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Original Research

Discordantly normal ApoB relative to elevated LDL-C in persons with metabolic disorders: A marker of atherogenic heterogeneity



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ABSTRACT

Objective: A significant proportion of persons with metabolic syndrome (MetS), prediabetes, or type 2 diabetes (T2D) do not develop atherosclerotic cardiovascular disease (ASCVD).

We sought to determine whether discordantly normal apolipoprotein B (ApoB) relative to elevated LDL-C may help to explain heterogeneity in ASCVD risk among persons with metabolic disorders.

Methods: There were 278 Bogalusa Heart Study participants with MetS (n=95), prediabetes (n=233), or T2D (n=31) and LDL-C ≥ 100 mg/dL who were free of carotid plaque at baseline (2001-02) and underwent carotid ultrasound at follow-up (2013-16). Multivariable modified Poisson regression estimated the long-term absence of carotid plaque for lower ApoB, continuously and categorically.

Results: Participants were on average 36.1 years old at baseline, 61.5% were women, and 31.7% were black. A total of 50.7% had discordantly normal ApoB (< 90 mg/dL) and the mean ApoB and LDL-C concentrations were 91.6 mg/dL and 137.7 mg/dL, respectively. In addition to having higher HDL-C and lower triglyceride values, individuals with ApoB < 90 were more likely to maintain persistent absence of plaque compared to those with ApoB ≥ 90 (73.1% versus 58.4%, $p=0.01$). Contrastingly, there was no significant difference in the proportion of individuals who remained free of plaque with increasing LDL-C ($p=0.45$). Independent of traditional risk factors including LDL-C, each 10 mg/dL lower ApoB (RR=1.11, 95% CI: 1.03-1.19) and ApoB < 90 (RR=1.22, 95% CI: 1.00-1.43) were significantly associated with the persistent absence of carotid plaque.

Conclusions: One-half of young persons with metabolic disorders and elevated LDL-C had discordantly normal ApoB and a low burden of carotid atherosclerosis over 13 years, suggesting that ApoB better represents the atherogenic lipid burden compared to LDL-C in this patient population. These results suggest a utility for assessing whether routine ApoB measurement can improve ASCVD risk stratification in young persons with metabolic disorders who have high triglycerides and low HDL-cholesterol.

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1. Introduction

An estimated 88 million (34.5%) and 34.2 million (10.5%) individuals living in the United States have prediabetes or type 2 diabetes, respectively [1]. An elevated fasting plasma glucose is also a pillar of metabolic syndrome, which affects approximately one-quarter of Americans and is a precursor to diabetes [2]. While metabolic syndrome, prediabetes, and type 2 diabetes increase the risk for atherosclerotic cardiovascular disease (ASCVD), a significant proportion of these individuals do not develop subclinical and/or clinical ASCVD over long-term (10-15 years) follow-up [3–5]. For example, approximately 40% of middle-aged persons with metabolic syndrome or type 2 diabetes have an absence of coronary artery calcium and a low risk for future ASCVD events [3,6]. Furthermore, though individuals with metabolic syndrome experience a higher risk of coronary heart disease for a given low-density lipoprotein cholesterol (LDL-C) concentration compared to the general population [7], the mechanisms that help to explain why a certain subgroup of persons with metabolic syndrome and elevated LDL-C do not develop significant atherosclerosis remains largely unexplored.

In certain scenarios, LDL-C may be an imprecise estimator of atherogenic particle concentration and thus ASCVD risk, particularly among persons with metabolic disorders who have elevated triglycerides and/or blood glucose [8]. Overall, a serum LDL-C measure reflects the *aggregate concentration* of cholesterol inside LDL, intermediate-density lipoprotein (IDL), and lipoprotein(a) [Lp(a)] particles [9]. Contrastingly, the apolipoprotein B (ApoB) measure captures the *number* of LDL, IDL, Lp(a), and very low-density lipoprotein (VLDL) particles, as there is one ApoB molecule for every lipoprotein particle [9]. Thus, ApoB is the primary apolipoprotein associated with non-high-density lipoprotein cholesterol and can be used as an estimate of atherogenic particle burden [10]. The discordance between ApoB and LDL-C may affect a considerable proportion of persons with metabolic disorders, as at least 7% of individuals with diabetes and 15% of those with metabolic syndrome have discordantly normal ApoB relative to LDL-C [11]. However, the association between discordantly normal ApoB and the long-term absence of atherosclerosis (healthy cardiovascular aging) [12] has not been studied among younger persons with metabolic disorders whom have elevated LDL-C.

Among participants free of carotid plaque at baseline but whom had metabolic syndrome, prediabetes, or type 2 diabetes and elevated LDL-C, we therefore sought to identify: 1) the proportion of persons with discordantly normal ApoB, 2) the proportion with persistent absence of carotid plaque across both ApoB and LDL-C thresholds, and 3) whether lower ApoB was independently associated with the long-term absence of carotid plaque. Identifying whether discordantly normal ApoB helps to explain a lower burden of atherosclerosis among certain individuals with elevated LDL-C and metabolic disorders may help improve precision in ASCVD risk assessment and preventive care.

2. Materials and methods

2.1. Study population

The Bogalusa Heart Study is an epidemiological study examining the natural history of cardiovascular disease across the lifespan among residents of Bogalusa, Louisiana. From 1973 to today, 9 surveys were conducted in children and adolescents aged 4 to 17 years, and 11 surveys were conducted among adults aged 18 to 51 years who had been examined previously as children [13]. There were 595 participants who underwent carotid ultrasound imaging at the baseline visit (2001-02) and follow-up visit (2013-16) with all available covariables of interest. Participants who had baseline carotid plaque (n=47), triglycerides >400 mg/dL (n=8), on lipid-lowering therapy (n=17), participants who did not have metabolic syndrome, prediabetes, or type 2 diabetes (n=165), and those with baseline LDL-C <100 mg/dL (n=80) were excluded resulting in a cohort of 278 individuals for the analysis (Supplemental Fig-

ure). All study participants provided written informed consent at each Bogalusa Heart Study examination, and study protocols were approved by the Institutional Review Board of the Tulane University Health Sciences Center.

