

Advanced Lipoprotein Testing Does Not Improve Identification of Subclinical Atherosclerosis in Young Adults: The Bogalusa Heart Study

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Background: The clinical value of advanced lipoprotein testing relative to traditional lipid testing remains controversial. To date, no studies have evaluated associations between advanced lipoprotein testing and subclinical atherosclerosis in healthy young adults.

Objective: To determine whether advanced lipoprotein testing using vertical-spin density-gradient ultracentrifugation better predicts carotid intima-media thickness, a validated measure of subclinical atherosclerosis, than does traditional lipoprotein testing in asymptomatic young adults.

Design: Cross-sectional community-based study.

Setting: Bogalusa, Louisiana.

Participants: 311 randomly selected adults from the Bogalusa Heart Study who were 20 to 38 years of age.

Measurements: The authors performed advanced lipoprotein testing using vertical-spin density-gradient ultracentrifugation, traditional testing using enzymatic methods, and Friedewald formula estimation of low-density lipoprotein cholesterol levels. A certified reader blinded to lipoprotein results determined carotid intima-media thickness by B-mode ultrasonography. C-statistics from area under the receiver-operating characteristic curves (AUCs) derived from multivariable regression models were compared.

Results: Lipid values obtained with advanced lipoprotein testing did not predict carotid intima-media thickness better than traditionally measured lipid values in 236 participants for whom all data were available. A model using traditional lipoprotein measures (AUC, 0.754 [95% CI, 0.690 to 0.812]) did not differ significantly from a model using advanced lipoprotein measures (AUC, 0.779 [CI, 0.662 to 0.871]) for prediction of carotid intima-media thickness ($P > 0.2$). Subclass pattern of LDL, lipoprotein(a) cholesterol, intermediate-density lipoprotein cholesterol, high-density lipoprotein cholesterol subclasses, and very-low-density lipoprotein subclasses did not improve the performance of models for prediction of carotid intima-media thickness.

Limitations: The study was cross-sectional, cardiac events were not determined, and only 1 method of advanced lipoprotein testing was used.

Conclusions: Advanced lipoprotein testing using vertical-spin density-gradient ultracentrifugation did not improve prediction of carotid intima-media thickness in young adults and may not be useful for assessing cardiovascular risk in this population.

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Dyslipidemia, a risk factor for atherosclerosis and cardiovascular events in middle-aged and older adults, is associated with subclinical atherosclerosis in children and young adults (1, 2). The National Cholesterol Education Program recommended early dyslipidemia screening and treatment for primary cardiovascular disease prevention (1). Variability in lipoprotein measurement, however, may limit the accuracy of standard lipid testing (3). With growing evidence that other lipoproteins not assessed by traditional lipid testing are associated with cardiovascular risk, there has been increasing interest in assays that can directly measure low-density lipoprotein (LDL) cholesterol and other variables, such as lipoprotein(a), remnants, and particle size (1, 4–6). These assays have been recommended for younger adults who may be at increased risk for premature coronary artery disease, as well as for more aggressive primary prevention (1, 7–9).

To date, no studies have specifically evaluated associations between advanced lipoprotein testing variables and subclinical atherosclerosis in young adults. Also unknown is whether advanced lipoprotein testing improves cardiovascular risk prediction compared with traditional lipoprotein testing in this population. Ultrasound measurement of carotid intima-media thickness, a validated marker of subclinical atherosclerosis and future cardiovascular risk, has

been used widely to study risk factors for atherosclerosis, including in young adults (10–18). The purpose of our study was to determine whether advanced lipoprotein testing by vertical-spin density-gradient ultracentrifugation predicts carotid intima-media thickness better than traditional lipoprotein testing in asymptomatic young adults.

METHODS

Study Participants and Design

The institutional review boards of Tulane University Health Sciences Center and University of Wisconsin Medical School approved this study. The Bogalusa Heart Study is a longitudinal study of the natural history of atherosclerosis in children and young adults in Bogalusa, Louisiana

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(10, 16). Seven cross-sectional studies of schoolchildren were conducted from 1973 to 1994, and 4 studies of young adults previously examined as children and still living in the community were conducted from 1983 to 1996. Included in baseline screening were all noninstitutionalized children who were attending kindergarten through ninth grade and whose parents consented to their participation.

