Introducing Obesity, Insulin Resistance, and Very Low-Density Lipoprotein Subclass Profile in Japanese School Children

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Introduction

Childhood obesity is one of the most serious public health problems worldwide. Obese children, especially children who have abdominal obesity, are likely to develop metabolic syndrome (MetS) during childhood [1-3], which is defined by the clustering of several metabolic abnormalities, such as dyslipidemia, hypertension, and hyperglycemia, and is associated with a higher risk of cardiovascular diseases and type 2 diabetes in later life [4,5]. In Japan, cross-sectional analysis showed that the prevalence of childhood obesity has gradually decreased since the early 2000s, with the highest prevalence in the late 1990s to early 2000s; [6] however, the prevalence of MetS is not lower in pre-teen Japanese children who are overweight in comparison with overweight adolescents in the United States [7]. In a cohort study of Japanese adults without MetS, new onset of MetS was observed in 9.32% over a 5-year period, and the presence of obesity and a high triglyceride (TG) level at baseline was associated with the highest hazard ratio for risk to develop MetS [8]. Furthermore, in Japanese men, an increased number of very low-density lipoprotein (VLDL) particles is strongly associated with coronary heart disease, independent of intermediate-density lipoprotein or small, dense low-density lipoprotein (LDL) [9].

VLDL particles are heterogeneous in size, density, and composition. In adult studies, central obesity has been reported to be strongly associated with larger VLDL subclasses [10,11]; however, little information on VLDL subclasses in children is available. Based on the Bogalusa Heart Study, [12] the relationship between obesity and specific VLDL subclasses was investigated in black and white children, and waist circumference was shown to be associated with large VLDL, but not with small VLDL. In addition, racial differences were observed; specifically, the concentration of large VLDL in white children was 2.5 times higher than black children and showed a 6-fold stronger association with waist circumference in white children. Furthermore, insulin resistance is an independent factor determining the VLDL subclass profile [13,14]. Insulin-
resistant children have higher VLDL concentrations, and visceral adiposity, race, and insulin resistance are independent determinants of VLDL size [15]. Taken together, these studies have shown that the relationships between abdominal adiposity, insulin resistance, and VLDL subclass profile in children depend on the characteristics of the study population, especially ethnicity. In the present study, we analyzed VLDL subclasses in Japanese school children and investigated the association between abdominal adiposity, insulin resistance, and TG levels in each VLDL subclass.

**Methods**

The subjects were 164 children (79 boys and 85 girls), 10.9 ± 1.6 years of age (mean ± SD; range = 9-13 years) who attended one of the two schools selected for this study and participated voluntarily in this study. All of the children were free from diseases other than dyslipidemia or obesity. Each child’s standing height and weight were measured, and the body mass index (BMI; kg/m²) was calculated. Obesity is defined as having a percentage of overweight greater than 20%, calculated according to the standard weight for sex, age and height: [(body weight - standard weight)/standard weight] X 100 [16]. Waist circumference was measured at the level of the umbilicus, and the waist-to-height ratio (WHtR) was calculated. Abdominal obesity is defined as a WHtR ≥ 0.5 [17].

Blood samples were obtained in the morning after an overnight fast. Total cholesterol (TC) and TG concentrations were measured by enzymatic methods. Serum lipoprotein analyses were performed by HPLC with gel permeation columns (LipoSEARCH; Skylight-Biotec, Inc., Akita, Japan), and LDL-cholesterol (C) and high-density lipoprotein-cholesterol (HDL-C) concentrations, and TG concentrations in three VLDL subclasses (large, medium, and small) were measured [18]. Non-HDL-C is defined as TC minus HDL-C. Plasma insulin and glucose concentrations were determined, and the homeostasis model of assessment ratio (HOMA-R) was obtained using Matthews’ formula as an index of insulin resistance [19].

In the present study, we employed the diagnostic criteria of MetS in Japanese children, as having abdominal obesity (waist circumference ≥ 80 cm and/or WHtR ≥ 0.5) plus two or more of the following: (i) dyslipidemia: high TG ≥ 120 mg/dl and/or low HDL-C < 40 mg/dl; (ii) elevated SBP (≥ 125 mmHg) and/or DBP (≥ 70 mmHg); (iii) elevated fasting glucose level (≥ 100 mg/dl). In case of 6 grade or younger school children, the cut-off value of waist circumference is 75 cm [20].

Informed consent was obtained from each child and the child’s parents. The study protocol was approved by the local Ethics Committee, which consists of members of the school’s Health Education Committee. The Health Education Committee also includes members of the local Board of Education and representatives from Nihon University Itabashi Hospital.

All of the data are expressed as the mean ± SD. The group differences were assessed using an unpaired t-test. Single and multiple linear regression analyses were used to assess the correlation between variables. A P-value < 0.05 was considered statistically significant. All of the statistical analyses were conducted using the JMP statistical package (v9.0; SAS Institute, Inc., Cary, NC, USA).

