

Distribution of LDL Particle Size in a Population-Based Sample of Children and Adolescents and Relationship with Other Cardiovascular Risk Factors

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Background: Smaller, denser LDL particles are associated with an increased risk for cardiovascular diseases (CVD). In youths, data on the distribution of LDL particle size and on its association with other CVD risk factors are limited.

Methods: We determined LDL peak particle size by nondenaturing 2%–16% gradient gel electrophoresis in a representative sample of 2249 youths 9, 13, and 16 years of age who participated in a school-based survey conducted in 1999 in the province of Quebec, Canada. Standardized clinical measurements and fasting plasma lipid, glucose, and insulin concentrations were available.

Results: The LDL peak particle size distribution was gaussian. The 5th, 50th (median), and 95th percentiles by age and sex were 255.5–258.6, 262.1–263.2, and 268.1–269.5 Å, respectively. The prevalence of the small, dense LDL phenotype (LDL peak particle size ≤ 255 Å) was 10% in participants with insulin resistance syndrome (IRS), in contrast to 1% in those without IRS. In a multiple regression analysis, the association of LDL size with other CVD risk factors [apolipoprotein B, HDL-cholesterol (HDL-C), triglyceride (TG), and insulin concentrations, and body mass index] was strongest with TG and HDL-C concentrations: a 1 SD increase in \log_e -transformed TG concentration was associated with

a 1.2 Å reduction in LDL size, and a 1 SD increase in HDL-C was associated with a 1.1 Å increase in LDL size.

Conclusions: Although the small, dense LDL phenotype is less prevalent in youths than adults, its prevalence is clearly increased in childhood IRS. Metabolic correlates of LDL size are similar in youths and adults.

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LDL particles are a heterogeneous mixture of lipoproteins differing in density, size, lipid composition, electrical charge, and pathologic properties (1). The LDL subclass distribution is influenced by several factors, including age, sex, estrogen therapy, diet, abdominal adiposity, insulin resistance, diabetic status, and genetic factors (2–12).

Nondenaturing polyacrylamide gradient gel electrophoresis (PAGGE)⁶ (13, 14) is the most widely used technique to estimate LDL particle size. In epidemiologic studies, the most common index to describe LDL particle size is the diameter of the most abundant subspecies, which has been named the LDL peak particle size. Using mathematical modeling that separated LDL size tracings on PAGGE into gaussian curves, Austin and Krauss (11) identified 2 subclass patterns: pattern A with a predominance of large, buoyant LDL particles >255 Å in diameter; and pattern B with a predominance of small, dense LDL with diameters ≤ 255 Å. Although PAGGE is suitable for large-scale clinical determination of LDL particle size (15), it has the drawback of being labor-intensive. As a

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⁶ Nonstandard abbreviations: PAGGE, polyacrylamide gradient gel electrophoresis; LDL-C and HDL-C, LDL- and HDL-cholesterol, respectively; apo, apolipoprotein; TC, total cholesterol; TG, triglyceride; CVD, cardiovascular disease; IRS, insulin resistance syndrome; QCAHS, Quebec Child and Adolescent Health and Social Survey; BMI, body mass index; BP, blood pressure; FFA, free fatty acid; CI, confidence interval; and BHS, Bogalusa Heart Study.

result, several lipid indexes have been proposed as alternatives to estimate LDL size. Surrogate markers of LDL size include the ratio of LDL-cholesterol (LDL-C) concentration to apolipoprotein B (apoB) concentration (16, 17), the ratio of total cholesterol (TC) to HDL-cholesterol (HDL-C) (18), the ratio of apoB to HDL-C (19), the ratio of triglyceride (TG) concentrations to HDL-C concentrations or log(TG/HDL-C) (19–22), and the TG concentration itself. Their use as proxy measures of LDL size remains controversial.

Data from case-control and prospective studies have suggested that small, dense LDL is associated with an increased risk of cardiovascular disease (CVD) (23, 24). However, the relationship between small, dense LDL and CVD may not be independent of other risk factors, such as plasma TG and HDL-C concentrations. In fact, small, dense LDL is a hallmark of the dyslipidemia characteristic of the insulin resistance syndrome (IRS), which includes increased TG and decreased HDL-C concentrations (8, 25–27). The increased atherogenicity of small, dense LDL might be related to its greater propensity for uptake by arterial tissue (28), its decreased LDL-receptor-mediated uptake in favor of an increased binding to scavenger receptor (29, 30) and increased proteoglycan binding (31), and its greater susceptibility to oxidative stress (32, 33).

Most of the studies examining LDL size and its relationships with other CVD risk factors have been conducted in adults. Limited information on LDL size and its metabolic correlates is available for children and adolescents (18, 34–42). To gain more information on this subject would be important because it is recognized that the process of atherosclerosis begins in childhood (43, 44) and that IRS is frequent in youths (45). The objectives of this study thus were (a) to describe the distribution of LDL particle sizes in a population-based sample of children and adolescents; (b) to examine the relationship between LDL particle size as estimated by PAGGE and other surrogate markers; (c) to determine the prevalence of the small, dense LDL phenotype in youths with IRS compared with those without IRS; and (d) to study the association between LDL particle size and other CVD risk factors.

Materials and Methods

STUDY POPULATION

The study population included participants in the Quebec Child and Adolescent Health and Social Survey (QCAHS), a school-based survey conducted between January and May 1999 in the province of Quebec, Canada. The survey design and methods have been reported previously (46) and are only summarized here. The QCAHS used a cluster sampling design to draw 3 independent provincially representative samples of youths 9, 13, and 16 years of age (1 sample per age). The sampling frame represented 97% of all youths targeted. Response percentages were 83.4% (1267 of 1520 eligible children), 79.2% (1186 of 1498), and 77.6% (1160 of 1495) for ques-

tionnaire and anthropometric measures and 51.5% (783 of 1520), 54.6% (818 of 1498), and 58.5% (874 of 1495) for blood sampling among 9-, 13-, and 16-year-olds, respectively. French Canadians comprised 79.6% of the sample. Of 2475 blood specimens available, 226 were excluded because 107 parents refused consent for analyses other than glucose and lipids, and 119 samples were thawed on arrival at the laboratory or were of insufficient amount. Age-specific comparisons of youths who provided blood samples ($n = 2249$) with those for whom samples were not available ($n = 1364$) revealed no statistically significant differences in sex, cigarette smoking, mean body mass index (BMI), parental income, and parental education. The study was approved by the Ethics Review Board of Ste-Justine Hospital. Written informed assent and consent were obtained from the participants and their legal guardians.

