text{ cm}$ for men; (2) triglycerides $\geq 150 \text{ mg/dL}$; (3) HDL-C $<50 \text{ mg/dL}$ for women or $<40 \text{ mg/dL}$ for men; (4) blood pressure measurement $\geq 130/85 \text{ mmHG}$; or (5) fasting blood glucose $\geq 100 \text{ mg/dL}$.

STATISTICAL ANALYSIS

Relationships between apo B, LDL-C and non-HDL-C were evaluated continuously using Spearman correlations and categorized into quintiles for cross-tabulation and weighted kappa analysis. Apo B, LDL-C, and non-HDL-C were categorized into tertiles for evaluation in regression models. Ranges of measurements included in each tertile for the biomarkers were as follows: apo B, <96.9 , $96.9\text{--}118.0$ and $>118.0 \text{ mg/dL}$; LDL-C, <106.0 , $106.0\text{--}129.0$, $>129.0 \text{ mg/dL}$; and non-HDL-C, <129.0 , $129.0\text{--}136.0$, $>136.0 \text{ mg/dL}$. Concordance/discordance was evaluated using two approaches chosen for comparability to methods utilized in previously published studies: (1) differences of >5 , 10 and 15 percentile points; and (2) residuals. For percentile differences, participants' percentile rankings for each lipid marker were tabulated then LDL-C and non-HDL-C percentiles were subtracted from the apo B percentile ranking. Using three different cut-points of either >5 , 10 or 15 percentile points different, participants were assigned to one of three categories: (1) discordant high, apo B $>5/10/15$ percentile points higher than LDL-C or non-HDL-C; (2) concordant, apo B within 5/10/15 percentile points of LDL-C or non-HDL-C; or (3) discordant low, apo B 5/10/15 percentile points lower than LDL-C or non-HDL-C. Three different percentile cut-points were chosen in order to evaluate whether the degree of discordance between apo B and LDL-C or non-HDL-C influenced associations. Finally, residuals from linear regression models of the difference between observed (measured) and expected for apo B compared to LDL-C and non-HDL-C were calculated. Discordance measured by residuals was defined as the following: apo B discordant low, $<25^{\text{th}}$ percentile residual; concordant, $25^{\text{th}}\text{--}75^{\text{th}}$ percentile residual; discordant high, $>75^{\text{th}}$ percentile residual. Univariate association between lipid marker tertiles, categories of apo B concordance/discordance and population characteristics were evaluated using Wald χ^2 test for categorical variables and ANOVA for continuous variables. Univariate associations for lipid markers, discordance and population characteristics were also assessed among those included versus excluded in the prospective analysis.

Prevalence ratio regression models were used to tabulate prevalence of $\text{CAC}>0$, $\text{CAC}>100$ and $\text{CAC}>400$ compared to $\text{CAC}=0$ by individual lipid markers and categories of apo B concordance/discordance. Apo B, LDL-C and non-HDL-C were evaluated both as tertiles and continuously. Continuous models were also conducted stratified by levels of discordance defined by percentile differences. Two different approaches were used for category definitions. The first used three categories with discordant low and discordant high as separate groups, in addition to the concordant group. This approach was undertaken because

having discordant low and discordant high levels may be differentially associated with health outcomes as the groups would represent those with lower and higher particle numbers, respectively. The second approach combined the discordant groups into a single discordant category, as done previously by Otvos et al [23]. Prevalence ratios (PR) were calculated per standard deviation (SD) (from overall population) increase (apo B SD=26.2 mg/dL, LDL-C SD=31.1 mg/dL, non-HDL-C SD=35.7 mg/dL). Residual discordance categories were evaluated as independent predictors of CAC. Four models were conducted for each association: (1) unadjusted (data not presented); (2) age, sex and race/ethnicity adjusted (data not shown); (3) “minimally-adjusted” model 2 + smoking status (never, former, current) and hypertension (yes/no); and (4) “fully-adjusted” model 3 + diabetes status (yes/no), BMI and triglycerides (log-transformed). Potential covariates were chosen *a priori* for known associations with lipid marker measurements and CAC with the goal to elucidate both clinical relevance and potential biological mechanisms. Covariates included in fully adjusted models were those factors that remained statistically significantly associated with CAC when included in models with all other covariate variables. Associations between lipid biomarkers and CAC or ASCVD risk may differ by race/ethnicity [24–26], metabolic syndrome [27–29], and age [12, 30, 31]. Therefore, analyses of individual markers (tertiles) and discordance by residuals were additionally conducted stratified by race/ethnicity (white, black, Chinese, Hispanic), metabolic syndrome (yes/no), and age categories (45–65, 55–64, 65–74, 75–84 years). Potential interactions between race/ethnicity, metabolic syndrome, or age and lipid markers on CAC prevalence were evaluated by including a cross-product term in models.

