Original Article

Usefulness of Serum Adiponectin Level as a Diagnostic Marker of Metabolic Syndrome in Obese Japanese Children

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This study aimed 1) to investigate the relationship between serum adiponectin levels and metabolic disorders and 2) to clarify the usefulness of serum adiponectin level as a diagnostic marker of metabolic syndrome in obese Japanese children. One hundred obese boys aged 8 to 13 years were examined. Serum adiponectin levels were measured by radioimmunoassay using a commercial kit. Abdominal fat thickness (maximum preperitoneal fat thickness: $P_{\max}$; minimum subcutaneous fat thickness: $S_{\min}$) was measured by ultrasonography. The relationships between adiponectin and clinical characteristics were analyzed by simple regression. The relationships between anthropometric measurements and metabolic disorders were analyzed among three groups divided according to adiponectin percentile. The prevalence of metabolic syndrome was also analyzed, with metabolic syndrome defined as the presence of three or more complications of obesity. The criteria for metabolic syndrome by adiponectin were subjected to a receiver operating characteristic (ROC) analysis. Body weight, waist circumference, $P_{\max}$, alanine aminotransferase and fasting serum insulin were all inversely correlated with adiponectin. There were significant differences in the prevalence of severe obesity, the accumulation of visceral adipose tissue, hyperinsulinemia, high serum low density lipoprotein-cholesterol, the number of complications of obesity and the prevalence of metabolic syndrome among the three groups. The area under the ROC curve for adiponectin was 0.672 ± 0.055 and the cut-off value was $6.65 \mu\text{g/ml}$. Hypoadiponectinemia was associated with visceral fat accumulation and metabolic syndrome in obese Japanese boys. Evaluation of adiponectin might contribute to an early intervention for obese children with metabolic syndrome. (*Hypertens Res* 2005; 28: 51–57)

Key Words: adiponectin, metabolic syndrome, obesity, children, visceral fat accumulation

Introduction

The prevalence of obesity in Japanese school children has increased threefold in the last three decades (1), and is currently about 10% (2). In children as well as adults, obesity is the major risk factor for hypertension, dislipidemia and atherosclerosis (3, 4). The incidence of type 2 diabetes mellitus in children increased 2.6-fold between the measurement peri-

ods of 1982–1986 and 1992–1996, and approximately 80% of the children with type 2 diabetes mellitus were obese (5). Forty percent of obese children at 7 years of age and 70–80% of obese adolescents are still obese as adults (6). It is important to intercede on behalf of these obese children to prevent adult obesity.

It is well known that obesity is often associated with metabolic syndrome, which is characterized by such complications as hypertension, glucose intolerance, dyslipidemia and hyper-

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Fig. 1. Measurement of abdominal fat thickness. Maximum preperitoneal fat thickness (P_{max}) and minimum subcutaneous fat thickness (S_{min}) were measured directly from the screen with electronic calipers.

insulinemia. In individuals with this syndrome, visceral fat accumulation induces hypertension, dyslipidemia and type 2 diabetes *via* insulin resistance and hyperinsulinemia (7, 8). Recently, adipocytokines such as leptin and adiponectin, which are secreted from adipose tissue, have been suggested to play an important role in metabolic syndrome (9). Adipocytokines mediate obesity-associated metabolic disorder independently of other risk factors for atherosclerosis.

Adiponectin, one of the adipocytokines, is a secreted protein that is expressed specifically and abundantly in adipose tissue (10). In adults, plasma concentrations of adiponectin are lower in the obese state than in the non-obese state, despite the presence of adipose-specific proteins (11), and adiponectin has a strong relation to type 2 diabetes mellitus and coronary artery disease (12, 13). A few studies have investigated the relation between adiponectin and symptoms of metabolic syndrome such as hypertension, visceral fat accumulation, hyperinsulinemia, dyslipidemia and liver dysfunction in obese children (14–17).

The purpose of this study was to investigate the relationship between serum adiponectin levels and the prevalence of metabolic disorders such as hypertension, hyperinsulinemia, liver dysfunction and dyslipidemia in obese boys. Our elucidation of a relationship between serum adiponectin levels and metabolic disorders suggests that serum adiponectin level would be a useful diagnostic marker of metabolic syndrome in obese children.

