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Original

High Density Lipoprotein Particle Size in Children: Relation to Atherogenic Dyslipidemia

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Abstract. Atherosclerosis begins in childhood. Protection from atherosclerosis is provided by high-density lipoprotein (HDL), a heterogeneous particle, which includes several subclasses differing in size, density and apolipoprotein content. The objective of this study was to document the relevance of assessing HDL particle size as another feature of dyslipidemia related to the develpment of atheosclerosis during childhood. For that purpose, HDL particle size in 268 community-based children (137 boys and 131 girls), 7−13 years old, was measured by gradient gel electrophoresis, and relationships of HDL particle size to plasma lipids parameters and the anthropometric indices were analyzed. There was no gender difference in HDL particle diameter. The results of analysis revealed significant positive correlations between HDL particle diameter and HDL-cholesterol level ($r=0.363$, $p<0.01$), apolipoprotein AI level ($r=0.310$, $r<0.05$) and low-density lipoprotein particle (LDL) size ($r=0.290$, $p<0.05$), while there was an inverse correlation with atherogenic index ($r=-0.316$, $p<0.05$). There was no significant correlation between HDL particle size and triglyceride levels in the overall analysis ($n=268$), however, when this relation was analyzed in the limited HDL size range below 11 nm, a significant inverse relation appeared between particle size and TG levels ($r=-0.546$, $P<0.01$, $n=75$). These findings indicate that the general shift toward smaller HDL particle size was associated with dyslipidemia characterized by higher atherogenic index and triglyceride level, lower HDL-C level and smaller LDL particle size. Therefore, HDL size may represent another relevant marker of atherogenic lipid metabolism.

Key words: HDL particle size, HDL-cholesterol, triglyceride, dyslipidemia, atherosclerosis, insulin resistance, gradient gel electrophoresis

Introduction

Numerous prospective epidemiological studies have firmly established that high-density lipoprotein (HDL), as assessed by its cholesterol content, is inversely correlated with the incidence and prevalence of coronary heart disease due to atherosclerosis (1). The anti-atherogenic action of HDL is probably related to reverse cholesterol transport by which excess peripheral cholesterol is returned to the liver (2). However, HDL is heterogeneous, encompassing several subclasses differing in size, density and apolipoprotein content. Several approaches, such as ultracentrifugation, precipitation, immunoaffinity chromatography, and various types of

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electrophoresis, have been used to isolate and characterize HDL subpopulations. As previous studies have shown, in agarose gel electrophoresis, most HDL has α-mobility, while some shown pre-β mobility. By gradient gel electrophoresis, pre β-HDL can be further separated into pre β_1-HDL, pre β_2-HDL and pre β_3-HDL, while α-HDL can be separated into five distinct subpopulations, HDL_{3c}, HDL_{3b}, HDL_{3a}, HDL_{2a} and HDL_{2b}, according to increasing particle size (3, 4).

During the past few years, several reports have indicated that patients with hyperlipidemia/dyslipidemia often have altered HDL subclass distributions. Recent studies using this classification have found relationships between HDL subclasses and different metabolic and anthropometric parameters. That is, it has been demonstrated that patients with hyperlipidemia have increased concentrations of small HDL particles (HDL_{3b} and HDL_{3c}) and decreased concentrations of large HDL particles (HDL_{2b} and HDL_{2a}) (5). Furthermore, it has been demonstrated that there are positive correlations between increased body mass index (BMI) and higher levels of HDL_{3b} and lower levels of HDL_{2b}, and inverse correlerations between fasting plasma insulin concentrations and plasma HDL_{3a}, HDL_{2a}, and HDL_{2b} (6). A very recent report proposed that lipoprotein subclass parameters can be used to manage risk more effectively and prevent cardiovascular disease in patients with insulin resistance (7). It is widely recognized that the insulin resistant state is associated with dyslipidemia and increased cardiovascular disease risk (8, 9).