CLINICAL VARIABLES

Height, weight, and blood pressure (BP) were measured according to standardized protocols (46). BMI was calculated as weight in kilograms divided by height in meters squared. Age- and sex-specific percentile cut point values for insulin, TG, and HDL-C concentrations were estimated from sample-specific distributions of the variables of interest. Children were categorized as overweight if they had a BMI at or above the age- and sex-specific 85th percentile value according to the US CDC growth charts (47). High/borderline systolic BP was defined as a value at or above the age-, sex-, and height-specific 90th percentile according to the National High Blood Pressure Education Program (48). Although IRS is a well-recognized clinical entity, there is no internationally accepted definition of childhood IRS. For the purpose of our analyses, an individual was classified as having IRS if she or he had a fasting plasma insulin at or above the 75th percentile (Table 1) and at least 2 of 5 other characteristics: overweight, high/borderline systolic BP, TG concentration at or above the 75th percentile, HDL-C concentration at or below the 25th percentile, and glucose ≥ 6.1 mmol/L (45). Current smokers 13 and 16 years of age were defined as those who responded positively to the following question: During the past 30 days, did you smoke cigarettes, even just a few puffs? This question was not asked of 9-year-

Table 1. Values for the age- and sex-specific 75th percentiles for insulin and TG concentrations, and for the age- and sex-specific 25th percentiles for HDL-C concentrations.

	Age, years	Insulin, pmol/L	TG, mmol/L	HDL-C, mmol/L
Boys	9	35.01	0.85	1.22
	13	60.04	1.05	1.11
	16	50.70	1.08	1.00
Girls	9	40.64	0.96	1.20
	13	69.92	1.07	1.13
	16	62.76	1.18	1.13

olds. Only 2.1% of this age responded positively to the question: Have you ever smoked a whole cigarette? Therefore, all 9-year-olds were classified as nonsmokers. Participants who consumed alcohol regularly were defined as those who responded "about once a week" to the question: During the last 12 months, how often did you drink alcohol? (just to taste, less than once a month, about once a month, about once a week).

BIOCHEMICAL ANALYSES

Overnight fasting blood samples were collected in collection tubes containing 1 g/L EDTA and were kept on ice until centrifugation. Plasma was separated on site within 45 min of collection, frozen on dry ice, and sent within 24 h to the laboratory, where specimens were stored at -80°C until analysis. Fasting plasma insulin, glucose, TC, TG, HDL-C, and free fatty acid (FFA) concentrations were measured as described previously (45, 46, 49). LDL-C was calculated by the Friedewald formula (50). ApoA1 and -B were determined by nephelometry (Beckman Array Protein System).

LDL peak particle size was measured by nondenaturing PAGE. Electrophoresis of 5 μL of plasma was carried out at 4°C in 2%–16% polyacrylamide gradient gels at 125 V for 24 h in a buffer containing 0.09 mol/L Tris, 0.08 mol/L boric acid, and 0.003 mol/L disodium EDTA (pH 8.3). The application of the samples was preceded by a prerun at 125 V for 15 min. Samples were loaded in loading buffer containing 200 g/L sucrose and 1 g/L bromphenol blue, and the voltage was set at 70 V for 20 min before the main migration at 125 V for 24 h. Gels were stained for proteins in a solution containing 500 mL/L ethanol, 100 mL/L acetic acid, and 1 g/L Coomassie Blue R-250 and destained in multiple changes of a solution containing 200 mL/L ethanol and 100 mL/L acetic acid. Gels were scanned by a densitometry image analyzer (Fluorchem; Alpha Innotech) and analyzed by the ImageMaster 1D Prime (Ver. 2.01) software (Pharmacia Biotech). We estimated the diameter of the major peak in each sample (LDL peak particle size) based on the migration of pooled plasma calibrators of known diameter (7). Sample application and migration differences were monitored with 2 protein internal standards, ferritin (122 Å) and thyroglobulin (170 Å; Amersham Pharmacia Biotech). Intergel CVs assessed with 2 plasma samples were 0.44% at 264.0 Å and 0.42% at 258.5 Å ($n = 112$ and 113, respectively). Duplicate measurements of a systematic random sample of 1 in 20 specimens analyzed on different days showed a median CV across specimens of 0.21% (5th–95th percentiles, 0.03%–0.62%; $n = 112$).

STATISTICAL ANALYSES

We used the values of the sample percentiles to estimate the values of the population percentiles of interest. Non-parametric confidence intervals (CIs) for the cut points of interest were constructed using the algorithm described by Hutson (51). When comparing percentile values be-

tween sexes or across ages, we concluded that they were significantly different if their respective 95% CIs did not overlap. We used χ^2 statistics to compare the prevalence of the small, dense LDL phenotype (≤ 255 Å) by age, sex, BMI category, and IRS status. Pearson correlation coefficients were computed to assess the associations between LDL size and its surrogate markers and between LDL size and other metabolic variables.

We next used mixed multiple linear regression to study the independent associations between LDL size and other metabolic variables. Independent variables were treated as fixed effects, and clustering among children in the same school was treated as a random effect. Because we pooled all participants, age- and sex-specific Z-scores for apoB, HDL-C, TG, and insulin concentrations and BMI were used in regression analyses. Z-scores were estimated from the study distributions. To take the complex sampling design into account, we estimated sampling weights and clustering effects and incorporated them into all of our computations except for correlations and regressions. The distributions of TG, insulin, and FFA concentrations and BMI were not gaussian; these variables were therefore \log_e -transformed for statistical analyses. Statistical analyses were performed with SAS statistical software (SAS Institute, Inc) and SUDAAN (Research Triangle Institute).

