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Correlation analysis of serum obestatin expression with insulin resistance in childhood obesity

M.Y. Zhang*, F. Li* and J.P. Wang

Department of Pediatrics, Shaoxing Second Hospital, Shaoxing, Zhejiang, China

*These authors contributed equally to this study.
Corresponding author: J.P. Wang
E-mail: jupingwangd@sina.com

ABSTRACT. Childhood obesity is a serious public health concern condition, as excess body fat can negatively affect a child's health. Obestatin is a hormone that regulates body weight by suppressing appetite and reducing food intake; fasting obestatin level is negatively correlated with basal insulin level. This study aims to investigate the role of obestatin in insulin resistance. A total of 54 children with simple obesity and 57 healthy controls were recruited. Levels of serum insulin, fasting blood glucose, cholesterol, triglyceride, low-density lipoprotein, and high-density lipoprotein were measured. Serum obestatin level was determined using an enzyme-linked immunosorbent assay. We found that body mass index and waist-hip ratio of obese children were significantly higher, while obestatin level in the obese group was significantly lower (P < 0.001), as compared to those of controls. In addition, obese children exhibited higher levels of insulin, total cholesterol, triglyceride, and low-density lipoprotein (P < 0.05) as compared to the controls. Fasting blood glucose and high-density lipoprotein levels were similar between the two groups (P > 0.05). Leptin level in the obese group was also higher (P < 0.05) as compared to that of control group. Results showed that insulin resistance index

was positively correlated with body mass index, waist-hip ratio (r = 0.41, P < 0.001; r = 0.245, P < 0.001), and triglyceride level (r = 0.25, P = 0.04). Our study demonstrated that obestatin is negatively correlated with fasting insulin and leptin levels, and positively correlated with insulin resistance in obese children.

Key words: Childhood obesity; Obestatin; Insulin resistance; Correlation

INTRODUCTION

Childhood obesity is a chronic nutritional disorder, and is fast becoming a global public health problem. Modern social living standards and lifestyle changes leads to rising obesity rates, especially in urban areas. Obesity is associated with various genetic and environmental factors, such as excessive food intake, and sedentary lifestyles. Obese children not only have numerous psychological barriers, but are also more susceptible to various metabolic diseases, hypertension, and diabetes (Su et al., 2014). Obestatin is a hormone that is present in stomach tissues and circulation. Studies have shown that obestatin injections can affect food intake, prolong gastric emptying time, and reduce intestinal contraction frequency. Obestatin levels were reported to be reduced in obese individuals and were negatively correlated with body mass index (BMI) (Zou et al., 2009; Zhang et al., 2011). On the other hand, Holst et al. (2007) demonstrated that obestatin may affect food absorption in rats regardless of feelings of hunger . It has been postulated that obestatin can affect feeding behaviors and digestion by influencing pancreas and stomach ghrelin secretion (Chanoine et al., 2006).

Insulin resistance (IR) is defined as insensitivity of insulin on target organs. Aside from genetic factors, multiple environmental factors can also lead to IR, including obesity, long-term hyperglycemia, and tall dissociate fatty acid hematic disease. Research has shown that fat accumulation is correlated with IR and other metabolic diseases.

However, the relationship between obestatin and IR in obese children is still not well characterized. Therefore, the aim of the present study was to investigate the role of obestatin in IR by measuring serum obestatin level and IR in obese children.

MATERIAL AND METHODS

Study subjects

A total of 54 children with simple obesity were recruited between May 2015 and April 2016 in Shaoxing Second Hospital. Subjects included 31 males and 23 females, ranging between 5-14 years of age. All subjects received abdominal ultrasound and endocrine function tests to exclude other endocrine diseases, genetic diseases, and nervous system induced secondary obesity. Obesity was diagnosed via criteria published by Li et al. (2010), which defined BMI cut off points for 2-18-year-old children in China. Another 57 healthy children (33 males and 24 females) receiving physical examinations at the same hospital were selected as controls, and ranged between 5-14 years of age. Gender, age, height, weight, blood pressure, waistline, and hipline were recorded.

$$\text{Body mass index (BMI)} = \text{weight (kg)/height}^2 \text{ (m}^2) \qquad \text{(Equation 1)}$$

$$\text{Waist-hip ratio (WHR)} = \text{waistline (cm)/hipline (cm)} \qquad \text{(Equation 2)}$$

The study protocol was approved by the Research Ethics Committee of Shaoxing Second Hospital, and informed consents were obtained from parents/guardians of all enrolled subjects.

