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Lipoprotein particle number and size predict vascular structure and function better than traditional lipids in adolescents and young adults

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BACKGROUND: In adults, dyslipidemia is associated with higher carotid thickness and arterial stiffness, predictors of cardiovascular events. In young subjects, lipid concentrations have not been consistently associated with vascular measures.

OBJECTIVE: The objective of the study was to compare nuclear magnetic resonance (NMR) measures of lipoprotein particle number (low-density lipoprotein [LDL] particle, low-density lipoprotein [HDL] particle, very low-density lipoprotein [VLDL] particle) and size (LDL size, HDL size, and VLDL size) to determine if they were associated with vascular measures more strongly than lipid concentrations (LDL cholesterol, HDL cholesterol, and triglyceride [TG]).

METHODS: We evaluated 214 lean (L), 228 obese (O), and 214 diabetic (T2DM) subjects aged 10 to 24 years (33% male and 39% Caucasian). Cardiovascular risk factors, vascular structure, and arterial stiffness were measured. General linear models were constructed including demographics, risk factors, and traditional or NMR lipid parameters. A composite vascular function score was developed as the outcome in receiver operator characteristic scores for determining which lipid parameter was superior in predicting vascular damage.

RESULTS: Risk factors worsened from L to O to T. However, LDL cholesterol was similar in O and T, whereas LDL size differentiated the 3 groups (T > O > L, $P \leq .0001$). Models demonstrated the superiority of NMR values, which entered for all but 1 vascular outcome and explained more of the variance than traditional lipid concentrations. Receiver operator characteristic curves demonstrated that NMR values were superior in predicting vascular outcomes. Models stratified by race were similar but cutpoints predicting vascular outcomes differed by race for TG, TG/HDL, and VLDL.

CONCLUSION: Lipoprotein particle number and size are more strongly related to vascular structure and function than traditional lipid values. NMR lipid measures may be a better indicator of risk for target organ damage than traditional lipid measures in adolescents and young adults.

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Introduction

In the late 1950s, the Framingham Heart Study established the relationship between elevated cholesterol levels and cardiovascular (CV) disease.[1] Subsequent data demonstrate that a substantial number of individuals who develop CV disease have normal low-density lipoprotein (LDL) cholesterol (LDL-C) levels.[2] Recently, direct measurement of lipoprotein particle size with nuclear magnetic resonance (NMR) has become available for clinical and research use.[3] The use of NMR allows for measurement of number and size of LDL particles, which may be better predictors of CV events[4] and early noninvasive atherosclerotic target organ damage (carotid intima media thickness [cIMT])[5] in adults than traditional cholesterol concentration.

Few studies have measured lipoprotein particle size and number in adolescents and young adults[6,7] but none have compared their usefulness in predicting target organ damage in young subjects. Therefore, we measured traditional and NMR lipid values and cIMT and arterial stiffness in adolescents and young adults. We hypothesized that NMR lipid values would be more strongly associated with target organ damage than traditional lipids even after adjusting for other CV risk factors.

Materials and methods

Population

The population consisted of 674 subjects mean aged 18 years (10–24 years, 33% male, 39% Caucasian) recruited for a study of the effects of type II diabetes mellitus (T2DM) on CV health. There were 12 Hispanics: 6 subjects who identified as White and Hispanic, 1 Black and Hispanic, 4 other Hispanic, and 1 more than 1 race Hispanic. Of the non-Hispanics, 259 were White, 394 Black, 1 American Indian, 8 more than 1 race. Because there were so few Hispanics and races other than White or Black, for analyses, the races/ethnicities were compressed into White and Black without regards to ethnicity. Subjects with T2DM were matched by age, race (Caucasian or African American), and sex to both lean and obese controls. T2DM ($T = 214$) was determined using American Diabetes Association criteria.[8] Obese controls ($O = 228$) had body mass index (BMI) ≥95th percentile by Centers for Disease Control (CDC) criteria with normal oral glucose tolerance test. Lean subjects ($L = 214$) had BMI ≤85th percentile. Pregnant women were excluded. Written informed consent was obtained from individuals aged >18 years or the parent or guardian for individuals aged <18 years. Written assent was also obtained for individuals aged <18 years according to the guidelines established by the Institutional Review Board at Cincinnati Children's Hospital Medical Center.