Results

The characteristics of the participants are listed in Table 2. In this pediatric population, the distribution of LDL peak particle size was gaussian (Fig. 1) and remarkably tight with a CV of 1.3% across all age and sex groups. Mean LDL size was significantly higher in girls than boys (263.0 vs 262.6 Å; $P = 0.018$) and lower for the 16-year-olds compared with the 9- and 13-year-olds (262.4 vs 263.0 Å and 263.0 Å, respectively; $P = 0.047$ and 0.041, respectively).

The means and values of selected percentiles for LDL peak particle size are presented by age and sex in Table 3. For each percentile examined, no difference was detected between 9-year-old boys and girls. Thirteen-year-old girls had higher values than same-age boys for the 25th and 50th percentiles, whereas in 16-year-olds, girls had a higher value than boys for the 5th percentile only. Comparisons across ages revealed lower 25th, 50th, and 75th percentile values for 16-year-olds compared with 9-year-olds in boys only.

Of the children studied, 2% had a small, dense LDL phenotype (LDL peak particle size ≤ 255 Å). There were no differences by age or sex (Table 4). Seven percent of individuals with a BMI equal to or greater than the 85th percentile and 10% of individuals with IRS had a small, dense LDL phenotype, which was significantly different from individuals with a BMI below the 85th percentile or without IRS, respectively.

Because it would be useful to have an estimate of LDL size based on commonly measured lipid concentrations, we investigated the association between surrogate mark-

Table 2. Characteristics of participants.^a

	Boys			Girls		
	9 years (n = 342)	13 years (n = 371)	16 years (n = 377)	9 years (n = 369)	13 years (n = 353)	16 years (n = 437)
BMI, kg/m ²	17.3 (3.2)	20.3 (3.8)	22.5 (3.9)	17.6 (3.5)	20.7 (4.3)	22.2 (4.1)
BP, mmHg						
Systolic	103.1 (9.9)	112.4 (11.7)	123.6 (13.5)	101.8 (9.2)	111.1 (11.3)	114.0 (11.0)
Diastolic	56.4 (6.2)	58.2 (6.6)	60.9 (7.1)	56.4 (5.9)	59.1 (7.1)	61.7 (7.4)
TC, mmol/L	4.06 (0.66)	3.86 (0.65)	3.68 (0.70)	4.22 (0.72)	4.04 (0.69)	4.14 (0.82)
LDL-C, mmol/L	2.33 (0.55)	2.20 (0.55)	2.12 (0.58)	2.48 (0.62)	2.33 (0.61)	2.37 (0.71)
ApoB, g/L	0.64 (0.14)	0.62 (0.15)	0.63 (0.17)	0.69 (0.16)	0.65 (0.16)	0.70 (0.21)
HDL-C, mmol/L	1.41 (0.27)	1.27 (0.23)	1.15 (0.20)	1.36 (0.23)	1.29 (0.24)	1.33 (0.25)
ApoA1, g/L	1.26 (0.18)	1.17 (0.16)	1.09 (0.14)	1.23 (0.16)	1.18 (0.16)	1.23 (0.18)
TG, mmol/L	0.71 (0.36)	0.87 (0.42)	0.90 (0.51)	0.84 (0.42)	0.91 (0.37)	0.96 (0.42)
FFA, mmol/L	0.49 (0.23)	0.41 (0.20)	0.33 (0.16)	0.54 (0.23)	0.42 (0.17)	0.43 (0.21)
Glucose, mmol/L	5.17 (0.31)	5.30 (0.36)	5.31 (0.39)	5.00 (0.37)	5.18 (0.35)	5.04 (0.38)
Insulin, pmol/L	29.4 (21.1)	50.1 (37.4)	47.0 (36.4)	35.4 (45.9)	58.5 (36.4)	51.0 (25.2)
Current smoker, %	NA ^b	12.5	32.8	NA	19.8	40.1
Regular alcohol intake, %	3.1	4.5	22.6	1.3	1.6	15.8

^a Data are the mean (SD) except for smoking status and alcohol intake status, which are percentages.

^b NA, not asked; however, only 2.1% had ever smoked a whole cigarette.

ers of LDL size suggested in the literature (16–22) and both the LDL size estimated by PAGGE and the small, dense LDL phenotype. We observed moderate correlations between LDL size and the TG/HDL-C molar ratio, the logarithm of this ratio, and the logarithm of TG concentrations: correlation coefficients by age and sex ranged from -0.50 to -0.57 , from -0.47 to -0.56 , and from -0.39 to -0.50 , respectively (Table 5). The associations between LDL size and other proxy measures (LDL-C/apoB, TC/HDL-C, and apoB/HDL-C ratios) were weaker. Various cut points for the TG/HDL-C molar ratio suggested to identify adults with the small, dense LDL phenotype (19, 21, 22) include 0.9, 1.33, and 1.7. In our pediatric population, the sensitivities and specificities of

these cut points were 85.9% and 78.2%, 76.1% and 93.5%, and 67.3% and 97.6%, respectively.

We next examined the association between LDL size and other CVD risk factors (Table 6). The strongest correlations were between LDL size and both TG (negative) and HDL-C (positive) concentrations: correlation coefficients by age and sex ranged from -0.39 to -0.50 and from 0.29 to 0.47, respectively. Significant but weaker negative associations were observed between LDL size and apoB concentration (correlation coefficients, -0.14 to -0.33 by age and sex). We observed weak negative associations between LDL size and TC and LDL-C concentrations only in 16-year-olds. BMI and insulin concentrations correlated negatively and weakly with LDL size

