Evaluation of adipocytokines in obese children with insulin resistance

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Obesity and overweight are among the most serious health problems in western societies and an increasing problem in developing countries. Recent studies indicate an important role of adipose tissue hormones, or "adipokines", in obesity-associated complications. To investigate the relation of two circulating adipokines (visfatin, adiponectin) with markers of insulin sensitivity and obesity in children, 40 obese children and 40 control children were recruited. Homeostasis model assessment for insulin resistance (HOMA-IR) and visfatin levels (4.99 ± 2.08 vs. 1.47 vs. 0.7, p<0.001; 31.3 ± 11.1 vs. 18.5 ± 10.7, p<0.001, respectively) were significantly elevated and adiponectin levels (2.01 ± 1.02 vs. 12.5 ± 6.2, p<0.001) were significantly lower in the obese group. Comparisons of the clinical and metabolic characteristics between insulin-resistant and noninsulin-resistant groups in obese children are summarized. The insulin-resistant group had higher visfatin levels (36 ± 9.7 vs. 22.9 ± 7.6, p<0.001) and lower adiponectin levels (1.7 ± 1.05 vs. 2.5 ± 0.77, p: 0.016). Visfatin was correlated positively and adiponectin was correlated negatively with body mass index standard deviation score (BMI-SDS) and HOMA-IR. The role of various adipokines as connectors between obesity and diabetes mellitus has been better elucidated in recent years. Based on the findings of this study, visfatin and adiponectin levels can be used as specific markers for insulin sensitivity.

Key words: visfatin, adiponectin, insulin resistance.

Obesity and overweight are among the most serious health problems in western societies and an increasing problem in developing countries[1]. Although genetic factors contribute to the pathogenesis of obesity, there is no doubt that in modern societies, obesity is accounted for mainly by excessive food intake and inadequate physical activity. Overweight and obesity are associated with increased risk of major cardiovascular diseases (arterial hypertension, atherosclerosis, ischemic heart disease, and heart failure), metabolic abnormalities (hyperlipidemia, diabetes mellitus, hyperuricemia), arterial and venous thrombosis, gallstone disease, and certain forms of cancer[2].

In particular, obesity is usually associated with at least some components of the metabolic syndrome. Initially, it was suggested that most of the abnormalities observed in the metabolic syndrome result from insulin resistance and/or hyperinsulinemia[3]. However, more recent studies indicate an important role of adipose tissue hormones, or "adipokines", in obesity-associated complications. Adiponectin is exclusively expressed and secreted by the adipose tissue and is involved in glucose and lipid metabolism. Hypoadiponectinemia has been shown to be associated with insulin resistance in animal and human studies[4]. Plasma adiponectin levels are decreased in subjects with obesity and insulin resistance or type 2 diabetes mellitus, and are inversely correlated with visfatin and fasting insulin levels[5,6]. Both tissue expression and plasma levels of visfatin increase in parallel with

obesity. It has insulin-mimetic effects and lowers plasma glucose levels[7].

We hypothesized that the two circulating adipokines mentioned above are linked to markers of insulin sensitivity and obesity in children.

Material and Methods

Patients

Forty children [obese group: 20 girls and 20 boys, mean age: 10.91 ± 2.65, mean body mass index standard deviation score (BMI-SDS): 2.31 ± 0.14] were recruited from among the children who attended the outpatient clinic of the Department of Pediatric Endocrinology for obesity between 2007 and 2008. Control subjects (20 girls and 20 boys, mean age: 11 ± 3 years, mean BMI-SDS: -0.04 ± 0.8) were enrolled in the study through healthy children. Children were excluded if they had a prior major illness, including type 1 or type 2 diabetes, took medications, or had a condition known to influence body composition, insulin action, or insulin secretion (e.g. glucocorticoid therapy, hypothyroidism, Cushing's disease). All subjects were in good health and had normal thyroid function. Patients with secondary obesity syndromes and acute illnesses were excluded from the study. Each child underwent a complete physical examination, including anthropometric measures. Height and weight were measured with an empty bladder in postabsorptive conditions. Height was measured to the nearest 0.5 cm on a standard height board, and weight was determined to the nearest 0.1 kg on a standard physician's beam scale with the subject dressed only in light underwear and no shoes. BMI was calculated as weight (in kilograms) divided by height (in meters squared). The degree of obesity was quantified using Cole's least mean square method, which normalizes BMI skewed distribution and expresses BMI-SDS. This measure gives age- and sex-specific estimates of the distribution median, the coefficient of variation, and the degree of skewness by a maximum-likelihood fitting technique[8]. The study protocols were approved by the institutional review board of GATA Medical Faculty Ethical Committee. Signed informed consent forms were obtained from the parents of the children.