Methods

Subjects

Subjects of this study consisted of 100 obese boys aged 8 to 13 years. In 2001, all subjects received medical check-ups under the auspices of a program entitled “The Prevention of

Cardio- and Cerebrovascular Diseases in Childhood.” The Department of Pediatrics, Niigata University School of Medicine and the School Health Division of the local governments in Niigata Prefecture undertake this program every year. School nurses recommended the check-up to obese children whose percentage of overweight (POW, a percentage of the standard weight for age, height, and sex) was over 20%. All subjects were simply obese, and their fasting plasma glucose levels were below 110 mg/dl, with hemoglobin A1c (HbA1c) levels below 5.6%. None had endocrine disorders or renal or hereditary diseases. All were nonsmokers and were not taking any regular medication. The body height, body weight, waist circumference, POW, percentage of body fat (PBF), systolic blood pressure (SBP), diastolic blood pressure (DBP), minimum subcutaneous fat thickness (S_{min}), maximum preperitoneal fat thickness (P_{max}), fasting serum adiponectin, fasting serum insulin, alanine aminotransferase (ALT), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), triglyceride (TG), fasting plasma glucose and HbA1c were measured for each boy. Informed consent was obtained from the parents and guardians of all subjects. The Ethical Committee of the Niigata University Graduate School of Medicine and Dental Sciences approved this study.

Anthropometric Measurements

Body height and weight were measured with a portable stadiometer and a digital scale to the nearest 1 mm and 0.1 kg, respectively. POW was calculated by using the standard body weight of Japanese children (18, 19). Blood pressure (BP) was measured three times in the seated position on the right arm using an automated BP recorder (Dinamap Model 8104; Critikon Inc., Tampa, USA). The third measurement was used

Table 1. Clinical Characteristics of the Subjects (n=100)

	Mean±SD	Range
Age (years)	11.0±1.3	8.6–13.5
Body height (cm)	148.1±10.2	125.8–171.3
Body weight (kg)	62.2±15.0	37.2–122.0
Percentage of overweight (%)	+54.2±15.1	+28.8–+110.0
Waist circumference (cm)	88.6±9.8	69.0–121.5
Percentage of body fat (%)	34.8±4.6	23–46
Systolic blood pressure (mmHg)	120.7±11.3	101–154
Diastolic blood pressure (mmHg)	59.3±8.7	41–80
<i>S</i> _{min} (mm)	14.3±4.4	5.9–28.3
<i>P</i> _{max} (mm)	12.7±2.9	5.5–19.4
ALT (IU/l)	51.0±42.8	12–221
Fasting serum HDL-C (mg/dl)	52.2±9.5	32–83
Fasting serum LDL-C (mg/dl)	117.5±24.8	63–185
Fasting serum TG (mg/dl)	116.1±63.5	34–443
Fasting plasma glucose (mg/dl)	87.8±7.0	70–108
Hemoglobin A1c (%)	4.69±0.25	4.2–5.4
Fasting serum insulin (U/ml)	22.3±10.6	7.9–55.6
Fasting serum adiponectin (g/ml)	7.30±2.94	2.4–18.9

*S*_{min}, minimum subcutaneous fat thickness; *P*_{max}, maximum preperitoneal fat thickness; ALT, alanine aminotransferase; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglyceride.

in this study. PBF was measured by the biological impedance method using an RJL Spectrum body composition analyzer (RJL Systems; Detroit, USA). *P*_{max} and *S*_{min} were measured by ultrasonography using ultrasound equipment (TOSHIBA Model SSA-250A; Toshiba Corp., Tokyo, Japan) and a linear-array probe (7.5 MHz). The methods used to measure *P*_{max} and *S*_{min} are shown in Fig. 1 (20). Subjects were kept in the supine position, the linear-array probe was kept perpendicular to the skin on the upper median abdomen, and a longitudinal scan was undertaken from the xyphoid process to the navel along the linea alba. Scanning was performed at the optimal position, and the surface of the liver was kept almost parallel to the skin by having the subjects hold their breath. The probe was touched to the skin as lightly as possible so that the fat layers were not compressed. *P*_{max} and *S*_{min} were determined as the visceral fat thickness of the hepatic falciform membrane, and the subcutaneous fat thickness of the upper abdomen, respectively. *P*_{max} and *S*_{min} were measured directly from the screen with electronic calipers. *P*_{max} and *S*_{min} were both related with visceral fat accumulation and subcutaneous fat accumulation in the upper abdomen as reported in a previous study (21).