The childhood origin of atherosclerosis is well recognized, as is the expression in childhood of characteristics that place individuals at increased risk for atherosclesois later in life. Atherosclerosis begins during childhood (10, 11). Although the relationships of low-density lipoprotein (LDL) particle subclass and particle size to the insulin resistant state or atherogenic metabolic state have received considerable attention even in children (12–14), there have been relatively few studies regarding HDL subclass or particle size in children (15–19).

This study was carried out to document the relevance of assessing HDL particle size as another feature of atherogenic dyslipidemia found in children, using a recently described method (20). The relationship between HDL particle size and various parameters was analyzed. Findings obtained in this study could provide additional information for understanding the pathogenesis of atherosclerosis in children.

Methods

Subjects

The study subjects were 268 school children (137 boys and 131 girls) 7 to 13 yr of age, residing in S-cho, Chiba, Japan. Parents or guardians of all the children gave informed consent for participation in the study. Children with diabetes mellitus, thyroid disease, chronic renal disease, or hepatobilliary disease were excluded from this study.

After an overnight fast, morning venous blood samples were collected from the children at school. The obesity index was calculated as follows: Obesity index = [(body weight − ideal weight)/ideal weight)] × 100. Children whose obesity index was over 20% were considered to be obese. The body mass index (BMI) was also calculated from the weight and height.

Lipid measurements

In regard to individual plasma lipid parameters, total cholesterol (TC) and triglycerides (TG) were determined enzymaticaly. HDL-C was measured by precipitation of other lipoproteins using the phosphowolframic acid method. LDL-C was calculated using Friedewald's formula (21). Apolipoproteins (ApoA1 and ApoB) were quantified by turbidimetric immunoassay. The atherogenic index (AI) was calculated as follows: AI = [TC − HDL-C] ÷ HDL-C. An AI value

below 3 is considered normal (22).

Gradient gel electrophoresis of plasma samples

The HDL particle diameter was determined according to the method described by Perusse et al (20), by gradient gel electrophoresis using 4 to 25% polyacrylamide. After the gels had been equilibrated at 120 V for 60 min, electrophoresis was performed for each gel containing the serum sample diluted 1:2 with sample buffer (consisting of 40% sucrose, 0.06% EDTA-2Na, and 0.01% BPP), to a volume of 20 μL. Each gel also contained thyroglobulin, ferritin, catalase, and lactate dehydrogenase as reference standards of known diameter. The gels were then electrophoresed at 15 V for 15 min, followed by migration at 70 V for 20 min, at 75 V for 16 h, and finally at 125 V for 8 h. The gels were then stained for lipid overnight with Sudan Black, and for reference standards with Coomassie Brilliant Blue for 45 min. They were then decolorified with ethanol and kept immersed in acetic acid. The decolorified gels were then scanned with an image scanner (Epson GT-6500: Seiko Epson Corporation, Nagano, Japan) and analyzed using an image processing and analysis program for Macintosh (NIH Image 1.61: National Institutes of Health, United States). Migration distances were determined. The HDL particle diameter was then calculated by comparing the mobility of the sample with that of the three calibrated standards on each gel. A higher integrated HDL size indicated a greater proportion of "large" HDL particles, whereas a low HDL size suggested an increased prevalence of "small" HDL particles. Inter- and intra-assay coefficients of variation for the integrated HDL size assessed by this method were < 3% (n=10) and <2% (n=9), respectively.

Low-density lipoprotein (LDL) particle size was also measured, using the present blood samples, according to the gradient gel electrophoresis method reported by Krauss (23) and Arisaka (11); type of solutions, duration time of electrophoresis, electric voltage, type of lipid staining, and reference standard substances differed from the present HDL electrophoresis. The data for LDL particle size has been reported elsewhere (14).

Statistical analysis

The relationships between HDL particle size and various parameters were analyzed. An unpaired Student's t-test was done to compare boys and girls for various parameters. Relationships between HDL particle size and the individual parameters of the lipid profile were analyzed using Spearman's non parametric rank correlation. $p<0.05$ was considered to denote a statistically significant difference.

Results

1) Table 1 shows the characteristics of subjects' profiles by sex. There was no statistically significant difference regarding obesity index and various lipid levels between boys and girls.