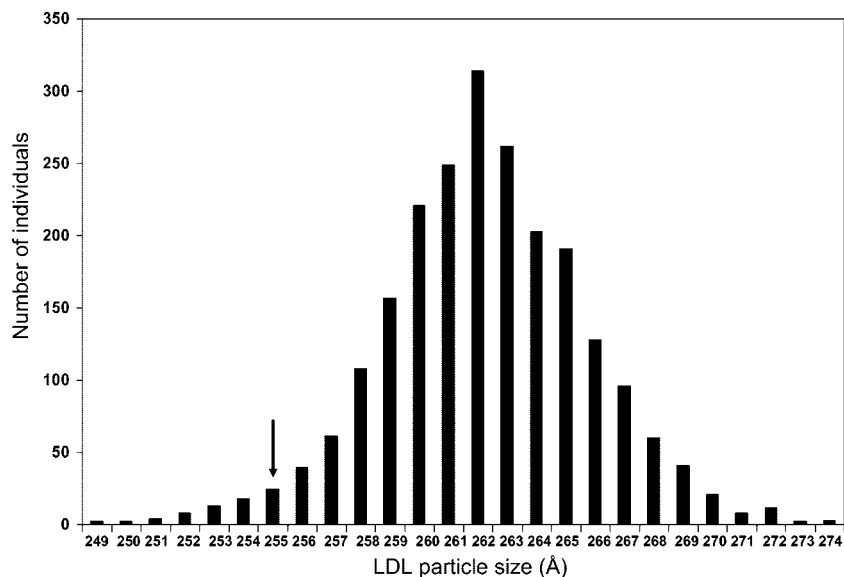


Fig. 1. Frequency distribution of LDL peak particle size in a representative sample of Quebec children and adolescents 9, 13, and 16 years of age.

The arrow indicates the cut point (≤ 255 Å) used in adults to define the small, dense LDL phenotype.

Table 3. Mean and selected percentile values for LDL peak particle size by age and sex.

	LDL peak particle size, Å					
	Boys			Girls		
	9 years (n = 342)	13 years (n = 371)	16 years (n = 377)	9 years (n = 369)	13 years (n = 353)	16 years (n = 437)
5th percentile	257.3	257.0	256.1	255.5	258.6	257.6
95% CI	256.7–258.2	256.1–258.4	254.0–257.1	254.1–256.8	256.6–259.3	257.1–258.4
25th percentile	260.8	260.3	260.1	260.5	261.3	260.8
95% CI	260.3–261.4	260.0–260.8	259.6–260.4	260.0–261.4	261.0–261.6	260.3–261.1
50th percentile	262.9	262.4	262.1	263.1	263.2	262.6
95% CI	262.6–263.4	262.1–262.8	261.7–262.6	262.8–263.5	262.9–263.6	262.3–263.0
75th percentile	265.6	264.8	264.1	265.3	265.4	264.8
95% CI	265.0–266.1	264.0–265.2	263.7–264.8	264.7–265.7	265.0–265.9	264.4–265.4
95th percentile	268.6	268.5	268.1	268.9	269.5	268.3
95% CI	267.9–269.5	267.6–269.5	267.2–268.8	267.8–270.4	268.4–270.3	267.5–269.0
Mean	263.1	262.6	262.1	262.8	263.4	262.7
95% CI	262.7–263.5	262.2–263.0	261.7–262.5	262.4–263.3	263.1–263.8	262.4–263.1

except in 13- and 16-year-old girls, for whom we found no significant associations. Finally, we found no significant correlations between LDL size and glucose or FFA concentrations.

Because many CVD risk factors correlated with LDL peak particle size are also intercorrelated, we performed multiple regression analyses to examine their independent contributions to variation in LDL size. We included in multiple regression analyses variables that had a significant association with LDL size in univariate analyses (Table 6). ApoB was highly correlated with TC and LDL-C, and apoA1 was highly correlated with HDL-C (all correlation coefficients ≥ 0.8); therefore, only apoB and HDL-C were tested as potential independent variables in multiple regression analyses. We did not detect significant associations between LDL size and alcohol intake or cigarette smoking status ($P = 0.715$ and 0.370 , respectively); thus, they were not included in multiple regres-

sion analyses. Because there was no significant heterogeneity in the effects of the independent variables (apoB, HDL-C, TG, insulin, BMI) by age and sex (all interaction P values > 0.1 with the exception of the interaction BMI \times sex, for which the P value was 0.023), age and sex groups were pooled in multiple regression analyses. After adjustment for age, sex, apoB, HDL-C, insulin, and BMI, LDL size remained strongly negatively associated with TG concentration: a 1 SD increase in \log_e TG was associated with a 1.2 Å decrease in LDL size (Table 7). Similarly, after adjustment for all other variables in the model, HDL-C remained strongly positively associated with LDL size: a 1 SD increase in HDL-C concentration was associated with a 1.1 Å increase in LDL size. In contrast, after adjustment, the association between BMI and LDL size was no longer significant. It is interesting to note that in the multivariable analysis, the association between insulin concentration and LDL size stayed significant but was positive. Finally, similar to univariate association analyses, girls had a mean LDL size slightly larger than boys in multivariable analysis, and 16-year-olds had smaller mean LDL size than 13- and 9-year-olds.

Table 4. Distribution of participants with small dense LDLs (≤ 255 Å) by sex, age, BMI category, and IRS status.

	Proportion with small dense LDL, % (n = 2249)	P^a
Sex		
Boys	2.0	
Girls	2.1	0.813
Age		
9-year-olds	2.2	
13-year-olds	1.0	
16-year-olds	2.8	0.085
BMI category		
BMI < 85 th percentile	1.2	
BMI ≥ 85 th percentile	6.8	< 0.001
IRS status		
No	1.0	
Yes	10.0	< 0.001

^a P for comparison between categories.

Discussion

To date, limited information is available on the distribution of LDL size in population-based samples of children (34, 36, 38, 39) and adolescents (39). Moreover, methods used to assess LDL size vary across studies, which limits comparisons among populations. Arisaka et al. (34), Shimabukuro et al. (38), and the present study (QCAHS) used PAGGE to determine particle size, whereas the investigators of the Bogalusa Heart Study (BHS) (39) and Shea et al. (36) used nuclear magnetic resonance spectroscopy. Agreement between methods is far from perfect and therefore the methods are not interchangeable (52). In spite of these methodologic differences, significantly higher mean LDL size was found in females compared

Table 5. Pearson correlations between gradient gel electrophoresis measurements of LDL peak particle size (Å) and surrogate estimates by age and sex.