Metabolic syndrome was defined using the National Cholesterol Education Program ATP III guidelines [14], characterized by any three out of the five following traits: 1) waist circumference >102 cm in men or >88 cm in women; 2) serum triglycerides \geq 150 mg/mL or drug treatment for elevated triglycerides; 3) serum HDL-C <40 mg/dL in men or <50 mg/dL in women; 4) blood pressure \geq 130/85 mmHg or drug treatment for elevated blood pressure; and 5) fasting blood glucose \geq 100 mg/dL or drug treatment for elevated blood glucose. Prediabetes was defined as an HbA1c between 5.7% to 6.4%, or a fasting plasma glucose between 100 mg/dL to 125 mg/dL [15]. Type 2 diabetes was defined as an HbA1c \geq 6.5%, fasting plasma glucose \geq 126 mg/dL or utilization of glucose-lowering therapy [15]. An elevated LDL-C (\geq 100 mg/dL) was defined according to the American Diabetes Association/American College of Cardiology (ADA/ACC) Consensus Treatment Goals in Patients with Cardiometabolic Risk and Lipoprotein Abnormalities [16].

2.2. General clinical and laboratory variables

All covariate data were collected at baseline. Rigorous protocols were employed to collect clinical and sociodemographic data on BHS participants [17]. Validated questionnaires were used to obtain sociodemographic variables including, age, race, and sex. Waist circumference was measured in triplicate from the lowest rib to the superior border of the iliac crest using a flexible tape [18]. Systolic and diastolic pressure were measured in triplicate using mercury sphygmomanometers on the right arm of study participants in a seated position, and the average of the three readings was used.

Participants were instructed to undergo a 12-hour fast prior to measurement of blood lipids and glucose. Total cholesterol and triglycerides were assessed using enzymatic procedures (Abbott VP laboratories, Chicago, IL). Serum HDL-C and ApoB were measured using agar-agarose gel electrophoresis and heparin-calcium precipitation procedures [19]. Serum values for LDL-C were calculated using the Friedewald equation [20].

2.3. Carotid ultrasonography

Experienced and trained technicians completed sonographic examinations on participants in the supine position and head rotated 10° away from the side examined. Images were captured using a Toshiba ultrasound instrument (Xario, SSA-660A, Toshiba America Medical Systems, Tustin, CA) with a linear array transducer of 7.5 MHz, following a detailed protocol [21]. Ultrasound B-mode measurements included maximum intima-media thickness (IMT) at diastole from the far walls of the common carotid artery, carotid bulb and internal carotid artery segments bilaterally. The mean of the maximum carotid IMT readings of the 3 left and 3 right far walls of the common, bulb, and internal segments were used for analysis. The presence of a carotid artery atherosclerotic plaque was defined by a distinct focal wall thickening >1.5 mm [22].

2.4. Statistical Analysis

For study sample characteristics, continuous variables were presented as means and standard deviations and categorical variables were presented as percentages. Differences between categorical variables were evaluated through the Chi-square test. Differences in normally and non-normally distributed continuous variables were assessed through the Student's t-test and Wilcoxon signed-rank test, respectively. Fisher's exact test was used to assess differences in categorical variables when cell sizes had <5 observations.

Predictor variables, including ApoB and LDL-C, were evaluated both continuously and as ideal or non-ideal categorical variables to assess

Table 1
Characteristics of 278 BHS Participants with metabolic disorders, LDL-C ≥ 100 mg/dL and baseline absence of significant carotid artery atherosclerosis.

Variable	All (n=278)	Apolipoprotein-B <90 mg/dL (n=141)	Apolipoprotein-B ≥ 90 mg/dL (n=137)	P-Value
Sociodemographic				
Age, mean \pm SD, years	36.1 \pm 4.5	35.8 \pm 4.6	36.5 \pm 4.5	0.19
Women, %	61.5	70.9	51.8	0.001
African American, %	31.7	36.2	27.0	0.10
Cardiovascular Imaging				
Baseline Carotid Intima-Media Thickness, mean \pm SD, mm	0.8 \pm 0.1	0.8 \pm 0.1	0.8 \pm 0.1	0.01
Persistent Absence of Carotid Plaque, %	65.8	73.1	58.4	0.01
ASCVD Risk				
10-year ASCVD Risk, median (Q1, Q3), % \dagger	1.2 (0.6, 2.7)	0.9 (0.4, 1.7)	1.8 (0.9, 3.9)	<0.001
Never Smokers, %	61.9	66.0	57.7	0.15
Waist Circumference, mean \pm SD, cm	96.6 \pm 16.5	94.1 \pm 16.9	99.3 \pm 15.8	0.008
Systolic Blood Pressure, mean \pm SD, mmHg	116.8 \pm 13.3	114.4 \pm 11.7	119.2 \pm 14.4	0.003
Diastolic Blood Pressure, mean \pm SD, mmHg	79.2 \pm 9.7	77.4 \pm 8.9	81.1 \pm 10.3	0.002
Antihypertensive Medication, %	9.0	7.1	11.0	0.26
Total Cholesterol, mean \pm SD, mg/dL	201.5 \pm 32.7	184.2 \pm 21.9	219.4 \pm 32.3	<0.001
HDL Cholesterol, mean \pm SD, mg/dL	45.8 \pm 10.4	47.0 \pm 9.7	44.5 \pm 11.0	0.04
LDL Cholesterol, mean \pm SD, mg/dL	137.7 \pm 26.9	124.1 \pm 17.7	151.7 \pm 27.6	<0.001
LDL Cholesterol ≥ 130 mg/dL, %	51.4	28.4	75.2	<0.001
LDL Cholesterol ≥ 160 mg/dL, %	18.0	2.8	33.6	<0.001
LDL Cholesterol ≥ 190 mg/dL, %	5.0	0.0	10.2	<0.001
Apolipoprotein B, mean \pm SD, mg/dL	91.6 \pm 19.8	76.5 \pm 8.5	107.1 \pm 15.8	<0.001
Triglycerides, median (IQR), mg/dL	112.5 (80.0, 150.0)	87.0 (67.0, 113.0)	141.0 (112.0, 205.0)	<0.001
Lipid-Lowering Medication at Follow-Up, %	18.0	10.6	25.6	0.001
Fasting Blood Glucose, median (IQR), mg/dL	83.0 (76.0, 91.0)	82.0 (75.0, 90.0)	84.0 (77.0, 93.0)	0.18
Glycated Hemoglobin A1c, median (IQR), %	5.9 (5.8, 6.1)	5.9 (5.8, 6.2)	5.9 (5.8, 6.1)	0.14
Pre-Diabetes, %	83.8	87.9	80.0	0.06
Type 2 Diabetes, %	11.2	12.1	10.2	0.63
Glucose-Lowering Medication, %	2.2	2.1	2.2	>0.99
Metabolic Syndrome, %	34.2	23.4	45.3	<0.001

