

Originals

## Relation of Adiposity Rebound age to Serum Small Dense Low-density Lipoprotein in Young Childhood

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### SUMMARY

The number of patients with childhood obesity is increasing worldwide. The age of adiposity rebound (AR), the age when body mass starts to rise after transient decrease after birth, is thought to have a predictive value of obesity in adulthood. In order to elucidate whether the early gain of weight is related to the future occurrence of metabolic syndrome, the author examined the relationship between the time of AR and the prevalence of children having atherogenic small dense LDL (SDLDL) in serum at 12 years of age in Fujioka town in Japan. A total of 215 children (114 boys and 101 girls) who were born between 1995 and 1996 have been enrolled in this study as a prospective cohort. Annual measurements of body-mass index (BMI) from 4 months to 12 years were carried out. We defined the age of AR as the age when the lowest BMI occurred during this period. At 12 years of age, serum concentrations of lipids including of SDLDL and lipoproteins were measured. As results children who exhibited an earlier AR had the higher BMI value at 12 years of age ( $p < 0.01$ ) in both sexes. The prevalence of SDLDL decreased progressively from 15.0% in children in whom AR occurred before the age of 4 years to 0% in those in whom AR occurred after 6 years. Furthermore, the earlier AR was associated with elevated triglyceride ( $p < 0.05$ ) and apolipoprotein B ( $p < 0.01$ ) levels and decreased HDL-cholesterol levels ( $p < 0.05$ ). In conclusion the present longitudinal population-based study indicates that children who exhibit AR at a younger age seem to be predisposed towards the future development of metabolic syndrome. Thus, it is possible to identify high-risk children with metabolic syndrome prospectively by measuring early adiposity rebound.

**Key Words** : body-mass index, adiposity rebound, obesity, small dense LDL, metabolic syndrome, insulin resistance

### INTRODUCTION

The prevalence of obesity in children is increasing worldwide. Also in Japan, due to the recent Westernization of the lifestyle, childhood obesity currently affects 10–15% of school children<sup>1~3</sup>. The adverse health consequences of obesity in childhood include dyslipidemia, elevated blood pressure, and insulin resis-

tance. Clustering of these atherogenic risk factors in early life is considered to play a critical role in the development of atherosclerosis since childhood<sup>4,5</sup>. Metabolic syndrome, components of which include hypertension, glucose intolerance, hypertriglyceridemia, decreased high-density lipoprotein cholesterol (HDL-C) level and central abdominal obesity, confers an excessively high risk of atherogenic cardiovascular disease, with an increased prevalence in overweight children<sup>6~8</sup>. Evidence suggests that stunted fetal growth or early infant weight gain may predispose towards the development of obesity or metabolic syndrome in later life<sup>9,10</sup>.

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The diagnosis of obesity in children and adults is based on the calculation of BMI by dividing the weight in kilograms by the height in meters squared ( $\text{kg}/\text{m}^2$ ). On average, a rapid increase of the BMI occurs during the first year of life. The BMI subsequently declines and reaches a nadir at around 6 years of age, and then increases again throughout childhood. This second BMI gain is called the adiposity rebound (AR). Recent reports indicated that an early AR is related to the development of later obesity<sup>11,12</sup>.

Recently, Errikson *et al.* reported that the number of patients with type 2 diabetes increased in persons in whom AR occurred early in infancy<sup>13</sup>. In relation to this observation, Bahrgava *et al.* demonstrated that a higher increase in BMI from 2 to 12 years of age is associated with increased risk of impaired crossing-into higher categories of body-mass index after the age of two years is associated with impaired glucose tolerance or diabetes in young adulthood<sup>14</sup>. These reports imply that those who exhibit weight gain in earlier life are at significantly higher risk for the development of metabolic syndrome and, subsequently diabetes or cardiovascular disease in adulthood.

These aspects motivated us to elucidate the relationship between the timing of weight gain during infancy and the metabolic consequences caused by changes in adiposity. Therefore, we examined the relationship between serial changes of BMI and the presence of circulating small dense low-density lipoprotein (SDLDL). Since a specific feature of metabolic syndrome and insulin resistance is the increased occurrence of small LDL particles, SDLDL has been accepted as an important predictor of metabolic syndrome<sup>15,16</sup>.

Our study was designed as a prospective, population-based study and all subjects undertook a total of 13 serial measurements of weight and length from 4 months until 12 years of age. To our knowledge, there have been no studies that have examined the relation of AR to SDLDL.