Surrogate marker	Correlation ^a							
	Boys				Girls			
	9 years (n = 342)	13 years (n = 371)	16 years (n = 377)	Total (n = 1090)	9 years (n = 369)	13 years (n = 353)	16 years (n = 437)	Total (n = 1159)
LDL-C/apoB ratio, mmol/g	0.20	0.30	0.23	0.26	0.37	0.28	0.30	0.31
TC/HDL-C ratio	-0.42	-0.42	-0.47	-0.45	-0.48	-0.38	-0.35	-0.39
ApoB/HDL-C ratio, g/mmol	-0.42	-0.41	-0.45	-0.44	-0.53	-0.40	-0.39	-0.44
TG/HDL-C ratio	-0.50	-0.56	-0.54	-0.54	-0.57	-0.51	-0.51	-0.52
Log _e (TG/HDL-C) ratio	-0.49	-0.56	-0.55	-0.54	-0.55	-0.47	-0.50	-0.50
Log _e TG, mmol/L	-0.41	-0.50	-0.46	-0.46	-0.47	-0.39	-0.45	-0.43

^a P values for all correlations are <0.001.

with males in the BHS (39), the QCAHS, and in Japanese children (38). However, these differences by sex are small, and their clinical significance remains to be determined. LDL size showed no consistent trend with age among 10- to 17-year-old black and white youths in the BHS (39), whereas 16-year-olds had significantly decreased LDL size compared with 13- and 9-year-olds in the QCAHS. Only 7- to 13-year-old children were evaluated in the Japanese studies (34, 38), and the effect of age on LDL size was not discussed. Whereas studies concur on differences by sex in LDL size in youth, differences by sex that are also observed in adults (53, 54), further investigations are needed to confirm differences by age in children and adolescents.

The prevalence of the small, dense LDL phenotype (pattern B, ≤ 255 Å) was only 2% in our sample and was similar between sexes and across ages. Arisaka et al. (34) reported a prevalence of 9.3%, which was similar by sex, in prepubertal Japanese children, whereas Shimabukuro et al. (38) reported a prevalence of 10.8% in prepubertal

Japanese boys and 4.4% in prepubertal Japanese girls. In the BHS (39), the prevalence of the nuclear magnetic resonance spectroscopy-determined small, dense LDL phenotype (mean LDL size ≤ 205 nm) ranged from 5% in black boys and girls to 13% in white boys. In contrast, the prevalence of the small, dense LDL phenotype as assessed by PAGGE in the Framingham Offspring Study was 33% in adult men but was markedly lower in premenopausal (5%) and in postmenopausal (13%) women (54). Because the conformational alterations characteristic of small, dense LDL are likely associated with increased atherogenicity (1, 23), the low prevalence of this trait in youths is reassuring. However, a sizeable proportion of youths who are overweight (6.8%) or who have IRS (10%) might have already experienced the burden of small, dense LDL.

Our data are concordant with studies conducted in adults (19–22) showing that the TG/HDL-C molar ratio (or the log-transformed ratio) is the simplest, best lipid index to predict LDL particle size, better than either log_e-transformed TG or HDL-C concentrations alone. In

Table 6. Pearson correlations between LDL peak particle size (Å) and other metabolic variables by age and sex.

Metabolic variables	Correlation							
	Boys				Girls			
	9 years (n = 342)	13 years (n = 371)	16 years (n = 377)	Total (n = 1190)	9 years (n = 369)	13 years (n = 353)	16 years (n = 437)	Total (n = 1159)
TC, mmol/L	0.06	-0.01	-0.12 ^a	<0.01	-0.09	-0.01	-0.11 ^a	-0.08 ^b
LDL-C, mmol/L	-0.02	-0.02	-0.12 ^a	-0.04	-0.12 ^a	-0.05	-0.11 ^a	-0.10 ^c
ApoB, g/L	-0.14 ^b	-0.18 ^c	-0.25 ^d	-0.19 ^d	-0.33 ^d	-0.20 ^c	-0.27 ^d	-0.27 ^d
HDL-C, mmol/L	0.44 ^d	0.44 ^d	0.46 ^d	0.45 ^d	0.47 ^d	0.40 ^d	0.29 ^d	0.37 ^d
ApoA1, g/L	0.29 ^d	0.19 ^c	0.27 ^d	0.27 ^d	0.26 ^d	0.26 ^d	0.05	0.16 ^d
TG, ^e mmol/L	-0.41 ^d	-0.50 ^d	-0.46 ^d	-0.46 ^d	-0.47 ^d	-0.39 ^d	-0.45 ^d	-0.43 ^d
FFA, ^e mmol/L	<0.01	0.08	-0.13 ^a	0.02	<0.01	-0.04	0.02	<0.01
Glucose, mmol/L	<0.01	0.08	0.04	0.03	-0.07	0.06	0.09	0.04
Insulin, ^e pmol/L	-0.12 ^a	-0.15 ^b	-0.11 ^a	-0.15 ^d	-0.20 ^d	-0.08	-0.02	0.08 ^b
BMI, ^e kg/m ²	-0.19 ^c	-0.21 ^d	-0.17 ^b	-0.22 ^d	-0.21 ^d	-0.08	-0.04	-0.10 ^b

^a P \leq 0.05.

^b P \leq 0.01.

^c P \leq 0.001.

^d P \leq 0.0001.

^e Variables were log_e-transformed for statistical analyses.

Table 7. Mixed multiple regression analysis showing the independent associations between LDL peak particle size (Å) and other metabolic variables, age, and sex.