**Results**

Ten boys (12.7%) and eleven girls (12.9%) were diagnosed as obese, and thirteen boys (16.5%) and nine girls (10.6%) had abdominal obesity. Compared to children without abdominal obesity, children with abdominal obesity exhibited higher TG and insulin concentrations and HOMA-R, and lower HDL-C concentrations, regardless of gender. LDL-C and non-HDL-C concentrations in boys with abdominal obesity were also higher than boys without abdominal obesity; however, no difference was observed in LDL-C or non-HDL-C concentrations in girls with and without abdominal obesity.

In children without abdominal obesity, girls had higher TC, LDL-C, TG, non-HDL-C, insulin concentrations, and HOMA-R than boys. In the study subjects, we found ten boys and twenty-eight girls with insulin resistance (HOMA-R ≥ 2.5), and only one boy with metabolic syndrome.

**VLDL subclass profile measured using the HPLC method**

Children with abdominal obesity had higher large, medium, and small VLDL-TG concentrations than children without abdominal obesity, regardless of gender (Table 1).

In children without abdominal obesity, girls had higher medium VLDL-TG and small VLDL-TG concentrations than boys. In children with abdominal obesity, the TG distribution in VLDL was shifted to a larger subclass in boys and girls.

**Table 1:** Characteristics of the subjects.

<table>
<thead>
<tr>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n = 79</strong></td>
<td><strong>n = 85</strong></td>
</tr>
<tr>
<td>without obesity</td>
<td>abdominal</td>
</tr>
<tr>
<td>n = 66</td>
<td>n = 13</td>
</tr>
<tr>
<td>Age (year)</td>
<td>11.0 ± 1.6</td>
</tr>
</tbody>
</table>
## Relationship between TG concentration in each VLDL subclass and variables

Total VLDL-TG concentration had a significant positive relationship with WHtR (boys: $r = 0.5662$, $p < 0.0001$; girls: $r = 0.3693$, $p = 0.0005$) and HOMA-R (boys: $r = 0.5184$, $p < 0.0001$; $r = 0.6141$, $p < 0.0001$).

The relationship between TG concentration in each VLDL subclass and WHtR and HOMA-R were analyzed using single regression analyses (Table 2).
Based on multiple regression analyses (Table 3), both the WHtR and HOMA-R were independent determinants of large, medium, and small VLDL-TG concentrations in boys. In contrast, HOMA-R was an independent determinant of all VLDL-TG subclasses in girls, unlike the WHtR.

Table 2: Relationship between WHtR, HOMA-R and VLDL-TG.

<table>
<thead>
<tr>
<th>Variables</th>
<th>WHtR</th>
<th>HOMA-R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>0.5662</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>large VLDL-TG</td>
<td>0.5800</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>medium VLDL-TG</td>
<td>0.5285</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>small VLDL-TG</td>
<td>0.4902</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Girls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>0.3693</td>
<td>0.0005</td>
</tr>
<tr>
<td>large VLDL-TG</td>
<td>0.3841</td>
<td>0.0003</td>
</tr>
<tr>
<td>medium VLDL-TG</td>
<td>0.3320</td>
<td>0.0019</td>
</tr>
<tr>
<td>small VLDL-TG</td>
<td>0.0263</td>
<td>0.8112</td>
</tr>
</tbody>
</table>

Table 3: Multiple regression analysis of the relationship between VLDL subclass profile and WHtR and HOMA-R.

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Independent variables</th>
<th>Boys</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r2</td>
<td>β</td>
<td>s.e.</td>
<td>p</td>
<td>r2</td>
<td>β</td>
<td>s.e.</td>
<td>p</td>
</tr>
<tr>
<td>VLDL-TG</td>
<td>WHtR</td>
<td>0.38</td>
<td>0.4</td>
<td>117</td>
<td>31.31</td>
<td>0.4</td>
<td>-6.54</td>
<td>51.51</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>HOMA-R</td>
<td>5.3</td>
<td>0.93</td>
<td>1.938</td>
<td>0</td>
<td>9.817</td>
<td>17.43</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>large VLDL-TG</td>
<td>WHtR</td>
<td>0.37</td>
<td>0.4</td>
<td>74</td>
<td>17.76</td>
<td>0.4</td>
<td>2.575</td>
<td>29.09</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>HOMA-R</td>
<td>2.4</td>
<td>0.001</td>
<td>1.1</td>
<td>0</td>
<td>5.412</td>
<td>0.985</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>medium VLDL-TG</td>
<td>WHtR</td>
<td>0.36</td>
<td>0.3</td>
<td>33</td>
<td>10.91</td>
<td>0.3</td>
<td>-7.08</td>
<td>18.53</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>HOMA-R</td>
<td>2.1</td>
<td>0.001</td>
<td>0.675</td>
<td>0</td>
<td>3.362</td>
<td>0.627</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>small VLDL-TG</td>
<td>WHtR</td>
<td>0.35</td>
<td>0.3</td>
<td>9.5</td>
<td>3.867</td>
<td>0.3</td>
<td>-2.04</td>
<td>6.247</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>HOMA-R</td>
<td>0.9</td>
<td>0.001</td>
<td>0.239</td>
<td>0</td>
<td>1.042</td>
<td>0.211</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Japanese school children with abdominal obesity, both boys and girls, had elevated concentrations of large, medium, and small VLDL-TG, as well as insulin resistance. In addition, abdominal adiposity was a significant independent determinant of the VLDL subclass profile in boys, but not girls. HOMA-R was a significant independent determinant of VLDL subclass profile in boys and girls.