Prospective evaluations of associations between baseline lipid markers and apo B discordance and CAC measurement through exam 5 (median follow-up=8.8 years) were conducted among participants who had at least one follow-up CAC measurement. Two separate analyses were conducted, CAC incidence and CAC progression. Relative risk regression was used to evaluate incident CAC at any exam through exam 5 by tertiles of lipid markers and apo B concordance/discordance (residuals) among participants with CAC=0 at baseline (N=2,216). Discordance by percentile stratified models were not conducted in the incidence models due to limited sample size for stratification. CAC progression analyses included all participants with at least one follow-up measurement using multinomial logistic regression. For CAC progression, CAC measurements (absolute change between exams) were standardized by tabulating increase per year and participants were categorized (due to non-normal distribution of data) as no progression, >0 - <75th percentile, or >75th percentile CAC increase/year (75th percentile cut-point: 16.44 Agatstons/year). In percentile difference discordance analyses only >5 and >10 percentile differences were utilized due to limited numbers of participants with >15 percentile difference discordance. For both incident and progression models, unadjusted, age, sex and race/ethnicity adjusted and multivariable adjusted models with the same covariates previous outlined were run. Statins and metabolic syndrome were additionally evaluated as covariates, but did not change study findings, so presented results do not include these models.

Sensitivity analyses were also conducted excluding participants >70 years of age (N=1,103 excluded) (cross-sectional and prospective analyses) and excluding participants who experienced a coronary heart disease event prior to exam 5 (N=273) (prospective analysis).

Findings did not change, so these participants were included in presented results. Statistical analysis was conducted using SAS (version 9.4, SAS Institute Inc., Cary, NC).

RESULTS

Baseline characteristics by categories of discordance defined by residual differences are presented on Table 1. Generally, those classified as having discordant low apo B relative to either LDL-C or non-HDL-C were more likely to be female, have higher education, have higher HDL-C and lower apo B and triglycerides. Conversely, those classified as discordantly high apo B were more likely to have hypertension or be taking anti-hypertensive medication, to have diabetes or metabolic syndrome, have a higher BMI, have higher triglyceride and apo B levels and lower HDL-C levels. Hispanics were also more likely to have discordantly high apo B levels. LDL-C, non-HDL-C and total cholesterol were statistically significantly associated with categories of residual-defined discordance, but there was no clear pattern to the associations. CAC prevalence was generally lowest among those with discordant low apo B, but were similar among participants with concordant measures or discordant high apo B.

Apo B, LDL-C and non-HDL-C were all highly correlated ($p < 0.001$) and weighted kappa analysis indicated moderate agreement of apo B relative to both LDL-C (k-statistic=0.65, 95% CI: 0.64, 0.67) and non-HDL-C (k-statistic=0.78, 95% CI: 0.77, 0.79) (Table 2). Cross-tabulations of quintiles of apo B, LDL-C and non-HDL-C demonstrate most participants were within 1 quintile different between the biomarkers and variation was greater in the middle quintiles than in the highest/lowest quintiles (Supplementary Tables 1–3). Relative to LDL-C, prevalence of discordant low apo B (defined as >15 percentile points difference) was $N=645$ (14.0%) and discordant high apo B was $N=654$ (14.1%) and compared to non-HDL-C, discordant low apo B prevalence was $N=251$ (5.4%) and discordant high was $N=260$ (5.6%). Using median cut-points, $N=416$ (9.0%) were discordant low (apo B $<$ median, LDL-C $\geq$ median) and $N=387$ (8.4%) were discordant high (apo B $\geq$ median, LDL-C $<$ median) when compared to LDL-C. Relative to non-HDL-C medians, $N=267$ (5.8%) were discordant low (apo B $<$ median, non-HDL-C $\geq$ median) and $N=220$ (4.8%) were discordant high (apo B $\geq$ median, non-HDL-C $<$ median). Associations between tertiles for individual biomarkers and discordance by residuals with prevalent CAC >0 are presented on Table 3. All individual biomarkers were associated with prevalent CAC >0 , regardless of covariates included in models. In minimally adjusted models, individuals with discordant high apo B relative to LDL-C were modestly more likely to have CAC >0 while individuals with discordant low apo B relative to non-HDL-C had modestly lower prevalence of CAC >0 . In models additionally adjusted for triglycerides, diabetes and BMI, only discordantly low apo B relative to non-HDL-C remained statistically significantly associated. Stratifying by age, metabolic syndrome or race/ethnicity did not change results and tests for interaction were not statistically significant ($P > 0.05$) (data not shown). Similarly, sensitivity analyses excluding participants >70 years of age did not change results (data not shown). Continuous associations between individual biomarkers and prevalent CAC >0 overall, and by categories of discordance by percentile differences are presented on Supplementary Table 4 and Supplementary Table 5. Individual markers were significantly associated with CAC >0 at baseline, however, when stratified by discordance categories, apo

B, LDL-C and non-HDL-C were only statistically significant associated with CAC>0 among individuals with concordant apo B and LDL-C or non-HDL-C levels.

