Peer reviewed article

Plasma visfatin concentrations in childhood obesity: relationships to insulin resistance and anthropometric indices

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Summary

Background: The aim of this study was to investigate the relationships between plasma visfatin, insulin resistance, lipid profile and anthropometric measurements in obese children.

Subjects and methods: Plasma levels of visfatin, insulin, glucose, lipid profile and anthropometric indices were determined in 30 obese children and compared with those in 30 age- and gender-matched non-obese children. Visfatin was measured with enzyme-linked immunosorbent assay and logarithmically transformed to log visfatin for parametric comparisons.

Results: The obese group had significantly elevated plasma visfatin, fasting glucose and insulin and homeostasis model assessment (HOMA) values, as well as elevated lipid concentrations, compared with non-obese children. In the obese group log visfatin correlated positively with weight ($p = 0.007$), waist circumference ($p = 0.007$), hip circumference ($p = 0.034$), BMI ($p = 0.005$), insulin ($p = 0.041$) and HOMA ($p = 0.044$). No correlation was found between visfatin and lipid profile in obese children ($p > 0.05$). Linear regression analysis revealed significant positive relationships between log visfatin and BMI ($p = 0.005$), insulin and BMI ($p < 0.001$), and between HOMA and BMI ($p < 0.001$) in the obese group but not in the control group. Multivariate regression analysis with log visfatin as a dependent variable showed that only BMI ($p = 0.005$) and bodyweight ($p = 0.014$) correlated positively with log visfatin in obese children.

Conclusions: An increased visfatin concentration may be associated with BMI and insulin resistance in obese children. Although these findings may lay a foundation for further hypotheses, the limited sample size in the present study means that longitudinal studies with more patients are needed.

Key words: children; visfatin; obesity; insulin resistance; lipids

Introduction

The prevalence of childhood obesity is increasing dramatically in developed countries [1], and obese children are at greater risk of becoming obese adults in the future. Hence research into childhood obesity is of paramount importance in preventing obesity-related mortality and morbidity in adults.

Nowadays adipose tissue is recognised as an endocrine organ which releases many cytokines such as tumour necrosis factor alpha, interleukin 6, leptin, adiponectin, and resistin [1]. The production, release and serum levels of these cytokines have been investigated in obesity [2–4]. Very recently, Fukuhara et al. [5] identified a novel cytokine, visfatin. Visfatin is predominantly secreted from visceral adipose tissue, its plasma level correlates with the amount of visceral fat in humans [5], and increased visceral body fat is closely linked to insulin resistance in adults [6]. Growing evidence indicates a role for visfatin in glucose homeostasis [7]. Visfatin may link with diabetes, owing to its insulin-mimetic action, by binding to the insulin receptor [5, 8]. Visceral obesity is known to be an important risk factor for

No financial support to declare.

the development of insulin resistance, type 2 diabetes mellitus and cardiovascular diseases in adults [8–10]. These findings indicate that hyperinsulinaemic insulin resistance and visceral adiposity can be considered early signs of metabolic syndrome.

Previous studies on obesity, visfatin, lipid parameters and insulin resistance produced conflicting results in adults and children [7, 11–13]. Some reports found increased visfatin levels in children [1, 7, 12–14]. In contrast, one study reported reduced plasma visfatin levels in obese adult patients [15]. One study reported a significant positive correlation between plasma visfatin and BMI [13], but other studies reported no correlation between visfatin and anthropometric measurements [1, 7, 16].

Due to the lack of a consensus on visfatin concentrations and their relationship to anthropometric and metabolic parameters in children, our aim in the present study was to investigate the role of visfatin in childhood obesity. The relationships between plasma visfatin, HOMA and lipid profile were also investigated.