Blood Samples

Venous blood samples were obtained to measure plasma glucose and insulin levels in the morning at 08:00 a.m. by venipuncture after an overnight fasting. After clotting, the serum was separated and immediately explored for analyses. Plasma glucose was determined by the glucose oxidase method. Plasma insulin was measured using IMMULITE immunoassay (IMMULITE Diagnostic Products Corporation, Los Angeles, CA). Plasma concentrations of total cholesterol, triglycerides and high-density lipoprotein-cholesterol (HDL-C) were measured using routine enzymatic methods with Olympus 2700 Analyzer. Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald formula. Plasma adiponectin levels were determined by radioimmunoassay (Linco Research, St. Charles, MO). Determination of visfatin levels was performed by enzyme immunoassay (visfatin C-terminal [human] EIA; Phoenix Pharmaceuticals, Belmont, CA).

Insulin Sensitivity Indices

Insulin resistance was estimated using the homeostasis model assessment for insulin resistance (HOMA-IR; fasting insulin X fasting glucose/22.5)[9]. Insulin resistance in children is defined as HOMA-IR levels greater than 3.16[10].

Statistical Analysis

Data were expressed as mean ± SD. Differences in the means of variables were tested using both parametric and non-parametric tests depending on the distribution of the variables. Correlation analyses were conducted using Spearman or Pearson correlation coefficients depending once again on the distribution of the variables. A probability value of less than 0.05 was considered significant. SPSS version 17 (SPSS, Chicago, IL) was used for analysis.

Results

The characteristics of the 40 obese adolescents and 40 control subjects are summarized in Table I. The obese and control groups showed no significant difference in terms of age, total cholesterol and LDL-C. Subjects in the obese group had significantly higher BMI-SDS than control subjects (2.31 ± 0.14 vs. -0.04 ± 0.83,

Table I. Clinical and Laboratory Characteristics of the Study Population

	Obese children	Controls	p
N (F/M)	20/20	20/20	
Age (years)	10.91 ±2.65	11.02±3	0.8
BMI-SDS	2.31±0.14	-0.04 ±0.83	<0.001
Total cholesterol (mg/dl)	175±36	166±25	0.17
Triglycerides (mg/dl)	129±70	82±41	0.001
HDL-cholesterol (mg/dl)	45±9	55±13	<0.001
LDL-cholesterol (mg/dl)	105±32	93±24	0.059
HOMA-IR	4.99±2.08	1.47±0.7	<0.001
Adiponectin (μg/ml)	2.01±1.02	12.5±6.2	<0.001
Visfatin (ng/ml)	31.3±11.1	18.5±10.7	<0.001

Data are given as mean±SD; difference at p<0.05 level.

BMI-SDS: Body mass index-standard deviation score. **HDL:** High-density lipoprotein. **LDL:** Low-density lipoprotein. **HOMA-IR:** Homeostasis model assessment for insulin resistance (fasting insulin (μU/mL) X fasting glucose (mg/dl)/22.5).

p<0.001). Triglyceride levels (129 ± 70 vs. 82 ± 41 mg/dl, p: 0.001) were significantly elevated and HDL-C levels (45± 9 vs. 55 ± 13) were significantly lower compared to the obese group. HOMA-IR and visfatin levels (4.99 ± 2.08 vs. 1.47 vs. 0.7, p<0.001 and 31.3 ± 11.1 vs. 18.5± 10.7 ng/ml, p<0.001, respectively) were significantly elevated and adiponectin levels (2.01 ± 1.02 vs. 12.5 ± 6.2 μg/ml, p<0.001) were significantly lower in the obese group (Table I).

Comparisons of clinical and metabolic characteristics between the insulin-resistant and noninsulin-resistant groups in obese children are summarized in Table II. The insulin-resistant group had higher visfatin levels (36 ±9.7 vs. 22.9 ±7.6 ng/ml, p<0.001) and lower adiponectin levels (1.7 ± 1.05 vs. 2.5 ±0.77 μg/ml, p: 0.016). There was no significant difference in lipid profiles between the two groups (Table II).

Table III shows the correlation of adipocytokines and lipids with BMI-SDS and HOMA-IR. Visfatin was positive correlated with BMI-SDS and HOMA-IR (r: 0.61, p<0.001 and r: 0.63, p<0.001) and adiponectin was negative correlated with BMI-SDS and HOMA-IR (r: -0.46, p: 0.002 and r:-0.44, p: 0.004). There was no correlation in lipid profiles with BMI-SDS and HOMA-IR (Table III).