## SUBJECTS AND METHODS

### 1. Subjects

The subjects of this study consisted of 215 children (114 males and 101 females) who were born from 1995 to 1996 in Fujioka town of Tochigi Prefecture. The population of this town was 18,000, half of them were

farmers and the remaining half commuted to nearby large cities. This town has 4 elementary schools and 2 junior high schools. All of the children who were born in this town were continuously followed up by infant health check in the health center during the preschool period and the data were stored at the regional health center. During the school age period, children underwent annual physical examination in the schools, and the measurement data were also kept at the regional health center. Therefore, the physical examination data of children who were born in F town were collected while they are in elementary and junior high schools, and managed by the town government. In addition, children aged 12 underwent blood examination. Regarding the use of individual data of physical examination and blood tests, written informed consent was obtained from the guardian and this study was approved by the town council and elementary schools.

### 2. Methods

#### 2.1. Identifying of adiposity rebound time by BMI

We obtained serial measurements of body weight and height at the age of 4 months, 1y, 2y, 3y, 4y, 5y, 6y, 7y, 8y, 9y, 10 y, 11y and 12y. All subjects underwent a total of 13 measurements. At 12 years of age, blood sampling was performed. We defined the age at AR, as the age between 1 and 12 years at which the lowest BMI occurred. The subjects were then divided into 5 groups according the age of AR as follows : group 1 : AR below 4 years ( $\leq 4y$ ) ; group 2 : 5y ; group 3 : 6y ; group 4 : 7y ; group 5 :  $\geq 8$  years.

#### 2.2. Measurements of lipoproteins

Morning venous blood samples were collected from the children at school. The BMI was calculated based on the weight and height of each subject. In regard to individual lipoproteins, total cholesterol (TC) [cholestest CHO, Daiichi Pure Chemicals] and triglycerides (TG) [aqua-auto TG-II, Kainos] were determined by enzymatic procedures. HDL-C was measured by precipitation of other lipoproteins using the direct method [Cholestest N HD, Daiichi Pure Chemicals]. LDL-C was calculated using Friedewald's formula. Apolipoproteins A1 (ApoA1) and apolipoprotein B (ApoB) were quantified by turbidimetric immunoassay [apoA-I auto N, apoB auto N, Daiichi Pure Chemicals]. The

**Table 1** Characteristics of subjects at 12 years of age according to sex

	Boys (n = 114)	Girls (n = 101)	Total (n = 215)	p values
Body-mass index	19.9 (3.9)	20.2 (3.7)	20.1 (3.8)	NS
Total cholesterol (mg/dL)	164.6 (27.4)	171.0 (22.1)	167.5 (25.3)	NS
LDL-cholesterol (mg/dL)	92.9 (21.5)	95.5 (21.8)	94.1 (21.6)	NS
HDL-cholesterol (mg/dL)	62.1 (11.3)	63.8 (11.6)	62.9 (11.4)	NS
Triglyceride (mg/dL)	62.6 (27.5)	66.6 (26.5)	64.4 (27.1)	NS
Apolipoprotein A1 (mg/dL)	152.2 (23.0)	154.4 (14.0)	153.3 (18.9)	NS
Apolipoprotein B (mg/dL)	79.3 (17.0)	82.1 (17.7)	80.6 (17.4)	NS
Atherogenic index	1.6 (0.5)	1.7 (0.5)	1.6 (0.5)	NS

Values are expressed as mean (SD)

NS : Not significant

atherogenic index (AI) was calculated as follows :  $AI = [TC - HDL - C] \div HDL - C^{17)}$ .

### 2.3. Determination of LDL particle size

The LDL particle diameter was determined according to the method described by Krauss *et al.*<sup>18)</sup>, by gradient gel electrophoresis on 2.5 to 16 % polyacrylamide. After the gels were equilibrated at 120 V for 20 minutes, electrophoresis was performed for each gel containing the serum sample diluted 1 : 2 with sample buffer (consisting of 31 % sucrose, 0.06 % EDTA-2Na, and 0.01 % BPP), in a volume of 20 mL. Each gel also contained thyroglobulin, apoferritin and latex beads as reference standards of known diameter. The gels were then electrophoresed at 120 V for 19 hours, and they were stained for lipid with Oil Red O that was heated to 55°C for 24 hours, and for protein with Coomassie Brilliant Blue for 15 minutes. They were then decolorified with ethanol and immersed in acetic acid. The 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 LDL particle diameter was then calculated by comparing the mobility of the sample with the mobility of three calibrated standards on each gel. SLDL was defined as LDL with a particle diameter  $\leq 25.5$  nm, based on the criteria proposed by Austin *et al.*<sup>19~21)</sup>.

