Low-Density Lipoprotein Cholesterol versus Particle Number in Middle School Children

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Objectives To characterize lipids and lipoproteins in a diverse school-based cohort and identify features associated with discordance between low-density lipoprotein cholesterol (LDL-C) and LDL particle (LDL-P).

Study design Sixth-grade children enrolled in the HEALTHY trial (n = 2384; mean age 11.3 ± 0.6 years; 54.2% female) were evaluated for standard lipids, lipoprotein particles measured by nuclear magnetic resonance, and homeostatic model of insulin resistance. Characteristics of subgroups with values of LDL-C and LDL-P discordant by >20 percentile units, an amount reasoned to be clinically significant, were compared.

Results Four-hundred twenty-eight (18%) of children were in the LDL-P < LDL-C subgroup and 375 (16%) in the LDL-P > LDL-C subgroup. Those with LDL-P > LDL-C had significantly greater body mass index, waist circumference, homeostatic model of insulin resistance, triglycerides, systolic and diastolic blood pressure, and reflected a greater Hispanic ethnic composition but fewer of black race than both the concordant (LDL-P ≅ LDL-C) and opposite discordant (LDL-P < LDL-C) subgroups.

Conclusions There is as much lipoprotein cholesterol compositional heterogeneity in sixth graders as has been described in adults and a discordant atherogenic phenotype of LDL-P > LDL-C, common in obesity, is often missed when only LDL-C is considered. Conversely, many children with moderate-risk cholesterol measures (75th to 99th percentile) have a lower LDL-P burden. *(J Pediatr 2013;163:355-62)*.

One of the modifiable risk factors for cardiovascular disease (CVD) is dyslipidemia, but the optimal biomarker(s) to capture this risk is debated. Decades of evidence support the role of cholesterol infiltration into the vascular wall in atherogenesis, with uptake of ectopic lipid leading to foam cell and fatty streak formation. Cholesterol enters the arterial wall in apolipoprotein B (apoB)-containing lipoproteins, predominantly low-density lipoprotein (LDL), but the cholesterol content of LDL particles (LDL-Ps) varies widely such that LDL cholesterol (LDL-C) is not always an accurate estimate of LDL-P burden. Non-high-density lipoprotein cholesterol (HDL-C, measured as total cholesterol [TC] minus HDL-C), captures the cholesterol content within all lipoprotein particles considered to be atherogenic, correlates more strongly with LDL-P than LDL-C, and is currently recommended as an alternate measure of atherosclerotic risk, especially in hypertriglyceridemic adults[1] and children.[2] LDL-lowering treatment in children is of proven benefit when LDL-C levels are extreme,[3] but the continued substantial burden of CVD suggests that the full spectrum of lipoprotein-related risk for optimal primary prevention is neither adequately identified or managed.

There is incomplete prediction of risk with either LDL-C or non-HDL-C[4] and persistent cardiovascular risk in the face of agressive cholesterol-lowering therapies.[5] Both may be explained at least partially by the disagreement between lipoprotein particle and cholesterol measures. LDL-P concentration can be modest in the face of elevated LDL-C (when LDL-Ps are particularly cholesterol-rich) and conversely can be substantial despite low LDL-C concentrations when LDL-Ps are cholesterol-depleted. In adult longitudinal studies, increased carotid intima media thickness and incident CVD events are more strongly predicted by baseline LDL-P assessed by apoB, nuclear magnetic resonance (NMR), or ion mobility than by either LDL-C or non-HDL-C.[6,7]

Although levels and correlates of LDL-P have been recently described in small cohorts of children,[8] data from a population-based pediatric evaluation of

apoB	Apolipoprotein B	LDL-C	Low-density lipoprotein cholesterol
BMI	Body mass index		
CVD	Cardiovascular disease	LDL-P	Low-density lipoprotein particle
HDL-C	High-density lipoprotein cholesterol	NMR	Nuclear magnetic resonance
		TC	Total cholesterol
HDL-P	High-density lipoprotein particle	TG	Triglycerides
HOMA-IR	Homeostatic model of insulin resistance	VLDL	Very-low-density lipoprotein
		VLDL-P	Very-low-density lipoprotein particle
LDL	Low-density lipoprotein		

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sufficient size to permit assessment of discordance between cholesterol and lipoprotein particle measures have not been variable. This report evaluates the lipid and lipoprotein particle characteristics in a well-characterized, diverse, school-based cohort of sixth graders[9] and characterizes the clinical traits that are associated with the LDL-P burden.