Blood sample collection and laboratory detection

Peripheral venous blood (5 mL) was drawn following an 8-hour (at least) fast. Serum insulin (FINS), fasting blood glucose (FBG), as well as lipid profiles, including cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), and high density protein of cholesterol (HDL-C) were measured, as previous described (Li et al., 2012). The homeostatic model assessment for insulin resistance (HOMA-IR) was used as a measure of insulin resistance.

$$\text{IR} = \text{FINS x FBG/22.5} \qquad \text{(Equation 3)}$$

Serum obestatin level was determined by enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Data analysis was carried out with the SPSS16.0 software. Measurement data are reported as means ± standard deviation (SD), and were subjected to the Student's *t*-test or the rank-sum test. Enumeration data were tested by the chi-square test with the inspection level α = 0.05. P value < 0.05 was considered to be statistically significant.

RESULTS

Gender, age, BMI, and WHR comparison

No statistical differences in gender and age were observed between the obese and control groups. However, obese children presented significantly higher BMI, waist-to-hip ratio (WHR), and lower obestatin level as compared to those of controls (P < 0.001) (Table 1).

Table 1. Gender, age, body mass index (BMI), and waist-to-hip ratio (WHR) of obese and control children.

Index	Obese group	Controls	P value
Male/female	31/23	33/24	0.96
Age (year)	9.03 ± 4.23	8.92 ± 3.24	0.88
BMI (kg/m^2)	25.02 ± 4.21	17.21 ± 3.21	P < 0.001
WHR	0.95 ± 0.13	0.84 ± 0.09	P < 0.001
Obestatin level (ng/L)	401.25 ± 120.14	495.34 ± 90.35	P < 0.001

Biochemical criterion comparison

Serum levels of FINS, FBG, TC, TG, LDLC, and HDLC were measured in all individuals. Results showed that insulin level in the obese group (118.24 ± 47.21) was significantly higher as compared to that in the control group (57.67 ± 50.65) (P < 0.05). However, FBG levels were similar between the two groups (P > 0.05). Leptin (Ln) (IR) level

was higher in the obese group as compared with that in the controls (P < 0.05). In addition, the obese group demonstrated markedly higher TC, TG, and LDL-C levels (P < 0.05) as compared to those in controls; HDL-C levels were comparable between the two groups (Figure 1).
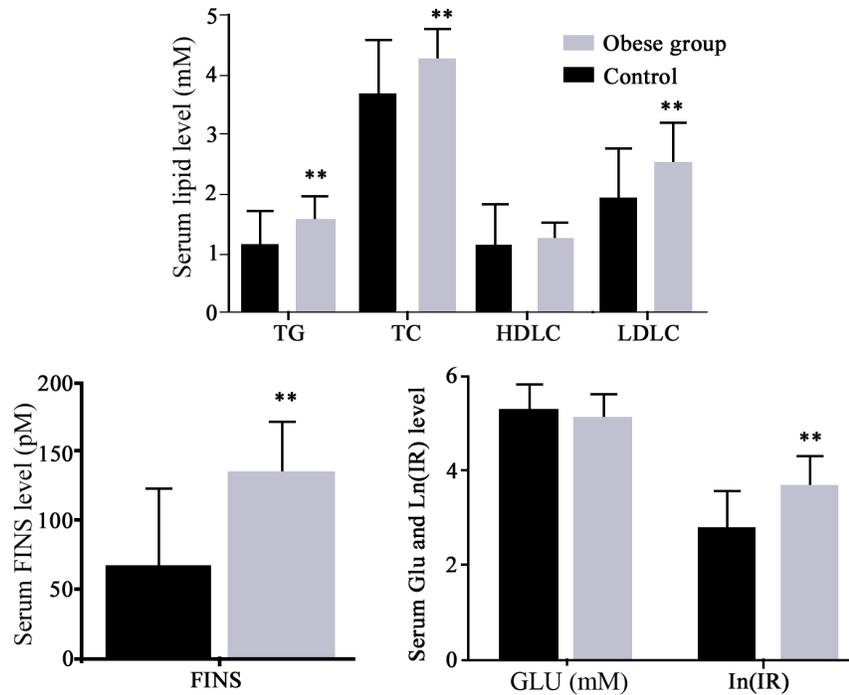

Figure 1. Biochemical criterion comparison. **P < 0.05 as compared with controls.

Correlation analysis of HOMA-IR and blood lipid

Correlation analysis was performed on HOMA-IR with BMI, WHR, and blood lipid indexes. We found that HOMA-IR was positively correlated with BMI, WHR, and serum TG (P < 0.05), suggesting that BMI, WHR, and TG may be involved in the development and pathogenesis of IR (Table 2).

Table 2. Correlation analysis of HOMA-IR with BMI, WHR, and blood lipid.