2) The HDL particle diameter was 11.4 (mean) ± 0.61 (SD) nm in boys and 11.4 ± 0.50 nm in girls, not a statistically significant difference. Even though particle size was analyzed by dividing children into two age groups, those above 10 yr old (11.8 ± 0.55 nm, n=162) and those below 10 yr (11.5 ± 0.62, n=122), there was no difference between boys and girls in either age group.

3) Relationships between HDL particle diameter and known individual risk factors for atherosclerosis were evaluated for boys and girls combined, boys alone, and girls alone. There were no apparent gender differences. Therefore, we analyzed the data from boys and girls together (Table 2, Figs. 1–5).

The HDL particle diameter showed a significant positive correlation with HDL-C ($r=0.363$, $p<0.001$) and ApoA1 ($r=0.310$, $p<0.05$), while it showed an inverse correlations with AI ($r=-0.316$, $p<0.05$) and BMI ($r=-0.138$, $p<0.05$). No statistically significant correlation was noted between HDL particle size and TC, LDL-C, ApoB,

Table 1 Characteristics of subjects by sex

	Boys (n=137)	Girls (n=131)	p value
Age (yr)	11.79 ± 0.15	11.87 ± 0.14	NS
Obesity Index (%)	10.50 ± 0.83	4.00 ± 0.27	NS
TC (mg/dl)	182.00 ± 2.33	156.83 ± 2.71	NS
LDL-C (mg/dl)	100.40 ± 2.03	102.75 ± 2.31	NS
HDL-C (mg/dl)	63.38 ± 1.23	62.40 ± 1.37	NS
TG (mg/dl)	91.09 ± 3.86	90.98 ± 3.94	NS
ApoA1 (mg/dl)	152.46 ± 1.70	150.69 ± 1.91	NS
ApoB (mg/dl)	79.83 ± 1.35	82.00 ± 1.61	NS
Atherogenic Index	2.00 ± 0.06	2.08 ± 0.07	NS

Mean ± SE, NS: Not significant.

Table 2 Relationship between HDL particle size and individual lipid levels, anthropometric indices and blood pressure in 268 children

	r	p-value
Total cholesterol	0.128	NS
LDL-cholesterol	−0.015	NS
HDL-cholesterol	0.363	p<0.01
Triglyceride	−0.112	NS
HDL≤11 nm	−0.546	p<0.01
ApoA1	0.310	p<0.05
ApoB	−0.069	NS
LDL particle size	0.290	p<0.05
Atherogenic index	−0.316	p<0.05
Body mass index	−0.138	p<0.05
Obesity index	−0.127	NS
Systolic blood pressure	−0.102	NS
Diastolic blood pressure	−0.04	NS

NS: Not significant.

TG, obesity index or blood pressure.

4) There was no significant correlation between HDL particle size and TG levels in the overall analysis (n=268), however, when this relation was analyzed in the limited HDL size range below 11 nm, a significant inverse relation appeared between particle size and TG levels ($r=-0.546$, $p<0.01$, n=75) (Fig. 2).

5) There was a statistically significant correlation between HDL particle size and LDL particle size ($r=0.290$, $p<0.05$).

Discussion

Changes in HDL particle size, in association with changes in HDL subclasses distribution are considered to reflect the metabolic process of uptake and transport of cholesterol by circulating HDL. Also, changes in HDL particle size may be related to altered lipid metabolism caused by insulin resistance, which is the background of atherosclerosis (24–26).

Several factors regulating HDL particle size are considered. Lipoprotein lipase is the rate-limiting enzyme in the clearance of TG-rich lipoproteins from plasma and is involved in the formation of HDL, whereas hepatic lipase plays a role in the catabolism of HDL. Therefore, abnormalities in the regulation of TG metabolism by lipoprotein lipase and hepatic lipase may reduce HDL levels. Furthermore, cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) are two other factors involved in the metabolism of HDL (26, 27). PLTP and CETP are released from adipose tissues and appear to directly modulate lipoprotein metabolism: enhanced PLTP activity results in a lower HDL-C fraction accompanied by a relative increase in small pre β-HDL. CETP also catalyzes the transfer of cholesteryl ester from HDL to TG-rich lipoprotein in exchange for TG and leads to an enrichment of HDL and LDL with TG. TG-enriched