All variables presented are baseline values unless otherwise noted.

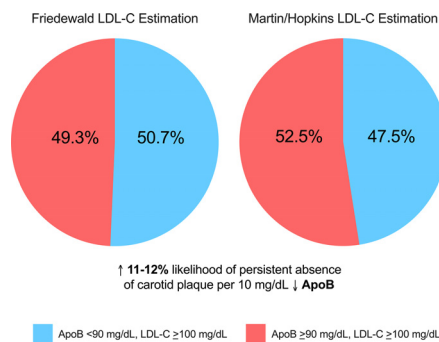
their association with persistent absence of carotid plaque. Categories included, never smoking, waist circumference <102 cm for men and <88 cm for women, systolic blood pressure <120 mm Hg and diastolic blood pressure <80 mm Hg, fasting blood glucose <100 mg/dL, fasting serum triglycerides <150 mg/dL, high-density lipoprotein-cholesterol ≥ 40 mg/dL in men and ≥ 50 mg/dL in women, and the absence of primary hypercholesterolemia (LDL-C <160 mg/dL). Normal ApoB (<90 mg/dL) was defined according to the ADA/ACC Consensus Treatment Goals in Patients with Cardiometabolic Risk and Lipoprotein Abnormalities [16]. Participants on antihypertensive medication and/or glucose-lowering therapy were categorized as non-ideal regardless of respective ASCVD risk factor values. Due to the young average age of sample population (<40 years), an age of 40 for all Bogalusa participants was used to calculate the pooled cohort equations 10-year risk for an ASCVD event at baseline [23].

Modified Poisson regression with robust error variance [24] estimated the likelihood of long-term absence of carotid plaque according to lower ApoB, independent from traditional ASCVD risk factors. Covariates adjusted for included age, sex, race, smoking status, waist circumference, fasting blood glucose, serum triglycerides, HDL-C, LDL-C, systolic blood pressure, diastolic blood pressure and glucose lowering- and blood pressure-lowering medications. We performed two sensitivity analyses: 1) performing analyses using the Martin/Hopkins equation to calculate LDL-C [25], and 2) additionally adjusting for lipid-lowering medication at follow-up.

Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC). All hypothesis tests were two-sided. We used an alpha threshold of 0.05 for detecting differences in descriptive statistics and for detecting significant relative risk ratios in regression models.

3. Results

Participants were on average 36.1 years old at baseline, 171 (61.5%) were women, 88 (31.7%) were African American, and 183 (65.8%) remained free of carotid plaque over 12.8 years of follow-up (Table 1).



Central Illustration. Proportion of discordantly normal ApoB relative to elevated LDL-C according to the Friedewald and Martin/Hopkins equations.

There were 95 (34.2%) persons with metabolic syndrome, 233 (83.8%) with prediabetes, and 31 (11.2%) with type 2 diabetes. The mean ApoB and LDL-C concentrations of the sample were 91.6 \pm 19.8 mg/dL and 137.7 \pm 26.9 mg/dL, respectively. There were 141 (50.7%) of participants with elevated LDL-C who had ApoB <90 mg/dL, of whom 31 (22%) had ApoB <70 mg/dL (Fig. 1A). Among the 137 (49.3%) participants with ApoB >90 mg/dL, 12 (8.8%) had ApoB >130 mg/dL (Fig. 1B). The proportion of participants who had discordantly normal ApoB relative to LDL-C was similar when using the Martin/Hopkins equation (47.5%) versus the Friedewald estimation of LDL-C (50.7%) (Central Illustration and Supplemental Table 1).

Persons with ApoB <90 mg/dL had significantly higher HDL-C (47.0 mg/dL versus 44.5 mg/dL, $p=0.04$) and lower serum triglycerides (87.0 mg/dL versus 141.0 mg/dL, $p<0.001$) compared to those with ApoB >90 mg/dL. There were no significant differences in the proportion with prediabetes or type 2 diabetes between those with concordant versus discordantly normal ApoB. Participants with ApoB <90 mg/dL were less likely to have metabolic syndrome (23.4% versus 45.3%, $p<0.001$) and had a lower 10-year ASCVD risk at follow-up (0.9% versus 1.8%, $p<0.001$).