Assays

Fasting blood samples were obtained from the antecubital vein of the subjects in the morning and were centrifuged at 3,000 rpm for 10 min. The serum was frozen and stored at

–20°C until analysis. ALT, HDL-C, LDL-C, TG, fasting plasma glucose and HbA1c were measured using an automatic analyzer. Serum insulin levels were measured by a commercial radioimmunoassay using a commercial kit (LS ‘Eiken’ Insulin; Eiken Chemical Co., Ltd., Tokyo, Japan). Serum adiponectin levels were measured by radioimmunoassay using a commercial kit (Human Adiponectin ELISA Kit; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). The intraassay coefficients of variation were 3.95% and <10% for insulin and adiponectin, respectively.

Statistical Analysis

First, we analyzed the relationship between serum adiponectin levels and clinical characteristics by simple regression.

Second, we divided the subjects into three groups according to their serum adiponectin percentile. Nonparametric Kruskal-Wallis one-way analysis of variance (ANOVA) was then used to analyze differences in age, anthropometric measurements, and numbers of complications of obesity among the three groups. Post hoc comparisons between pairs of means were made using Wilcoxon’s rank-sum test. In this study, the complications of obesity consisted of hypertension (according to the guidelines for hypertension in children) (22), liver dysfunction (ALT level > 30 IU/l), hyperinsulinemia (fasting serum insulin level ≥ 15 U/ml), high serum LDL-C (serum LDL-C level ≥ 140 mg/dl), hypertriglyceridemia (fasting serum TG level ≥ 120 mg/dl) and low serum HDL-C (serum HDL-C level < 40 mg/dl). The criteria of each complication of obesity were decided according to the definition of “obesity disease” in Japanese children by the Committee for Research on the Appropriate Body Build in Children (23). Comparisons of the prevalence of severe obesity (POW ≥ 50%), accumulation of visceral adipose tissue (waist circumference ≥ 80 cm), acanthosis nigricans and each complication of obesity were analyzed among the three groups using χ^2 test. In this study, the subjects with three or more complications of obesity were defined as having metabolic syndrome, and the prevalence of metabolic syndrome was also analyzed among the three groups using χ^2 test.

The above statistical evaluations were performed using the Stat View program for Windows (version 5.0; Abacus Concepts, Berkeley, USA). For all analyses, probability (*p*) values below 0.05 were considered to indicate statistical significance.

The receiver operating characteristic (ROC) analysis of the criteria for metabolic syndrome by serum adiponectin level was performed using SPSS version 11.0 J software (SPSS Inc., Chicago, USA).

Results

Clinical characteristics of all subjects in this study are shown in Table 1. The mean POW of all subjects was 54.2%, and the mean serum adiponectin level was 7.30 g/ml.

Table 2. Simple Regression Analysis between Serum Adiponectin Levels and Clinical Characteristics

	<i>r</i>	<i>p</i>
Age (years)		NS
Body height (cm)		NS
Body weight (kg)	-0.200	<0.05
Percentage of overweight (%)		NS
Waist circumference (cm)	-0.243	<0.05
Percentage of body fat (%)		NS
Systolic blood pressure (mmHg)		NS
Diastolic blood pressure (mmHg)		NS
<i>S</i> _{min} (mm)		NS
<i>P</i> _{max} (mm)	-0.220	<0.05
ALT (IU/l)	-0.218	<0.05
Fasting serum HDL-C (mg/dl)		NS
Fasting serum LDL-C (mg/dl)		NS
Fasting serum TG (mg/dl)		NS
Fasting plasma glucose (mg/dl)		NS
Hemoglobin A1c (%)		NS
Fasting serum insulin (U/ml)	-0.374	<0.01

*S*_{min}, minimum subcutaneous fat thickness; *P*_{max}, maximum preperitoneal fat thickness; ALT, alanine aminotransferase; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TG, triglyceride; NS, not statistically significant.

The results of simple regression analysis between serum adiponectin levels and clinical characteristics are presented in Table 2. Body weight, waist circumference, *P*_{max}, ALT and fasting serum insulin showed negative correlations with the serum levels of adiponectin. Fasting serum insulin was significantly more related to serum adiponectin levels than any other parameter ($p < 0.01$, $r = -0.374$).