Independent variable	β^a	SE	P^b
Z-score ^c			
ApoB (1 SD)	-0.22	0.07	0.002
HDL-C (1 SD)	1.10	0.07	<0.001
TG (1 SD)	-1.21	0.08	<0.001
Insulin (1 SD)	0.26	0.07	<0.001
BMI (1 SD)	0.02	0.07	0.810
Age ^d			
16 years	-0.57	0.22	0.009
13 years	0.03	0.20	0.893
Sex ^e			
Girls	0.39	0.12	0.001

^a β , regression coefficient.
^b P , probability for the regression coefficient.
^c Regression coefficients (β) represent the change in mean LDL peak particle size per unit (1 SD) increase in Z-scores for apoB or HDL-C or TG or insulin or BMI, after adjustment for all other variables in the model.
^d Regression coefficients (β) represent the change in mean LDL particle size for the 16-year-olds or 13-year-olds compared with the 9-year-old group, after adjustment for all other variables in the model.
^e Regression coefficient (β) represents the change in mean LDL particle size for females compared with the male group, after adjustment for all other variables in the model.

adults, correlations between LDL particle size and TG/HDL-C molar ratio (or the log-transformed ratio) ranged from -0.64 to -0.78 (19–22), which is stronger than correlations observed in the present study. As expected, this surrogate marker has limitations in its ability to predict the small, dense LDL phenotype. In the QCAHS, even when we used the most sensitive cut point suggested in adults (0.9), the false-negative rate was 14% and the false-positive rate was 22%.

Similar to results reported in studies of adults (7, 8, 25, 55–59), the fasting plasma TG concentration was the strongest independent correlate (negative) of LDL peak particle size in the present pediatric study. This is consistent with the prevailing model for the generation of small LDL particles, which proposes that increased hepatic production of TG-rich lipoproteins (i.e., VLDL) associated with insulin resistance leads to TG-enriched LDL particles through cholesteryl ester transfer protein-mediated exchange of cholesteryl esters from LDL to VLDL particles and of TG from VLDL to LDL particles. TG-enriched LDL then becomes a good substrate for hepatic lipase, which hydrolyzes the TG in LDL, producing small, dense LDL (1). The weak correlation between LDL size, which depends on core lipid content, and apoB concentration, which is related to particle number, underscores that both LDL composition and LDL particle number should be considered in the assessment of CVD risk.

An unexpected finding of our study was that after adjustment for the other metabolic components of IRS (apoB, TG, and HDL-C concentrations and BMI), we detected a positive association between fasting insulin

concentration and LDL size, suggesting that hyperinsulinemia is associated with a larger LDL size. This appears inconsistent with the known association between IRS and a small LDL size. However, in most adult studies, insulin sensitivity was not independently associated with LDL size, and the effect of insulin resistance on LDL size could be explained by the effect of insulin resistance or hyperinsulinemia on VLDL metabolism (7, 8, 25, 55–59). Moreover, plasma insulin concentrations reflect both pancreatic insulin secretion and peripheral insulin resistance. Our data suggest that beyond the effect of insulin resistance on VLDL production/secretion, which leads to smaller LDL size, insulin concentrations could also influence VLDL subclass distribution and, thus, LDL subclass distribution. In fact, it has been proposed that there is a channeling within the VLDL–intermediate-density lipoprotein–LDL delipidation cascade such that parallel processing pathways generate different intermediate-density lipoprotein and LDL products from different TG-rich lipoprotein precursors (1). Large TG-rich VLDL-1 are thought to be the precursors of small, dense LDL. Furthermore, in normolipidemic individuals and in insulin-sensitive obese individuals, it has been reported that acute hyperinsulinemia lowered the concentration of large TG-rich VLDL-1 but had little effect on VLDL-2 (60, 61). There thus is experimental evidence supporting our observation of a positive association between LDL size and insulin concentration after adjustment for the other metabolic components of IRS. This will require confirmation in other large population-based samples of youths and adults.

In our pediatric population, age, sex, and the metabolic variables explained 30% of the variation in LDL peak particle size, which is similar to values reported for Japanese schoolchildren (22.9%–28.1%) (38) but lower than those reported in the Framingham Offspring Study (50%–57%) (54). This suggests that the relative importance of genetic and environmental determinants of LDL size differs between children and adults.

In conclusion, the metabolic correlates of LDL size are similar between youths and adults. Although the small, dense LDL phenotype is less prevalent in youths than adults, its prevalence is clearly increased in childhood IRS, which supports the need for early prevention of CVD risk factors.

