Characterization of Low-Density Lipoprotein Subclasses in Children

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Low-density lipoprotein (LDL) particles are heterogeneous in density, size, and chemical composition, and this heterogeneity is thought to be genetically influenced. In the present study, plasma LDL subclasses in 248 children aged 7 to 13 years were analyzed by gradient gel electrophoresis. The prevalence of small dense LDL (SDLDL), a potent atherogenic LDL, was 9.3%, which is lower than that reported in adults. Furthermore, children with this LDL subclass showed increased body fatness and dyslipidemia, including elevated plasma triglyceride and apolipoprotein (apo) B concentrations and decreased plasma high-density lipoprotein (HDL) cholesterol and apo A-I concentrations, compared with children without this phenotype. These findings suggest that in addition to genetic factors, environmental factors that affect these cardiovascular risk factors may also influence expression of the SDLDL subclass.

AN ELEVATED PLASMA low-density lipoprotein (LDL) cholesterol level is a risk factor for atherosclerotic cardiovascular disease.[1] The existence of subclasses of LDL characterized by variations in density, size, and chemical composition of the LDL particles is also widely recognized.[2-3] A number of case-control and cross-sectional studies have demonstrated that individuals with a predominance of small dense LDL (SDLDL) particles are at high risk of developing cardiovascular disease.[4-7] Chemically, SDLDL particles are apolipoprotein (apo) B–enriched and cholesterol ester–poor.[8] Furthermore, SDLDL particles undergo oxidative modification readily, which is an important step in the development of arterial fatty streaks.[9]

Such LDL heterogeneity may be genetically controlled,[10-12] but it is possible that environmental influences are related to expression of the LDL subclass phenotype. The prevalence of the SDLDL subclass in adult populations was found to be 44% in one study,[13] but to our knowledge, data on children have not been documented. The purpose of the present study was to determine the LDL subclass pattern and its relationship to atherogenic risk factors such as body fatness and plasma lipid, lipoprotein, and apolipoprotein levels in children.

SUBJECTS AND METHODS

Subjects

The subjects were 246 prepubertal school children (128 boys and 118 girls) aged 7 to 13 years living in a rural town (total population, 8,600) in Chiba, Japan. Ninety percent of the children recruited participated in the study. All of the children's parents signed informed-consent forms for this examination.

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Anthropometric Measurements

The standing height and weight of each child (with shoes off) were measured, and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Blood Pressure

Blood pressure was measured with the child in a seated position in a quiet environment with the right arm at heart level, using an automated device (Desital Blood Pressure Monitor UA-743; AND, Tokyo, Japan). This device measures blood pressure by oscillometric techniques rather than Korotkoff sounds. Diastolic blood pressure as measured by the device is, on average, closer to the fifth than to the fourth Korotkoff sound.

Lipids, Lipoproteins, and Apolipoproteins

After an overnight fast, venous blood samples were obtained by venipuncture and analyzed. Total cholesterol and triglyceride levels were measured enzymatically by routine laboratory methods. High-density lipoprotein (HDL) cholesterol level was measured in the clear supernatant obtained after precipitation of very–low-density lipoprotein and LDL by magnesium chloride and dextran sulfate, respectively. Apo A-I and apo B levels were measured by the single radioimmunodiffusion method. LDL cholesterol level was calculated using the Friedewald formula: LDL cholesterol = total cholesterol − HDL cholesterol − triglyceride/5 (mg/dL).[14]

LDL Subclass

The size distribution of LDL particles in an aliquot of venous blood from each individual was analyzed by gel electrophoresis on 2.5% to 16% polyacrylamide gels using a modified method of Krauss and Burke[2] and Austin et al.[4] The gels were fixed and stained for lipid with 0.04% Oil Red 0 (Sigma, St Louis, MO) 60% ethanol, and the center of the most prominent band was marked on the gel. Three standards of known diameter, apoferritin (12.2 nm), thyroglobulin (17.0 nm), and latex beads (37 nm), were included on each gel. A densitometric scan at the appropriate wavelength identified LDL subclass peaks and standard peaks in the lanes. Migration distances (from the top of the gel to the absorbance maxima) were determined, and then the LDL particle diameter corresponding to each of these peaks was calculated from a calibration curve using three standards of known diameter.[8] LDL subclasses were classified as SDLDL (diameter < 25.5 nm) and non-SDLDL (diameter ≥ 25.5 nm) based on LDL particle size[15] (Fig 1).

Statistical Analysis

The data (values for the group with SDLDL subclass phenotype *v* non-SDLDL subclass phenotype) were compared using the Mann-Whitney *U* test. All results are presented as the mean ± SD.