During the last 6 months of the 1995–1996 survey, 519 of the 1420 participants were selected by a random-number generator to undergo assessment of carotid intima-media thickness. The mean age of these 519 participants was 32 years, 39% were men, and 71% were of white ethnicity. They were similar to the overall cohort in ethnicity, sex, body mass index, systolic blood pressure, high-density lipoprotein (HDL) cholesterol level, LDL cholesterol level, and triglyceride level ($P > 0.2$ for all of the preceding variables) but were approximately 4 years older ($P < 0.001$) (10, 16, 19, 20). Roughly 33% ($n = 449$) of the 1995–1996 survey group were randomly selected to undergo advanced lipoprotein testing. Three hundred eleven participants had both advanced lipoprotein testing and carotid intima-media thickness measurements. Independent, random selection for each test should have produced a smaller sample size. Our groups overlapped more than would be expected by chance alone, probably because the overall sample may have been enriched with participants from the last half of the 1995–1996 survey, when carotid intima-media thickness was first measured. Numbers of patients chosen for measurement of carotid intima-media thickness and advanced lipoprotein were based on resources and funding.

Study Procedures

Detailed study methods have been described elsewhere (10, 16, 19–22). Participants fasted for at least 12 hours before assessments. Blood pressure, serum samples, family history, health-habit information, anthropometric information, and carotid intima-media thickness data were collected sequentially. Height and weight were measured in duplicate and were averaged to calculate body mass index (kg/m^2). Waist circumference was measured midway between the rib cage and the superior border of the iliac crest. Blood pressure in the right arm was measured in triplicate while participants were seated. Blood samples were collected by venipuncture and were transported to the New Orleans core laboratory in a cold-packed box on the same day. Serum glucose levels were measured by a glucose oxidase method.

Lipid and Lipoprotein Testing

Traditional lipoprotein testing was performed as follows. Serum total cholesterol and triglycerides were measured enzymatically (Abbott VP Analyzer, Abbott Laboratories, Abbott Park, Illinois). Intraclass correlation coefficients between blinded, duplicate values (59 pairs) were 0.98 for each measurement (20). In contrast to previous Bogalusa Heart Study reports in which LDL cholesterol

Context

Traditional lipoprotein testing methods do not directly measure low-density lipoprotein cholesterol, lipoprotein(a), lipid remnants, or particle size. Does measurement of these laboratory values improve prediction of atherosclerosis?

Contribution

This cross-sectional study of 311 healthy young adults used vertical-spin density-gradient ultracentrifugation to directly measure multiple lipoprotein subclass patterns, lipoprotein(a), and intermediate-density lipoprotein cholesterol. These values did not predict carotid intima-media thickness better than traditionally measured lipid values.

Cautions

Carotid intima-media thickness is an imperfect measure of clinically important atherosclerosis. Associations between lipoproteins and carotid intima-media thickness may differ between healthy people and older people with known atherosclerosis.

—The Editors

terol was measured directly, we estimated LDL cholesterol by using the Friedewald formula to represent standard clinical practice.

Advanced lipoprotein testing was conducted by vertical-spin density-gradient ultracentrifugation (Vertical Auto Profile-I, Atherotech, Inc., Birmingham, Alabama), as described elsewhere (21, 23). Lipoproteins were separated by a single vertical-spin density gradient (21, 23). Cholesterol distribution was measured across the gradient by continuous sampling from the tube bottom using an automated analyzer, with subsequent deconvolution of the peaks. When this procedure is used, measurements of LDL cholesterol and HDL cholesterol do not include cholesterol carried by lipoprotein(a) or intermediate-density lipoprotein cholesterol. By this method, LDL cholesterol is the sum of lipoprotein(a), intermediate-density lipoproteins, and the remainder, which is termed “real” LDL cholesterol. On the basis of the relative position of the peak concentration of LDL cholesterol in the density gradient, the LDL subclass pattern was characterized as large-buoyant, intermediate, or small-dense. For data analysis, a numerical value (1, 2, or 3, respectively) was assigned on the basis of the dominant subclass pattern. Subclasses of HDL cholesterol (large and small) were measured similarly. Very-low-density lipoprotein (VLDL)-3 cholesterol represents cholesterol carried in the smallest measured VLDL subclass (21, 23). Intraclass correlation coefficients between blinded duplicate values (12 pairs) were 0.97 (lower limit of 95% CI, 0.92) for LDL cholesterol, 0.89 (lower limit of CI, 0.74) for large HDL cholesterol, 0.81 (lower limit of CI, 0.56) for small HDL cholesterol, 0.81 (lower limit of CI, 0.56) for VLDL-3, and 0.95 (lower limit of CI, 0.87)

for intermediate-density lipoprotein cholesterol (21). The subclass pattern assignment for LDL cholesterol showed 83% concordance (lower limit of CI, 62%) (21).