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References

1. Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity [Review]. *J Lipid Res* 2002;43:1363–79.
2. McNamara JR, Campos H, Ordovas JM, Peterson J, Wilson PWF, Schaefer EJ. Effect of gender, age, and lipid status on low-density lipoprotein subfraction distribution. *Arteriosclerosis* 1987;7:483–90.
3. Campos H, Sacks FM, Walsh BW, Schiff I, O'Hanesian MA, Krauss RM. Differential effects of estrogen on low-density lipoprotein subclasses in healthy postmenopausal women. *Metabolism* 1993;42:1153–8.
4. Mauger JF, Lichtenstein AH, Ausman LM, Jalbert SM, Jauhiainen M, Ehnholm C, et al. Effect of different forms of dietary hydrogenated fats on LDL particle size. *Am J Clin Nutr* 2003;78:370–5.
5. Sjogren P, Rosell M, Skoglund-Andersson C, Zdravkovic S, Vessby B, de Faire U, et al. Milk-derived fatty acids are associated with a more favorable LDL particle size distribution in healthy men. *J Nutr* 2004;134:1729–35.
6. Terry RB, Wood PD, Haskell WL, Stefanick ML, Krauss RM. Regional adiposity patterns in relation to lipids, lipoprotein cholesterol, and lipoprotein subfraction mass in men. *J Clin Endocrinol Metab* 1989;68:191–9.
7. Tchernof A, Lamarche B, Prud'Homme D, Nadeau A, Moorjani S, Labrie F, et al. The dense LDL phenotype. Association with plasma lipoprotein levels, visceral obesity, and hyperinsulinemia in men. *Diabetes Care* 1996;19:629–37.
8. Reaven GM, Chen YDI, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense, low density lipoprotein particles. *J Clin Invest* 1993;92:141–6.
9. Selby JV, Austin MA, Newman B, Zhang D, Quesenberry C Jr, Mayer EJ, et al. LDL subclass phenotypes and the insulin resistance syndrome in women. *Circulation* 1993;88:381–7.
10. Feingold KR, Grunfeld C, Pang M, Doerrler W, Krauss RM. LDL subclass phenotypes and triglyceride metabolism in non-insulin-dependent diabetes. *Arterioscler Thromb* 1992;12:1496–502.
11. Austin M, Krauss RM. Genetic control of low-density-lipoprotein subclasses. *Lancet* 1986;2:592–5.
12. Bosse Y, Perusse L, Vohl MC. Genetics of LDL particle heterogeneity: from genetic epidemiology to DNA-based variations [Review]. *J Lipid Res* 2004;45:1008–26.
13. Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoprotein in normal humans. *J Lipid Res* 1982;23:97–104.
14. Williams PT, Krauss RM, Nichols AV, Vranizan KM, Wood PDS. Identifying the predominant peak diameter of high-density and low-density lipoproteins by electrophoresis. *J Lipid Res* 1990;31:1131–9.
15. Krauss RM, Blanche PJ. Detection and quantitation of LDL subfractions. *Curr Opin Lipidol* 1992;3:377–83.
16. Wägner AM, Jorba O, Rigla M, Alonso E, Ordóñez-Llanos J, Pérez A. LDL-cholesterol/apolipoprotein B ratio is a good predictor of LDL phenotype B in type 2 diabetes. *Acta Diabetol* 2002;39:215–20.
17. Tallis GA, Shephard MD, Sobel S, Whiting MJ. The total apolipoprotein B/LDL-cholesterol ratio does not predict LDL particle size. *Clin Chim Acta* 1995;240:63–73.
18. Kang H-S, Gutin B, Barbeau P, Litaker MS, Allison J, Le N-A. Low-density lipoprotein particle size, central obesity, cardiovascular fitness, and insulin resistance syndrome markers in obese youths. *Int J Obes Relat Metab Disord* 2002;26:1030–5.
19. Boizel R, Benhamou PY, Lardy B, Laporte F, Foulon T, Halimi S. Ratio of triglycerides to HDL cholesterol is an indicator of LDL particle size in patients with type 2 diabetes and normal HDL cholesterol levels. *Diabetes Care* 2000;23:1679–85.
20. Dobiasova M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apo B-lipoprotein-depleted plasma (FER_{HDL}). *Clin Biochem* 2001;34:583–8.
21. Maruyama C, Imamura K, Teramoto T. Assessment of LDL particle size by triglyceride/HDL-cholesterol ratio in non-diabetic, healthy subjects without prominent hyperlipidemia. *J Atheroscler Thromb* 2003;10:186–91.
22. Hanak V, Munoz J, Teague J, Stanley A Jr, Bittner V. Accuracy of the triglyceride to high-density lipoprotein cholesterol ratio for prediction of the low-density lipoprotein phenotype B. *Am J Cardiol* 2004;94:219–22.
23. Kwiterovich PO Jr. Clinical relevance of the biochemical, metabolic, and genetic factors that influence low-density lipoprotein heterogeneity [Review]. *Am J Cardiol* 2002;90:30i–47i.
24. Sacks FM, Campos H. Low-density lipoprotein size and cardiovascular disease: a reappraisal [Review]. *J Clin Endocrinol Metab* 2003;88:4525–32.
25. Howard BV, Mayer-Davis EJ, Goff D, Zaccaro DJ, Laws A, Robbins DC, et al. Relationships between insulin resistance and lipoproteins in nondiabetic African Americans, Hispanics, and non-Hispanic Whites: the Insulin Resistance Atherosclerosis Study. *Metabolism* 1998;47:1174–9.
26. Friedlander Y, Kidron M, Caslake M, Lamb T, McConnell M, Bar-On H. Low density lipoprotein particle size and risk factors of insulin resistance syndrome. *Atherosclerosis* 2000;148:141–9.
27. Hulthe J, Bokemark L, Wikstrand J, Fagerberg B. The metabolic syndrome, LDL particle size, and atherosclerosis: the Atherosclerosis and Insulin Resistance (AIR) Study. *Arterioscler Thromb Vasc Biol* 2000;20:2140–7.
28. Bjornheden T, Babyi A, Bondjers G, Wiklund O. Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. *Atherosclerosis* 1996;123:43–56.
29. Campos H, Arnold KS, Balestra ME, Innerarity TL, Krauss RM. Differences in receptor binding of LDL subfractions. *Arterioscler Thromb Vasc Biol* 1996;16:794–801.
30. Galeano NF, Al-Haideri M, Keyserman F, Rumsey SC, Deckelbaum RJ. Small dense low density lipoprotein has increased affinity for LDL receptor-independent cell surface binding sites: a potential mechanism for increased atherogenicity. *Lipid Res* 1998;39:1263–73.
31. Anber V, Millar JS, McConnell M, Shepherd J, Packard CJ. Interaction of very-low-density, intermediate-density, and low-density lipoproteins with human arterial wall proteoglycans. *Arterioscler Thromb Vasc Biol* 1997;17:2507–14.
32. Chait A, Brazg RL, Tribble DL, Krauss RM. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am J Med* 1993;94:350–6.
33. Tribble DL, Rizzo M, Chait A, Lewis DM, Blanche PJ, Krauss RM. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. *Am J Med* 2001;110:103–10.
34. Arisaka O, Kojima M, Yamazaki Y, Kanazawa S, Koyama S, Shimura N, et al. Relationship between the presence of small, dense low-density lipoprotein and plasma lipid phenotypes in Japanese children. *J Atheroscler Thromb* 2004;11:220–3.
35. Kaitosaari T, Ronnema T, Raitakari O, Talvia S, Kallio K, Volanen I, et al. Effect of 7-year infancy-onset dietary intervention on serum lipoproteins and lipoprotein subclasses in healthy children in the prospective, randomized Special Turku Coronary Risk Factor Intervention Project for Children (STRIP) study. *Circulation* 2003;108:672–7.
36. Shea S, Aymong E, Zybert P, Berglund L, Shamoon H, Deckelbaum RJ, Basch CE. Fasting plasma insulin modulates lipid levels and