Laboratory

After a minimum 10-hour overnight fast, participants had questionnaire, anthropometric, blood pressure (BP), laboratory, and vascular structure and function data collected. Experienced personnel obtained 2 measures of height using a calibrated stadiometer (Veeder-Root, Elizabethtown, NC) and 2 measures of weight using a Health-O-Meter electronic scale. The average of each was used. Three measures of BP were obtained with a mercury sphygmomanometer according to published pediatric standards,[9] and the average was used. Fasting plasma glucose, insulin, glycosylated hemoglobin (HbA1c), and high sensitivity C-reactive protein were measured by standard techniques.[10] Assays of fasting plasma lipids were carried out in an National Heart Lung & Blood Institute & Centers for Disease Control (NHLBI-CDC) standardized laboratory. LDL-C concentration was calculated using the Friedewald equation as previously described.[10]

The lipoprotein particle analysis was performed with a 400-MHz proton NMR analyzer at LipoScience (Raleigh, NC) as previously described.[3] In brief, the number of particles of lipoprotein subclasses of different size is derived from the measured amplitudes of the distinct lipid methyl group NMR signals they emit. The intensity of each signal is proportional to the quantity of the subclass, which is converted to particle concentration units (nmol/L for LDL particle [LDL-P] and very low-density lipoprotein cholesterol particle [VLDL-P] and μmol/L for high-density lipoprotein cholesterol particle [HDL-P]).

Although this technique can separate VLDL, LDL, and HDL into 10 subclasses, average particle numbers were counted (VLDL-P, LDL-P, and HDL-P) and sizes (VLDL-S, LDL-S, and HDL-S) were computed as the sum of the diameter of each subclass multiplied by its relative mass percentage as estimated from the amplitude of its methyl NMR signal. NMR lipoprotein particle analyses were done on frozen samples that had been stored at −80°C. Previous studies have demonstrated that NMR lipoprotein particle analyses are unaffected by frozen storage and multiple freeze-thaw cycles.[11] Reproducibility of the NMR-measured lipoprotein particle parameters determined by replicate analyses of plasma pools found between-run coefficients of variability for low-normal concentrations were <4% for total LDL-P and HDL-P concentrations, <0.5% for LDL-S and HDL-S, <8% for large and small LDL subclasses, and <5% for large and small HDL subclasses.[11] For all NMR analyses, samples were handled in a fully blinded fashion such that investigators had no knowledge of subject characteristics.

Vascular testing

Carotid ultrasound was performed using B-mode ultrasonography with a GE Vivid 7 ultrasound imaging system (GE Medical Systems, Wauwatosa, WI) with a high-resolution linear array vascular ultrasound centered at 7.5 MHz. For each subject, the far wall of each carotid segment bilaterally was examined independently from continuous angles to identify the thickest cIMT for the right and left common, bulb (bifurcation), and internal

carotid arteries. Multiple digital image loops were digitally transmitted using the Amicas Vericys Medical System (Chicago, IL) for offline reading and analyses. All images were read by a single experienced and research-trained ARDMS Registered Vascular Sonographer who was blinded to subject group. The mean of right and left carotid segments were used in analyses for all parameters. A manual tracing technique was used to measure the maximum carotid thickness from the leading edge (lumen-intima) to the leading edge (medial-adventitia). This technique has coefficients of variation for repeat readings of 5.3% to 8.0%.[12] M-mode measurements of the distal common carotid were also performed 1 cm proximal to the beginning of the carotid bulb. The maximal and minimal lumen diameters were read from the M-mode tracing for calculation of carotid stiffness (Peterson's elastic modulus [PEM]).[13]