Carotid Ultrasonography

Images of common carotid, bulb, and internal carotid arterial segments were recorded by using a Toshiba Sono-layer SSH 160A (Toshiba Medical, Tokyo, Japan) and a 7.5-MHz linear array transducer (10, 16). Carotid artery segments were interrogated and measured by using protocols from the Atherosclerosis Risk in Communities Study (24). One certified reader blinded to lipoprotein results determined far-wall carotid intima-media thickness on B-mode ultrasonographic images by using a semi-automated measurement program (16). Three right and 3 left mean values were determined separately for each carotid segment. Measurements were averaged to determine segmental and composite values. Bilateral images could not be obtained in 2.6% of persons for the common carotid segment, in 11.7% of persons for the bulb segment, and in 17.9% of persons for the internal carotid arterial segments; image acquisition becomes more difficult in anatomically deeper and more tortuous segments (16). Complete bilateral images were available for 74.5% of participants. Fifty-two individuals had repeated examination within 10 to 12 days. Intraclass correlations were 0.45, 0.54, and 0.52 for common, bulb, and internal carotid artery segments, respectively, and 0.55 for composite carotid intima-media thickness, comparable to other epidemiologic studies (22, 25). The mean absolute value of repeated-measure differences was 0.06 mm (lower limit of CI, 0.05 mm), with a median of 0.04 mm for the mean carotid intima-media thickness of all 6 segments.

Statistical Analyses

Statistical analyses were performed by using SAS, version 8.2 (SAS Institute, Inc., Cary, North Carolina). Descriptive variables were described by using means and SDs. Wilcoxon signed-rank tests for paired data were used to assess differences, and Spearman rank correlations were computed to assess correlations between advanced and traditional lipoprotein testing values. In univariate and multivariable analyses, the dependent variable was composite carotid intima-media thickness in the highest quartile of the study sample. Univariate associations were determined by using logistic regression with each lipoprotein value considered independently.

Independent variables considered in multivariable analysis, in addition to traditional and advanced lipoprotein values, were age, sex, ethnicity, body mass index, alcohol use, smoking status, systolic and diastolic blood pressures, glucose, insulin, and waist circumference, which previously were shown to predict carotid intima-media thickness in this cohort (16). Collinearity was evaluated. Variables that had variance inflation factors greater than 2.0 and were similar in magnitude were considered collinear (26). These included the following pairs: body mass

index and waist circumference, diastolic and systolic blood pressures, traditional total cholesterol and triglyceride levels, and advanced lipoprotein total cholesterol and LDL cholesterol values. Less clinically pertinent or less precisely measured variables among collinear pairs were removed (body mass index, diastolic blood pressure, traditional and advanced total cholesterol levels). The remaining independent variables were retained in all multivariable models.

To determine discriminative value between predictive models, we constructed multivariable *c*-statistic models. In binary models, the *c*-statistic is analogous to the area under the receiver-operating characteristic curve (AUC) (27). A baseline model was constructed by using traditional lipoprotein values and other noncollinear, nonlipid variables. To determine whether advanced lipoprotein data improved prediction of carotid intima-media thickness, alternate models were created in which each traditional lipoprotein in the baseline model was substituted for by its comparable advanced lipoprotein testing variable or variables.

Individuals with incomplete data were excluded by list-wise deletion. Because results using segmental carotid intima-media thicknesses were similar to those using composite values, only composite carotid intima-media thickness analyses are reported. Alternate models were compared by testing differences in *c*-statistics; an α value less than 0.05 was considered significant (28).

Role of the Funding Sources

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RESULTS

Baseline Characteristics

Among 311 participants who underwent carotid intima-media thickness assessments and had advanced lipoprotein testing using vertical-spin density-gradient ultracentrifugation; 42.4% were men; 67.5% were white; and the mean age was 32.1 years, SD 2.9 (range, 20 to 38 years) (Table 1). Mean composite carotid intima-media thickness was 0.735 mm, SD 0.104. A composite carotid intima-media thickness of 0.781 mm or greater defined the highest quartile in this study.