Among individuals with CAC=0 at baseline, baseline levels of the individual biomarkers were associated with development of CAC through exam 5 (Table 4). In contrast to associations with prevalent CAC, discordantly low apo B relative to LDL-C was inversely associated and discordantly high apo B relative to non-HDL-C was positively associated with CAC development in minimally adjusted models, respectively. In models additionally adjusted for triglycerides, diabetes status, and BMI, apo B discordance with LDL-C and non-HDL-C were no longer statistically significantly associated with CAC development after baseline. Overall, associations between apo B, LDL-C and non-HDL-C with CAC development were similar across categories of apo B discordance with LDL-C and non-HDL-C (Supplementary Table 5, Supplementary Table 7). Odds of CAC progression, regardless of baseline CAC measurement, were statistically significantly higher with increasing apo B, LDL-C and non-HDL-C levels (Table 5). In minimally adjusted models, discordantly low apo B relative to LDL-C and non-HDL-C were not associated with CAC progression, but discordantly high apo B levels were associated with increased odds of CAC progression. When additionally adjusted for triglycerides, diabetes and BMI, only discordantly high apo B compared to non-HDL-C levels were statistically significantly positively associated with odds of CAC progression.

DISCUSSION

Among non-statin users in this age, gender and racially/ethnically heterogeneous MESA population, concordance between apo B and LDL-C and non-HDL-C was high, particularly between apo B and non-HDL-C. All lipid markers were individually associated with CAC prevalence, incidence, and progression. Modest associations were observed between discordant apo B levels relative to LDL-C or non-HDL-C and prevalent or incident CAC in minimally adjusted models. When additionally accounting for factors associated with both discordance and CAC, triglycerides, diabetes status and BMI, associations were generally no longer statistically significant. However, discordantly high apo B relative to non-HDL-C and LDL-C were both associated CAC progression in minimally-adjusted models. These associations remained after accounting for triglycerides, diabetes and BMI among individuals with discordantly high apo B relative to non-HDL-C, but not relative to LDL-C.

An ongoing debate in ASCVD risk assessment involves which combination of risk factors and biomarkers best predict risk of a future ASCVD event, and little is known about the associations of these biomarkers with measures of atherosclerotic disease, such as CAC development [1]. Additionally, cholesterol was the first lipid measurement used for CHD risk assessment [32], but it has since been discovered that there may be better or additional markers for assessing risk. These markers include apo B, a protein component of LDL-P and other atherogenic lipoprotein particles, including intermediate density lipoprotein (IDL), very low-density lipoprotein (VLDL), and lipoprotein (a) particles. Each particle contains one apo B molecule making it an ideal marker for assessing particle number. It is theorized that the number of particles (higher number more atherogenic) [4, 5] and the size of particles (smaller size more atherogenic) [2, 3, 6] are more influential on risk and that measurement

of apo B may better reflect this than cholesterol measurement. While closely related, LDL-C, non-HDL-C and apo B represent slightly different aspects of atherogenic lipoproteins, and while subtle, these differences appear to matter. Results from several recent cholesteryl ester transfer protein (CETP) inhibitor trials appear to demonstrate the importance of these subtle differences [33–37]. While CETP inhibitors were designed to increase HDL-C, several also substantially lowered LDL-C, but did not lower ASCVD risk. Two Mendelian randomization analyses by Ference *et al* followed up on the clinical trial results by evaluating associations between genetic variants associated with LDL-C levels and ASCVD risk [33, 38]. In these analyses, associations were lower than expected for variants associated with lower LDL-C when apo B was discordantly higher. The results from these analyses indicated that the benefits of lowering measured cholesterol levels in atherogenic lipoproteins depends on lowering the actual particle number, which is reflected in the measurement of apo B, and provide support for the potential benefit of measuring apo B.

The results of the present study are consistent with previous reports on the individual measures of apo B, LDL-C and non-HDL-C [7–16, 39], but are generally smaller than associations reported in previous investigations of apo B discordance [7, 8, 10–13, 15, 16]. This may be due to a number of differences between these studies, including differences in populations, outcome variables, or degree of concordance among markers. Definitions of concordance/discordance have varied in previous studies, typically using medians [8, 12, 15], percentile differences [7] or residuals [10, 12]. Some possible explanations for the contradictory findings are as follows: Most previous studies were conducted in populations >40 years old, representing a similar age group to MESA, but were typically majority White, and several included only men or only women. Medians for lipid markers in the present study were also largely similar to prior studies, except for CARDIA, which is not surprising given that lipids were measured at a much younger age (<25 years) in CARDIA. Not all previous studies have reported detailed assessment of the degree of concordance among the assessed biomarkers, but Spearman correlations and agreement measured by k-statistics were high in the MESA population and were generally higher than the correlations and k-statistics reported in several prior studies [11, 15]. Possibly the most important difference is that all but one previous study evaluated discordance in relation to ASCVD outcomes rather than subclinical disease. While CAC is highly predictive of ASCVD risk, it is a marker of subclinical disease rather than an ASCVD outcome. Perhaps discordance is associated with ASCVD through a mechanism that is not directly related to CAC or that it is more weakly associated with CAC, which may be supported by the apo B discordance with non-HDL-C results from the incidence and progression analyses using residual-defined discordance. The results for apo B compared to non-HDL-C trended in directions consistent with previous studies, while generally not reaching statistical significance. It is possible that an association may have been observed with a larger sample size, although associations were still observed in the smaller CARDIA study (N=2,794). It may also be that discordance in the age range of this population is more an indicator of the presence of subclinical disease rather than predictive of risk for subclinical disease (i.e. CAC presence), and thus, more predictive of higher risk of ASCVD events within 10-years than risk of CAC development. Much of the results presented in the current study are for prevalent rather than incident CAC, which could also have contributed to differences in observed results.