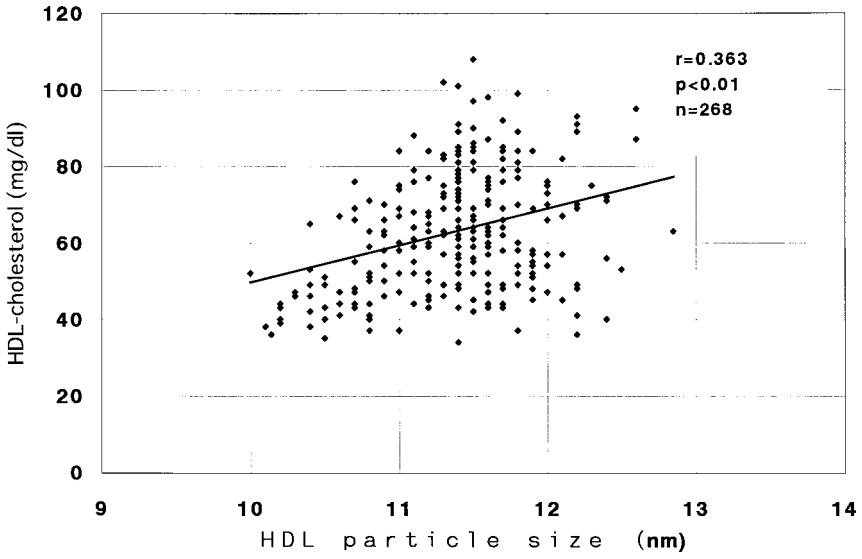

Fig. 1 Relationship between HDL particle diameter and HDL-cholesterol level.

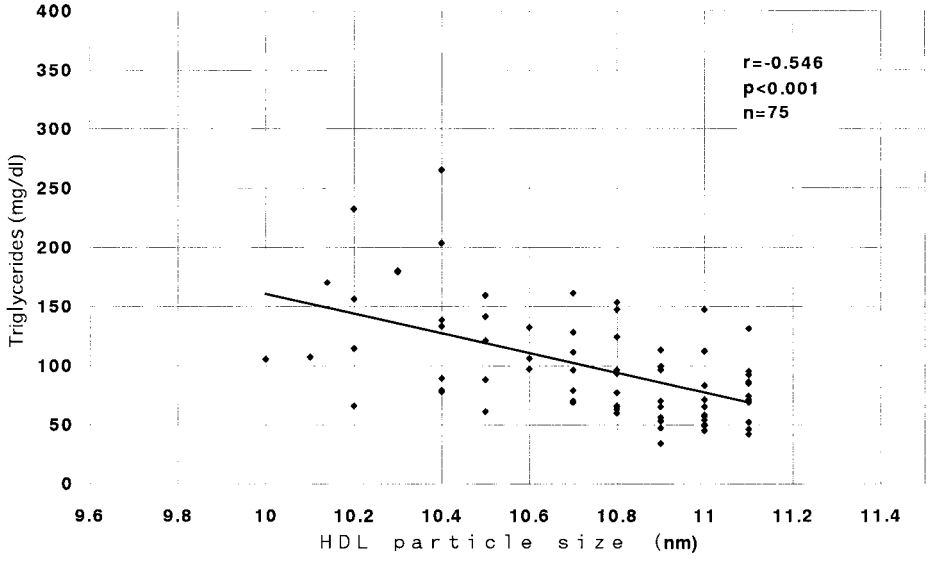

Fig. 2 Relationship between HDL particle diameter (range below 11 nm) and triglycerides level.

HDL and LDL particles can be subjected to further lipolysis by hepatic lipase, leading to the formation of small, dense HDL and LDL particles (28, 29). In obesity, increased synthesis of these lipid transfer proteins by the enlarged mass of adipose tissue occurs and it has been demonstrated that weight reduction is associated with a decrease in plasma CETP activity (30–32). Factors that promote the conversion of HDL and LDL into populations of smaller particles is thought to be related to insulin resistance (19, 32).