Seventy-five participants had incomplete data on carotid intima-media thickness. Age, sex, ethnicity, waist circumference, systolic blood pressure, LDL cholesterol level, HDL cholesterol level, and triglyceride level were similar in the 236 participants who had complete data on both

Table 1. Baseline Characteristics of 311 Participants Undergoing Measurement of Carotid Intima–Media Thickness and Advanced Lipoprotein Testing

Characteristic	Value
Age, y	32.1, SD 2.9
Men, %	42.4
White ethnicity, %	67.5
Waist circumference, cm	88.3, SD 15.9
Systolic blood pressure, mm Hg	112.5, SD 11.3
Fasting glucose, mmol/L (mg/dL)	4.5, SD 0.6 (81.0, SD 11.9)
Current alcohol use, %	23.3
Current smoker, %	36.0
Composite carotid intima–media thickness, mm	0.735, SD 0.104

carotid intima–media thickness and lipoprotein testing (Figure) and in those with incomplete data on carotid intima–media thickness ($P > 0.2$ for all variables except age, for which $P = 0.08$).

Traditional versus Advanced Lipoproteins

Total cholesterol, HDL cholesterol, and LDL cholesterol values were higher and triglyceride values were lower when measured traditionally than when measured by using vertical-spin density-gradient ultracentrifugation. Correlations between traditional and respective advanced lipoprotein correlates were positive and statistically significant (Table 2).

Univariate Associations with Carotid Intima–Media Thickness

Traditionally measured LDL cholesterol level was positively associated with carotid intima–media thickness in

the 75th percentile or greater, although with borderline significance ($P = 0.117$). “Real” LDL cholesterol was also positively associated with borderline significance ($P = 0.070$) (Table 3).

Multivariable Models

In multivariable logistic regression models, substituting advanced lipoprotein testing values for corresponding traditional lipoprotein values did not improve prediction of the highest quartile of carotid intima–media thickness (Table 4). The baseline predictive model using traditional lipoprotein testing produced an AUC of 0.754 (CI, 0.690 to 0.812). Consistent with univariate analyses, the only traditional lipid value that contributed significantly to the model was LDL cholesterol ($P = 0.008$). Other significant contributors were age ($P = 0.022$), systolic blood pressure ($P = 0.001$), and current use of alcohol ($P = 0.043$).

In comparison, the best advanced lipoprotein testing model, which was obtained by substituting comparable advanced lipoprotein testing values for each of the traditional lipoprotein values in the baseline model, did not significantly improve identification of participants in the highest quartile of carotid intima–media thickness (AUC, 0.782 [CI, 0.718 to 0.841]; $P > 0.2$) (Table 4, model A). The only significant advanced lipoprotein testing value that contributed to this model was “real” LDL cholesterol ($P = 0.013$); however, current alcohol use also contributed significantly ($P = 0.003$). In addition, separate models individually substituting traditional lipoprotein values for corresponding advanced lipoprotein values also did not yield significantly better prediction (Table 4). Among the ad-

Figure. Flow of study participants.