Index	BMI (body mass index)	WHR (waist-to-hip ratio)	TC (cholesterol)	TG (triglyceride)	LDLC (low density lipoprotein cholesterol)	HDLC (high density protein of cholesterol)
r value	0.41	0.245	0.10	0.25	0.07	0.12
P value	P < 0.001	P < 0.001	0.23	0.04	0.65	0.15

Correlation analysis of obestatin

Correlation analysis demonstrated that obestatin was negatively correlated with BMI and Ln (IR), but not WHR and other blood lipid indexes (Table 3).

Table 3. Correlation analysis of obestatin.

Index	BMI (body mass index)	WHR (waist-to-hip ratio)	Ln (IR) (Leptin)	FINS (Serum insulin)	TC (cholesterol)	TG (triglyceride)	LDL-C (low density lipoprotein cholesterol)	HDL-C (high density protein of cholesterol)
r value	-0.215	-0.145	-0.223	-0.345	0.058	0.067	0.076	0.065
P value	P < 0.001	0.45	P < 0.001	P < 0.001	0.24	0.45	0.42	0.29

DISCUSSION

The main reason for simple obesity in children is energy metabolism imbalance. Adipose accumulation often causes abnormal metabolic processes, and can lead to metabolic syndromes. Obesity arises due to elevated fatty acid levels, leading to excess lipid accumulation and activation of inflammatory pathways, which results in IR (Makki et al., 2013). Previous studies showed that obesity could easily lead to free fatty acid deposition in storage organs such as the liver and muscle tissues. Abnormal fat deposition is one of the main factors for pathogenesis of IR (Consitt et al., 2009; Samuel, 2011). Therefore, IR often occurs in obese people. Our results confirmed that HOMA-IR was positively correlated with BMI, which was consistent with a previous report by Barseem and Helwa (2015). Furthermore, our results demonstrated that obese children were prone to blood lipid disorders, as they showed significantly higher serum TC, TG, and LDL-C levels as compared with controls.

Obestatin is an active peptide with multiple biological functions, such as stimulating cell proliferation, differentiation, and sugar and lipid metabolism (Gesmundo et al., 2013). Obestatin can prevent IR occurrence by influencing insulin secretion. Ghrelin and obestatin, two gastrointestinal peptides formed via post-translational processing of the common precursor-preproghrelin, play important physiological roles in obesity and hypertension (Wang et al., 2014). Our results showed that peripheral obestatin level in obese children was significantly lower than that of normal controls, and was negatively correlated with BMI, which was consistent with previous reports by Shen et al. (2013). Obestatin declination in obese patients leads to decreased suppression of appetite and subsequent weight gain (Arslan et al., 2010). TG reduction was also found to be associated with obestatin levels (Agnew et al., 2011). While most studies suggest that obestatin is negatively correlated with IR, the specific mechanisms driving this phenomenon is still unclear. It is thought that obestatin can regulate β cells and expression of insulin related genes, thus affecting insulin secretion and sensitivity.

Various studies have suggested that that BMI and basal insulin is negatively correlated with fasting obestatin levels (Lippl et al., 2008; Wali et al., 2014); TG and TC are also associated with IR. IR leads to down-regulation of esterase synthesis and activity, resulting in reduced TG hydrolysis and overall elevation of TG. Our study showed that IR leads to changes in BMI, WHR, and TG levels, suggesting that these factors may be reliable markers for predicting the occurrence and development of IR. In obese children, obestatin showed negative correlation with Ln (IR) and FINS, which was consistent with previous reports (Nakahara et al., 2008). Obestatin injections in Wistar rats can significantly reduce the occurrence of pancreatic edema (Ceranowicz et al., 2009). Obestatin can also treat cerulein-induced acute pancreatitis (Bukowczan et al., 2016). Interestingly, obstatin and insulin show interdependent relationship; insulin can reduce obestatin level in insulin sensitive patients (Anderwald-Stadler et al., 2007), suggesting that insulin and insulin sensitivity are important factors affecting obestatin secretion. Similarly, metabolic abnormalities resulting in altered insulin secretion may affect obestatin levels as well (Huang et al., 2012).

However, the exact mechanisms driving the correlation between obestatin and IR in obese children are not fully understood. Whether the correlation between obestatin and IR solely occur in simple obesity or other metabolic disorders, and whether this relationship holds true in the adult population remain to be investigated.

To conclude, weight control in children is a serious public health concern, and can result in IR, which is associated with multiple cardiovascular and cerebrovascular diseases. Obestatin plays an important role in childhood obesity, and may participate in IR. However, further investigation is required to determine the exact mechanism by which obestatin regulates insulin.

Conflicts of interest

The authors declare no conflict of interest.

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