Relationship between the Presence of Small, Dense Low-density Lipoprotein and Plasma Lipid Phenotypes in Japanese Children

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To clarify the relationship between the expression of atherogenic small, dense low-density lipoprotein (SDLDL) and underlying lipid metabolic abnormalities, the prevalence of SDLDL in relation to the serum lipid phenotype was analyzed in 229 children. The LDL particle size was measured using gradient gel electrophoresis, and a particle size of less than 25.5 nm was considered to represent SDLDL. The overall prevalence of SDLDL in the sample population was 8.2% (19/229; 11/117 for boys and 8/112 for girls).

Hyperlipidemia phenotype IIb (elevated concentrations of both triglyceride [TG] and total cholesterol [TC]) was strongly associated with SDLDL in 83% (5/6) of the subjects. An elevated TG concentration (phenotype IV) was associated with SDLDL in 55% (10/18) of the subjects. The association between hyperlipidemia phenotype IIa (elevated TC but a normal TG concentration) and SDLDL was quite low (2%; 1/56), but SDLDL was detected in 5% (8/155) of the subjects who presented with normolipidemia. Therefore, these findings suggest that the expression of SDLDL is largely related to lipid abnormalities characterized by phenotype IIb or IV, the underlying metabolic abnormality of which is suspected to be insulin resistance; however, an additional mechanism for the formation of SDLDL that functions independently of plasma lipid abnormalities also seems to exist. J Atheroscler Thromb, 2004; 11: 220–223.

Key words: Small, Dense LDL, Hyperlipidemia phenotype IIb, Hypertriglyceridemia, Insulin resistance, Atherosclerosis, Children

Introduction

Low-density lipoprotein (LDL) particles are heterogeneous in size, density, and composition. Small, dense LDL (SDLDL) particles readily undergo oxidative modifications, which is an important step in the development of the arterial fatty streaks that lead to atherosclerosis (1). SDLDL is strongly associated with premature atherosclerotic coronary artery disease (2, 3), and can be detected even in children (4). SDLDL production is thought to be an integral feature of insulin resistance conditions caused by genetic and environmental factors, and the presence of SDLDL is thought to be a biochemical marker for a metabolic syndrome that is strongly related to the development of atherosclerosis (5, 6).

Children presenting with SDLDL are thought to be highly susceptible to future atherosclerotic disease. Screening for SDLDL using routine lipid measurements seems to be a meaningful test for targeting intervention and preventing future atherosclerotic diseases. Therefore, the present study attempted to clarify whether metabolic abnormalities represented by the expression of SDLDL are related to a characteristic plasma lipid phenotype pattern.
Subjects and Methods

The children in the present study were selected from a population-based sample used in a previous study that was part of a community-wide health education program for the prevention of cardiovascular disease risk factors in Shibayama town (a rural town with a total population of 8,600 people) (7). Two hundred and twenty-nine school children, aged 10 to 13 years (117 boys and 112 girls), were enrolled in this study. Children with diabetes mellitus, thyroid disease, chronic renal disease, or hepatobiliary disease were excluded from the study. The parents or guardians of all the children provided informed consent for participation in the study. After an overnight fast, venous blood samples were obtained by venipuncture and analyzed.

With regard to individual serum lipid parameters, total cholesterol (TC), HDL-C and triglycerides (TG) were determined by enzymatic procedures (8). LDL-C was calculated using Friedewald’s formula (9). Apolipoproteins (ApoA1 and ApoB) were quantified by turbidimetric immunoassay (10). The atherogenic index (AI) was calculated as follows: AI = TC ÷ HDL-C. An AI value below 3 is considered to be normal (11).

Using a calculation based on anthropometric measurements, children whose body weight was more than 20% of their ideal weight were judged to be obese; the prevalence of obesity in the present cohort was 18% (42 out of 229 subjects).

The distribution of LDL particle sizes in an aliquot of venous blood from each individual was analyzed using gel electrophoresis on 2.5–16% polyacrylamide gels (Bioclaft SDG-501) according to a modification of the method described by Krauss and Burke (12). The gels were fixed and stained for lipid, using 0.04% Oil Red O (Sigma, St. Louis, USA) in 60% ethanol, and the center of the most prominent band was marked on the gel. Three standards of known diameters, (apoferritin, 12.2 nm; thyroglobulin, 17.0 nm; and latex beads, 37 nm), were included on each gel. A densitometric scan at an appropriate wavelength allowed the identification of LDL subclass peaks and standard peaks in the lanes. Migration distances (from the top of the gel to the absorbance maxima) were determined, and the LDL particle diameter corresponding to each of these peaks was then calculated from a calibration curve plotted using the three standards of known diameters. LDL subclasses were classified into SDLDL (diameter < 25.5 nm) and non-SDLDL (diameter ≥ 25.5 nm) based on the LDL particle size.

To classify dyslipidemia according to phenotype, we used a tentative criteria based on the National Cholesterol Education Program (NCEP) Expert Panel’s findings (13, 14): type IIa, TC ≥ 200 mg/dl, TG < 150 mg/dl; type IIb, TC ≥ 200 mg/dl, TG ≥ 150 mg/dl; type IV, TC < 200 mg/dl, TG ≥ 150 mg/dl; and normolipidemia, TC < 200, TG < 150. Each phenotype group was then divided into two subgroups according to the HDL-C value: either more or less than 40 mg/dl.

Results

1) Table 1 shows the characteristics of the subjects’ profiles according to sex. With regard to lipid levels, there was no significant difference between boys and girls.