Relatively few studies have examined HDL

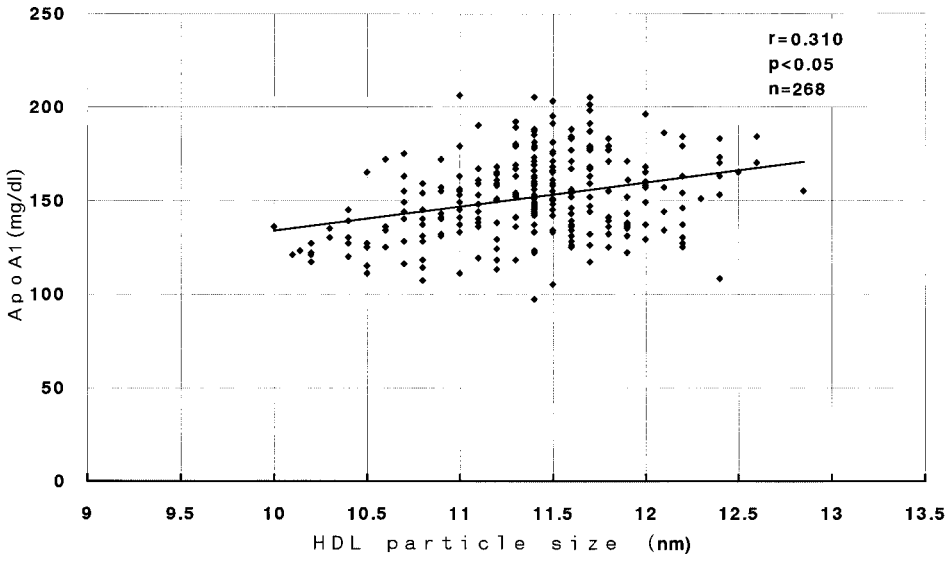

Fig. 3 Relationship between HDL particle diameter and ApoA1 level.

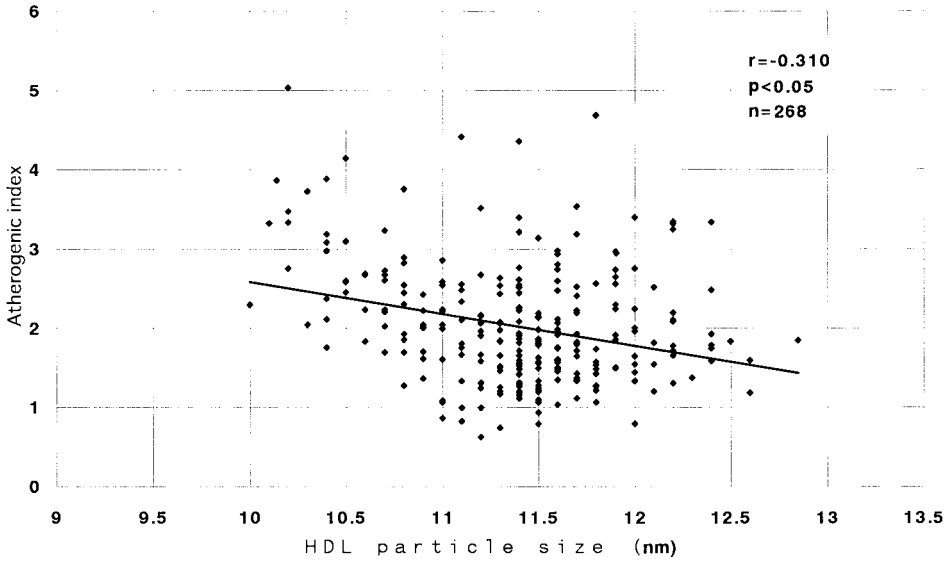

Fig. 4 Relationship between HDL particle diameter and the atherogenic index.

