Research Papers

Relationship between Insulin Resistance and Serum Levels of Adiponectin and Resistin with Childhood Obesity

Chao Chun Zou, Li Liang and Fang Hong

From the Children's Hospital of Zhejiang University School of Medicine, 57 Zhugan Xiang, Hangzhou 310 003, China.

Correspondence to: Dr. Li Liang, The Children's Hospital of Zhejiang University School of Medicine, 57 Zhugan Xiang, Hangzhou 310 003, China. E-mail: zou108cc@yahoo.com

Objective: *The aim is to study the relationship between insulin resistance and serum adiponectin and resistin in obese children.* ***Methods***: *A total 113 obese and 37 nonobese children were enrolled and serum adiponectin and resistin were measured.* ***Results***: *Compared with controls, higher insulin resistance by homeostasis model (HOMA-IR) and lower whole body insulin resistance index (WBISI) were found in obese children (all P <0.05). The adiponectin levels in obese children and controls were 3.63 and 5.79 μg/L with a significant difference (P<0.001) while the difference of resistin levels was not significant (P = 0.948). Significant correlations between insulin resistance parameter and age, sexual development, BMI, serum triglyceride, ApoB, LDL-cholesterol, HDL-cholesterol, alanine aminotransferase, uric acid, or adiponectin levels (all P <0.05) were noted. Stepwise multiple regression analysis showed that BMI and adiponectin levels were independent determinants of WBISI.* ***Conclusions***: *These results suggest that adiponectin may play a protective role in obese children through decreasing insulin resistance.*

Key words: *Adiponectin, Child, Insulin resistance, Obesity, Resistin.*

INSULIN resistance (IR) with consequent hyperinsulinemia has a central role in the pathogenesis of many diseases including obesity, diabetes mellitus, hypertension and dyslipidemia. Recent investigations have been focused on a family of adipose derived cellular mediators (adipocytokines), including TNF-α, IL-6 and leptin. The importance of these agents is that they are produced by fat cells and are known to play a key role in the complex interorgan communication network, which appears to modulate intermediate metabolism and energy balance(1-3). Recently, another two adipocytokines (adiponectin and resistin) are described as secretory products of adipose tissue. Some animal model and several human studies suggested that resistin were related to obesity and glucose metabolism while some studies in human did not find such relationship(4-7). Some previous studies have investigated the role of adiponectin in obesity(7-9), but rare in insulin resistance. Here, we measured serum adiponectin and resistin levels in obese and nonobese children to study their role in obesity, especially in the mechanism of insulin resistance.

Subjects and Methods

A total of 113 obese children from our unit were enrolled in this study from January 1 2005 to November 31 2005. The criteria for obese diagnosis was defined that the body mass index (BMI) was over 95% percentile of the same gender and age. They included 35 girls and 78 boys aged from 6.0 to 16.0 years (mean 11.1 ± 2.3 years). In obese group, 55 cases were prepuberal (Tanner I) and 58 were puberal (51 of Tanner II and 7 of Tanner III) and the mean BMI was 27.71 ± 3.88 kg/m^2. Hyperlipemia in 57 cases (50.44%), nonalcoholic fatty liver disease (NAFLD) in 63 cases (55.75%), impaired

glucose tolerance (IGT) in 11 cases (9.73%), hypertension in 31 cases (27.43%) were noted in obese group. The control group consisted of 37 nonobese children without other endocrine, metabolic or kidney disease, including 17 boys and 20 girls aged from 7.3 to 14.9 years (mean 10.3 ± 1.8 years). In controls, 25 cases were prepuberal (Tanner I) and 12 were puberal (11 of Tanner II and 1 of Tanner III) with a mean BMI of 15.52 ± 1.53 kg/m^2.

Blood samples were obtained for adiponectin, resistin, and other biochemical measurements in the morning after an overnight fasting. All sera were stored frozen at -80°C until measurement. The sera were diluted by 100 or 5-fold for adiponectin or resistin measurement before assay. Serum levels of adiponectin and resistin were assayed by an enzyme linked immunosorbent assay (ELISA). Quantikine kits purchased from R&D Systems Inc (USA) were used to detect serum adiponectin and resistin according to the manufacturer's protocol. Negative and positive controls were used. The intra-and interassay CVs of adiponectin were ranged from 2.5 to 4.7% and 5.8 to 6.9% respectively. The intra- and interassay CVs of resistin were ranged from 3.8 to 5.3% and 7.8 to 9.2% respectively. The assay sensitivities were ranged from 0.079-0.891μg/L (0.246 μg/L) for adiponectin and 0.010-0.055 μg/L (0.026 μg/L) for resistin respectively.

