Estimation of LDL Particle Size Using Lipid Indices: A Population-Based Study of 1578 Schoolchildren

Yaeko Watabe, MD,¹ Osamu Arisaka, MD, PhD,¹ Noriko Miyake, MD, PhD,² Go Ichikawa, MD, PhD,¹
Satomi Koyama, MD, PhD,¹ and Naoto Shimura, MD, PhD¹

Abstract

Background: Low-density lipoprotein (LDL) is atherogenic and LDL particles are reduced in diameter in the presence of insulin resistance, forming small, dense LDL. This study was conducted to assess the relationship between commonly used lipid indices and LDL particle size and furthermore to clarify the best surrogate lipid markers that could conveniently be used to estimate LDL particle size in children.

Methods: We determined LDL particle diameter by gradient gel electrophoresis in 1578 children aged 10–12 years. At the fasting state, the relationships between measured LDL particle size and lipid variables [total cholesterol (TC), triglycerides (TG), LDL-cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), atherogenic index [(TC-HDL-C)/HDL-C, TG/HDL-C, LDL-C/HDL-C, and LDL-C/apolipoprotein B (Apo B) and non-HDL-C (TC-HDL-C)] were analyzed.

Results: The LDL particle diameter was $26.64 (\text{mean}) \pm 0.48 (\text{SD})$ nm in boys ($n = 820$) and 26.66 ± 0.49 nm in girls ($n = 758$); there was not a statistically significant difference. There were statistically significant correlations between LDL particle size and TG or HDL-C concentrations ($r = 0.28 \sim 0.37$), but the correlations with LDL-C and ApoB were very weak. The combined lipid measures, such as atherogenic index, TC/HDL-C, TG/HDL-C, and LDL-C/HDL-C showed moderate correlations ($r = 0.33 \sim 0.38$) with LDL particle size; however, the correlation of non-HDL-C with LDL particle size was weak ($r = 0.18 \sim 0.19$). Simple HDL-C measure appeared to be of comparable value to combined lipid measures.

Conclusions: Our data indicate that various lipid indices are not superior to HDL-C levels alone as a clinical tool for estimating LDL particle size. Non-HDL-C was less valuable in this aspect.

Introduction

LOW-DENSITY LIPOPROTEIN (LDL) particles are heterogeneous in density, size, lipid composition, and pathogenic properties.^{1–3} The smaller and denser they are, the stronger their atherogenic impact, possibly because of the low binding affinity of small, dense LDL particles to the LDL receptor, their low resistance to oxidative stress, their prolonged half-life in plasma, and their easy penetration of the arterial intima due to small particle size.^{4,5} It has been demonstrated in many studies that predominance of small dense LDL (sdLDL; LDL particle size of ≤ 25.5 nm) is associated with increased risk of coronary artery disease and the presence of sdLDL is a predictor of cardiovascular events.^{6,7} As cardiovascular risk factors persist over time from childhood to adulthood,^{8,9} the presence of sdLDL in childhood is predictive of

coronary heart disease in adulthood. In addition, since smaller LDL particle diameter is associated with increases in insulin resistance,^{10–13} detecting sdLDL in the plasma is recognized as a useful tool for the prediction and screening of lifestyle-related diseases that appear to be associated with insulin resistance.^{14,15} It has been reported that higher fasting insulin level was also correlated with smaller mean LDL particle size in boys.¹⁶ However, data on the distribution of LDL particle size and its association with cardiovascular risk factors are limited in children.

The gold standard for assessing LDL particle size is gradient electrophoresis, a sophisticated technique that cannot be used on a regular basis in clinical laboratories.^{1,2} Therefore, the measurement of LDL particle diameter is not routinely performed in the clinical setting. As surrogate markers of LDL particle size, the relationships between LDL particle size and several lipid indices easily derived

¹Department of Pediatrics, Dokkyo Medical University, Mibu, Kitakobayashi, Japan.

²Department of Clinical Pathology, Juntendo Nerima Hospital, Tokyo, Japan.

from a lipid panel, such as LDL-C/apolipoprotein B (Apo B),¹⁷ atherogenic index,¹⁸ total cholesterol (TC)/high-density lipoprotein cholesterol (HDL-C),¹⁹ triglycerides (TG)/HDL-C,²⁰ and non-HDL-C,²¹ have been proposed in adults with inconsistent results; however, investigations in children have been limited.