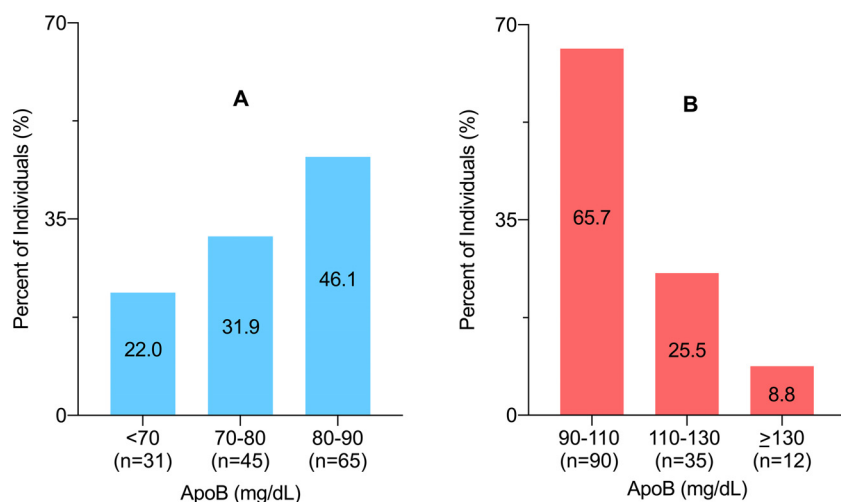


Fig. 1. A. Distribution of ApoB among persons with metabolic disorders, elevated LDL-C and ApoB <90. **Fig. 1. B.** Distribution of ApoB among persons with metabolic disorders, elevated LDL-C and ApoB ≥90.

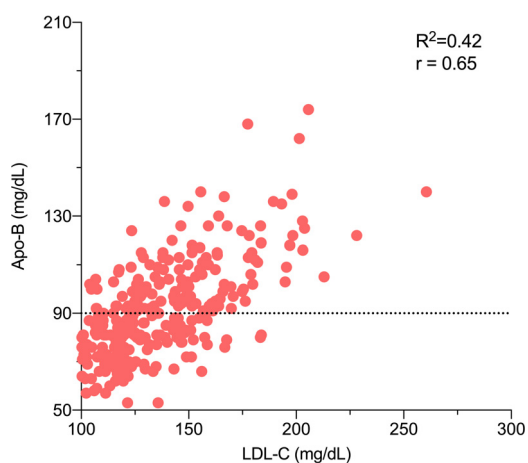


Fig. 2. Correlation between ApoB and LDL-C.

Overall, LDL-C explained 42% of the variability in ApoB as the two lipoprotein measures shared only a moderate positive correlation ($r=0.65$; Fig. 2). Individuals with ApoB <90 mg/dL were more likely to have persistent absence of carotid plaque compared to those with ApoB ≥90 (73.1% versus 58.4%, $p=0.01$; Fig. 3A). Contrastingly, there was no significant difference in the proportion of persons who remained free of carotid plaque with increasing LDL-C (Fig. 3B).

After adjusting for individual ASCVD risk factors, including LDL-C, each 10 mg/dL lower ApoB (RR=1.11, 95% CI: 1.03-1.19, $p=0.008$) was significantly associated with persistent absence of carotid plaque (Table 2). Similarly, individuals with an ApoB <90 mg/dL were 22% more likely to maintain long-term absence of plaque compared to those with ApoB ≥90 mg/dL (RR=1.22, 95% CI: 1.00-1.49, $p=0.05$). Contrastingly, lower LDL-C (RR=0.99, 95% CI: 0.94-1.03, per 10 mg/dL lower) and LDL-C <160 (RR=0.93, 95% CI: 0.71-1.22, $p=0.61$) were not significantly associated with the non-development of carotid plaque. Consistent with models using the Friedewald estimation of LDL-C, lower ApoB significantly predicted the persistent absence of carotid plaque (RR=1.12, 95% CI: 1.03-1.21, $p=0.007$, per 10 mg/dL lower) after adjusting for LDL-C calculated by the Martin/Hopkins equation and other ASCVD risk factors (Supplemental Table 2). Point estimates and significance values for lower/normal ApoB with persistent absence of plaque also remained consistent after additionally adjusting for lipid-lowering therapy at follow-up (Supplemental Table 3).

4. Discussion

Among individuals with LDL-C ≥100 mg/dL and metabolic syndrome, prediabetes, or type 2 diabetes, we found that one-half had ApoB <90 mg/dL and an associated lower burden of subclinical atherosclerosis through early adulthood. While LDL-C did not associate with persistent absence of carotid plaque, lower ApoB independently conferred a higher likelihood of non-development of carotid plaque. Furthermore, there was only a moderate correlation between the lipoprotein markers in this cohort of participants, with LDL-C explaining 42% of the variability in ApoB. These results provide further evidence supporting that ApoB better represents the atherogenic lipid burden among persons with metabolic disorders compared to LDL-C. Overall, our findings suggest a utility for assessing whether routine ApoB measurement can help to improve ASCVD risk stratification in young persons with metabolic disorders who may have high triglycerides and low HDL-cholesterol.