Samples for fasting glucose and insulin (FG, FI) after an overnight fasting were obtained. Plasma glucose levels were detected by glucose oxidase method (Beijing North Biotechnology Invest, China) with intra-assay and interassay CVs of 2.1% and 4.4%. Insulin serum levels were determined by radioimmunoassay (Beijing North Biotechnology Invest, China). The intra-assay and interassay CVs were 6.4% and 9.7% respectively. Insulin resistance and ß cell function index were calculated as follow: insulin resistance by homeostasis model (HOMA-IR) = FI $\times$ FG/22.5(10), whole body insulin resistance index (WBISI) = 10000/[FI (mLU/L) $\times$ FG (mg/dL) $\times$ mean insulin (mIU/L) $\times$ mean glucose (mg/dL)]1/2 [11]. Serum triglyceride (TG), alanine aminotransferase (ALT), and uric acid were measured in the clinical laboratories of our unit. Plasma apolipoprotein (Apo) A1, ApoB, high-density lipoproteins-cholesterol (HDL-C) and low-density lipoproteins-cholesterol (LDL-C) levels were detected as well.

Statistical analyses were conducted by using SPSS software (10.0). Pearson Chi-square was used to measure the enumeration data between subgroups. Quantitative data were presented as mean $\pm$ SD. Because the data for adiponectin, resistin, TG, ALT and insulin, HOMA-IR and WBISI were skewed, they were presented as median (mix-max). The statistical significance was estimated by unpaired Student's t test or two independent nonparametric tests (Mann-Whitney U method). Bivariate correlation analysis for insulin resistance parameter (WBISI, HOMA-IR) and other factors (including adiponectin and resistin) were done using Spearman correlation. Stepwise multiple linear regression models were used to examine the determinant of WBISI after these skewed parameters were transformed logarithmically. A 2-tailed P <0.05 was considered significant.

Results

The characteristics of controls and obese children were shown in *Table I*. Compared with controls, higher HOMA-IR and lower WBISI were found in obese children (all P <0.001). Moreover, the adiponectin level in obese children was 3.63 μg/L, which was significantly lower than that of controls (5.79 μg/L, P <0.001). However, the serum resistin levels were not significantly different between obese children and controls (P = 0.948).

When analyzing the correlation between the insulin resistance parameter (WBISI, HOMA-IR) and other factors, significant correlations between insulin resistance parameter and age, stage of sexual development, BMI, TG, ApoB, LDL-C, HDL-C, ALT, uric acid, or adiponectin levels (all P <0.05) were found. However, no significant correlation was found between insulin resistance parameter and gender (treated female as 0, male as 1), ApoA1 or resistin (all P >0.05), as shown in *Table II*.

Stepwise multiple regression analysis for WBISI (log-transformed) included all factors which P <0.1 in Spearman correlation was performed. We found that BMI and log-transformed adiponectin levels [log(adiponectin)] were the independent

TABLE I–*Characteristics of Control and Obese Groups*

	Controls (n = 37)	Obese group (n = 113)	Z value	P value
HOMA-IR	1.26 (0.77-2.52)	2.07 (0.83-11.71)	5.743	<0.001
WBISI	5.59 (2.02-12.61)	1.68 (0.05-9.65)	7.478	<0.001
Adiponectin (µg/L)	5.79 (2.25-13.15)	3.63 (1.43-9.42)	4.700	<0.001
Resistin (µg/L)	4.25 (2.06-10.25)	4.18 (1.43-10.38)	0.065	0.948

HOMA-IR: insulin resistance by homeostasis model; WBISI: whole body insulin resistance index.