Table 3 shows the relationships of anthropometric measurements, prevalence of severe obesity, prevalence of accumulation of visceral adipose tissue, prevalence of acanthosis nigricans, prevalence of each complication of obesity, the number of complications of obesity and prevalence of metabolic syndrome among the three groups divided according to their serum adiponectin percentile. The mean adiponectin levels were as follows: for group 1 (adiponectin level cut points >8.1 g/ml), 10.6 g/ml; for group 2 (5.8 to 8.0 g/ml), 6.8 g/ml; and for group 3 (<5.7 g/ml), 4.6 g/ml. There was no significant difference in age among the three groups.

There were significant differences in prevalence of severe obesity, accumulation of visceral adipose tissue, hyperinsulinemia and high serum LDL-C among the three groups. There were also significant differences in the number of complications of obesity and the prevalence of the metabolic syndrome among the three groups.

Figure 2 depicts the ROC curve for the criteria of metabolic syndrome according to the serum adiponectin level. The area under the ROC curve for serum adiponectin level was

0.672 ± 0.055 . The cut-off value was 6.65 g/ml, the sensitivity was 63.9%, and the specificity was 66.7%.

Discussion

Serum adiponectin levels were inversely correlated with waist circumference and *P*_{max} by simple regression in these obese boys. On the other hand, serum adiponectin levels were not correlated with POW, PBF or *S*_{min}. Waist circumference and *P*_{max} were related with visceral fat accumulation, and *S*_{min} was related with subcutaneous fat accumulation as reported in previous studies (21, 24). Nishina *et al.* revealed a significant relationship between visceral fat accumulation and insulin in obese Japanese children (25). Serum adiponectin levels had a closer association with waist circumference than *P*_{max} in our study. Kikuchi *et al.* reported that waist circumference was more useful than *P*_{max} in a screening of metabolic syndrome in obese children (26). The results of our study suggest that serum adiponectin levels are inversely correlated with visceral fat accumulation, and are less associated with subcutaneous fat accumulation in obese boys. There have been only a few epidemiological studies investigating the relation between adiponectin and visceral fat accumulation in children. Asayama *et al.* reported that serum adiponectin level was lower in obese children than in non-obese children (6.4 ± 0.6 vs. 10.2 ± 0.8 mg/l), and serum adiponectin levels were inversely correlated with the visceral adipose tissue area, which was measured by computed tomography (14). In a study by Atzmon *et al.*, although both visceral fat and subcutaneous fat secreted adiponectin in rats, the expression of adiponectin in visceral fat was about 4-fold higher than that in subcutaneous fat (27). And Milan *et al.* showed that adiponectin expression was significantly lower in the visceral adipose tissue of genetically obese rats than lean rats, but no differences were observed when subcutaneous adipose tissues of the same animals were compared (28). Together, these results indicate that serum adiponectin levels may become a useful marker of visceral fat accumulation, which is closely related to metabolic syndrome in obese children.

Serum adiponectin levels were inversely correlated with serum insulin levels by simple regression in these obese boys. In a study by Weyer *et al.*, serum adiponectin levels were more closely associated with insulin resistance by two-step hyperinsulinemic euglycemic clamp than with PBF (29). In another study, high concentrations of adiponectin were associated with a substantially reduced relative risk of type 2 diabetes after adjustment for age, sex, waist-to-hip ratio, body-mass index, smoking, exercise, alcohol consumption, education and HbA1c in adults (30). Hotta *et al.* reported that serum adiponectin levels declined in the early phase of obesity and remained decreased after the development of type 2 diabetes in rhesus monkeys (31). On the other hand, Mohlig *et al.* reported that hyperinsulinemia caused a significant decrease of serum adiponectin levels under euglycemic conditions in humans (32).