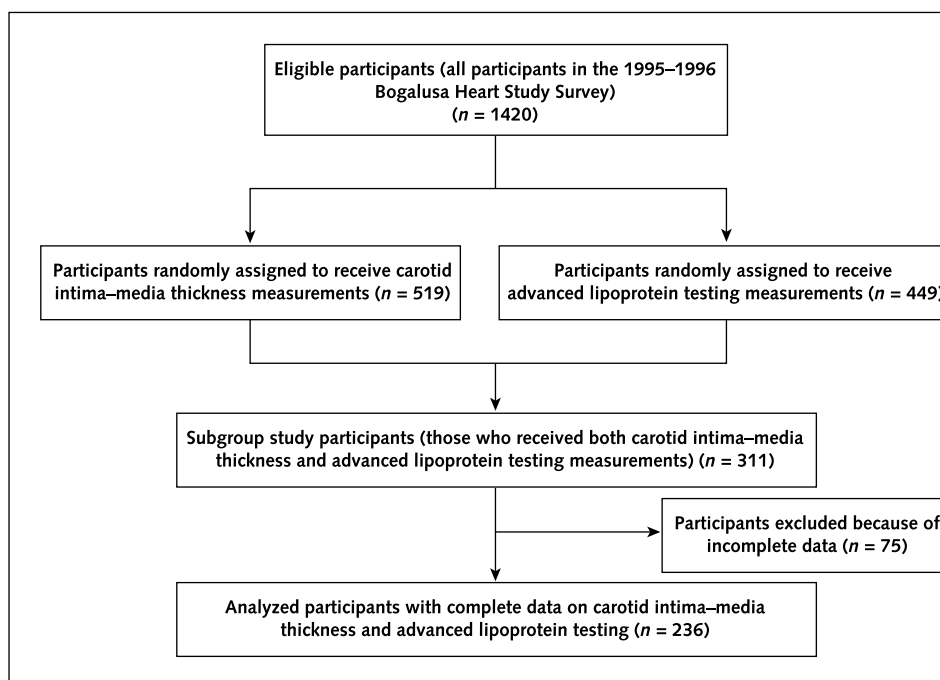


Table 2. Lipid Values in 311 Participants according to Traditional and Advanced Lipoprotein Testing*

Lipid	Value according to Traditional Testing, mmol/L (mg/dL)	Value according to Advanced Testing, mmol/L (mg/dL)	Mean Difference [95% CI], mmol/L (mg/dL)†	Correlation Coefficient‡
Total cholesterol	5.0, SD 1.0 (193.5, SD 38.9)	4.85, SD 0.97 (187.4, SD 37.7)	0.15 [0.12 to 0.19] (5.9 [4.7 to 7.5])	0.94
HDL cholesterol	1.25, SD 0.42 (48.4, SD 16.2)	1.11, SD 0.36 (42.9, SD 13.9)	0.14 [0.12 to 0.16] (5.5 [4.8 to 6.1])	0.91
Large HDL cholesterol	–	0.26, SD 0.22 (10.1, SD 8.6)	–	0.72
Small HDL cholesterol	–	0.83, SD 0.19 (32.2, SD 7.5)	–	0.71
LDL cholesterol	3.17, SD 0.91 (122.5, SD 35.3)	3.02, SD 0.78 (116.8, SD 30.2)	0.15 [0.1 to 0.19] (5.7 [4.0 to 7.3])	0.90
“Real” LDL cholesterol	–	2.45, SD 0.63 (94.9, SD 24.5)	–	0.86
Lipoprotein(a) cholesterol	–	0.19, SD 0.11 (7.5, SD 4.1)	–	0.16‡
IDL cholesterol	–	0.41, SD 0.21 (15.7, SD 8.0)	–	0.64
LDL subclass§	–	1.9, SD 0.7	–	0.21
Triglycerides	1.26, SD 0.97 (111.3, SD 85.7)	1.53, SD 1.04 (135.7, SD 92.0)	–0.28 [–0.22 to –0.33] (–24.4 [–19.2 to –29.4])	0.87
VLDL-3 cholesterol	–	0.30, SD 0.16 (11.5, SD 6.0)	–	0.63

* HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; VLDL = very-low-density lipoprotein.

† $P < 0.001$ for all comparisons unless otherwise indicated.

‡ $P = 0.003$.

§ Ranges from pattern 1 (large–buoyant) to pattern 2 (intermediate) to pattern 3 (small–dense).

vanced lipoprotein testing models with at least 1 traditional lipoprotein value, the highest AUC was seen in model E, in which “real” LDL cholesterol, lipoprotein(a), intermediate-density lipoprotein cholesterol, and LDL subclass pattern were substituted for traditional LDL cholesterol. However, this model did not significantly improve the baseline model (AUC, 0.779 [CI, 0.662 to 0.871]; $P > 0.2$). Of note, “real” LDL cholesterol was again the only advanced lipoprotein value that contributed significantly to the model ($P = 0.015$). Substituting HDL subclasses for traditional HDL cholesterol values resulted in AUC values ranging from 0.742 to 0.744 for predicting carotid intima–media thickness in the 75th percentile or greater (Table 4).

In another model, substituting VLDL-3 cholesterol and triglycerides from advanced lipoprotein testing for traditional lipoprotein triglyceride values did not improve prediction of carotid intima–media thickness (AUC range, 0.728 to 0.741) (Table 4).