- particle sizes in 2- to 3-year-old children. *Obes Res* 2003;11:709–21.
37. Ohta T, Kakiuti Y, Kurahara K, Saku K, Nagata N, Matsuda I. Fractional esterification rate of cholesterol in high density lipoprotein is correlated with low density lipoprotein particle size in children. *J Lipid Res* 1997;38:139–46.
 38. Shimabukuro T, Sunagawa M, Ohta T. Low-density lipoprotein particle size and its regulatory factors in school children. *J Clin Endocrinol Metab* 2004;89:2923–7.
 39. Freedman DS, Bowman BA, Otvos JD, Srinivasan SR, Berenson GS. Levels and correlates of LDL and VLDL particle sizes among children: the Bogalusa Heart Study. *Atherosclerosis* 2000;152:441–9.
 40. Williams PT, Krauss RM. Associations of age, adiposity, menopause, and alcohol intake with low-density lipoprotein subclasses. *Arterioscler Thromb Vasc Biol* 1997;17:1082–90.
 41. Arisaka O, Fujiwara S, Yabuta K, Mokuno H, Mitugi Y, Miyake N. Characterization of low-density lipoprotein subclasses in children. *Metabolism* 1997;46:146–8.
 42. Steinbeck KS, Birmingham MA, Mahajan D, Baur LA. Low-density lipoprotein subclasses in children under 10 years of age. *J Paediatr Child Health* 2001;37:550–3.
 43. Williams CL, Hayman LL, Daniels SR, Robinson TN, Steinberger J, Paridon S, et al. Cardiovascular health in childhood: a statement for health professionals from the Committee on Atherosclerosis, Hypertension, and Obesity in the Young (AHOY) of the Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation* 2002;106:143–60.
 44. McGill HC Jr, McMahan CA, Herderick EE, Malcom GT, Tracy RE, Strong JP. Origin of atherosclerosis in childhood and adolescence [Review]. *Am J Clin Nutr* 2000;72:1307S–15S.
 45. Lambert M, Paradis G, O'Loughlin J, Delvin EE, Hanley JA, Levy E. Insulin resistance syndrome in a representative sample of children and adolescents from Quebec, Canada. *Int J Obes Relat Metab Disord* 2004;28:833–41.
 46. Paradis G, Lambert M, O'Loughlin J, Lavallee C, Aubin J, Berthiaume P, et al. The Quebec Child and Adolescent Health and Social Survey: design and methods of a cardiovascular risk factor survey for youth. *Can J Cardiol* 2003;19:523–31.
 47. US Department of Health and Human Services. CDC growth charts: United States. DHHS publication no. 2000-1250. December 2000:28 pp. <http://www.cdc.gov/nchs/data/ad/ad314.pdf> (accessed April 14, 2005).
 48. Update on the 1987 task force report on high blood pressure in children and adolescents: a working group report from the National High Blood Pressure Education Program. *Pediatrics* 1996;98:649–58.
 49. Allard P, Delvin EE, Paradis G, Hanley JA, O'Loughlin J, Lavallee C, et al. Distribution of fasting plasma insulin, free fatty acids, and glucose concentrations and of homeostasis model assessment of insulin resistance in a representative sample of Quebec children and adolescents. *Clin Chem* 2003;49:644–9.
 50. Friedewald WF, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 1977;18:499–502.
 51. Hutson AD. Calculating nonparametric confidence intervals for quantiles using fractional order statistics. *J Appl Stat* 1999;26:343–53.
 52. Witte DR, Taskinen MR, Perttunen-Nio H, Van Tol A, Livingstone S, Colhoun HM. Study of agreement between LDL size as measured by nuclear magnetic resonance and gradient gel electrophoresis. *J Lipid Res* 2004;45:1069–76.
 53. Freedman DS, Otvos JD, Jeyarajah EJ, Shalurova I, Cupples LA, Parise H, et al. Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. *Clin Chem* 2004;50:1189–200.
 54. Campos H, Blijlevens E, McNamara JR, Ordovas JM, Posner BM, Wilson PW, et al. LDL particle size distribution. Results from the Framingham Offspring Study. *Arterioscler Thromb* 1992;12:1410–9.
 55. Lahdenpera S, Sane T, Vuorinen-Markkola H, Knudsen P, Taskinen MR. LDL particle size in mildly hypertriglyceridemic subjects: no relation to insulin resistance or diabetes. *Atherosclerosis* 1995;113:227–36.
 56. Slyper AH, Zvereva S, Schectman G, Hoffmann RG, Mueller RA, Walker JA. Insulin resistance is not a major determinant of low-density lipoprotein particle size. *Metabolism* 1997;46:1275–80.
 57. Mykkanen L, Haffner SM, Rainwater DL, Karhapaa P, Miettinen H, Laakso M. Relationship of LDL size to insulin sensitivity in normoglycemic men. *Arterioscler Thromb Vasc Biol* 1997;17:1447–53.
 58. Ambrosch A, Muhlen I, Kopf D, Augustin W, Dierkes J, Konig W, et al. LDL size distribution in relation to insulin sensitivity and lipoprotein pattern in young and healthy subjects. *Diabetes Care* 1998;21:2077–84.
 59. Fagerberg B, Hulthe J, Bokemark L, Wikstrand J. Low-density lipoprotein particle size, insulin resistance, and proinsulin in a population sample of 58-year-old men. *Metabolism* 2001;50:120–4.
 60. Malmstrom R, Packard CJ, Watson TD, Rannikko S, Caslake M, Bedford D, et al. Metabolic basis of hypotriglyceridemic effects of insulin in normal men. *Arterioscler Thromb Vasc Biol* 1997;17:1454–64.
 61. Bioletto S, Golay A, Munger R, Kalix B, James RW. Acute hyperinsulinemia and very-low-density and low-density lipoprotein subfractions in obese subjects. *Am J Clin Nutr* 2000;71:443–9.