Methods

Patients

The study population consisted of 30 obese children aged 9–14 years. Obese children were recruited from the Paediatric Outpatient Clinic of our hospital. Thirty age- and sex-matched non-obese children were included as the control group. Control subjects were selected among the children who were admitted to the hospital for routine checkups or routine follow-up examinations and had no clinical or laboratory signs of systemic illness. All obese children included in the study were diagnosed as having simple obesity, without additional diseases such as diabetes mellitus, hypertension and hypothyroidism. Children with secondary obesity were excluded from the study.

A careful history and physical examination including anthropometric measurements were obtained in all subjects. Bodyweight, height, waist circumference and hip circumference were measured, and body mass index (BMI) was calculated (in kg/m^2) as an index of overall adiposity. Height and weight were determined using precision stadiometers and scales to the nearest 0.1 cm and 0.1 kg respectively. The values were compared with median age-related Turkish standards [17]. Waist circumference was measured midway between the inferior margin of the last rib and the crest of the ileum in a horizontal plane. Hip circumference was measured round the pelvis at the point of maximal protrusion of the buttocks. Circumferences were measured to the nearest 1 mm. Pubertal development was assessed by physical examination according to the criteria of Tanner [18]. The cut-off values for BMI recommended by Cole et al. [19] were applied in the diagnosis of obesity among the subjects included. Children with BMI over their age- and sex-specific 95th percentile values were defined as obese children, and those with BMI <85 percentiles were considered non-obese. Children with a BMI between the 85th and 95th percentiles were defined as overweight and excluded from the study. The study protocol was approved by the local Ethics Committee and informed consent was obtained from the parents of all participants.

Blood samples

The fasting blood samples were collected in the morning between 8:00 AM and 10:00 AM following an overnight fast. Blood samples were centrifuged (4000 rpm) at room temperature for 10 minutes. The separated plasma was then stored at –70 °C until the time of the assays for visfatin and insulin levels.

Biochemical parameters including glucose, triglycerides (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) were measured on the same day by Behring RXL autoanalyser (Germany). Very low-density lipoprotein cholesterol (VLDL-C) was calculated by the formulation of triglyceride/5 and LDL-C was calculated by the Friedwald formula, LDL-C (mg/dl): TC-[HDL-C + TG/5] [20].

Serum insulin was measured by a commercial chemiluminescence assay kit (DPC Diagnostics) using the hormone autoanalyser (Immulite 2000, DPC, USA). The intra-assay and inter-assay coefficients of variance (CV) for insulin were 5.4% and 4.3% respectively.

The insulin resistance index was estimated from fasting serum insulin and serum glucose levels using the homeostasis model assessment (HOMA) = fasting serum insulin (μIU/mL) × fasting serum glucose (mg/dL)/405 [21].

Visfatin measurement

Plasma visfatin was measured by a commercial visfatin C-terminal enzyme-linked immunosorbent assay kit (Alpco Diagnostics, Salem, NH) using the microplate reader (sensitivity 30 pg/mL). The intra- and inter-assay CV were 5.04% and 6.67% respectively. A monoclonal antibody specific for human visfatin had been pre-coated onto a 96-well microplate in this ELISA kit.

Statistical analysis

All results were expressed as mean plus/minus standard deviation. The single sample Kolmogorov-Smirnov test was used to estimate the variables' distribution characteristics. The differences between the patients and the control groups were estimated using the Chi-square (for gender distribution) and two-tailed unpaired t-test (for normally distributed data). Non-parametric Mann-Whitney U test was used for comparison of visfatin values between patients and the controls. Visfatin values were logarithmically transformed to log visfatin in order to perform parametric analyses. Pearson's correlation analysis, linear and multivariate regression analysis were performed to evaluate the relationships between metabolic, hormonal, and anthropometric parameters. Statistical analyses were performed using SPSS 12.0 software package (SPSS, Inc., Chicago, IL). A p value of less than 0.05 was accepted as significant.

Results

The mean age of obese (11.7 ± 1.8 years) and non-obese children (11.2 ± 0.6 years), their male/female ratios and pubertal stages were similar (table 1). The obese group had significantly higher weight, height, BMI and waist and hip circumferences compared with healthy controls (p <0.001 for each parameter). Anthropometric and demographic data of obese and control children are shown in table 1.