The novelty of the current study is that our findings demonstrate that normal ApoB may be an upstream negative risk factor that can predict the absence of atherosclerosis and provide useful information for ASCVD risk stratification *prior* to arterial imaging in middle age. While elevated ApoB (≥130 mg/dL in the general population) has traditionally been regarded as a risk enhancer [26], we show that normal ApoB (<90 mg/dL) may be a potential negative risk factor among young persons with metabolic disorders. Negative risk factors may be valuable to help guide the initiation and intensity of lifestyle and/or primary prevention pharmacotherapy among younger persons at-risk for premature atherosclerosis because of the strong reliance of current clinical ASCVD risk equations on age. In particular, negative risk factors provide a meaningful reduction in risk and may be used as additional data points to support lifestyle management rather than pharmacotherapy for the primary prevention of ASCVD [27]. Though CAC=0 is the most robust negative risk factor for ASCVD events and mortality [28], the absence of carotid plaque may also provide a meaningful post-test reduction in risk, as the absence of carotid plaque confers a lower risk of incident ASCVD events [29]. The mean baseline age of the current study was 36, an age group that is not included in the pooled cohort equations ASCVD risk calculations and where the measurement of CAC may be less helpful due to the very low prevalence of vascular calcification [30].

As the current approaches for ASCVD risk assessment in persons with metabolic disorders continue to evolve, our study is among the first to assess the prognostic utility of normal ApoB on subclinical atherosclerosis outcomes in this patient population. While statin therapy will undoubtedly remain a cornerstone of primary prevention, our findings demonstrate that there may be more subtleties to risk stratification

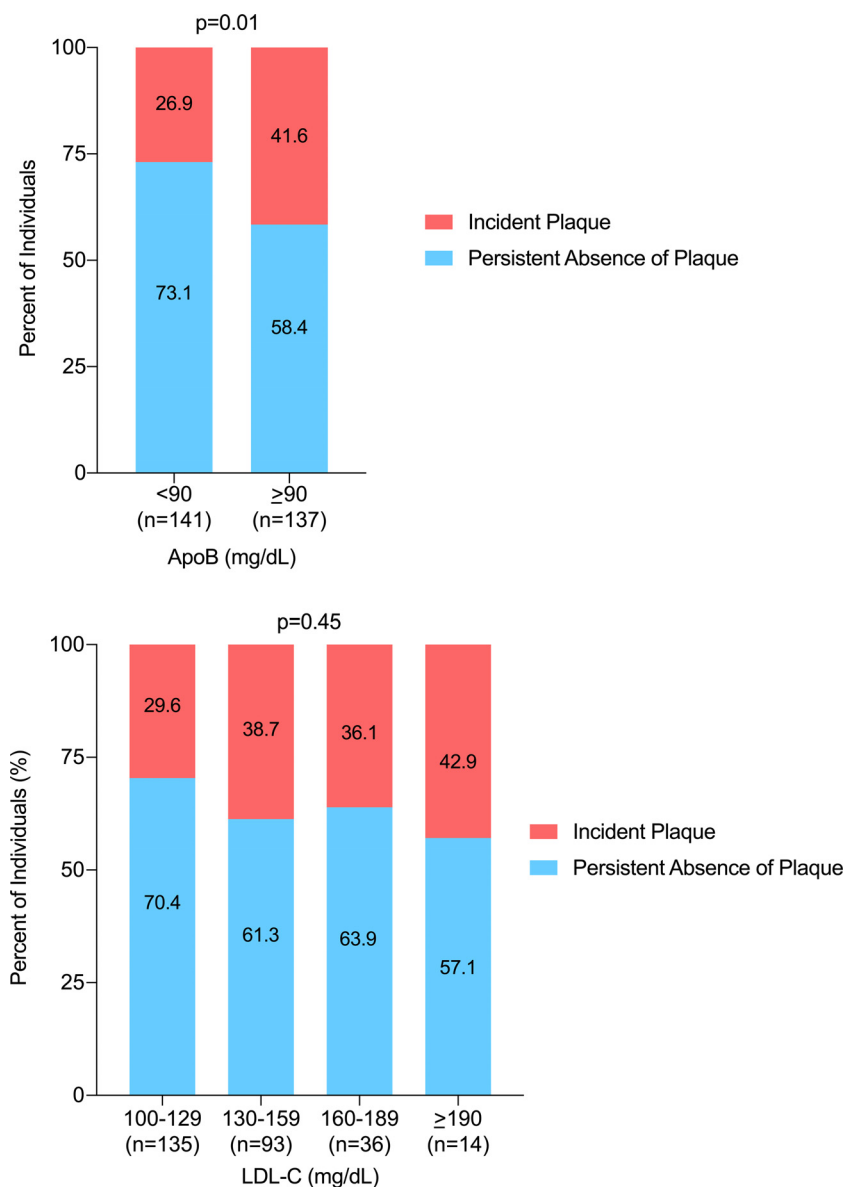


Fig. 3. A. Proportion of persistent absence of carotid plaque according to ApoB. Fig. 3. B. Proportion of persistent absence of carotid plaque according to LDL-C thresholds.

Table 2
Associations of ApoB and LDL-C with the persistent absence of carotid plaque in persons with elevated LDL-C and metabolic disorders.

Model	All (n=278)		Metabolic Syndrome* (n=95)		Prediabetes or Type 2 Diabetes* (n=264)	
	Relative Risk (95% CI)	P-Value	Relative Risk (95% CI)	P-Value	Relative Risk (95% CI)	P-Value
Continuous Predictor Model[†]						
ApoB, per 10 mg/dL lower	1.11 (1.03, 1.19)	0.008	1.18 (1.02, 1.38)	0.03	1.12 (1.03, 1.21)	0.005
LDL-C, per 10 mg/dL lower	0.99 (0.94, 1.03)	0.54	0.95 (0.87, 1.02)	0.17	0.99 (0.95, 1.04)	0.78
Categorical Predictor Model[†]						
ApoB <90 mg/dL	1.22 (1.00, 1.49)	0.05	1.40 (0.99, 1.98)	0.06	1.24 (1.00, 1.53)	0.04
LDL-C <160 mg/dL	0.93 (0.71, 1.22)	0.61	0.88 (0.59, 1.33)	0.56	0.90 (0.68, 1.19)	0.47

* Not mutually exclusive.

