Early Adiposity Rebound and Small Dense Low-Density Lipoprotein in Childhood Obesity

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Abstract

Aim: The adiposity rebound (AR) corresponds to the second rise in the body mass index (BMI) curve that occurs between ages 5 and 7 years. The goal of this study was to determine whether age at AR is related to the presence at 12 years old of small dense low-density lipoprotein (SDLDL), an atherogenic lipoprotein produced as a metabolic consequence of AR.

Methods: A longitudinal population-based prospective study was performed in 215 children. Serial measurements of BMI were conducted at ages 1, 1.5, 2 and yearly thereafter until 12, based on which age at AR was calculated. The subjects were divided into 5 groups according to age at AR of ≤4, 5, 6, 7 and ≥8 years. Plasma lipids and SDLDL were measured at 12 years of age. SDLDL (LDL particle size <25.5 nm) was determined by non-denaturing 2-16% gradient gel electrophoresis.

Results: The prevalences of SDLDL were 15.0% in children with age at AR ≤4 y, 8.1% in those with age at AR 5 y, and 0% in all other groups (AR at ≥6 years). An earlier AR was significantly associated with higher BMI, increased plasma triglyceride ($p < 0.05$), increased atherogenic index ($p < 0.05$), and decreased HDL-cholesterol ($p < 0.05$) at 12 years of age.

Conclusion: Children with AR before 4 years old showed a high prevalence of atherogenic SDLDL, indicating a predisposition for future cardiovascular disease.

Keywords: Childhood obesity; Adiposity rebound; Insulin resistance; Small dense LDL; Cardiovascular disease

Introduction

Low-density lipoprotein (LDL) particles are heterogeneous in density, size, lipid composition, and pathogenetic properties. Smaller and denser LDL particles have a stronger atherogenic impact, possibly because of low binding affinity of these particles to the LDL receptor, low resistance to oxidative stress, a prolonged half-life in plasma, and easier penetration of the arterial intima due to the small particle size [1,2]. LDL particles are reduced in diameter because of alteration in lipoprotein metabolism caused by insulin resistance, producing small dense LDL (SDLDL) particles of size ≤ 25.5 nm [3,4]. Many studies have shown that circulating SDLDL is associated with increased occurrence of atherogenic coronary artery events in adults [5,6]. Therefore, the presence of SDLDL in adolescence is a risk factor for future coronary heart disease [7,8].

Excessive weight gain in early childhood is thought to lead to adult obesity-related morbidity and mortality [9]. Adiposity rebound (AR) is defined as the second rise in the body mass index (BMI) curve that normally occurs between ages 5 and 7 years; on average, a rapid increase in BMI occurs during the first year of life, subsequently declines and reaches a nadir at around 6 years of age, and then increases again throughout childhood [10]. Recent studies have shown that early AR (<4 years) is related to a risk for later obesity and metabolic consequences caused by insulin resistance [10-12]. These observations motivated us to examine the relationship between the timing of AR and production of SDLDL as a metabolic consequence of changes in adiposity during infancy.

Methods

Subjects

A longitudinal population-based study was performed in 215 children (114 boys and 101 girls) in a birth cohort in Tochigi Prefecture, Japan. All of the children were followed up in infant health checks at a health center during the preschool period, and data were stored at a regional health center. During the school-age period, children underwent an annual physical examination at school, and the resulting data were also kept at the regional health center. At 12 years of age, all the children underwent a blood examination at high school. Written informed consent was obtained from parents or guardians for the physical examinations. The study was approved by the ethics committee of Dokkyo Medical University.

Identification of age at adiposity rebound using BMI

Serial measurements of body weight and height were obtained yearly from ages 1 to 12 years, giving a total of 12 measurements in all subjects. Age at AR was defined as the age between 1 and 12 years at which the lowest BMI occurred before the second BMI rise. The

subjects were then divided into 5 groups based on an age at AR ≤ 4 y, 5 y, 6 y, 7 y, and ≥8 y.