TABLE II–*Spearman Correlation between Insulin Resistance and Other Factors*

	HOMA-IR		WBISI	
	r	P value	r	P value
Gender	–0.019	0.814	0.028	0.738
Age	0.290	<0.001	–0.375	<0.001
Sexual development	0.232	0.004	–0.302	<0.001
BMI	0.505	<0.001	–0.648	<0.001
TG	0.282	0.001	–0.317	<0.001
HDL-C	–0.270	0.001	0.294	<0.001
LDL-C	0.164	0.047	–0.196	0.017
ApoA1	0.016	0.848	–0.088	0.289
ApoB	0.199	0.015	–0.255	0.002
ALT	0.288	<0.001	–0.359	<0.001
Uric acid	0.378	<0.001	–0.456	<0.001
Adiponectin	–0.247	0.002	0.392	<0.001
Resistin	–0.123	0.275	0.078	0.489

HOMA-IR: insulin resistance by homeostasis model; WBISI: whole body insulin resistance index; BMI: body mass index; TG: triglyceride; HDL-C: high-density lipoproteins-cholesterol; LDL-C: low-density lipoproteins-cholesterol; ALT: alanine aminotransferase; Apo A1: apolipoprotein A1; ApoB: apolipoprotein B.

determinants of WBISI in children, which explained 46% of the variance. log(WBISI) was inversely associated with BMI (P <0.001) and positively associated with log (adiponectin) levels (P <0.001). Age, stage of sexual development, log (TG), log(ALT), HDL-C, LDL-C, ApoB, uric acid were excluded in the equations (all P >0.05), as shown in *Table III*.

Discussion

Obesity and insulin resistance have been recognized as leading causes of major health issues, particularly diabetes type 2 and metabolic syndrome. Although obesity, defined as excess body fat, is frequently accompanied by insulin resistance, diabetes, metabolic syndrome and cardiovascular disease, the molecular basis for the link between obesity and those diseases has not yet been clarified. Adipose tissue expresses various secretory proteins, including leptin, TNF-α, and adiponectin, which may be involved in the regulation of energy expenditure, lipid metabolism and insulin resistance.

In this study, we noted lower adiponectin level in obese children, which is consistent with previous studies(7,8). Animal model and some human studies showed resistin may be associated with diet-

TABLE III–*Regression Analysis of WBISI (log-transformed)*

	Beta	SE	t	P value	R^2 (adjusted)
Constant	6.745	1.132	6.378	<0.001	
BMI	-0.214	0.033	−6.364	<0.001	
log(Adiponectin)	0.032	0.013	3.573	<0.001	0.453 (0.431)

BMI: body mass index.

induced obese(4,5). However, the resistin in obese children in this study was not different from that of controls. This is similar with previous reports and suggests adiponectin, not resistin plays role in obesity(6-7). It is still unclear that why adiponectin levels decreased with increased adipose tissue mass. However, it is clear that low levels of adiponectin decrease fatty acid oxidation and higher fatty acid is associated with insulin resistance(12-13). Recent data have demonstrated that adiponectin effects are mediated by the interaction with muscle and hepatic receptors through activation of AMP kinase, the cellular "fuel gauge", which in turn inhibits acetyl CoA carboxylase and increases fatty acid beta-oxidation(13-14).

Although many studies have been done about the insulin resistance in obese, the mechanism is not clear. As like some other studies, insulin resistance correlated with BMI, TG, ApoB, ALT and uric acid (9). We here seek to describe the relation between insulin resistance and the two serum adipocytokines (adiponectin and resistin) in obese children. We found that serum adiponectin levels were positively and significantly correlated with WBISI. This suggested that adiponectin, as an adipocytokine, might play a role in the pathophysiology of insulin resistance and obesity.

Recent studies found that circulating adiponectin is predominantly present as several characteristic multimers, including low molecular weight adiponectin (LMW form), middle molecular weight adiponectin (MMW form) and high molecular weight adiponectin (HMW form)(15-16). More-over, different forms of adiponectin might activate different signal transduction pathways and exert distinct functions on its target tissue. HMW complex is the most active form of adiponectin in depressing blood glucose levels(17-

20). In the present study, although low correlation between WBISI and serum adiponectin was found, whether different ratio of the different forms which might exist have an influence on insulin resistance is still unknown, hence further study is required.

In summary, our data that adiponectin correlated with insulin resistance support that adiponectin may play a protective role in obese children. Since our samples are small and association studies are susceptible to various biases, confirmatory studies using larger population are needed to prove these results.

Acknowledgments

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What this Study Adds

- Obese children have increased insulin resistance and lower serum adiponectin levels.
- Serum adiponectin levels are the independent determinant of WBISI in children.

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