[†] Age, sex, race, never smoker, glucose-lowering medication, blood pressure-lowering medication, and continuous values for waist circumference, fasting blood glucose, serum triglycerides, systolic blood pressure, diastolic blood pressure, HDL-C, LDL-C, and ApoB.[‡] Age, sex, race, never smoker, glucose-lowering medication, blood pressure-lowering medication, waist circumference <102 cm in men and <88 cm in women, fasting blood glucose <100 mg/dL, serum triglycerides <150 mg/dL, systolic blood pressure <120 mmHg and diastolic blood pressure <80 mmHg, HDL-C ≥40 mg/dL in men and ≥50 mg/dL in women, LDL-C <160 mg/dL, and ApoB <90 mg/dL.

among those who harbor traditional risk factors, including elevated LDL-C and early signs of insulin resistance. Given that the absence of carotid plaque is associated with a low risk for a future ASCVD event through older age [29], future interventional studies are required to determine whether the subgroup of younger persons with metabolic disorders who have ApoB <90 mg/dL may be initially managed with aggressive lifestyle modification. However, a significant proportion (27%) of individuals with metabolic disorders and ApoB <90 in the current study still developed incident carotid plaque over the 12.8-year follow-up, therefore it is unknown as to whether this ApoB cut-point can be used clinically to guide primary ASCVD prevention care.

Individuals with ApoB <90 mg/dL were 22% more likely to maintain persistent absence of carotid plaque through middle age, even in the setting of chronically elevated LDL-C. Furthermore, there was an 11% higher likelihood of long-term absence of carotid plaque per 10 mg/dL lower ApoB. Our findings add to previous studies that have demonstrated an association between discordantly normal ApoB relative to LDL-C and CAC as well as ASCVD events in various populations. For example, 75% of young adults in the CARDIA study with ApoB <88.9 mg/dL and LDL-C >107.0 mg/dL had CAC=0, and there was no significant difference in the risk of CAC >0 for participants with elevated versus non-elevated LDL-C among those with low ApoB [9]. In women with elevated LDL-C but normal ApoB, the risk of ASCVD events was overestimated such that this latter group had a 41% lower hazard of ASCVD compared to women with concordantly high LDL-C and ApoB [31]. We build on such studies and show that one-half of individuals with metabolic syndrome, prediabetes, or type 2 diabetes have discordantly normal ApoB in the setting of elevated LDL-C.

We observed a similar proportion of participants who remained free of carotid plaque across common LDL-C thresholds (100-130, \geq 130, \geq 160, and \geq 190) and that lower LDL-C did not significantly predict the persistent absence of carotid plaque independent of ApoB. These results suggest that LDL-C values may not necessarily capture the variance in atherogenic potential and the differences in subclinical carotid atherosclerosis initiation among young persons with prediabetes, diabetes, and/or metabolic syndrome. Overall, our findings support evidence showing that LDL-C is not independently associated with downstream residual risk and/or all-cause mortality, and that ApoB is a superior predictor of myocardial infarction compared to LDL-C among statin-treated individuals [32].

Our results reinforce the superior utility of ApoB compared to LDL-C in capturing the physiology between lipids and atherogenesis, as well as for risk stratification in patients who experience a heterogeneously increased probability of future ASCVD events. For example, a significant proportion of persons with type 2 diabetes have residual ASCVD risk despite LDL-C lowering with statin pharmacotherapy [33]. Remnant lipoprotein particles, originating from VLDL-C and chylomicrons, may contribute to residual ASCVD risk in persons with insulin resistance and/or metabolic syndrome [33]. Furthermore, recent analyses have demonstrated that ApoB-containing lipoproteins including VLDL-C account for approximately 50% of the myocardial infarction risk in an at-risk elderly population not on lipid-lowering therapy, while ApoB-containing lipoproteins including LDL-C and IDL-C collectively explain less than one-third of the risk [34]. Although remnant lipoproteins may all vary in size, each molecule contains an ApoB particle and this risk may therefore be captured with serum measurement of ApoB.

Although ApoB and LDL-C have commonalities, the two molecules represent distinctive aspects of atherogenic lipid particles. There is one ApoB molecule per non-HDL-C particle (chylomicron, VLDL-C, IDL-C, and LDL-C) [10]. Of note, discordantly high ApoB relative to LDL-C in persons with metabolic syndrome, prediabetes, and type 2 diabetes also presents as a diagnostic challenge because insulin resistance increases VLDL production and leads to a depletion of LDL-C and smaller LDL-C particle number through cholesterol ester transfer protein [35]. Elevated ApoB in the setting of metabolic disorders confers an increased risk of

subclinical atherosclerosis [36]. However, LDL-C catabolism and LDL-C receptor expression are also reduced in the presence of insulin resistance, which may increase the circulating pool of LDL-C [37]. Given our interests in identifying negative risk markers among those with metabolic disease, our sample and analysis were constructed with the goal of identifying individuals with discordantly normal ApoB relative to elevated LDL-C. Overall, our findings in the setting of previous observations [35] suggest that LDL-C may not be the most optimal measure for assessing future atherogenic burden and overall ASCVD risk in this patient population.