To our knowledge, CARDIA is the only other study to examine discordance between apo B and cholesterol measurements and CAC [8]. While the results from CARDIA differ from the findings in this MESA population, an important difference between these studies is the population age. CARDIA investigators measured participants' apo B, LDL-C, and non-HDL-C between ages 18-30 (mean age: 25 ± 3.6 years), and CAC measurements were taken twenty-five years later. Conversely, the MESA study population was 45-84 years at time of baseline lipid marker and prevalent CAC measurements and was approximately 56-96 years at the time of the exam 5 CAC measurement. Previous studies have reported that the association between apo B and ASCVD risk decreases with age [12, 30, 31], although sensitivity analyses excluding participants >70 years of age did not change our study results. The disparate results between CARDIA and MESA may indicate that apo B provides risk prediction benefit of CAC development in mid-life among younger individuals. Conversely, in a population that represents the target age range for ASCVD risk screening [19], and with high CAC prevalence (>45%), additional measurement of apo B beyond LDL-C or non-HDL-C may provide modest additional benefit in predicting CAC. However, discordance appeared to be of limited utility in predicting CAC after accounting for diabetes status, obesity and triglyceride levels confirming these as important risk factors associated with discordance. Whether the association between discordance and CAC is due to its correlation with these factors or discordance itself contributes to CAC development is a mechanistic question of interest that warrants further research. This observation further supports screening at early ages to reduce lifetime ASCVD risk, which was emphasized in the 2018 ACC/AHA/Multisociety cholesterol guideline [1]. Consistent with the findings in the present study, previous analyses in MESA observed that apo B did not improve CHD net reclassification when added to established CHD risk factors in the pooled cohort equation [14, 40].

Strengths and Limitations

The primary strength of this study is the use of the MESA population with its rigorous, standardized data collection and measurement procedures from multiple locations throughout the United States. In particular, the diversity of age and race/ethnicity and balance of male/female sex is a major strength of the MESA cohort. Specific to the present study, this is the first to evaluate associations between discordance using two different approaches for defining discordance and CAC in a population with ages particularly relevant for understanding risk from measured lipid biomarkers. There are several benefits of evaluating associations of potential risk/protective factors relative to CAC, including the larger number of outcomes available to evaluate and the importance of CAC as strong indicator of future ASCVD events [18, 41]. The ability to evaluate both prevalent and incident CAC, which is an important risk factor for future ASCVD, is another strength. While it is important to evaluate standard ASCVD outcomes, using CAC provided a larger number of event outcomes with which to evaluate associations. It also provides useful information on the disease process, by assessing whether discordance is present early on, whether it is associated with intermediate risk factors (e.g. CAC) and whether it may be relevant for targeting in early assessments and monitoring of ASCVD risk. While CAC is reflective of overall atherosclerotic burden and a predictor of risk for ASCVD events, it may

be that it is there are other subclinical measures associated with discordance and ASCVD risk that are more relevant and should be evaluated in future studies.