Therefore, the objectives of this study were to investigate the relationship between measured LDL particle size and various lipid parameters in a large population-based sample of children, and to then clarify the best surrogate markers to estimate LDL particle size.

Methods

Subjects

In total 1,578 children (820 boys and 758 girls), fourth grade elementary school students (10 years) and first year junior high school students (12 years), participated in this school-based health survey of lifestyle-related disease, conducted between 2008 and 2012 in Fujioka town in the province of Tochigi, Japan. The population of this town is 18,000, with one-half of the people working as farmers and one-half commuting to nearby large cities. The town has four elementary schools and two junior high schools.

The examinations were carried out in September-October at the school. In order to obtain overnight fast blood samples, students were allowed to bring a packet box breakfast, which they took after the health examination at the schoolroom.

Written informed consent was obtained from the guardian for the physical examination. The study was approved by the ethics committee of Dokkyo Medical University.

Measurements of lipids and blood pressure

Fasting blood sampling and standardized measurement of systolic blood pressure and diastolic blood pressure were performed at school. Blood pressure was measured in the sitting position after a 5 min rest by trained observers with an electronic sphygmomanometer (H-55, TERUMO) using a cuff that was appropriate to the size of the child's upper right arm.

Blood samples collected in collection tubes containing 1 g/L EDTA were sent within 3 h to the laboratory. Plasma was separated and stored at -80°C until measurement of LDL particle size.

Levels of TC (Cholestest CHO, Daiichi Pure Chemicals Co. Ltd.) and TG (Aqua-auto TG-II, Kainos Laboratories, Inc.) were determined using enzymatic methods. HDL-C was measured by precipitation of other lipoproteins with the

direct method (Cholestest N HD, Daiichi Pure Chemicals Co. Ltd.). Low-density lipoprotein cholesterol (LDL-C) was calculated by using the Friedewald formula. Apolipoprotein B (ApoB) was quantified by turbidimetric immunoassay (ApoB auto N, Daiichi Pure Chemicals Co. Ltd.). Among the total cohort of 1578 children, 381 boys and 353 girls underwent measurement of Apo B due to insufficient amount of blood sampling. The atherogenic index (AI) was calculated as follows: $\text{AI} = [\text{TC} - \text{HDL-C}] / \text{HDL-C}$.²²

Determination of LDL particle size

The LDL particle diameter was determined according to the method described by Krauss et al.,¹ by gradient gel electrophoresis on 2.5% to 16% polyacrylamide. After the gels were equilibrated at 120 V for 20 min, electrophoresis was performed for each gel containing the serum sample diluted 1:2 with sample buffer (comprised of 31% sucrose, 0.06% EDTA-2Na, and 0.01% BPP) to a volume of 20 mL. Each gel also contained thyroglobulin, apoferritin, and latex beads as reference standards of known diameter. The gels were then electrophoresed at 120V for 19 h and then stained for lipid with Oil Red O that was heated to 55°C for 24 h, and for protein with Coomassie Brilliant Blue for 15 min. They were then destained with ethanol and immersion in acetic acid. The gels were then scanned with an image scanner (Epson GT-6500: Seiko Epson Corporation) and analyzed using an image processing and analysis program for Macintosh (National Institutes of Health Image 1.61). Migration distances were determined. The LDL particle diameter was then calculated by comparing the mobility of the sample with the mobility of three calibrated standards on each gel. Small dense LDL was defined as LDL with a particle diameter ≤ 25.5 nm, based on the criteria proposed by Austin et al.²³

Statistical analysis

All statistical analyses were performed using SPSS 15.0 for Windows. Unpaired Student's *t*-test was used to compare various parameters between the groups. The relationships between LDL particle size and variables were analyzed using parametric tests; relationships between the variables are reported as Pearson correlation coefficients. A *P* value < 0.05 was considered statistically significant.

Results

The baseline characteristics of the study population are shown in Table 1.