The main strength of this analysis was the measurement of ApoB in a relatively young sample with elevated ASCVD risk. The longitudinal measurement of carotid atherosclerosis with a follow-up time of approximately 13 years can also be considered as a strength. Furthermore, given the young age of our cohort, we were able to successfully exclude the low number of participants on lipid-lowering therapy and thus captured true physiological patterns in ApoB and LDL-C that were not affected by long-term statin therapy. Furthermore, our results remained consistent when using the Martin/Hopkins calculation of LDL-C and after adjusting for lipid-lowering therapy at follow-up. Our study also included a large proportion of both women (61.5%) and African American (31.7%) participants, two populations that have been traditionally underrepresented in research. As the current landscape of ASCVD risk stratification evolves to incorporate more precision in primary prevention, our study results may prove to be timely with regards to improving patient-provider discussions regarding prevention options for those with metabolic syndrome, prediabetes, and type 2 diabetes.

Our study was limited by a relatively small sample size due to our interest in identifying negative risk markers among those with elevated LDL-C and underlying metabolic disorders. For example, we were unable to separately analyze individuals with type 2 diabetes from prediabetes and future follow-up studies are required. Likewise, this small sample size may have limited our ability to detect statistically significant differences across LDL-C thresholds. Nevertheless, we present hypothesis generating findings that may help to explain the lower risk of subclinical and/or clinical ASCVD among a certain subgroup of individuals with metabolic syndrome, prediabetes, or type 2 diabetes with normal ApoB. Furthermore, even though we used recommended cut-points for normal ApoB (<90 mg/dL) and elevated LDL-C (>100 mg/dL) there may have been limitations to our discordance analysis. For example, a patient with an LDL-C of 101 mg/dL and ApoB of 89 mg/dL would be considered have discordant lipid values in the current study design, which may not necessarily translate to a clinically significant discordance. Lastly, we were unable to adjust for metabolic syndrome or type 2 diabetes duration due to a lack of data availability. However, this time period was likely a very short duration given the young age of the cohort. Thus, future similar studies with longer follow-up will be required to assess how this sample of individuals progresses in regard to subclinical ASCVD outcomes.

5. Conclusions

In conclusion, one-half of participants with elevated LDL-C (>100 mg/dL) and metabolic syndrome, prediabetes, or type 2 diabetes had discordantly normal ApoB (<90 mg/dL). Persons with ApoB <90 were 22% more likely to remain free of carotid plaque over 13 years follow-up compared to those with ApoB \geq 90. Furthermore, an 11% higher likelihood of long-term absence of carotid plaque was observed per 10 mg/dL lower ApoB on the continuous scale. Contrastingly, lower LDL-C did not significantly predict the persistent absence of carotid plaque independent of ApoB and a similar proportion of individuals remained free of plaque across various LDL-C thresholds. These findings suggest a utility for further assessing whether routine ApoB measurement can help to improve ASCVD risk stratification in young persons with metabolic disorders who may have high triglycerides and low HDL-cholesterol.

Declaration of Competing Interest

None.

CRediT authorship contribution statement

Alexander C. Razavi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft, Writing - review & editing. **Lydia A. Bazzano:** Funding acquisition, Validation, Writing - review & editing. **Jiang He:** Validation, Writing - review & editing. **Marie Krousel-Wood:** Validation, Writing - review & editing. **Kirsten S. Dorans:** Validation, Writing - review & editing. **Michael A. Razavi:** Validation, Writing - review & editing. **Camilo Fernandez:** Methodology, Validation, Writing - review & editing. **Seamus P. Whelton:** Methodology, Validation, Writing - review & editing. **Tanika N. Kelly:** Supervision, Project administration, Resources, Methodology, Validation, Writing - review & editing.