DISCUSSION

Levels of LDL cholesterol determined both by traditional and advanced lipoprotein testing helped identify young asymptomatic adults with carotid intima–media thickness in the highest quartile of the study sample. When compared with models using traditional lipoprotein mea-

Table 3. Lipoprotein Values in the Highest versus Lower 3 Quartiles of Carotid Intima–Media Thickness in 236 Participants*

Variable	Carotid Intima–Media Thickness		P Value†
	Top Quartile	Lower 3 Quartiles	
Traditional testing			
Total cholesterol, mmol/L (mg/dL)	5.15, SD 1.16 (199.1, SD 44.9)	4.95, SD 0.99 (191.6, SD 38.3)	>0.2
Triglycerides, mmol/L (mg/dL)	1.2, SD 0.86 (106.3, SD 76.4)	1.3, SD 1.09 (114.9, SD 96.8)	>0.2
LDL cholesterol, mmol/L (mg/dL)	3.34, SD 1.08 (129.3, SD 41.8)	3.11, SD 0.86 (120.4, SD 33.3)	0.117
HDL cholesterol, mmol/L (mg/dL)	1.23, SD 0.59 (47.7, SD 22.7)	1.24, SD 0.36 (48.0, SD 13.9)	>0.2
Advanced testing			
Total cholesterol, mmol/L (mg/dL)	4.97, SD 1.09 (192.0, SD 42.3)	4.82, SD 0.94 (186.5, SD 36.2)	>0.2
Triglycerides, mmol/L (mg/dL)	1.61, SD 1.18 (142.9, SD 104.4)	1.52, SD 0.99 (134.4, SD 87.7)	>0.2
LDL cholesterol, mmol/L (mg/dL)	3.15, SD 0.86 (121.8, SD 33.1)	2.97, SD 0.79 (114.8, SD 30.6)	0.142
“Real” LDL cholesterol, mmol/L (mg/dL)	2.56, SD 0.69 (99.0, SD 26.5)	2.41, SD 0.63 (93.2, SD 24.3)	0.070
Lipoprotein(a) cholesterol, mmol/L (mg/dL)	0.17, SD 0.09 (6.5, SD 3.5)	0.20, SD 0.11 (7.6, SD 4.1)	0.121
IDL cholesterol, mmol/L (mg/dL)	0.42, SD 0.24 (16.2, SD 9.1)	0.39, SD 0.18 (15.2, SD 7.1)	>0.2
LDL subclass‡	2.0, SD 0.8	1.9, SD 0.7	>0.2
HDL cholesterol, mmol/L (mg/dL)	1.06, SD (41.1, SD 17.6)	1.11, SD 0.33 (43.0, SD 12.9)	>0.2
Large HDL cholesterol, mmol/L (mg/dL)	0.25, SD 0.32 (9.5, SD 12.2)	0.27, SD 0.21 (10.4, SD 8.2)	>0.2
Small HDL cholesterol, mmol/L (mg/dL)	0.8, SD 0.23 (31.1, SD 9.0)	0.84, SD 0.19 (32.5, SD 7.4)	>0.2

* HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein.

† From logistic regression models.

‡ Ranges from pattern 1 (large–buoyant) to pattern 2 (intermediate) to pattern 3 (small–dense).

Table 4. Multivariable Models Evaluated for Predicting Carotid Intima–Media Thickness in the Highest versus Lower 3 Quartiles of Carotid Intima–Media Thickness in 236 Participants*

Model	Advanced Lipoprotein Terms	Traditional Lipoprotein Terms	AUC (95% CI)
Baseline	–	HDL cholesterol, LDL cholesterol, triglycerides	0.754 (0.690–0.812)
A	Large HDL cholesterol, small HDL cholesterol, IDL cholesterol, “real” LDL cholesterol, lipoprotein(a) cholesterol, LDL subclass, triglycerides, VLDL-3 cholesterol	–	0.782 (0.718–0.841)†
B	“Real” LDL cholesterol	HDL cholesterol, triglycerides	0.752 (0.681–0.810)
C	“Real” LDL cholesterol + lipoprotein(a) cholesterol	HDL cholesterol, triglycerides	0.777 (0.710–0.835)
D	“Real” LDL cholesterol + lipoprotein(a) cholesterol + IDL cholesterol	HDL cholesterol, triglycerides	0.778 (0.710–0.835)
E	“Real” LDL cholesterol + lipoprotein(a) cholesterol + IDL cholesterol + LDL subclass‡	HDL cholesterol, triglycerides	0.779 (0.662–0.871)†
F	Small HDL cholesterol	LDL cholesterol, triglycerides	0.744 (0.671–0.803)
G	Small HDL cholesterol, large HDL cholesterol	LDL cholesterol, triglycerides	0.742 (0.671–0.803)
H	VLDL-3 cholesterol	LDL cholesterol, HDL cholesterol	0.741 (0.671–0.803)
I	VLDL cholesterol	LDL cholesterol, HDL cholesterol	0.729 (0.660–0.793)
J	Triglycerides	LDL cholesterol, HDL cholesterol	0.728 (0.660–0.793)
K	VLDL-3 cholesterol, triglycerides	LDL cholesterol, HDL cholesterol	0.737 (0.666–0.798)