Nonultrasound-based vascular testing was conducted after 5 minutes of rest in the supine position. Three measures of brachial artery distensibility (BrachD) and mean arterial BP (MAP) were obtained with a DynaPulse Pathway instrument (Pulse Metric, Inc, San Diego, CA) as previously described.[10] DynaPulse derives brachial artery pressure curves from distensibility arterial pressure signals obtained from a standard cuff sphygmomanometer assuming a straight tube brachial artery and T-tube aortic system. Evaluation of repeated measures in our laboratory shows excellent reproducibility with coefficients of variability <9%.[10] Three measures of carotid-femoral pulse wave velocity (PWV) were obtained with tonometry with a SphygmoCor SCOR-PVx System (Atcor Medical, Sydney, Australia) according to the manufacturer's protocol. The coefficient of variability for repeat measures is <7%.[10] Augmentation index (AIx), which is influenced by arterial stiffness but is also a measure of wave reflections, was also collected in triplicate using tonometry on the radial artery using the generalized transfer function applied by the device and normalized to heart rate of 75 beats per minute. Reproducibility studies in our laboratory demonstrated intraclass correlation coefficients between 0.7 and 0.9 for all variables.[10]

Statistical methods

All analyses were performed with Statistical Analyses Software (SAS, version 9.3; Cary, NC, USA). Mean values for demographic, anthropometric, and laboratory data were obtained by group. Variance stabilizing procedures were used as needed. Analysis of variance was performed to look for differences by group and Chi-squared analyses were performed for categorical variables. Bivariate correlations were calculated between vascular outcome variables and lipid parameters overall and by group. Separate general linear models using stepwise selection process were constructed using important either traditional or NMR lipid parameters and other important covariates from correlation analyses to elucidate if traditional or NMR lipid parameters were independent determinates of CV outcomes after correcting for other CV risk factors. Variables that were available to enter the models included age, race, sex, group, BMI z-score (height for AIx only), systolic BP and diastolic BP z-score for cIMT or MAP for arterial stiffness, heart rate, log fasting glucose, insulin, HbA1c, C-reactive protein, and either traditional or NMR lipid parameters. Regression diagnostics were performed, and no model had a condition index >3.2 with values <10 indicating lack of significant collinearity. Highest variance inflation factor was 2.48 with values <5 considered lack of significant collinearity. Model fit was deemed robust with Mallow's Cp no more than 1 greater than P (number of regression parameters).

In an effort to compare traditional lipids to NMR lipid parameters in predicting vascular structure and function in our cohort, we developed a composite measure of vascular target organ damage. We calculated z-scores using means and standard deviations from the lean, healthy subjects in our population for all 3 cIMT measures, PWV, AIx, and BrachD. The z-scores of all measures were summed to produce the vascular score (VS). A higher score representing thicker and or stiffer vessels. Receiver operator characteristic (ROC) scores were then developed for each of the traditional and NMR lipid parameters to determine which had the most sensitivity and specificity in predicting elevated VS (≥90th% for lean). The nonparametric approach of DeLong[14] was then used to determine if differences in area under the curve (AUC; C-statistic) were statistically significant between traditional and NMR lipid values.

Results

The study included more non-Caucasians in the obese nondiabetic control group (O) but there were no differences in age or sex among groups (Table 1). Lean subjects (L) had lower BMI than O and T2DM group (T) with other CV risk factors increasing in severity across the groups. Traditional lipid parameters also tended to worsen across groups (Table 1) with significantly increasing total cholesterol, triglycerides (TGs), and significantly declining HDL-C from L to O to T (all $P \leq .05$). LDL-C did not differ between O and T. In contrast, LDL-P increased and LDL-S decreased from L to O to T such that subjects with T2DM had the largest number of the smallest and most dense LDL particles. VLDL-P was only higher in T although size increased from L to O to T. HDL-P did not differ but HDL-S did decline across the groups. These results demonstrate an increasingly more atherogenic lipid profile across groups.