### 2.4. Statistical analysis

An unpaired Student's t-test was used to compare

various parameters between the groups. Tests for trend and the association between the age at AR and variables of BMI, TC, LDL-C, HDL-C, TG, ApoA1, and AI were examined by multivariate logistic regression analysis. P-values < 0.05 were considered statistically significant.

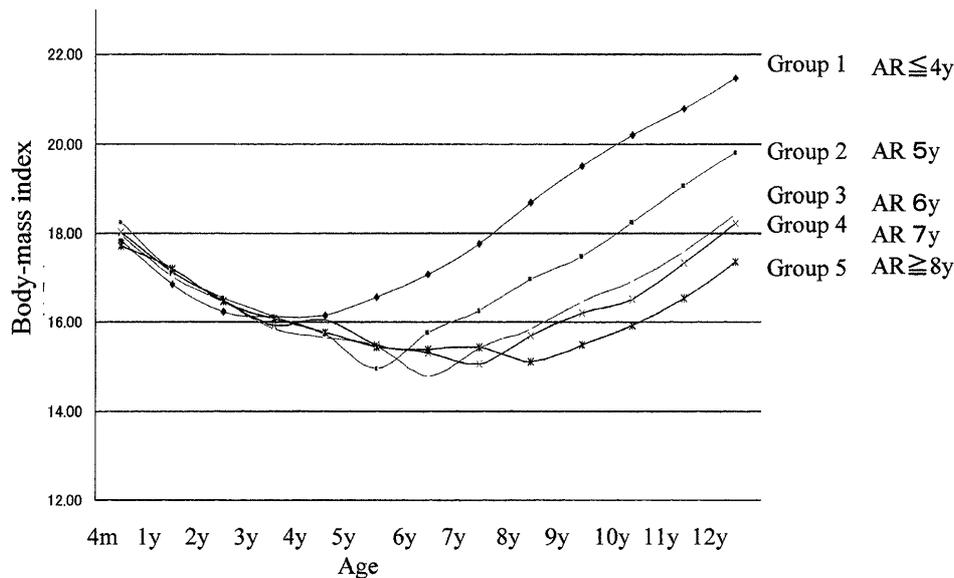
## RESULTS

Table 1 shows the characteristics of the 215 subjects in each sex separately. No statistically significant differences in the various parameters were observed between the boys and the girls.

Figure 1 shows the serial changes in BMI between 12 months and 12 years according to the age of AR in 215 children. Children who had an AR at an earlier age exhibited higher BMI values at 12 years of age and this tendency was the same in both sexes ( $p < 0.01$ ).

Table 2 shows the relationship between the age of AR and BMI and biochemical parameters at 12 years of age. It appeared that the earlier the timing of AR, the higher the TG and ApoB. An inverse relationship was found between the timing of AR and HDL-C levels.

Table 3 shows the prevalence of SLDL (proportion of children having SLDL) in relation to the age of AR. Children who exhibited an AR at an earlier age were associated with an increased prevalence of SLDL. The SLDL appeared only in children in group 1 (15.0 %) and group 2 (8.1 %), in which AR occurred earlier than 4 years and between 4 and 5 years, respectively.



**Figure 1** shows the serial changes in BMI between 4 months and 12 years according to the age of adiposity rebound in 215 children. Children who had an adiposity rebound at the earliest age, up to 4 years, had the highest BMI in later childhood.

**Table 2** Relationship between the age at adiposity rebound and various parameters in 215 children at 12 years of age

Age at adiposity rebound	BMI	TC	LDL-C	HDL-C	TG	Apo A1	Apo B	AI
Group 1 ( $\leq 4y$ ) n = 60	21.5 (4.3)	166.0 (24.9)	96.6 (21.5)	60.0 (11.6)	67.2 (30.2)	144.1 (17.4)	85.5 (17.8)	1.8 (0.6)
Group 2 (5y) n = 74	19.7 (3.1)	168.3 (24.2)	93.4 (21.9)	62.8 (9.2)	69.5 (26.9)	161.4 (15.9)	82.9 (15.1)	1.6 (0.4)
Group 3 (6y) n = 36	19.5 (3.4)	165.9 (22.5)	92.5 (20.2)	62.0 (7.6)	58.7 (20.1)	150.0 (11.4)	81.5 (9.3)	1.6 (0.4)
Group 4 (8y) n = 26	17.8 (1.7)	173.2 (33.8)	91.6 (24.5)	70.0 (13.6)	54.2 (18.8)	167.6 (22.8)	74.2 (14.9)	1.5 (0.4)
Group 5 ( $\geq 8y$ ) n = 19	17.1 (1.7)	168.9 (25.4)	89.0 (19.9)	69.8 (11.1)	51.8 (21.1)	155.8 (14.3)	63.1 (18.3)	1.5 (0.5)
P value for trend	< 0.01	NS	< 0.05	p < 0.05	p < 0.05	NS	p < 0.01	P < 0.05