Measurements of lipids

Fasting blood sampling in the morning was performed at school. To obtain blood samples after an overnight fast, students were allowed to bring a packet box breakfast, which they ate after undergoing a health examination in the schoolroom. Blood samples collected in tubes containing 1 g/L EDTA were sent to the laboratory. Plasma was separated and stored at -80°C until measurement of LDL particle size. Levels of total cholesterol (TC) (Cholestest CHO, Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) and triglycerides (TG) (Aqua-auto TG-II, Kainos Laboratories, Inc., Tokyo, Japan) were determined using enzymatic methods. High-density lipoprotein cholesterol (HDL-C) was measured by precipitation of other lipoproteins with the direct method (Cholestest N HD, Daiichi Pure Chemicals Co. Ltd.). Low-density lipoprotein cholesterol (LDL-C) was determined using the Friedewald formula. The atherogenic index was calculated from the formula: [TC– HDL-C]/HDL-C [13].

Determination of LDL particle size

The LDL particle diameter was determined using the method described by Krauss et al. [4,14] with 2.5 to 16% polyacrylamide gradient gel electrophoresis. Gels were equilibrated at 120 V for 20 min, and electrophoresis was then performed using serum samples diluted 1:2 with sample buffer (31% sucrose, 0.06% EDTA-2Na, and 0.01% BPP) to a volume of 20 mL. Each gel also contained thyroglobulin, apoferritin and latex beads, as reference standards of known diameter. The gels were electrophoresed at 120 V for 19 h, and then stained for lipids with Oil Red O with heating to 55°C for 24 h and for protein with Coomassie Brilliant Blue for 15 min. Gels were then decolorified with ethanol and immersion in acetic acid, scanned with an image scanner (Epson GT-6500; Seiko Epson Corp., 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 LDL particle diameter was calculated by comparing the mobility of the sample with the mobility of the three calibrated standards on each gel. SDLDL was defined as LDL with a particle diameter <25.5 nm, based on the criteria proposed by Austin et al. [15].

Statistics

An unpaired Student t-test was used to compare parameters between the groups. Tests for trends and for the association of age at AR with BMI, LDL-C, HDL-C, TG and atherogenic index were examined by multivariate logistic regression analysis. $P < 0.05$ was considered to be significant.

Results

Serial changes in BMI from 4 months to 12 years according to age at AR in the 215 children are shown in Figure 1. Children with AR at an earlier age had a higher BMI at 12 years of age. Relationships between age at AR and BMI and serum lipids at 12 years of age are shown in Table 1. Earlier AR appeared to be associated with higher TG and atherogenic index, whereas there was an inverse relationship between age at AR and HDL-C levels, and no relationship between the timing of AR and plasma levels of TC and LDL-C. Prevalences of SDLDL at 12 years of age in relation to age at AR are shown in Table 2. Children

with AR at an earlier age had an increased SDLDL, with prevalences of 15.0% and 8.1% in the AR ≤4 y and 5 y groups, respectively, but of 0% in the three higher age groups.

Age at adiposity redound	BMI (Kg/m^2)	TC (mg/dl)	LDC-C (mg/dl)	HDL-C (mg/dl)	TG (mg/dl)	Atherogenic Index
AR ≤ 4 y (n = 60)	21.5 (4.3)	166.0 (24.9)	96.6 (21.5)	60.0 (11.6)	67.2 (30.2)	1.8 (0.6)
AR 5 y (n = 74)	19.7 (3.1)	168.3 (24.2)	93.4 (21.9)	62.8 (9.2)	69.5 (26.9)	1.6 (0.4)
AR 6 y (n = 36)	19.5 (3.4)	165.9 (22.5)	92.5 (20.2)	62.0 (7.6)	58.7 (20.1)	1.6 (0.4)
AR 7 y (n = 26)	17.8 (1.7)	173.2 (33.8)	91.6 (24.5)	70.0 (13.6)	54.2 (18.8)	1.5 (0.4)
AR ≥ 8 y (n = 19)	17.1 (1.7)	168.9 (25.4)	89.0 (19.9)	69.8 (11.1)	51.8 (21.1)	1.5 (0.5)
	P < 0.01	NS	NS	P < 0.05	P < 0.05	P < 0.05

Values are expressed as mean (SD) NS: Not significant

Atherogenic index = [TC-HDL-C]/HDL-C

Table 1: Relationships between the age at adiposity rebound and variables in 215 children at 12 years of age.