Other important limitations of this study include that much of the results are cross-sectional. Cross-sectional studies inherently have temporality issues and lack of associations could be related to the concurrent timing of exposure and outcome measurements, although, results did not differ in the analysis assessing CAC incidence or progression. Additionally, given the age of this population, there is a very high prevalence of CAC. When combined with the highly correlated nature of the lipid markers, resulting in a low prevalence of discordance, this likely limited the ability to detect associations due to smaller numbers of participants in discordant categories. However, the results still provide important data on the relationship of discordance between apo B, LDL-C and non-HDL-C and CAC prevalence, incidence and progression. Loss to follow-up due to lack of CAC measurements led to the exclusion of 521 participants, which can lead to bias when these participants differ substantially compared to those included in the analysis. There were some differences, with those excluding being more likely to already have CAC and to be older and less healthy, however, lipid measures and discordance did not differ significantly. As those with CAC were already excluded from the incidence analysis, it is more likely to have influenced the CAC progression results and it is important to acknowledge that this may have influenced study findings. The lack of apo B measurements among statin users and the 'MESA 1000' sub-cohort is also a limitation as this resulted in a smaller sample size available for analysis and as discordance among statin users may have different implications, which we were unable to evaluate. This also means that study results only apply to individuals not taking statin therapy at the time of measurement of apo B, LDL-C and non-HDL-C, so further research would be needed to evaluate associations in statin users. However, it is important to note that part of the rationale for evaluating discordance and lipid markers is to ascertain individuals not classified as high risk using traditionally measurement markers, but who could benefit from the initiation of statins making this a relevant population within which to evaluate discordance. Finally, the inclusion of triglycerides, diabetes status and BMI as covariates may represent an over-adjustment. However, presenting results adjusted for these factors is important to account for other potential mechanisms through which diabetes and BMI may influence CAC development, and to account for factors already measured as part of clinical exams, as is the case for triglycerides. In acknowledgement of this possibility we present results both with and without adjustment for these factors.

Conclusions

Our results suggest that apo B is associated with CAC, but whether the additional measurement of apo B provides added information beyond LDL-C or non-HDL-C for prediction of prevalent or incident CAC or CAC progression among older adults not taking lipid lowering medications remains unclear. Apo B discordance may still be important for predicting ASCVD-associated risk as this study did not evaluate ASCVD outcomes, but rather CAC as a biomarker of atherosclerosis, and further research is needed to confirm findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Manuscript Highlights

- Apolipoprotein B was associated with of coronary artery calcium
- Discordance low in racially/ethnically diverse adults 45 years
- Modest association: discordant apolipoprotein B and coronary artery calcium

Table 1.

Baseline characteristics of the study population for apo B discordance categories defined by residuals in the Multi-Ethnic Study of Atherosclerosis (N=4,623)

	Apo B Discordant-Low ⁴	Apo B Concordant ⁴	Apo B Discordant-High ⁴	P-Value ¹
<u>Relative to LDL-C</u>				
Overall, n (%)	1194 (25.8)	2287 (49.5)	1142 (24.7)	---
Age, mean (SD)	61.5 (10.7)	62.1 (10.4)	61.7 (10.0)	0.21
Gender (female), n (%)	684 (57.3)	1166 (51.0)	576 (50.4)	<0.001
Race/Ethnicity, n (%)				<0.001
White	463 (38.8)	833 (36.4)	390 (34.2)	
Black	425 (35.6)	685 (30.0)	220 (19.3)	
Chinese	145 (12.1)	248 (10.8)	163 (14.3)	
Hispanic	161 (13.5)	521 (22.8)	369 (32.3)	
Education, n<High School (%)	167 (14.1)	415 (18.2)	278 (14.3)	<0.001
Smoking, n (%)				0.12
Never	641 (54.0)	1136 (49.8)	559 (49.2)	
Former	397 (33.4)	848 (37.2)	425 (37.4)	
Current	150 (12.6)	296 (13.0)	153 (13.5)	
Pack-years, n (%)				0.12
Never	654 (55.6)	1169 (51.9)	579 (51.5)	
<25 pack-years	354 (30.1)	709 (31.5)	347 (30.8)	
25 pack-years	168 (14.3)	373 (16.6)	199 (17.7)	
Metabolic syndrome, n (%)	193 (16.2)	641 (28.0)	740 (64.9)	<0.001
Hypertension, n (%)	507 (42.5)	815 (35.6)	266 (23.3)	<0.001
Diabetes, n (%)	84 (7.0)	234 (10.2)	183 (16.0)	<0.001
Obese, n (%) ³	304 (25.5)	722 (31.6)	428 (24.7)	<0.001
Body mass index (kg/m ²), mean (SD)	27.3 (5.4)	28.2 (5.5)	29.2 (5.3)	<0.001
LDL-C (mg/dL), mean (SD)	120.7 (30.5)	118.4 (30.3)	121.1 (33.2)	0.02
Non-HDL-C (mg/dL), mean (SD)	139.4 (37.8)	140.4 (33.0)	160.4 (34.2)	<0.001
HDL-C (mg/dL), mean (SD)	57.7 (14.9)	51.7 (14.9)	43.3 (11.6)	<0.001
Triglycerides (mg/dL), median (IQR)	74.0 (58.0, 98.0)	103.0 (79.0, 133.0)	189.0 (149.0, 233.0)	<0.001 ²
Apo B (mg/dL), mean (SD)	96.1 (24.0)	106.1 (22.6)	127.0 (25.5)	<0.001
Total Cholesterol (mg/dL), mean (SD)	197.2 (37.6)	192.0 (33.3)	203.7 (35.6)	<0.001
CAC				
Median (IQR)	0.0 (0.0, 52.8)	0.0 (0.0, 72.0)	2.7 (0.0, 88.8)	---
>0 (%)	513 (43.0)	1082 (49.5)	590 (51.7)	<0.001
100 (%)	243 (20.4)	501 (21.9)	267 (23.4)	0.21
400 (%)	97 (8.1)	203 (8.9)	107 (9.4)	0.56
<u>Relative to non-HDL-C</u>				
Overall, n (%)	1162 (25.1)	2307 (49.9)	1154 (25.0)	---
Age, mean (SD)	61.5 (10.3)	62.1 (10.7)	61.8 (9.8)	0.18