Previously, the relationship between waist circumferences and VLDL subclasses has been investigated in children, demonstrating that waist circumference is associated with large VLDL, but not small VLDL [12]. In another study, obese adolescents with intrahepatic fat accumulation had a marked increase in large VLDL, and to a lesser extent, medium VLDL, but no differences in small VLDL compared with obese adolescents without hepatic fat accumulation [21]. In the present study, we confirmed that Japanese school children with abdominal obesity, both boys and girls, had elevated concentrations in VLDL-TG, and the subclass distribution was shifted to larger particles.

It is known that hepatic fatty acid availability and insulin are two major modulators of VLDL production and secretion [22]. The acute effects of insulin on VLDL kinetics have been investigated in healthy human subjects [23]. Insulin infusion down-regulates VLDL1 secretion and increases VLDL2 secretion, resulting in a shift in the balance from VLDL1 to VLDL2; however, in an insulin resistance state, hyperinsulinemia promotes lipogenesis and suppression of VLDL1 secretion is impaired. Our results regarding alterations
in the VLDL subclass profile observed in children with abdominal obesity and the relationship between HOMA-R and VLDL subclass are consistent with the underlying mechanism. Therefore, insulin resistance is an important factor determining VLDL subclass profile in Japanese school children.

Another important finding of the present study was the gender difference in the relationship between abdominal adiposity and VLDL-TG subclass distribution. The previous study investigating the impact of abdominal adiposity on VLDL subclass profile in black and white children demonstrated the racial difference; however, the gender difference was not evaluated [12]. Because of the hyperlipolytic state, the liver of abdominal obese patients is exposed to an increased flux of adipose-derived fatty acids, which contributes to an increased production and secretion of VLDL particles. In a human adult study, gender and obesity were shown to have independent and different effects on VLDL-TG kinetics [24]. In lean healthy subjects, women have a higher rate of VLDL-TG production and a higher rate of VLDL-TG clearance than men. In obese subjects, the rate of VLDL-TG production increases in men, while no change occurs in women, and the rate of VLDL-TG clearance decreases in women, while no change occurs in men. Thus, the plasma VLDL-TG concentration in obese adults is determined primarily by the production rate in men, but by the clearance rate in women. The reduced VLDL-TG clearance is associated with insulin resistance. A human adult study has demonstrated that muscle LPL activity is correlated with the serum TG concentration, and is also related to the fasting insulin concentration and insulin sensitivity, suggesting that the hyperinsulinemia associated with insulin resistance may have a down-regulating effect on muscle LPL activity [25]. In addition, estrogen may decrease lipoprotein lipase activity by a post-transcriptional modification of protein levels in premenopausal women [26]. Our results in Japanese girls demonstrate that HOMA-R, but not the WHtR, is an independent determinant of all VLDL-TG subclasses based on multiple regression analyses, suggesting that impaired insulin actions, rather than an increased flux of fatty acids, is a main mechanism explaining TG elevation combined with the changes in VLDL-TG subclass profile in girls. In our previous study investing HDL-C subclass alteration in abdominal obese children [27], gender difference was also demonstrated; cholesterol levels in small and very small HDL were lower in girls than boys. Furthermore, we evaluated the LDL subclass profile in this study subjects, showing that LDL subclass alteration associated with abdominal obesity was exhibited in boys, but not in girls (Supplementary table). The sex hormonal status should be evaluated in future studies.

Our study had limitations. We investigated VLDL subclasses using HPLC methods. Therefore, we could not compare our data with previous reports that used other methods, [12,15,21] and the racial difference regarding the impact of abdominal obesity on the alteration in VLDL subclass profile could not be analyzed; however, we confirmed that the VLDL subclass profile was altered in Japanese school children with abdominal obesity. Second, we could not obtain information on nutritional intake. Dietary intake, especially fish consumption, is related to a favorable subclass distribution of VLDL [28,29]. Further detailed studies, including dietary assessment, should be conducted to elucidate the mechanisms of VLDL subclass alterations associated with abdominal obesity.

Conclusions

It is now recognized that a fundamental defect in the dyslipidemia component of MetS is an overproduction of large VLDL particles, resulting in a higher concentration of small dense LDL and a lower concentration of HDL-C.20 In Japanese school children, we found that abdominal obesity and insulin resistance were associated with the VLDL subclass profile, which suggests that VLDL subclass analysis may provide a better guide for the management of MetS, even in school children.

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Author Disclosure Statement

No competing financial interests exist.

References