Age at adiposity rebound	Prevalence of SDLDL
AR ≤ 4 y (n = 60)	9 (15%)
AR 5 y (n = 74)	6 (8.1%)
AR 6 y (n = 36)	0
AR 7 y (n = 26)	0
AR ≥ 8 y (n = 19)	0
Total	15/215 (6.9%)

Table 2: Relationship between the age at adiposity rebound and the prevalence of small, dense LDL at 12 years of age.
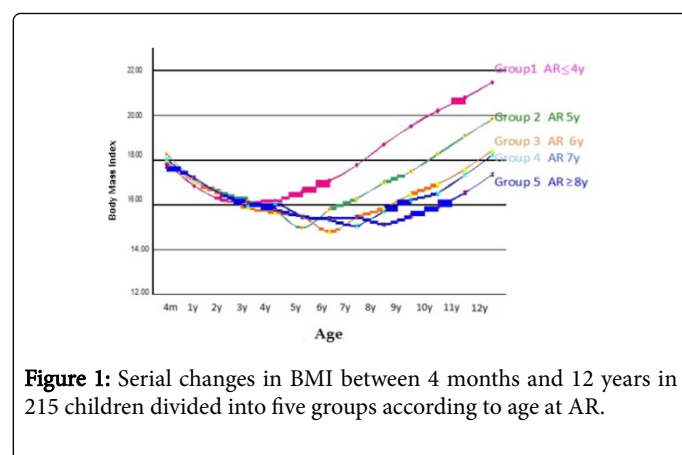

Figure 1: Serial changes in BMI between 4 months and 12 years in 215 children divided into five groups according to age at AR.

Discussion

In this study, children with early AR had higher BMI at 12 years of age, and circulating SDLDL was detected only in children with AR at <5 years. These children also exhibited atherogenic lipid profiles with elevated plasma levels of TG and atherogenic index and decreased plasma HDL-C, whereas TC and LDL-C, the metabolism of which is independent of insulin resistance [16], did not show significant changes related to the timing of AR.

A reduction in LDL particle size associated with an increase in TG and a decrease in HDL-C may result from alteration of lipoprotein metabolism caused by insulin resistance [1]. 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; thus, the cholesterol-depleted LDL then becomes smaller and denser. 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 [2-4]. The activities of CETP and HTGL are enhanced by increased adiposity or increased insulin resistance. Therefore, altered insulin secretion or insulin sensitivity caused by lifestyle-related dietary or physical activity may be metabolically responsible for the reduction in LDL particle size [16,17].

The prevalence of SDLDL in children has been found to be 2-11% [18-20], and the overall prevalence of SDLDL in the current study was 6.9%. Genetic and environmental factors may be responsible for the varying prevalences of SDLDL [2,16].

An association between early AR and presence of circulating SDLDL at 12 years of age was also shown in the present study. The mechanism underling the relationship of AR and SDLDL is complex, but metabolic programming leading to future insulin resistance may occur during early AR; changes of body composition associated with a BMI increase in this early period (<4 or 5 years of age) may play a pivotal role in development of insulin resistance, subsequently enhancing production of SDLDL [2,21]. Processes that regulate early body fat stores in early childhood have recently been shown to confer increased susceptibility to development of leptin resistance and insulin resistance [22-24].

Childhood plasma lipids levels and lipoprotein phenotypes representative of insulin resistance are known to correlate strongly with values measured in middle age [25]. This indicates that atherogenic lipoprotein SDLDL produced in adolescence tracks to adulthood, thus increasing the prevalence of cardiovascular diseases such as cardiac infarction and stroke and serving as a risk factor for overall mortality. Therefore, we propose that the most effective method for prevention of a future insulin-resistant state is to prevent an increase of BMI during early childhood, especially before 4 years old.

A limitation of the current study is that we were unable to investigate the levels of blood glucose and insulin, which are directly related to insulin resistance. However, SDLDL is considered to be a surrogate marker of insulin resistance and is actually responsible for the development of atherosclerosis [3,7].

Conclusion

In conclusion, the results of this longitudinal population-based cohort study indicate that children who exhibit AR before 4 years old have a high prevalence of atherogenic SDLDL, indicating a predisposition for future cardiovascular disease. Therefore, monitoring of AR may be effective for early identification of children at risk for cardiovascular disease in adulthood.

Acknowledgements

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