Obese children had significantly higher fasting glucose (p = 0.023), fasting insulin (p = 0.005) and HOMA values (p = 0.002) than control subjects (table 2). Although no difference was found between the HDL-C levels of the two groups (p >0.05), significantly higher TC (p <0.001), LDL-C (p = 0.001), VLDL-C (p = 0.006), and triglycerides (p = 0.009) levels were found in the obese group than in the control group (table 2). Visfatin (4.0 ± 3.0 ng/mL vs. 2.2 ± 1.5 ng/mL, p = 0.014) (fig. 1) and log visfatin (p = 0.006) values in obese children were significantly higher than in control subjects (table 2).

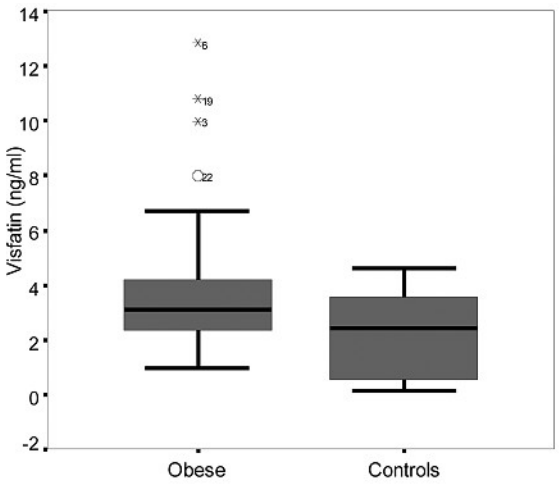

Figure 1

The difference in visfatin levels between obese and the control children (Percentiles for obese children and the controls respectively: 25th percentile, 2.26 vs. 0.50 ng/ml; 50th percentile, 3.19 vs. 2.37; 75th percentile, 4.74 vs. 3.54 ng/ml. Case numbers 3, 6, 19 and 22 are outliers in obese group) (P = 0.014).

Correlations

Significantly positive correlations were found between anthropometric indices and insulin (p <0.05), between anthropometric indices and HOMA (p <0.05), and between anthropometric indices and log visfatin (p <0.05) in the obese group (fig. 2) (table 3). There were also significant positive correlations between log visfatin and insulin (r = 0.381, p = 0.041) and between log visfatin and HOMA (r = 0.358, p = 0.044) in the obese group. However, no significant correlation was found between anthropometric indices, log visfatin and HOMA in non-obese children (table 3). None of the lipid parameters (TC, TG, HDL-C, LDL-C and VLDL-C) was found to be correlated with anthropometric measurements, hormonal parameters and with log visfatin in either the obese or the control group (data not shown).

Table 1

Anthropometric indices in obese and control children.

	Obese group (n = 30)	Control group (n = 30)	P
Male/Female ratio	16/14	14/16	NS
Age (years)	11.7 ± 1.8	11.2 ± 0.6	NS
Pubertal stage	2 (2–3)	2 (2–3)	NS
Weight (kg)	59.4 ± 16.1	31.4 ± 5.5	<0.001
Height (cm)	148 ± 13	138 ± 6	<0.001
Waist circumference (cm)	84.7 ± 10.3	59.4 ± 5.6	<0.001
Hip circumference (cm)	98.8 ± 11.5	69.4 ± 6.0	<0.001
BMI (kg/m2)	26.2 ± 3.4	16.3 ± 2.0	<0.001

BMI: body mass index; NS: not significant

Table 2

Laboratory values in obese children and the control group.