Table 3. Comparison of Anthropometric Measurements and Metabolic Disorders among Three Groups Divided According to Adiponectin Percentile

	Group 1 (n=32)	Group 2 (n=35)	Group 3 (n=33)	p
The mean adiponectin level (g/ml)	10.6	6.8	4.6	
Age (years)	10.8±1.3	11.0±1.4	11.2±1.3	NS
Body height (cm)	145.9±10.8	148.4±9.2	149.8±10.5	NS
Body weight (kg)	57.9±12.5	63.0±16.1	65.6±15.2	NS
Percentage of overweight (%)	+49.8±11.7	+55.0±17.5	+57.6±14.8	<0.05
Waist circumference (cm)	85.2±9.2	89.4±10.1	91.0±9.3	NS
Percentage of body fat (%)	35.5±4.2	35.4±4.4	33.4±4.9	NS
The number of complications of obesity	1.5±1.2 ^{#,*}	2.3±1.1	2.4±1.0	<0.01
Prevalence of each anthropometric and metabolic disorder				
Severe obesity (%)	37.5	54.3	72.7	<0.05
Accumulation of visceral adipose tissue (%)	65.6	88.6	93.9	<0.01
Acanthosis nigricans (%)	31.3	48.6	54.5	NS
Hypertension (%)	15.6	8.6	12.1	NS
Liver dysfunction (%)	43.8	65.7	66.7	NS
Hyperinsulinemia (%)	53.1	82.9	87.9	<0.01
Low serum HDL-C (%)	3.1	11.4	0.0	NS
High serum LDL-C (%)	6.3	17.1	33.3	<0.05
Hypertriglyceridemia (%)	31.3	40.0	39.4	NS
Metabolic syndrome (%)	18.8	40.0	57.6	<0.01

Data are mean±SD and prevalence (%). Subjects are divided into three groups according to adiponectin percentile. Adiponectin cut points are >8.1 g/ml (group 1), 5.8 to 8.0 g/ml (group 2), <5.7 g/ml (group 3). HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; NS, not statistically significant. Severe obesity: percentage of overweight≥50%; accumulation of visceral adipose tissue: waist circumference≥80 cm; hypertension: school children (grades 1–3), systolic blood pressure≥130 mmHg, and/or diastolic blood pressure≥80 mmHg; school children (grades 4–6), systolic blood pressure≥135 mmHg, and/or diastolic blood pressure≥80 mmHg; liver dysfunction: ALT level>30 IU/l; hyperinsulinemia: fasting serum insulin level≥15 U/ml; low serum HDL-C; serum HDL-C level<40 mg/dl; high serum LDL-C: serum LDL-C level≥140 mg/dl; hypertriglyceridemia: fasting serum TG level≥120 mg/dl; metabolic syndrome: three or more complications of obesity. Comparison of the mean among three groups are using Kruskal-Wallis one-way analysis of variance. Comparison of prevalence among three groups are using χ^2 test. [#] $p<0.05$ vs. group 2, * $p<0.05$ vs. group 3 using Wilcoxon's rank-sum test.

Fig. 2. Receiver operating characteristic curve for the criteria of metabolic syndrome by serum adiponectin level.

Adiponectin has been shown to directly protect against the development of insulin resistance and subsequent atherosclerosis in animal studies using mice (33–35). In adults, hypoadiponectinemia is also associated with atherosclerosis and

hypertension (36). However, there have been few studies investigating the relation between adiponectin and hypertension in children. In the present study, serum adiponectin levels were not correlated with SBP or DBP in obese boys. Further studies on the relationship between adiponectin and childhood hypertension will be needed.

In adults, epidemiological studies have shown that serum adiponectin levels were negatively correlated with various indices of metabolic syndrome, such as hypertension, insulin resistance, glucose intolerance, dyslipidemia and hyperuricemia (37, 38). A few previous studies in children have also reported that serum adiponectin levels were inversely correlated with hyperinsulinemia, insulin resistance, dyslipidemia, liver dysfunction and hyperuricemia (14–17). In the present study, serum adiponectin levels were considered to be associated with the number of complications of obesity and the prevalence of metabolic syndrome with three or more complications of obesity in obese boys. These findings are consistent with previous reports of linkage between adiponectin and metabolic syndrome. Hypoadiponectinemia is thus strongly associated with metabolic syndrome in children.

In conclusion, our study investigated the relationship among adiponectin, visceral fat accumulation, BP, insulin, serum lipids, ALT and metabolic syndrome in obese boys. In addition, hypoadiponectinemia in obese boys was associated with accumulation of visceral adipose tissue, hyperinsulinemia and metabolic syndrome. It is necessary to intercede on behalf of obese children with metabolic syndrome, because these children will develop atherosclerosis and type 2 diabetes mellitus as adults. Evaluation of serum adiponectin levels might contribute to an early intervention for obese children with metabolic syndrome.

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