* Covariates in all models were age, sex, ethnicity, fasting glucose and insulin levels, systolic blood pressure, waist circumference, current drinking, and current smoking. AUC = area under receiver-operating characteristic curve; HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; VLDL = very-low-density lipoprotein.

† $P > 0.2$ for AUC compared with baseline model.

‡ LDL subclass ranges from pattern 1 (large–buoyant) to pattern 2 (intermediate) to pattern 3 (small–dense).

tures, those using advanced lipoprotein measures obtained from vertical-spin density-gradient ultracentrifugation did not significantly improve prediction of subclinical atherosclerosis in young adults.

Previous studies of young adults have demonstrated clear relationships between traditional lipoprotein levels and increased carotid intima–media thickness (10–12, 16, 18, 29). Among Bogalusa Heart Study participants, LDL cholesterol and HDL cholesterol levels in childhood (4 to 17 years of age) predicted carotid intima–media thickness in young adulthood (25 to 37 years of age) (10). Similarly, childhood LDL cholesterol levels in the Cardiovascular Risk in Young Finns Study and total cholesterol levels in 8- to 18-year-olds in Muscatine, Iowa, predicted carotid intima–media thickness in young adulthood (11, 12). Thus, there is increasing evidence that cardiovascular risk factors for older individuals are also markers of subclinical atherosclerosis in younger individuals.

Small–dense LDL particles, lipoprotein(a) mass, and lipoprotein remnants have been associated with atherosclerosis in observational studies of middle-aged and older adults (1, 4, 5, 8, 9, 30–32). Recent studies showing associations between advanced lipoprotein test values and atherosclerosis in young adults (mean ages, 35 to 38 years) were conducted in patients with type 1 diabetes mellitus and used different advanced lipoprotein methods and outcomes, so they cannot be directly compared with this study (33, 34). One study demonstrated associations between incident coronary artery disease and HDL size and total VLDL concentration, but not LDL size (34). Another study found no association between particle sizes and cor-

onary calcium scores in diabetic individuals; however, smaller LDL and HDL particles and larger VLDL particles were associated with higher coronary calcium scores in nondiabetic controls (33).

In this study, total cholesterol, HDL cholesterol, and LDL cholesterol levels measured by vertical-spin density-gradient ultracentrifugation were lower and levels of triglycerides were higher than corresponding lipoprotein values obtained through traditional testing. Lower levels of triglycerides obtained by traditional methods probably contributed to higher traditional estimates of LDL cholesterol levels. Nevertheless, findings in univariate and multivariable models were consistent. In the former, traditional LDL cholesterol levels and “real” LDL cholesterol levels were associated with carotid intima–media thickness in the highest quartile, although the associations were of borderline significance. In multivariable analysis, “real” LDL cholesterol was the only variable from advanced lipoprotein testing that contributed significantly to prediction of increased carotid intima–media thickness, just as LDL cholesterol was the only significant contributor among the traditional lipoprotein testing variables. These findings are consistent with previous results from this cohort, in which directly measured LDL cholesterol levels predicted increased carotid intima–media thickness (16). Our current analyses demonstrate that advanced lipoprotein testing variables, when measured by using the methods described here, do not predict increased carotid intima–media thickness better than traditional lipoprotein values in young symptomatic adults.