	Obese group (n = 30)	Control group (n = 30)	P
Fasting glucose (mg/dl)	89.6 ± 9.2	84.6 ± 6.8	0.023
Fasting insulin (µIU/ml)	7.9 ± 6.1	4.3 ± 2.7	0.005
HOMA	1.74 ± 1.26	0.89 ± 0.59	0.002
Total cholesterol (mg/dl)	157 ± 31	129 ± 16	<0.001
HDL-C (mg/dl)	42 ± 9	44 ± 8	NS
LDL-C (mg/dl)	93 ± 28	72 ± 12	0.001
VLDL-C (mg/dl)	22.9 ± 12.6	15.6 ± 5.2	0.006
Triglycerides (mg/dl)	109 ± 55	78 ± 25	0.009
Visfatin (ng/ml)	4.0 ± 3.0	2.2 ± 1.5	0.014
Log Visfatin	0.50 ± 0.31	0.16 ± 0.46	0.006

HOMA: Homeostasis model assessment; HDL-C: High-density lipoproteins cholesterol; LDL-C: Low-density lipoproteins cholesterol; VLDL-C: Very low-density lipoproteins cholesterol; Log: logarithmically transformed; NS: not significant

Table 3

Pearson's correlation coefficients (r) between anthropometric, hormonal and lipid parameters in obese and non-obese children.

Correlation coefficients for obese children	Age	Weight	BMI	Waist circumference	Hip circumference
Glucose	0.098	–0.020	–0.190	–0.150	–0.051
Insulin	0.430*	0.550**	0.544**	0.602***	0.698***
HOMA	0.460*	0.552**	0.502**	0.600***	0.700***
TC	0.254	0.014	0.030	–0.170	0.209
TG	0.149	0.054	0.162	–0.002	–0.037
Log visfatin	0.250	0.489**	0.507**	0.491**	0.394*
Correlation coefficients for the control subjects					
Glucose	0.245	0.061	0.117	0.242	0.127
Insulin	0.069	0.207	0.042	–0.012	0.158
HOMA	0.077	0.223	0.077	0.029	0.182
TC	–0.039	–0.110	–0.039	–0.189	–0.191
TG	0.133	–0.097	–0.129	0.254	0.031
Log visfatin	–0.033	–0.013	0.030	–0.001	0.035

* P <0.05, ** P <0.01, *** P = 0.001 or P <0.001
HOMA: Homeostasis model assessment; TC: Total cholesterol; TG: Triglycerides; Log: logarithmically transformed

Table 4

Multivariate analysis between log visfatin as dependent variable and anthropometric measurements, age, gender and pubertal stage as independent variables.

Independent variable	β	SE	P value
BMI	0.638	0.014	0.005
Weight	1.012	0.007	0.014
Height	–0.557	0.010	NS
Waist circumference	0.404	0.008	NS
Hip circumference	0.112	0.007	NS
Age	–0.166	0.014	NS
Gender	0.086	0.113	NS
Pubertal stage	0.074	0.121	NS

NS: not significant

Table 5

Multivariate regression analysis between HOMA index as dependent variable and plasma visfatin concentration, body mass index and waist circumference as independent variables.

Independent variable	β	SE	P value
BMI (kg/m^2)	0.727	0.081	0.003
Visfatin (ng/ml)	–0.023	0.083	NS
Waist circumference (cm)	–0.004	0.026	NS

BMI: body mass index, NS: not significant

Regression analyses

When log visfatin was taken as a dependent variable and other parameters as independent variables, significant relationships of log visfatin to weight (r = 0.507; p = 0.004), waist circumference (r = 0.490; p = 0.006), hip circumference (r = 0.425; p = 0.019) and BMI (r = 0.528; p = 0.003) were found in the obese group (p <0.05) by linear regression analysis. Multivariate regression analysis revealed significant relationships of log visfatin as dependent variable to BMI (p = 0.005) and bodyweight (p = 0.014) as independent variables. Age, gender, pubertal stage, height and circumferences of waist and hip did not show a significant relationship to log visfatin in multivariate analysis (table 4). Finally, to clarify the relative contribution of BMI, waist circumference, and visfatin in the determination of insulin resistance, a multivariate regression analysis was performed. In this statistical model only BMI retained its significant relationship to HOMA, whereas visfatin and waist circumference did not (table 5).