Subjects with T2DM had evidence for target organ damage with higher cIMT, PEM, AIx, PWV and lower BrachD (Table 1). O had worse vascular structure and function compared with L for all parameters except common cIMT (all $P \leq .05$). Independent determinants of vascular

Table 1 Description of the study population (mean ± SD or frequency)

	Group = L	*N* = 232	Group = O	*N* = 228	Group = T	*N* = 214
Variable	Mean	SD	Mean	SD	Mean	SD
Age (y)	17.6	3.5	18.0	3.4	18.1	3.1
Race (% Caucasian)*	43		32		44	
Sex (% Male)	36		30		33	
Height (cm)†	165.5	10.6	166.3	9.7	168.7	10.0
Weight (kg)‡	59.0	11.6	103.1	21.5	106.0	28.5
BMI (kg/m^2)‡	21.3	2.6	37.2	7.0	37.1	9.0
SBP (mm Hg)§	107.6	10.3	116.6	11.3	121.5	12.1
DBP (mm Hg)‡	59.4	12.8	66.1	12.3	67.2	13.1
HR (beats/min)§	63.4	10.5	66.2	9.7	70.2	11.8
Glucose (mg/dL)†	89.5	6.2	92.4	7.6	150.5	79.9
Insulin (mU/mL)§	11.5	4.6	22.4	15.1	26.9	19.8
Glycosylated Hemoglobin (%)†	5.4	0.5	5.5	0.4	8.1	2.9
High-sensitivity C-reactive protein (mg/L)‡	1.0	1.7	4.4	4.2	4.6	4.1
Total cholesterol (mg/dL)§	160.7	28.4	171.6	32.5	179.8	40.6
LDL-C (mg/dL)‡	89.8	23.6	105.2	28.6	107.0	33.3
HDL-C (mg/dL)¶	56.5	13.1	47.4	10.1	44.4	11.3
Triglycerides (mg/dL)§	72.5	34.9	99.4	61.1	137.4	87.9
VLDL-P (nmol/L)†	42.7	24.8	46.5	25.3	56.5	37.3
LDL-P (nmol/L)§	849.7	248.7	1102.6	326.8	1257.9	437.9
HDL-P (nmol/L)	33.1	5.1	32.5	5.1	32.7	5.7
VLDL-S (nm)§	45.5	4.6	48.4	6.2	52.5	8.0
LDL-S (nm)¶	21.3	0.5	21.0	0.5	20.7	0.6
HDL-S (nm)¶	9.5	0.5	9.0	0.4	8.8	0.4
Common IMT (mm)†	0.50	0.08	0.49	0.08	0.53	0.10
Bulb IMT (mm)§	0.47	0.09	0.49	0.10	0.53	0.12
Internal IMT (mm)§	0.39	0.08	0.41	0.09	0.43	0.11
Peterson Elastic Modulus (mm Hg)‡	175.0	49.6	195.5	60.8	206.5	71.6
Augmentation index (%)§	−0.8	10.9	2.8	11.3	6.5	12.0
Brachial distensibility (%Δ/mm Hg)‖	6.9	1.2	5.5	1.1	5.3	1.0
Pulse wave velocity (m/s)§	5.3	0.7	6.3	1.0	6.6	1.1

BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HDL-S, HDL size; HR, heart rate; IMT, intima media thickness; LDL-S, LDL size; SBP, systolic blood pressure; SD, standard deviation; VLDL-P, very low-density lipoprotein particle.

*$P \leq .05$ for O > L&T.

†$P \leq .05$ for T > L&O.

‡$P \leq .05$ for T&O > L.

§$P \leq .05$ for T > O > L.

¶$P \leq .05$ for L > O > T.

‖$P \leq .05$ for L > O&T.

outcomes using NMR lipid parameters are presented in Table 2.