TABLE 1. BACKGROUND ANTHROPOMETRIC, BLOOD PRESSURE, AND LIPID DATA OF 1578 SCHOOLCHILDREN

	Boys (n = 820)	Girls (n = 758)	P value
Body mass index (Z-score)	0.1 ± 1.1	-0.1 ± 0.9	< 0.05
Systolic blood pressure (mmHg)	105.1 ± 10.9	104.0 ± 10.9	< 0.05
Diastolic blood pressure (mmHg)	57.9 ± 8.6	58.5 ± 8.4	n.s.
TC (mg/dL)	168.1 ± 27.0	171.4 ± 25.6	< 0.05
LDL-C (mg/dL)	92.4 ± 23.5	95.6 ± 22.2	< 0.005
HDL-C (mg/dL)	68.1 ± 14.1	67.2 ± 12.7	n.s.
TG (mg/dL)	58.9 ± 30.8	63.2 ± 30.1	< 0.01
Apo B (mg/dL)	69.6 ± 19.8 (n = 381)	72.6 ± 19.4 (n = 353)	< 0.05

Values are expressed as mean $\pm$ standard deviation.

Apo B, apolipoprotein B; HDL-C, high-density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol; TG, triglycerides.

TABLE 2. CORRELATION OF LDL PARTICLE SIZE AND BODY MASS INDEX, BLOOD PRESSURES, LIPIDS, AND VARIOUS LIPID INDICES

	Boys (n=820)		Girls (n=758)	
	r	P value	r	P value
Body mass index (Z-score)	-0.15	<0.0001	-0.08	<0.05
Systolic blood pressure	-0.001	n.s.	-0.01	n.s.
Diastolic blood pressure	-0.01	n.s.	-0.03	n.s.
TC	0.02	n.s.	0.01	n.s.
LDL-C	-0.12	<0.0001	-0.13	<0.0001
HDL-C	0.37	<0.0001	0.37	<0.0001
TG	-0.31	<0.0001	-0.28	<0.0001
ApoB	-0.13 (n=381)	<0.01	-0.06 (n=353)	n.s.
Atherogenic index	-0.35	<0.0001	-0.38	<0.0001
TC/HDL-C	-0.35	<0.0001	-0.38	<0.0001
TG/HDL-C	-0.37	<0.0001	-0.36	<0.0001
LDL-C/HDL-C	-0.33	<0.0001	-0.35	<0.0001
non-HDL-C	-0.18	<0.0001	-0.19	<0.0001
LDL-C/ApoB	-0.03 (n=381)	n.s.	-0.08 (n=353)	n.s.

n.s., not significant; TC, total cholesterol.

1. The LDL particle diameter was $26.64 (\text{mean}) \pm 0.48$ (Standard deviation) nm in boys ($n=820$) and 26.66 ± 0.49 nm in girls ($n=758$), which was not significantly different.
2. The prevalence of small, dense LDL (LDL with a particle diameter ≤ 25.5 nm) in was 0.6% (5/820) in boys and 2% in girls (15/758).
3. Correlations coefficients of the measured LDL particle diameter versus cardiovascular risk factors (i.e., lipids, blood pressure, and body mass index) are shown in Table 2. Varying degrees of statistically significant correlations between LDL particle size and cardiovascular risk factors were observed except for serum TC and blood pressure.
4. Correlations coefficients of measured LDL particle diameter versus surrogate markers [i.e., atherogenic index (AI) [(TC-HDL-C)/HDL-C], TC/HDL-C, TG/HDL-C, HDL-C/LDL-C, and LDL-C/Apo B, and non-HDL-C)] for estimating LDL particle diameter are shown in Table 2. The correlation between LDL particle size and AI is shown in Fig. 1.

Discussion

To date, limited information is available on the distribution of LDL particle size in large population-based samples of children and adolescents. This study used strict fasting blood samples collected from more than 1500 subjects, thus eliminating the effects of postprandial elevation of TG or insulin on lipoprotein metabolism and LDL particle size.^{4,18}

In terms of LDL particle size by sex, despite methodological differences, higher mean LDL particle sizes have been reported in females compared with males.^{3,24} We did not observe any such difference by sex in our study [26.64 nm (boys) vs. 26.66 nm (girls)]. Further investigations are needed to confirm sexual differences in children and adolescents.

The prevalence of the sdLDL phenotype was only 1.3% in our cohort and was similar between sexes and across ages. Shimabukuro et al.³ reported a prevalence of 10.8% in prepubertal Japanese boys and 4.4% in prepubertal Japanese

girls by the same gradient gel electrophoresis method. Freedman et al.²⁵ reported that the prevalence of the small, dense LDL phenotype ranged from 5% in black boys and girls to 13% in white boys, using nuclear magnetic resonance spectroscopy. These different observations may be caused by varying effects of genetic and environmental factors affecting LDL particle size.²⁶⁻²⁸

With regard to correlations between LDL particle size and simple lipid measures, moderate correlations were observed between LDL particle size and both TG and HDL-C concentrations; correlation coefficients by sex were -0.31 (boys) and -0.28 (girls) and 0.37 (both sex), respectively. In contrast, the correlations between LDL particle size and LDL-C and ApoB were weak but statistically significant.