Of note, small–dense LDL cholesterol levels did not

significantly predict increased carotid intima–media thickness in multivariable analysis. This finding is not surprising for this age group. Isolated elevations in small–dense LDL level are rare, especially in otherwise healthy individuals, and they frequently accompany characteristics that are linked to the metabolic syndrome. The prevalence of the metabolic syndrome was only 13% in our sample, which may be too small to allow detection of significant associations. Also, increased lipoprotein(a) cholesterol was not associated with increased carotid intima–media thickness. Although this finding may seem inconsistent with those of studies linking lipoprotein(a) to cardiovascular events, the previous studies measured lipoprotein(a) mass while we measured cholesterol carried by lipoprotein(a) (4, 35). The relationship between lipoprotein(a) cholesterol and cardiovascular events is not known.

Although this cohort reasonably represented young adults who might undergo lipoprotein screening, minorities other than black persons were underrepresented. The older age of the subgroup receiving carotid intima–media thickness testing may have led to larger values than would have been observed in the rest of the surveyed population. This could exaggerate an association between cholesterol levels and carotid intima–media thickness. However, preferential relationships with one type of lipoprotein testing should not have occurred without significant dyslipidemia, which was not seen in this study and would not be expected in young adults presenting for routine screening. Other limitations included the sample size, which was further reduced because of incomplete carotid intima–media thickness measurements and difficulty in reliably measuring carotid intima–media thickness in certain segments. Nevertheless, the groups that had carotid intima–media thickness measurements and advanced lipoprotein testing were selected randomly and independently of each other. Finally, carotid intima–media thickness is a surrogate end point for cardiovascular disease risk, so our findings cannot be considered definitive. Despite its limitations, carotid intima–media thickness is a validated predictor of prevalent and incident cardiovascular disease (36–38). The final sample, although relatively small, was sufficient to rule out important differences in predicting clinically significant carotid intima–media thickness in this unique population. The sample size was comparable to that of similar studies in young and older adults.

This study evaluated only 1 method of advanced lipoprotein testing. Other methods, such as nuclear magnetic resonance spectroscopy or gradient gel electrophoresis, have provided incremental power for identifying atherosclerosis and predicting cardiovascular events in middle-aged and older adults (36–38). Also, improved vertical-spin ultracentrifugation techniques may allow better separation of atherogenic from less atherogenic lipoproteins and may provide better discriminating power than in our study (6). This is the first study, to our knowledge, evaluating the ability of any advanced lipoprotein testing

technique to predict subclinical atherosclerosis in young healthy adults.

The inherent measurement variability of all lipoprotein tests should also be considered. Biological coefficients of variation range from 7% to 8% for LDL and HDL cholesterol to up to 24% for triglycerides (3). Technical and procedural differences further add 2% to 24% to total variability (3). When measurements are performed in standardized laboratories, analytic variability for directly measured LDL cholesterol is comparable to LDL cholesterol estimated by the Friedewald equation (coefficients of variation, 6% vs. 4%, respectively); however, independently operating clinical laboratories report coefficients of variation of 12% for LDL cholesterol (3). In highly regulated and standardized settings, direct lipoprotein assays may vary less. However, in the absence of definite clinical benefits, the additional costs of advanced lipoprotein testing may not be justified. These costs can only be estimated because of regional and contractual pricing differences, but advanced lipoprotein testing costs approximately \$20 to \$60 more per test than traditional testing. Future studies with newer lipoprotein testing methods, larger sample sizes, and other cardiovascular outcomes are needed.

In this study of young adults, advanced lipoprotein testing by vertical-spin density-gradient ultracentrifugation did not significantly improve identification of increased carotid intima–media thickness compared with traditional lipoprotein testing. “Real” LDL cholesterol was the most significant predictor of increased carotid intima–media thickness among advanced lipoprotein measures, but it did not improve the predictive value of LDL cholesterol estimated by traditional testing. Lipoprotein(a) cholesterol and LDL subclass pattern as determined by vertical-spin density-gradient ultracentrifugation were not significantly associated with increased carotid intima–media thickness. Although lipoprotein values are powerful predictors of cardiovascular events and are measured routinely in young adults, the method of advanced lipoprotein testing used in our study did not appreciably improve the identification of increased carotid intima–media thickness, a surrogate for cardiovascular risk. In comparison, conventional lipid testing provided equivalent predictive value at a lower cost. Before conclusions regarding lipid testing in young adults can be considered definitive, further studies must determine whether other methods of advanced lipoprotein testing are associated with subclinical atherosclerosis in a similar sample.

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