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Supplementary materials

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References

- Report NDSNational diabetes statistics report, 2020. Natl Diabetes Stat Rep 2020.
- Aguilar M, Bhuket T, Torres S, Liu B, Wong RJ. Prevalence of the metabolic syndrome in the United States, 2003-2012. *JAMA* 2015;313(19):1973-4.
- Razavi AC, Wong N, Budoff M, Bazzano LA, Kelly TN, He J, et al. Predicting long-term absence of coronary artery calcium in metabolic syndrome and diabetes. *JACC Cardiovasc Imaging* 2020;14(1):219-29.
- Grundy SM. Pre-diabetes, metabolic syndrome, and cardiovascular risk. *J Am Coll Cardiol* 2012;59(7):635-43.
- Malik S, Zhao Y, Budoff M, Nasir K, Blumenthal RS, Bertoni AG, et al. Coronary artery calcium score for long-term risk classification in individuals with type 2 diabetes and metabolic syndrome from the multi-ethnic study of atherosclerosis. *JAMA Cardiol* 2017;2(12):1332-40.
- Wong ND, Sciammarella MG, Polk D, Gallagher A, Miranda-Peats L, Whitcomb B, et al. The metabolic syndrome, diabetes, and subclinical atherosclerosis assessed by coronary calcium. *J Am Coll Cardiol* 2003;41(9):1547-53.
- Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol. *J Am Coll Cardiol* 2019;73(24):e258-350.
- Otvos JD, Mora S, Shalaurova I, Greenland P, MacKey RH, Goff DC. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. *J Clin Lipidol* 2011;5(2):105-13.
- Wilkins JT, Li RC, Sniderman A, Chan C, DM Lloyd-Jones. Discordance between Apolipoprotein B and LDL-cholesterol in young adults predicts coronary artery calcification the CARDIA study. *J Am Coll Cardiol* 2016;67(2):193-201.
- Sniderman AD, Thanassoulis G, Glavinovic T, Navar AM, Pencina M, Catapano A, et al. Apolipoprotein B particles and cardiovascular disease: a narrative review. *JAMA Cardiology* 2019;4(12):1287-95.
- Cao J, Nomura SO, Steffen BT, Guan W, Remaley AT, Karger AB, et al. 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). *J Clin Lipidol* 2020;203(2):587-92.
- Whelton SP, Silverman MG, McEvoy JW, Budoff MJ, Blankstein R, Eng J, et al. Predictors of long-term healthy arterial aging. *JACC Cardiovasc Imaging* 2015;8(12):1393-400.
- Berenson GSBogalusa Heart Study Investigators. Bogalusa heart study: a long-term community study of a rural biracial (Black/White) population. *Am J Med Sci* 2001;322(5):293-300 Nov.
- High Blood Cholesterol Evaluation Treatment Detection National Cholesterol Education Program Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. No. 2, National Cholesterol Education Program, National Heart, Lung, and Blood Institute, National Institutes of Health.
- Association AD Classification and diagnosis of diabetes. *Diabetes Care*; 2017.
- Brunzell JD, Davidson M, Furberg CD, Goldberg RB, Howard BV, Stein JH, et al. Lipoprotein management in patients with cardiometabolic risk. Consensus conference report from the american diabetes association and the american college of cardiology foundation. *J Am Coll Cardiol* 2008;51(15):1512-24.
- Foster TA, Berenson GS. Measurement error and reliability in four pediatric cross-sectional surveys of cardiovascular disease risk factor variables-The Bogalusa Heart Study. *J Chronic Dis* 1987;40(1):13-21.
- Freedman DS, Serdula MK, Srinivasan SR, Berenson GS. Relation of circumferences and skinfold thicknesses to lipid and insulin concentrations in children and adolescents: The Bogalusa Heart Study. *Am J Clin Nutr* 1999;69(2):308-17.
- Srinivasan SRBG. Serum lipoproteins in children and methods for study. *CRC Handb Electrophor* 1983;3:185-204.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18(6):499-502 Jun.
- Ruan L, Chen W, Srinivasan SR, Xu J, Toprak A, Berenson GS. Relation of serum phosphorus levels to carotid intima-media thickness in asymptomatic young adults (from the Bogalusa Heart Study). *Am J Cardiol* 2010;106(6):793-7.
- Stein JH, Korcarz CE, Hurst RT, Lonn E, Kendall CB, Mohler ER, et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the american society of echocardiography carotid intima-media thickness task force endorsed by the society for vascular medicine. *J Am Soc Echocardiogr*. Mosby Inc. 2008;21:93-111.
- Goff DC, Lloyd-Jones DM, Bennett G, Coady S, D'Agostino RB, Gibbons R, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American college of cardiology/American heart association task force on practice guidelines. *Circulation* 2014;63(25 Part B):2935-59.
- Zou G. A Modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol* 2004;159(7):702-6.
- Martin SS, Blaha MJ, Elshazly MB, Toth PP, Kwiterovich PO, Blumenthal RS, et al. Comparison of a novel method vs the Friedewald equation for estimating low-density lipoprotein cholesterol levels from the standard lipid profile. *JAMA - J Am Med Assoc* 2013;310(19).
- Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease. *J Am Coll Cardiol* 2019;74(10):e177-232.
- Blaha MJ, Blankstein R, Nasir K. Coronary artery calcium scores of zero and establishing the concept of negative risk factors. *J Am Coll Cardiol* 2019;12-14.
- Blaha MJ, Cainzos-Achirica M, Greenland P, McEvoy JW, Blankstein R, Budoff MJ, et al. Role of Coronary Artery Calcium Score of Zero and Other Negative Risk Markers for Cardiovascular Disease: The Multi-Ethnic Study of Atherosclerosis (MESA). *Circulation* 2016;133(9):849-58 Mar 1.
- Mortensen MB, Fuster V, Muntendam P, Mehran R, Baber U, Sartori S, et al. A Simple Disease-Guided Approach to Personalize ACC/AHA-Recommended Statin Allocation in Elderly People: The BiImage Study. *J Am Coll Cardiol* 2016;68(9):881-91.
- McClelland RL, Chung H, Detrano R, Post W, Kronmal RA. Distribution of coronary artery calcium by race, gender, and age: results from the multi-ethnic study of atherosclerosis (MESA). *Circulation* 2006;113(1):30-7.
- Mora S, Buring JE, Ridker PM. Discordance of low-density lipoprotein (LDL) cholesterol with alternative LDL-related measures and future coronary events. *Circulation* 2014;129(5):553-61.
- Johannesen CDL, Mortensen MB, Langsted A, Nordestgaard BG. Apolipoprotein B and Non-HDL cholesterol better reflect residual risk than LDL cholesterol in statin-treated patients. *J Am Coll Cardiol* 2021;77(11):1439-50.
- Chait A, Ginsberg HN, Vaisar T, Heinecke JW, Goldberg IJ, Bornfeldt KE. Remnants of the triglyceride-rich lipoproteins, diabetes, and cardiovascular disease. *Diabetes* 2020;69(4):508-14.
- Balling M, Afzal S, Varbo A, Langsted A, Davey Smith G, Nordestgaard BG. VLDL cholesterol accounts for one-half of the risk of myocardial infarction associated with apoB-containing lipoproteins. *J Am Coll Cardiol* 2020;76(23):2725-35.

- [35] Martin SS, Qasim AN, Mehta NN, Wolfe M, Terembula K, Schwartz S, et al. Apolipoprotein B but not LDL cholesterol is associated with coronary artery calcification in type 2 diabetic whites. *Diabetes* 2009;58(8):1887–92.
- [36] Mattsson N, Magnussen CG, Rönnemaa T, Mallat Z, Benessiano J, Julia A, et al. Metabolic syndrome and carotid intima-media thickness in young adults: roles of apolipoprotein B, apolipoprotein A-I, C-reactive protein, and secretory phospholipase A2: the cardiovascular risk in young finns study. *Arterioscler Thromb Vasc Biol* 2010;30(9):1861–6.
- [37] Vergès B. Pathophysiology of diabetic dyslipidaemia: where are we? *Diabetologia* 2015;58(5):886–99.