The most consistent covariates were age, sex, race, and BP. Presence of T2DM was important for the AIx and PWV. In the carotid bulb, there was a group by LDL-P interaction such that LDL-P was only significant in T2DM. BMI was significant for PWV and BrachD; metabolic variables for common cIMT, PEM, and BrachD. An NMR lipid variable entered the models for all outcomes except for common carotid. LDL-P was the most consistent contributor (bulb, internal cIMT, and AIx). The only traditional lipid parameter that entered any model was HDL-C for BrachD with a lower amount of the variance explained by the model with HDL-C than with NMR variables (BrachD = 1.9 + 0.067 × female + 0.083 × BMI z-score − 0.0024 × MAP − 0.0025 × heart rate − 0.032 × log insulin + 0.08 × HDL-C; $R^2 = 0.43$, $P \leq .0001$). Regression of internal cIMT (Fig. 1) and PWV (Fig. 2) on NMR and traditional lipid variables demonstrates the stronger relationship with NMR lipid measures. As seen in previous studies, only a small amount of the variance in cIMT was explained by the model[10] and the contribution on NMR lipid values was small. To further explore potential race differences in the relationship between NMR lipid values and VS, we repeated the models stratified by race (data not shown). The models for White and Black were similar to each other although it appeared that diabetes might be more important in Blacks and SBP more important in Whites. However, the stratified

Table 2 Independent determinants of vascular outcomes

	Common	Bulb	Internal	PEM	AIx	PWV	BrachD
Parameter	Higher worse						Lower worse
Intercept	−0.87	−0.89	−1.22	4.8	20.81	1.13	1.32
Age	0.0097	0.012	0.016	0.021	2.5	0.02	
Race (Caucasian reference)	0.041		0.048	0.05		0.079	
Sex (male reference)	−0.095	−0.054	−0.11			0.022	0.68
Group (T2DM reference)							
Lean		0.068*			−3.59	−0.098	
Obese		0.023*			−2.02*	−0.039	
Group by LDL-P interaction							
Lean		−0.00012					
Obese		−0.00056*					
BMI z-score						0.025	−0.085
Height					−0.31		
SBP z-score	0.014	0.024	0.031				
MAP				0.0047	0.18	0.0032	−0.0021
HR						0.0028	−0.0024
Insulin log				0.038			−0.39
Glycosylated haemoglobin	0.015						
LDL-P		0.000072	0.00011		0.0035		
LDL-S							0.041
HDL-S				−0.58		−0.033	
VLDL-S					0.15		
R2	0.18	0.11	0.22	0.17	0.17	0.57	0.44

Aix, Augmentation index; BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HDL-S, HDL size; HR, heart rate; IMT, intima media thickness; LDL-P, LDL particle; LDL-S, LDL size; MAP, mean arterial blood pressure; PEM, Peterson's elastic modulus; PWV, pulse wave velocity; SBP, systolic blood pressure; SD, standard deviation; T2DM, type II diabetes mellitus; VLDL-S, very low-density lipoprotein size.

All model $P \leq .0001$. All beta parameter estimates $P \leq .05$ except where indicated by *.

Nonsignificant terms not involved in an interaction were removed from the models and are represented by blank cells.

models had fewer significant terms and lower R^2 than the model with races combined, which may indicate lower power of the stratified analyses. When we explored differences in optimum cut-points to predict vascular dysfunction by race, not surprisingly, cutpoints for TG, TG/HDL ratio, and VLDL particle number differed by for Whites (103 mg/dL, 2.63 and 60.1 nmol/L) and Blacks (82 mg/dL, 1.8 and 36.7 nmol/L) respectively but the LDL and HDL parameters and non-HDL were similar.

The prevalence of elevated VS (VS, ≥90th% for lean subjects) was 30% overall (9.5% for L, 35.5% for O, 46.3% for T). When the C-statistics for the AUC for the ROC