	Apo B Discordant-Low ⁴	Apo B Concordant ⁴	Apo B Discordant-High ⁴	P-Value ¹
Gender (female), n (%)	643 (55.3)	1165 (50.5)	618 (53.6)	0.02
Race/Ethnicity, n (%)				<0.001
White	474 (40.8)	842 (36.5)	370 (32.1)	
Black	324 (27.9)	666 (28.9)	340 (29.5)	
Chinese	154 (13.3)	274 (11.9)	128 (11.1)	
Hispanic	210 (18.1)	525 (22.8)	316 (27.4)	
Education, n<High School (%)	182 (15.7)	436 (19.0)	242 (21.1)	0.01
Smoking, n (%)				0.03
Never	618 (53.3)	1153 (50.2)	565 (49.2)	
Former	377 (32.5)	861 (37.5)	432 (37.6)	
Current	165 (14.2)	283 (12.3)	151 (13.2)	
Pack-years, n (%)				0.33
Never	630 (54.7)	1190 (52.6)	582 (51.2)	
<25 pack-years	348 (30.2)	710 (31.4)	352 (31.0)	
25 pack-years	173 (15.0)	364 (16.1)	203 (17.8)	
Metabolic syndrome, n (%)	354 (30.5)	724 (31.4)	496 (43.0)	<0.001
Hypertension, n (%)	446 (38.4)	803 (34.8)	339 (29.4)	<0.001
Diabetes, n (%)	99 (8.5)	235 (10.2)	167 (14.5)	<0.001
Obese, n (%) ³	334 (28.7)	743 (32.2)	377 (32.7)	0.07
Body mass index (kg/m ²), mean (SD)	27.9 (5.4)	28.2 (5.5)	28.6 (5.5)	0.009
LDL-C (mg/dL), mean (SD)	118.4 (33.2)	117.1 (29.3)	126.0 (31.6)	<0.001
Non-HDL-C (mg/dL), mean (SD)	145.4 (39.1)	140.4 (32.9)	154.0 (35.8)	<0.001
HDL-C (mg/dL), mean (SD)	53.2 (15.1)	51.7 (14.9)	48.2 (14.9)	<0.001
Triglycerides (mg/dL), median (IQR)	106.0 (75.0, 162.0)	101.0 (72.0, 146.0)	129.0 (89.0, 178.0)	<0.001 ²
Apo B (mg/dL), mean (SD)	97.7 (24.8)	105.6 (22.7)	125.9 (26.1)	<0.001
Total Cholesterol (mg/dL), mean (SD)	198.6 (38.9)	192.1 (32.9)	202.2 (35.3)	<0.001
CAC				
Median (IQR)	0.0 (0.0, 55.1)	0.0 (0.0, 76.6)	0.0 (0.0, 78.5)	---
>0 (%)	496 (42.7)	1118 (48.5)	571 (49.5)	0.001
100 (%)	238 (20.5)	512 (22.2)	261 (22.6)	0.40
400 (%)	94 (7.8)	214 (9.3)	99 (8.6)	0.48

Abbreviations: apolipoprotein B, apo B; coronary artery calcium, CAC; high-density lipoprotein cholesterol, HDL-C; low-density lipoprotein cholesterol, LDL-C; interquartile range, IQR; non-high-density lipoprotein cholesterol, non-HDL-C; standard deviation, SD.

¹P-value from ANOVA for continuous variables and X² test for categorical.

²P-value from ANOVA using log-transformed triglyceride.

³Defined as body mass index ≥ 30 kg/m²

⁴Discordant low=residual difference <25th percentile; discordant high=residual difference >75th percentile; concordant=residual difference 25th-75th percentile

Table 2.

Agreement of measured Apo B with LDL-C and non-HDL-C

	Weighted Kappa		Spearman Correlation	
	k-statistic¹	95% CI	Spearman Correlation r	P-Value
Apo B vs. LDL-C	0.65	(0.64, 0.67)	0.85	<0.001
Apo B vs. non-HDL-C	0.78	(0.77, 0.79)	0.93	<0.001
LDL-C vs. non-HDL-C	0.73	(0.72, 0.74)	0.91	<0.001

Abbreviations: apolipoprotein B, apo B; low-density lipoprotein cholesterol, LDL-C; non-high-density lipoprotein cholesterol, non-HDL-C.

¹ k-statistic between 0.61-0.80 is considered moderate agreement.

² Chicchetti-Allison weights were utilized.

Table 3.