Discussion

Studies performed during the last decade indicate that adipose tissue is not only a site of triglyceride storage, but also a source of multiple biologically active mediators, including leptin, tumor necrosis factor-α, adiponectin,

Table II. Comparisons of Clinical and Metabolic Characteristics Between Insulin-Resistant and Noninsulin-Resistant Groups in Obese Children

	OBESE SUBJECTS		
	Insulin-resistant	Noninsulin- resistant	p
N (F/M)	11/14	6/9	
Age (years)	11.18±2.31	10.46 ±3.18	0.41
BMI-SDS	2.33±0.14	2.27±0.12	0.18
Total cholesterol (mg/dl)	171±28	183±48	0.34
Triglycerides (mg/dl)	139±75	114±61	0.29
HDL-cholesterol (mg/dl)	44±11	47±6.7	0.31
LDL-cholesterol (mg/dl)	101±24	112±43	0.31
Adiponectin (μg/ml)	1.7±1.05	2.5±0.77	0.016
Visfatin (ng/ml)	36±9.7	22.9±7.6	<0.001

Data are given as mean±SD; difference at p<0.05 level.

BMI-SDS: Body mass index-standard deviation score. **HDL:** High-density lipoprotein. **LDL:** Low-density lipoprotein.

Table III. Correlation of Adipocytokines and Lipids with BMI-SDS and HOMA-IR

		BMI-SDS		HOMA-IR	
		r	p	r	P
LIPIDS					
	Total cholesterol (mg/dl)	-0.69	0.67	-0.09	0.54
	Triglycerides (mg/dl)	0.06	0.71	0.18	0.26
	HDL-cholesterol (mg/dl)	-0.14	0.36	-0.13	0.4
	LDL-cholesterol (mg/dl)	-0.06	0.69	-0.11	0.48
ADIPOCYTOKINES					
	Adiponectin (μg/ml)	-0.46	0.002	-0.44	0.004
	Visfatin (ng/ml)	0.61	<0.001	0.63	<0.001

Data are given as mean±SD; difference at $p<0.05$ level.
BMI-SDS: Body mass index-standard deviation score. **HDL:** High-density lipoprotein. **LDL:** Low-density lipoprotein. **HOMA-IR:** Homeostasis model assessment for insulin resistance (fasting insulin (μU/mL) X fasting glucose (mg/dl)/22.5).

apelin, visfatin, vaspin, acylation-stimulating protein, resistin, interleukin-6, plasminogen activator inhibitor-1, and transforming growth factor-β[11,12]. They modulate insulin sensitivity and are new therapeutic targets in metabolic syndrome. Adipokines are an exciting new link between obesity and insulin resistance but also obesity and cardiovascular disease, hypertension, as well as hyperlipidemia[13]. In the present study, we found significantly higher visfatin and HOMA-IR levels and lower adiponectin levels in the obese group than control group. Hypoadiponectinemia has been shown to be associated with insulin resistance in animal and human studies[4]. Plasma adiponectin levels are decreased in subjects with obesity and insulin resistance or type 2 diabetes mellitus, and are inversely correlated with visfatin and fasting insulin levels[5,6]. Berndt et al.[14] showed that plasma visfatin correlates significantly with percent body fat, BMI and visfatin mRNA level in visceral adipose tissue, but not with visceral fat mass or waist-to-hip ratio, and no relationship was observed between plasma visfatin and fasting plasma insulin, fasting glucose and insulin sensitivity in nondiabetic subjects. In two recent studies[15,16], plasma visfatin was higher in patients with type 2 diabetes mellitus than in normoglycemic controls. However, it was unclear if the higher visfatin level was associated with the diabetes itself or with the greater amount of visceral adipose tissue in diabetic subjects[16]. Our findings showed that there were higher visfatin levels and lower adiponectin levels in the obese group than control group, and at the same time, the insulin-resistant obese group had higher visfatin and lower adiponectin levels than the noninsulin-resistant obese group. Additionaly, visfatin levels were positively and adiponectin levels were negatively correlated with HOMA-IR levels. As a result, insulin resistance rather than obesity had higher potential to be the cause of higher visfatin and lower adiponectin levels.

The limitation of this study is the small sample size. Therefore, for a more accurate significant conclusion, a greater number of patients and regression analysis are needed.

Increased body weight is tightly associated with insulin resistance and type 2 diabetes mellitus[17,18]. The role of various adipokines as connectors between obesity and diabetes mellitus has been better elucidated in recent years. Based on the findings of this study, visfatin and adiponectin levels can be used as specific markers for insulin sensitivity.

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