Metabolically, hypertriglyceridemia promotes TG transfer from very low-density lipoprotein (VLDL) to HDL. The TG-enriched HDL then transfers TG to LDL and removes cholesterol from LDL; thus, the cholesterol-depleted LDL

FIG. 1. The correlation between low-density lipoprotein (LDL) particle size and atherogenic index in both sexes.

then becomes smaller and denser. In the presence of hypertriglyceridemia, the cholesteryl ester transfer protein allows cholesteryl esters to be transferred from LDL in exchange for a TG molecule from VLDL. TG is then hydrolyzed by hepatic triglyceride lipase or lipoprotein lipase to produce smaller, denser LDL particles.^{2-4,18} The activities of cholesteryl ester transfer protein and hepatic triglyceride lipase are enhanced by increased adiposity or increased insulin resistance. A reduction in LDL particle size associated with a decrease in the HDL-C level may result from the alteration in lipoprotein metabolism caused by insulin resistance.^{10,13,29} Thus, TG and HDL-C concentrations are considered to correlate well with LDL particle size.

In our investigation the correlation between Apo B and LDL particle size was poor, but ApoB can be considered as a surrogate measurement of LDL particle number, because each LDL particle carries one apolipoprotein B-100 molecule. Assessment of abnormalities in LDL particle number in addition to LDL particle size is important for qualitative evaluation of the atherogenicity caused by LDL particles.^{15,30}

With regard to the correlations between LDL particle size and combined lipid measures, lipid ratios that include information on at least two measures might provide a more integrated and concentrated explanation than single lipid measures; even TG or HDL-C appeared to exhibit pretty good correlations with LDL particle size. Therefore, several lipid ratios have been proposed as convenient clinical indicators because of the integrative information provided by multiple variables.^{19,31,32} In our observations the same degree of moderate correlations (i.e., correlation coefficients: $-0.33 \sim -0.38$) between LDL particle size and the AI, TC/HDL-C, TG/HDL-C, and LDL-C/HDL-C were found. However, the correlations between LDL particle size and non-HDL-C, LDL/Apo B were weaker. Therefore, it appeared that simple HDL-C measurement had similar value to AI, TC/HDL-C, TG/HDL-C, and LDL-C/HDL-C.

Among these parameters, the TG/HDL-C ratio has been reported as an indicator of insulin resistance³³⁻³⁵ and the AI as a predictive value for cardiovascular disease.^{22,36} Recently, the TG/HDL-C ratio has been reported to have a good correlation with homeostasis model assessment for insulin resistance, and as a marker of insulin resistance that is an essential metabolic background of reduction of LDL particle size.³⁷⁻³⁹ However, in the present study TG/HDL-C appeared to not be superior to other lipid indices. It was considered that any lipid indices (combining lipid measures, including either TG or HDL-C) seemed to correlate with LDL particle size to the same degree. Similar observations have been reported in adults.⁴⁰

Recently, it has been reported that non-HDL-C, calculated as the difference between TC and HDL-C levels, is well associated with metabolic syndrome in youths, and screening for metabolic syndrome based on non-HDL-C concentration is thought to have an advantage in the pediatric setting because pretest fasting is not required.^{21,41} However, contrary to our expectation, the correlation between non-HDL-C and LDL particle size was the weakest among the lipid parameters investigated. A similar observation has been reported elsewhere.^{42,43} Although the underlying mechanisms linking non-HDL-C and pediatric metabolic syndrome remain to be elucidated, our data indicate that non-HDL-C is not a good tool for the prediction of metabolic syndrome.

In conclusion, our data indicate that various lipid indices are not superior to HDL-C levels alone as a clinical tool for estimating LDL particle size. As surrogate markers to predict LDL particle size, lipid indices (combined lipid measures), including TG or HDL-C had similar value, but non-HDL-C was less valuable.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Osamu Arisaka, MD, PhD

Department of Pediatrics

Dokkyo Medical University

880 Kitakobayashi, Mibu, Tochigi 321-0293

Japan

E-mail: arisaka@dokkyomed.ac.jp