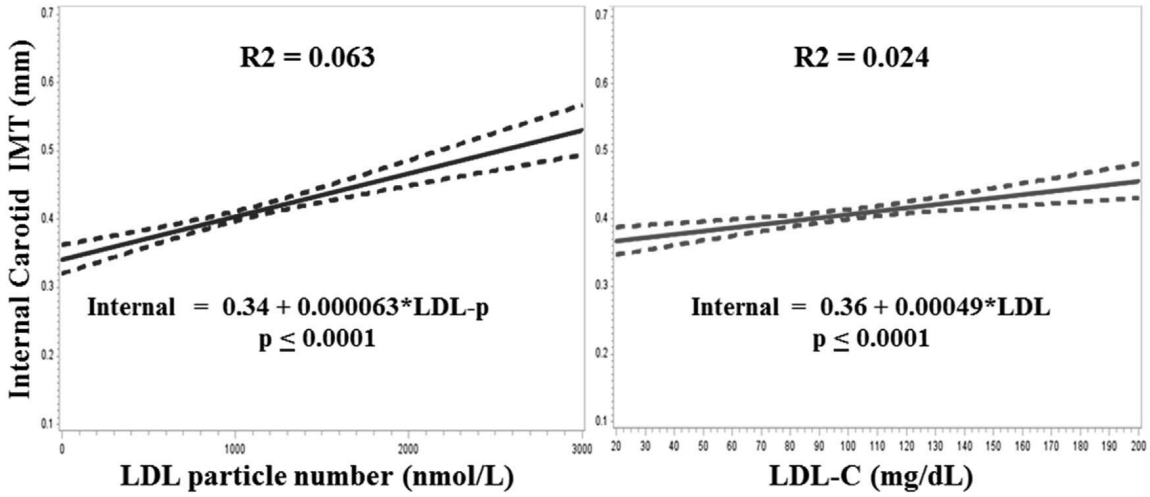

Figure 1 Regression of Internal cIMT on LDL parameters (solid line = regression, dashed line = confidence interval around the mean). cIMT, carotid intima media thickness; LDL, low-density lipoprotein.

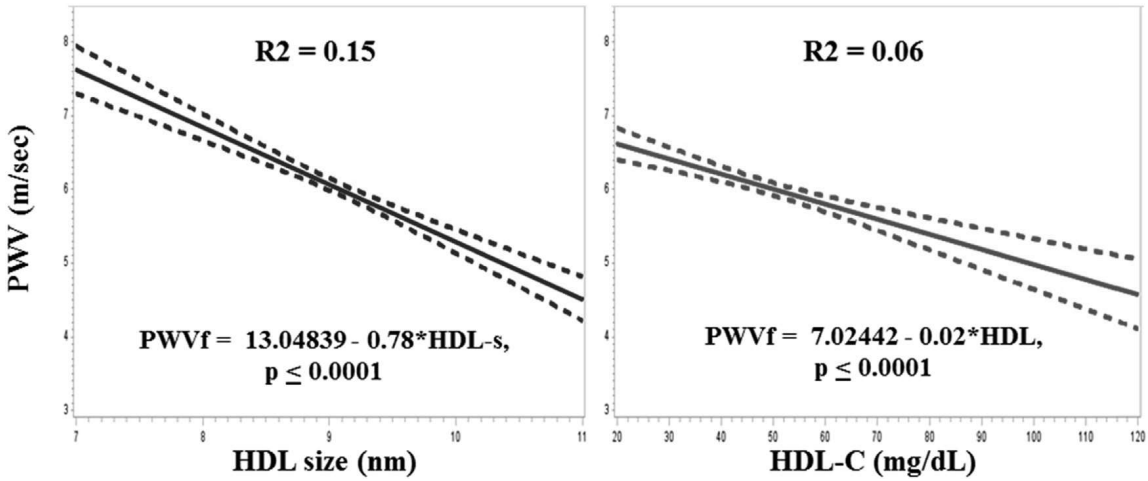

Figure 2 Regression of PWV on HDL parameters (solid line = regression, dashed line = confidence interval around the mean). HDL, high-density lipoprotein; PWV, pulse wave velocity.

curves were compared by the method of Delong[14] (Table 3), HDL-S had greater sensitivity and specificity compared with HDL-C with a significantly greater AUC ($P \leq .003$). Similarly, LDL-P was superior to LDL-C ($P \leq .001$) with a trend for superiority for LDL-S ($P = .11$). TG concentration had a greater AUC then VLDL-P or VLDL-S. TG to HDL ratio (TG/HDL-C) was greater than TG alone but performed similarly to HDL-S, and there was no significant difference in AUC between TG/HDL-C and HDL-S.