2) SDLDL was detected in 19 (8.2%) of the 229 children (11 [9.4%] in boys and 8 [7.1%] in girls). The prevalences of SDLDL for each hyperlipidemia phenotype are shown in Table 2. Five of the 6 (83%) subjects with phenotype IIb hyperlipidemia exhibited SDLDL. Five of the 12 (42%) subjects with hypertriglyceridemia (IV) exhibited SDLDL.

Table 1. Characteristics of subjects by sex.

<table>
<thead>
<tr>
<th></th>
<th>Boys (n = 117)</th>
<th>Girls (n = 112)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.79 ± 0.15</td>
<td>11.87 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Obesity Index (%)</td>
<td>12.96 ± 0.83</td>
<td>15.48 ± 0.27</td>
<td>NS</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>182.00 ± 2.33</td>
<td>156.83 ± 2.71</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>100.40 ± 2.03</td>
<td>102.75 ± 2.31</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>63.38 ± 1.23</td>
<td>62.40 ± 1.37</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>91.09 ± 3.86</td>
<td>90.98 ± 3.94</td>
<td>NS</td>
</tr>
<tr>
<td>ApoA1 (mg/dl)</td>
<td>152.46 ± 1.70</td>
<td>150.69 ± 1.91</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB (mg/dl)</td>
<td>79.83 ± 1.35</td>
<td>82.00 ± 1.61</td>
<td>NS</td>
</tr>
<tr>
<td>Atherogenic Index</td>
<td>2.00 ± 0.06</td>
<td>2.08 ± 0.07</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean ± SE
NS: not significant

Table 2. Prevalence of small, dense LDL in relation to plasma lipid phenotype in 229 children.

<table>
<thead>
<tr>
<th>Plasma lipid phenotype</th>
<th>Prevalence of SDLDL</th>
<th>HDL-C ≥ 40</th>
<th>HDL-C &lt; 40</th>
<th>Obesity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type IIa (TC ≥ 200, TG &lt; 150)</td>
<td>1/56 (2%)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Type IIb (TC ≥ 200, TG ≥ 150)</td>
<td>5/6 (83%)</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Type IV (TC &lt; 200, TG ≥ 150)</td>
<td>5/12 (42%)</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Normolipidemia (TC &lt; 200, TG &lt; 150, HDL-C ≥ 40)</td>
<td>8/155 (5%)</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>19/229 (8%)</td>
<td>8</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>
Eight of the 155 (5%) subjects with normolipidemia appeared to possess SDLDL. Eight of the 42 (19%) subjects with obesity exhibited SDLDL.

Discussion

The currently available data on the prevalence of SDLDL or LDL particle size in children is limited (4,15–17). Genetics, age, and gender may be related to the prevalence of SDLDL. In the present study, the plasma lipid pattern that appeared to be most closely related to the expression of SDLDL was phenotype IIb hyperlipidemia. Therefore, an elevated TG concentration (phenotype IIb or IV) may be responsible for the expression of SDLDL. In such cases, several metabolic pathways affected by genetic and nongenetic factors are considered to be involved in the formation of SDLDL (18,19).

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; the cholesterol-depleted LDL then becomes smaller and denser (20, 21). In the presence of hypertriglyceridemia, the cholesteryl ester transfer protein (CETP) allows cholesteryl esters to be transferred from LDL in exchange for a TG molecule from VLDL. TG is then hydrolyzed by hepatic triglyceride lipase (HTGL) or lipoprotein lipase to produce smaller, denser LDL particles (21–24). The activities of CETP and HTGL are well known to be enhanced by increased insulin resistance (25).

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. Altered insulin secretion and/or insulin sensitivity caused by lifestyle-related dietary or physical activity (environmental factors) may be metabolically responsible for the reduction in LDL particle size (4, 25).

Concerning genetic factors determining LDL particle size, a recent study investigating dyslipidemia in young Japanese children, which included an analysis of lipid levels in family members, reported that phenotype IIb was a characteristic feature of familial combined hyperlipidemia (FCHL) (14). FCHL is the most common inherited disorder of lipid metabolism and is estimated to cause about 10 to 20% of premature coronary heart disease during adulthood. One of the most striking metabolic features of FCHL is the presence of SDLDL; the principle pathogenesis of FCHL is a reduction in insulin sensitivity causing an alteration in lipoprotein metabolism and the formation of SDLDL (26–28). In the present study, SDLDL was also detected in some subjects exhibiting normolipidemia. This finding suggests that some genetic mechanism determining LDL particle size may function in a manner that is independent of the presence of plasma lipid abnormalities. Therefore, in children who are genetically affected by FCHL, children with SDLDL but without dyslipidemia may develop an overt hyperlipidemia phenotype IIb later.

Recent studies in adults have shown that patients with type 2 diabetes associated with microalbuminuria have smaller LDL than those with only microalbuminuria and young men with high-normal blood pressure had smaller LDL than those with normal blood pressure (29). Weight reduction, the treatment of diabetes by acarbose or troglitazone, and the treatment of hyperlipidemia by new statins as well as fibrates have been reported to be successful in increasing LDL particle size (29, 30).

In conclusion, SDLDL is considered to be associated with metabolic syndrome that centers on insulin resistance. The present study indicates that the presence of SDLDL is largely related to lipid abnormalities characterized by lipid phenotype IIb or hypertriglyceridemia, the underlying metabolic abnormality of which is suspected to be insulin resistance. Additional genetic factors determining LDL particle size may also act independently of plasma lipids during childhood.

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