Associations between baseline apo B, LDL-C, non-HDL-C and apo B concordance/discordance with LDL-C and non-HDL-C using residuals and medians with prevalent CAC >0 at baseline (N=4,623)

		Minimally-adjusted ¹		Fully-adjusted ²	
	N CAC>0/Total N	Prevalence Ratio (95% CI)	P	Prevalence Ratio (95% CI)	P
<i>Individual Measures</i>					
Apo B Tertiles					
<96.9 mg/dl	637/1538	Ref		Ref	
96.9 - 118.0 mg/dl	741/1540	1.13 (1.06, 1.20)	<0.001	1.10 (1.04, 1.17)	0.002
>118.0 mg/dl	807/1545	1.25 (1.17, 1.32)	<0.001	1.21 (1.13, 1.29)	<0.001
LDL-C Tertiles					
<106.0 mg/dl	648/1494	Ref		Ref	
106.0 - 129.0 mg/dl	735/1537	1.11 (1.04, 1.17)	<0.001	1.09 (1.03, 1.15)	0.005
>129.0 mg/dl	802/1592	1.19 (1.12, 1.26)	<0.001	1.17 (1.10, 1.24)	<0.001
Non-HDL-C Tertiles					
<129.0 mg/dl	639/1512	Ref		Ref	
129.0 - 136.0 mg/dl	733/1529	1.12 (1.05, 1.19)	<0.001	1.09 (1.02, 1.15)	0.007
>136.0 mg/dl	813/1582	1.21 (1.14, 1.29)	<0.001	1.17 (1.09, 1.25)	<0.001
<i>Discordance by Residual³</i>					
Apo B/LDL-C					
Discordant Low	513/1194	0.95 (0.90, 1.01)	0.12	0.98 (0.92, 1.04)	0.46
Concordant	1082/2287	Ref		Ref	
Discordant High	590/1142	1.08 (1.02, 1.14)	0.01	1.02 (0.95, 1.08)	0.61
Apo B/non-HDL-C					
Discordant Low	496/1162	0.93 (0.88, 0.99)	0.03	0.93 (0.87, 0.99)	0.02
Concordant	1118/2307	Ref		Ref	
Discordant High	571/4623	1.05 (0.99, 1.12)	0.07	1.03 (0.98, 1.10)	0.24

Abbreviations: apolipoprotein B, apo B; coronary artery calcium, CAC; high-density lipoprotein cholesterol, HDL-C; low-density lipoprotein cholesterol, LDL-C; non-high-density lipoprotein cholesterol, non-HDL-C.

¹Prevalence ratio regression model adjusted for age, sex and race/ethnicity (White, Black, Chinese, Hispanic), smoking status, and hypertension.

²Prevalence ratio regression model adjusted for age, sex, race/ethnicity (White, Black, Chinese, Hispanic), smoking status (never, former/current), hypertension (yes/no), body mass index, diabetes status (yes/no), triglycerides (log-transformed).

³Discordant low=residual difference <25th percentile; discordant high=residual difference >75th percentile; concordant=residual difference 25th-75th percentile

Table 4

Associations between baseline apo B, LDL-C, non-HDL-C, and apo B concordance/discordance with LDL-C and non-HDL-C with incident CAC > 0 through examination 5 among participants with CAC = 0 at the baseline (N = 2216)

		<u>Minimally-adjusted</u>		<u>Fully-adjusted</u>	
	N CAC > 0/Total N	RR (95% CI) [*]	P	RR (95% CI) [†]	P
Individual measures					
Apo B tertiles					
<96.9 mg/dL	203/811	Reference		Reference	
96.9–118.0 mg/dL	237/735	1.30 (1.12, 1.52)	<.001	1.27 (1.08, 1.48)	.003
>118.0 mg/dL	270/670	1.53 (1.32, 1.78)	<.001	1.48 (1.27, 1.74)	<.001
LDL-C tertiles					
<106.0 mg/dL	204/766	Reference		Reference	
106.0–129.0 mg/dL	229/723	1.21 (1.04, 1.41)	.01	1.20 (1.03, 1.39)	.02
>129.0 mg/dL	277/727	1.41 (1.22, 1.62)	<.001	1.37 (1.19, 1.58)	<.001
Non-HDL tertiles					
<129.0 mg/dL	197/782	Reference		Reference	
129.0–136.0 mg/dL	244/734	1.33 (1.15, 1.55)	<.001	1.29 (1.10, 1.51)	.001
>136.0 mg/dL	269/700	1.45 (1.25, 1.68)	<.001	1.38 (1.18, 1.62)	<.001
Discordance by residual [‡]					
Apo B/LDL-C					
Discordant low	168/617	0.85 (0.73, 0.99)	.04	0.86 (0.73, 1.01)	.06
Concordant	357/1098	Reference		Reference	
Discordant high	185/501	1.08 (0.94, 1.24)	.30	1.00 (0.84, 1.19)	.99
Apo B/non-HDL-C					
Discordant low	178/604	0.93 (0.79, 1.08)	.32	0.91 (0.78, 1.06)	.23
Concordant	336/1089	Reference		Reference	
Discordant high	196/523	1.17 (1.02, 1.34)	.03	1.13 (0.98, 1.30)	.09

Apo B, apolipoprotein B; CAC, coronary artery calcium; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol.