Methods

HEALTHY, a cluster randomized trial designed to investigate the effectiveness of an integrated lifestyle intervention in middle schools in the reduction of risk factors for type 2 diabetes, has been described in full previously.[9] Schools were the unit of randomization, intervention, and analysis. Major inclusion criteria for schools were at least 50% of children eligible for federally subsidized, free, or reduced-priced meals and/or at least 50% of its students whose race/ethnicity was black or Hispanic. The study was approved by the institutional review boards of all participating research institutions. All children for whom data were collected provided assent with parental consent. Baseline data on sixth graders incorporated into these analyses included anthropometric measures, blood pressure, fasting insulin, glucose, and lipid profiles.

Fasting blood draws were ensured using a 2-step procedure: (1) The evening before data collection, the study staff called the students scheduled for the next day's blood draws to remind them not to eat any food or drink anything except water after midnight and not to eat breakfast; and (2) At check-in, students were questioned about the last time they had anything to eat or drink and those who indicated they had not fasted were rescheduled but still received their incentive.

To rule out any confounding of nonfasting sampling on glucose, insulin, or triglyceride (TG) values, a full sensitivity analysis was performed in which the authors excluded any subjects with a baseline glucose >99 mg/dL and no study conclusions were altered. The principle outcome variable in this report, the LDL-P, is not affected by the fasting state. Pubertal status was individually self-reported in private by use of the validated Pubertal Development Scale[10] and converted to pubertal stage groups that are consistent with the 5 pubertal stages that have been outlined by Tanner. The homeostatic model of insulin resistance (HOMA-IR) was calculated to estimate insulin resistance using the formula: fasting glucose [mmol/L] × fasting insulin [μU/L]/22.5.

Plasma samples were collected in ethylenediaminetetraacetic acid after a 12- to 14-hour fast and were separated on the morning of collection by centrifugation (1200–1500 g, 4°C, 20 min). Lipid profiles, including TC, TG, and HDL-C were measured by Centers for Disease Control and Prevention–standardized direct assay. LDL-C was estimated by use of the Friedewald formula. Lipoprotein particle profiles were measured on archived frozen specimens by NMR spectroscopy with the LipoProfile–3 algorithm at LipoScience, Inc (Raleigh, North Carolina).[6] Very-low-density lipoprotein (VLDL) particle (VLDL-P), LDL particle (LDL-P), and HDL particle (HDL-P) subclasses were quantified from the amplitudes of their spectroscopically distinct lipid methyl group NMR signals. VLDL-P, LDL-P, and HDL-P are the totals of the particle number concentrations of their respective subclasses, and their weighted-average particle sizes were calculated from the sum of the diameter of each subclass multiplied by its relative mass percentage estimated from the amplitude of its methyl NMR signal.[11] Results reported are from the 2384 sixth-grade HEALTHY participants who provided informed consent for ancillary studies and for whom a frozen specimen was available for analysis.

Statistical Analyses

Means (±SD), medians (±quartile), or frequency distributions (for categorical variables) were used to summarize the characteristics for the complete sample. Percentile distributions of LDL-C and LDL-P were calculated and participants defined as having concordant or discordant levels if the difference between the 2 measures of LDL quantity were ≤20 or >20 percentile units, respectively. Any definition of discordance is unavoidably subjective; we considered a difference of >20 percentile units to be reflective of a clinically meaningful difference in LDL burden. For example, an LDL-C at 75th percentile, if associated with an LDL-P at 95th percentile, might reflect the risk associated with the 95th percentile of LDL-C, and visa versa. Regression models were fit for the association of concordance/discordance status with sex and race/ethnicity by the use of the PROC GLIMMIX procedure and with anthropometric and lab values using the PROC MIXED procedure.[12] To adjust for the clustering of participants within schools, a random effect was included in the models.

All models were adjusted for pubertal stage and sex was added as an additional covariate to all models except those assessing association between sex and discordance/concordance status. *P*-values along with adjusted means and 95% CIs are reported. Whenever exploratory statistically significant group differences were found ($P < .05$), Bonferroni-adjusted pair-wise comparisons were performed to determine where the actual differences lie. Because of skewness, insulin, cholesterol molecules per LDL-P, HDL-P, TG, and VLDL-P size were log transformed and LDL-P and VLDL-P were square root transformed to distribute data normally.

The distributions for LDL-P size and HDL-P size were nontransformable and could not be subjected to the regression models although means and 95% CIs are reported. When considered as a dichotomous variable above or below 75 nmol/L, however, small LDL-P associated significantly with LDL-P >75th percentile and with all variables associated with LDL-P (data not shown). Spearman rank correlations were estimated to assess the associations of LDL-C and LDL-P with clinical and laboratory characteristics, unadjusted for cluster of participants within schools. To illustrate how often discordant lipoprotein phenotypes might be missed by standard LDL or non-HDL-C values, a cross tabulation of LDL-P in the first quartile, second