Values are expressed as mean (SD)

NS : Not significant

**Table 3** Relation between the age at adiposity rebound and the prevalence of small dense LDL

Age at adiposity rebound	prevalence of SDDL
Group 1 ( $\leq 4y$ ) n = 60	9 (15.0%)
Group 2 (5y) n = 74	6 (8.1%)
Group 3 (6y) n = 36	0
Group 4 (7y) n = 26	0
Group 5 ( $\geq 8y$ ) n = 19	0
Cumulative	15/215 (6.9%)

## DISCUSSION

The importance of early infancy for predisposition to later obesity was proposed by Rolland-Cachera<sup>11)</sup>, who coined the term "adiposity rebound" to refer to

the second rise in BMI after birth. The occurrence of AR at a younger age (< 5 years old) is now recognized as an important predictor for increased BMI that tracks from childhood into adulthood<sup>3,10,11)</sup>. Therefore, AR time is considered to be a good indicator for the subsequent development of obesity; i.e., the early AR time suggests that some obesity promoting factors might be operative very early in life. Accumulating data indicate that individuals who exhibit AR at an earlier age are particularly prone towards developing obesity and insulin resistance in later life<sup>22~25)</sup>.

In the present study, children with early AR exhibited high BMI at 12 years of age, indicating that increasing adiposity is associated with early AR. Furthermore, SDDL appeared only in children belonging to groups 1 and 2 (Table 3), in which the timing of AR was  $\leq 5$  years. These two groups also exhibited atherogenic li-

poprotein profiles, with elevated plasma levels of TG and ApoB and decreased plasma levels of HDL-C (Table 2). An atherogenic index, which is considered to reflect a lipoprotein phenotype indicative of insulin resistance<sup>17,21)</sup>, was reduced in children with later AR time (Table 2).

In patients with metabolic syndrome, plasma TG and ApoB levels tend to increase and plasma HDL-C and Apo A1 levels tend to decrease. These alterations in the lipoprotein phenotype are caused by reduced activity of lipoprotein lipase due to insulin resistance<sup>16)</sup>. A specific lipoprotein feature of metabolic syndrome is increased serum concentration of SLDL<sup>26)</sup>. Cholesteryl ester transfer protein (CETP) probably has an important role in the remodeling of larger to smaller LDL particles by mediating TG enrichment of intermediate density lipoprotein and LDL<sup>15,16,22,27)</sup>.

The prevalence of SLDL in children remains obscure but it is estimated to be 4~7% in population-based studies<sup>20,21)</sup>. In the present study the whole prevalence of SLDL in children was 6.9%. An association between early AR and existence of SLDL was demonstrated in the present study. The mechanism by which AR at a younger age would be associated with the existence of SLDL at 12 years of age is obscure. However, the SLDL is likely to be related to the timing of BMI gain rather than to the BMI attained at any particular age, thereby indicating the importance of the dynamic process of rapid BMI gain in younger age. Some metabolic programming leading to future insulin resistance may occur during dynamic increases of BMI; programming can result in alterations in structures and functions of tissues (namely in pancreatic  $\beta$  cell, muscle, adipocytes, and liver)<sup>28~30)</sup>. Another possible interpretation is that if infants with early AR develop a high body mass, they can exhibit a disproportionately higher fat mass in relation to lean body mass, which would lead to insulin resistance through increased plasma fatty acids that could inhibit intracellular signal transduction by insulin. Thus, the processes that regulate early body fat stores may confer increased susceptibility to the development of insulin resistance<sup>25,30)</sup>.

In conclusion, the early AR, which is a dynamic increase in BMI at an earlier age, is thought to be an important indicator of risk for later obesity or metabolic

disease. Early AR during early childhood may lead to an enhanced response to environmental factors, such as overnutrition or reduced physical activity in later childhood, resulting in susceptibility for development of metabolic syndrome. Therefore, we propose that the primary prevention of future metabolic syndrome would be to prevent children from experiencing an increase of BMI during early childhood, especially before 5 years. They should be considered candidates for aggressive interventions to maintain a healthy lifestyle into and throughout adulthood.

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