Discussion

Our data demonstrated that fewer ideal levels of CV risk factors are present in adolescents and young adults with obesity and T2DM compared with lean adolescents and young adults. As previously demonstrated, the CV risk factors are associated with noninvasive measures of atherosclerotic target organ damage. Not surprisingly, lean subjects have the lowest LDL-C and TGs and the highest HDL-C levels. However, LDL-C levels were similar between O and T2DM groups, whereas NMR lipid evaluation was more sensitive, demonstrating significantly higher LDL-P in diabetic compared with both obese and lean groups. The most novel finding from our data is that NMR lipid parameters are stronger independent predictors of vascular damage than traditional lipid parameters. These data suggest the utility of using advanced lipid testing to assess risk for target organ damage in high-risk adolescents and young adults with obesity and T2DM. This may be especially important in young subjects with T2DM because LDL-P was an important determinant of thickness in the carotid bulb but only in the presence of diabetes.

In 1957, the Framingham Heart Study established the relationship between elevated serum total cholesterol level and incident coronary heart disease.[1] Since then, there have been many refinements in lipid measurements including the recent development of techniques that determine lipoprotein particle number and size. These improvements in technique are important because the total cholesterol and TG concentrations reported with traditional lipid testing include the total amount of cholesterol found in a variety of different lipoprotein particles.[2] Because the cholesterol content and atherogenic potential of lipoproteins may differ by lipoprotein class and size, the latter may be more strongly related to atherosclerosis than traditional cholesterol concentration. Therefore, it is not surprising that longitudinal adult studies have found that LDL-P had a higher hazard ratio for predicting incident CV events than LDL-C.[5] Furthermore, in subjects with discordant lipid values (ie, acceptable LDL-C but abnormal LDL-P), LDL-P, not LDL-C-predicted events.[4,5] In fact, 1 study found that LDL-P was related to coronary artery disease even after adjustment for Framingham risk score and

Table 3 AUC of ROC curves for prediction of vascular score >90th% of lean subjects

Lipid value	AUC	Sensitivity	Specificity	Best cutpoint
HDL-C	0.68	0.65	0.64	47
HDL-P	0.55	0.67	0.42	33.6
HDL-S*	**0.73**	0.68	0.70	8.9
LDL-C	0.63	0.68	0.53	94
LDL-P†	**0.70**	0.77	0.54	925
LDL-S	0.70	0.54	0.76	20.8
TG	0.69	0.67	0.63	83
VLDL-P	0.56	0.34	0.78	60.1
VLDL-S	0.67	0.55	0.76	49.2
TG/HDL-C	0.72	0.66	0.67	1.8

AUC, area under the curve; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; HDL-S, HDL size; LDL, low-density lipoprotein; LDL-P, LDL particle; LDL-S, LDL size; TG, triglyceride; VLDL-P, very low-density lipoprotein particle; VLDL-S, very low-density lipoprotein size. Bold items indicate highest AUC among concentration, particle number and particle size.

*$P \leq .003$ for AUC significantly different from HDL-C.

†$P \leq .003$ for AUC significantly different from LDL-C (by method of DeLong et al[14]).

LDL-C.[15] Similarly, small HDL size was also independently associated with coronary events for both sexes retaining significance after adjustment for lipid concentration and other CV risk factors in men.[16]

LDL-P has proven useful in predicting sub-clinical atherosclerosis, with a stronger relationship to carotid plaque than LDL-C in former NFL football players known to be at high risk for atherosclerosis.[17] LDL-P was also proven useful in healthy individuals. In the Multi-Ethnic Study of Atherosclerosis study, multivariable models containing LDL-P predicted increased cIMT and addition of LDL-C did not improve the results.[18] In the Bogalusa Heart Study, models using NMR lipoprotein size had a larger AUC for the ROC curve predicting elevated cIMT (0.782, [0.718–0.841]) although the result was statistically similar to the model using traditional lipid values (0.754 [0.690–0.812]).[19] In the Atherosclerosis Risk in Communities study, both LDL-C and LDL-P were significant predictors of carotid plaque volume in older adults (mean age 71 years), but the odds ratio of greater plaque burden was slightly higher for LDL-P.[20] LDL size is also important. LDL-S is inversely related to cIMT independent of CV risk factors in healthy middle-aged men.[21] LDL-S also predicted higher cIMT in subjects with familial combined hyperlipidemia where traditional lipids were not significant.[22] Small LDL-S is also associated with thicker cIMT in middle-aged subjects with metabolic syndrome[23] and in patients with coronary artery disease.[24,25] In subjects with T2DM, small LDL was more highly associated with cIMT than LDL-C[26] retaining significance even after adjustment for other risk factors.[27] The importance of LDL-P number and size are not entirely clear for subjects with type 1 diabetes mellitus as the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study found stronger relationship between cIMT and LDL-C than LDL-P or LDL-S.[28] Similar to our data, however, they found all lipid parameters related more strongly to the internal than the common carotid artery.