^{*} Relative risk regression model adjusted for age, sex, and race/ethnicity (white, black, Chinese, Hispanic), smoking status (never, former/current), hypertension (yes/no).

[†] Relative risk regression model adjusted for age, sex, and race/ethnicity (white, black, Chinese, Hispanic), smoking status (never, former/current), hypertension (yes/no), body mass index, diabetes (yes/no), triglycerides (log-transformed).

[‡] Discordant low = residual difference, <25th percentile; discordant high = residual difference >75th percentile; concordant = residual difference 25th–75th percentile.

Table 5

Odds ratios for associations of baseline apo B, LDL-C, non-HDL-C and apo B concordance/discordance with LDL-C and non-HDL-C with CAC progression (absolute change/year) relative to no progression through visit 5 (N=3947)

	Minimally adjusted				Fully adjusted			
	>0-<75th percentile*		>75th percentile*		>0 - <75th percentile*		>75th percentile*	
	N>0-<75th	N>75th	OR (95% CI) [†]	OR (95% CI) [†]	OR (95% CI) [‡]	OR (95% CI) [‡]	OR (95% CI) [§]	P [‡]
Individual measures								
Apo B tertiles								
<96.9 mg/dL	401	293	Ref	Ref	Ref	Ref	Ref	<.001
96.9–118.0 mg/dL	439	401	1.38 (1.14, 1.66)	1.84 (1.47, 2.29)	1.36 (1.12, 1.65)	1.72 (1.37, 2.16)		
>118.0 mg/dL	496	409	1.97 (1.63, 2.39)	2.58 (2.06, 3.24)	1.92 (1.56, 2.37)	2.37 (1.86, 3.03)		
LDL-C tertiles								
<106.0 mg/dL	387	323	Ref	Ref	Ref	Ref	Ref	<.001
106.0–129.0 mg/dL	440	376	1.33 (1.10, 1.61)	1.44 (1.16, 1.80)	1.31 (1.08, 1.59)	1.39 (1.11, 1.74)		
>129.0 mg/dL	509	404	1.81 (1.50, 2.19)	2.03 (1.63, 2.53)	1.76 (1.46, 2.14)	1.98 (1.58, 2.48)		
Non-HDL tertiles								
<129.0 mg/dL	385	311	Ref	Ref	Ref	Ref	Ref	<.001
129.0–136.0 mg/dL	447	383	1.49 (1.23, 1.80)	1.73 (1.39, 2.16)	1.46 (1.20, 1.78)	1.56 (1.24, 1.97)		
>136.0 mg/dL	504	409	1.91 (1.58, 2.32)	2.25 (1.80, 2.81)	1.87 (1.51, 2.31)	2.04 (1.60, 2.61)		
Discordance by residual [§]								
Apo B/LDL-C								
Discordant low	325	255	0.85 (0.71, 1.03)	0.92 (0.74, 1.14)	0.90 (0.75, 1.10)	1.03 (0.82, 1.29)		.69
Concordant	679	537	Ref	Ref	Ref	Ref		
Discordant high	332	311	1.13 (0.93, 1.38)	1.39 (1.11, 1.72)	1.00 (0.80, 1.25)	1.10 (0.85, 1.41)		.02
Apo B/non-HDL-C								
Discordant low	318	255	0.88 (0.73, 1.06)	0.95 (0.76, 1.18)	0.87 (0.72, 1.04)	0.93 (0.74, 1.16)		
Concordant	679	544	Ref	Ref	Ref	Ref		
Discordant high	339	304	1.21 (1.00, 1.46)	1.46 (1.17, 1.81)	1.16 (0.95, 1.41)	1.35 (1.08, 1.69)		

apo B, apolipoprotein B; CAC, coronary artery calcium; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol.

* 75th percentile cut-point = 16.44 Agatstons increase/year.

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[†] Multinomial logistic regression model with log-transformed CAC as dependent variable adjusted for age, sex, race/ethnicity (White, Black, Chinese, Hispanic), smoking status (never, former/current), hypertension (yes/no).

[‡] Wald χ^2 for overall type 3 analysis of effects.

[§] Discordant low = residual difference <25th percentile; discordant high = residual difference >75th percentile; concordant = residual difference 25th–75th percentile.

^{||} Multinomial logistic regression model with log-transformed CAC as dependent variable adjusted for age, sex, race/ethnicity (White, Black, Chinese, Hispanic), smoking status (never, former/current), hypertension (yes/no), body mass index, diabetes (yes/no).