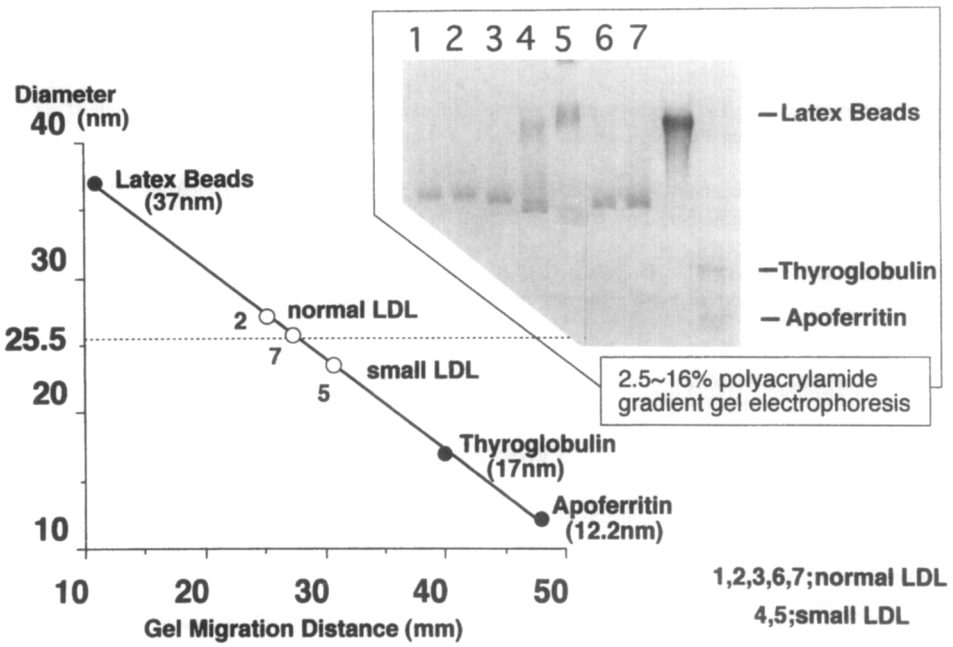

Fig 1. Gradient gel calibration using LDL particle diameter versus gel migration distance. Lanes 1 to 3, 6, and 7 show normal LDL; lanes 4 and 5 show small LDL.

RESULTS

The prevalence of SDLDL in LDL subclasses was 23 of 248 children (9.3%: 12 of 128 boys [9.4%] and 11 of 118 girls [9.3%]). Plasma triglyceride and apo B levels were higher (both $P < .01$) and HDL cholesterol and apo A-I were lower ($P < .01$ and $P < .05$, respectively) in children with the SDLDL versus the non-SDLDL subclass phenotype. The mean BMI of children with the SDLDL subclass was higher ($P < .01$) than that of those with the non-SDLDL subclass. Total cholesterol, LDL cholesterol, and blood pressure values for the two groups did not differ significantly (Table 1). These tendencies were consistent even when statistical analysis was performed on the data for boys and girls. None of the children had type III dyslipidemia, which affects measurement of the LDL subclass phenotype.

DISCUSSION

In the present study on children, the prevalence of the SDLDL phenotype was 9.3%, which is low compared with the prevalence (44%) reported in the population study of American adults.[13] The prevalence of SDLDL in 213 Japanese adults over 40 years of age who had no atherogenic cardiovascular disease was 34.7% in our hospital-based study.[16] Although the different genetic backgrounds must be taken into consideration, it is probable that the prevalence of the SDLDL phenotype is lower in children than in adults.

Table 1. Plasma Lipoprotein Levels, BMI, and Blood Pressure by LDL Subclass Phenotype in Children

Parameter	SDLDL (n = 23)	Non-SDLDL (n = 225)
Total cholesterol (mg/dL)	179 ± 31	175 ± 29
LDL cholesterol (mg/dL)	98 ± 23	93 ± 24
HDL cholesterol (mg/dL)	50 ± 11*	64 ± 14
Triglycerides (mg/dL)	153 ± 80*	94 ± 43
Apo A-I (mg/dL)	125 ± 25†	136 ± 21
Apo B (mg/dL)	93 ± 25*	73 ± 18
BMI (kg/m^2)	20.5 ± 0.7*	18.5 ± 1.6
Systolic blood pressure (mm Hg)	120 ± 14	118 ± 17
Diastolic blood pressure (mm Hg)	77 ± 11	75 ± 15

NOTE. Values are the mean ± SD.
*$P < .01$.
†$P < .05$.

Furthermore, as reported for adults, the SDLDL phenotype was found to be closely related to dyslipidemia and increased fatness: our subjects with the SDLDL phenotype had increased triglyceride and apo B plasma levels, high BMI values, and low HDL cholesterol and apo A-I plasma levels. These findings suggest that environmental factors that influence these cardiovascular risk factors may also influence expression of the SDLDL subclass. Although large family studies using complex segregation analysis, twin studies, and genetic-linkage studies have provided evidence for genetic influences on LDL subclasses, of equal importance may be the findings that both genetic and environmental influences are involved.[17,18] In fact, a report from the Framingham study showed that the LDL subclass changed in 15% of the participants during a 3- to 4-year follow-up period.[19] Therefore, it may be that environmental factors are related to the prevalence of the SDLDL subclass phenotype. Current studies also demonstrate that SDLDL subclass phenotype is an integral feature of the insulin resistance syndrome.[20] Altered insulin secretion and/or insulin sensitivity caused by life-style–related dietary and exercise effects (so-called environmental factors) might be related to the metabolic basis of the heterogeneous chemical composition of LDL.

The observed correlation between the prevalence of SDLDL and body fatness/dyslipidemia and the lower prevalence of SDLDL in children than in adults suggest that in addition to genetic factors, environmental factors may affect LDL subclasses.

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