particle size in children (15–19). In the present study, relationships among lipids, lipoproteins, anthropometric measures and HDL particle sizes in children were investigated. It was found that HDL particle size showed a general shift toward smaller size in association with decreased HDL-C and ApoA1, and increased atherogenic index, TG and BMI, indicating the features of atherogenic metabolic triad. Although circulating HDL-C concentration is recognized for its inverse association with the occurrence of cardiovascular disease, HDL-C is only moderately predictive of disease (1, 2). This is exactly because circulating HDL, as assessed by its cholesterol content (HDL-

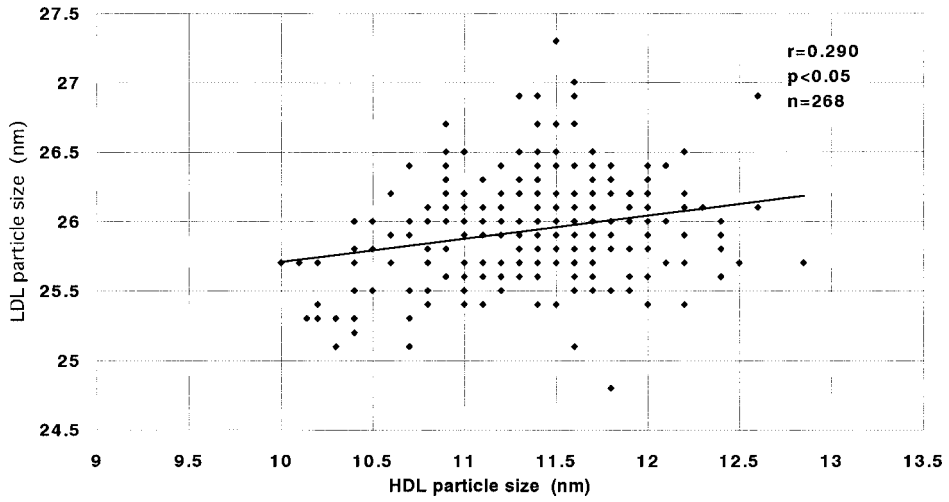

Fig. 5 Relationship between HDL particle diameter and LDL particle diameter.

C), does not reflect the heterogeneity of HDL particles (HDL subfractions). Therefore, it is reasonable that HDL particle size and HDL-C level are not strongly correlated, as shown in the present study. These results favor the idea that the low cardiovascular disease risk may be due in part to their anti-atherogenic HDL pattern, rather than the HDL-C concentration. In addition, no significant correlation was found between HDL particle size and either TC or LDL-C,providing further support for the previously published notion that the measurement of TC or LDL-C alone does not allow proper identification of individuals who are carriers of atherogenic dyslipidemia.

Regarding the relationship between HDL particle size and plasma TG levels, a tendency for an inverse relationship was recognized in the HDL size ranges below approximately 11 nm. However, in the diameter ranges above 11 nm, this association was not found. The association of reduced HDL particle size with elevated TG may be explained by the bidirectional exchange of TG between HDL and TG-rich lipoprotein: hypertriglyceridemia appears to promote TG transfer from TG-enriched lipoprotein to HDL particles under the action of CETP with a subsequent hydrolysis of TG-enriched HDL by hepatic lipase or lipoprotein lipase, producing small HDL particles (4, 28, 32, 34). Although it is thought that TG is a critical component of lipoprotein particles and plasma TG level affects lipoprotein size, both TG level -related metabolic effect and TG level-independent genetic effect may be operated for determining HDL particle size (4, 35).

We also noted a positive relationship between HDL particle size and LDL particle size, suggesting that the synergic reduction in the size of these two lipoproteins may be the consequence of a common metabolic alteration, probably related to insulin resistance (8, 9, 23, 24). Therefore, HDL size may represent another relevant marker of atherogenic dysmetabolism.

In conclusion, although the mechanisms by which HDL size could affect cardiovascular disease risk remain to be further investigated, the results of this study imply that a general shift toward smaller HDL particle size may be related to the pathogenesis of atherosclerosis in children. Therefore, HDL size may represent another relevant marker of atherogenic lipid metabolism. Prospective studies are clearly warranted to quantify the contribution of HDL particle size to the evaluation of the future risk of cardiovascular disease development.

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