Few studies have evaluated the relationship between lipids and arterial stiffness. Specifically, in adults with a mutation in the lecithin:cholesterol acyl transferase gene, affected subjects with very low HDL-C had higher PWV than nonaffected controls.[29] In healthy Japanese subjects, low HDL-C was also associated with higher brachial-ankle PWV.[30] No studies to date have evaluated the effect of lipoprotein particle size on arterial stiffness, we found that a higher TG/HDL-C ratio was associated with increased PWV and lower BrachD in adolescents and young adults.[31] Although measurement of HDL size was not available in that study, since TG/HDL-C ratio is highly correlated with lipoprotein particle size in adolescents and young adults,[32] it appears these results are concordant.

Limitations

This study has a number of limitations. First, our study is cross-sectional. Therefore, we cannot determine if the more adverse-sized lipoprotein particles are initiating the target organ damage. Also, by design, our study was enriched with individuals of non-Caucasian race, obesity, and T2DM. Although our statistical models controlled for these factors, the results of our study may not be generalizable to the general population. We also only measured traditional and NMR values at 1 time point so we cannot control for biologic or seasonal variation.[33] Because only a small amount of the variance in cIMT was explained by the model and the contribution on NMR lipid values was small, it is possible that our cohort was imaged at too young an age to have a sufficient exposure to high LDL particles to demonstrate a strong effect on vascular parameters. Other limitations include that NMR measures were performed on frozen samples, whereas the traditional lipids were performed on fresh blood. However, there was a correlation of 0.92 between TG measured by both techniques and a previous study found no difference in results after multiple freeze/thaw cycles[11] suggesting validity of the frozen samples. We also did not measure oxidized LDL, a biomarker known to have a stronger relationship to target organ damage than the traditional LDL-C measurement.[34] Because there are no longitudinal studies using BrachD to predict heart attack and stroke, we were unable to weight our vascular dysfunction score based on strength of prediction of hard CV events. Finally, lack of widespread availability (only a few laboratories in the US perform the testing) and increased cost may limit generalizability and clinical application of NMR lipid testing in the near future.

Conclusions

Adult studies demonstrate the superiority of lipoprotein particle size and number over traditional cholesterol concentration in predicting degree of atherosclerosis. Our study extends these observations to a younger population for the first time. We found that lipoprotein particle number and size are more strongly related to vascular structure and function than traditional lipid values in adolescents and young adults although the effect size for the lipid parameters was small. Because diet,[35] exercise programs,[36] and drugs[37–41] may affect lipoprotein particle size in different ways, particle size may become important in assessing the efficacy of treatment of lipids to reduce CV risk. This may be why 1 recent analysis found that managing LDL-C and LDL-P in comparison to LDL-C alone resulted in reduced costs and fewer CV events.[39] For this reason, we may see increased use of measures of lipoprotein particle size in the future to prevent heart attack and stroke.

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Authors' contributions: E.M.U., L.M.D., and T.R.K. designed the study, collected the data, participated in analyses and contributed to the article. C.E.M. analyzed the carotid ultrasounds, participated in analyses, and

contributed to the article. P.R.K. and Z.G. performed data analyses and contributed to the article. A.S.S. contributed to the analysis and completion of the article. All authors have approved this version and agree to be accountable for all aspects of the work.

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