Discussion

The increasing prevalence of obesity is becoming an important public health problem in childhood and presents numerous problems. Similarly to the risks of obesity in adulthood, childhood obesity is also a leading cause of paediatric hypertension associated with type II diabetes mellitus, and increases the risk of cardiovascular diseases [10, 16]. Because visceral adipose tissue is considered an important source of visfatin, studies on visfatin alterations in children may be useful in understanding some complications of obesity.

The body heights of our obese children were found to be significantly higher than those of non-obese children. Obese children often display increased linear growth. Obesity is characterised by high serum growth hormone (GH)-binding protein and normal to high IGF-1 levels. Obesity, as in tall stature, may suggest an increase in responsiveness to GH. Hence the increased linear growth in obese children can be partly explained by this increase in GH sensitivity [22].

In the present study the impact of childhood obesity on circulating visfatin and the relationships between visfatin, anthropometric measurements, insulin resistance and lipid profile were investigated in obese children. Significantly higher plasma visfatin and insulin levels in obese children were found when compared with the controls.

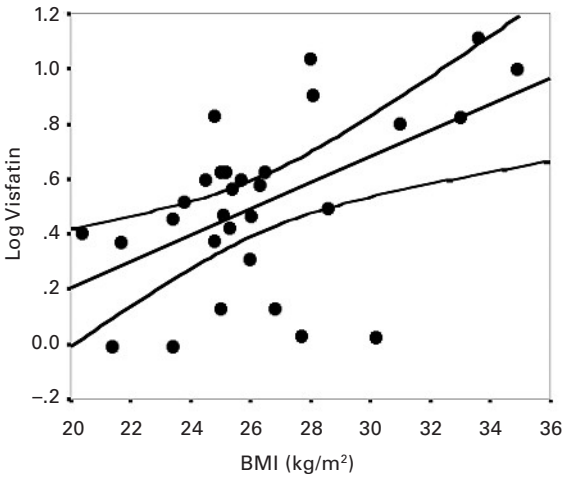

Figure 2

The relationship between log visfatin and body mass index in obese children (B = 0.058 [95% confidence interval, 0.017–0.098], Beta = 0.638, P = 0.005).

Positive correlations were observed between plasma visfatin levels and weight, BMI, waist circumference and hip circumference in obese children. However, with multivariate analysis only BMI and bodyweight were retained to be related to visfatin levels. In the original study of Fukuhara et al. [5] visfatin identified as secreted from visceral adipocytes and plasma visfatin levels were found to be significantly correlated with visceral fat tissue in adults. The results concerning visfatin upregulation in obesity are contradictory [3, 7, 13–15, 23]. Most previous studies have reported elevated visfatin concentrations in obesity [3, 7, 13, 14, 23], though Pagano et al. [15] have reported decreased plasma visfatin and its messenger RNA in subcutaneous adipose tissue of obese subjects.

In the present study significant positive correlations of plasma visfatin with anthropometric markers including weight, BMI and waist-hip circumferences were found in obese children. Multivariate analysis also revealed positive relationships between visfatin and BMI or bodyweight. However, similar relationships were not found in non-obese subjects. This situation may suggest the existence of a threshold in childhood obesity above which relationships would have been more prominent. Although a positive correlation between visfatin levels and BMI has been reported in adults [13], another study [1] showed a negative correlation between visfatin levels and BMI in a Chinese obese adult population. Moreover, Haider et al. [7] found no significant correlation between plasma visfatin concentrations and BMI in obese children. Two other studies [12, 23] likewise reported no correlation between serum concentrations of visfatin and anthropometric markers in male adult subjects and in both obese and lean women. The reasons for these conflicting results may be ethnic heterogeneity, different population characteristics (children, men, women) and confounding factors such as gender, diabetes mellitus, gestation etc. [1]. Discrepancies in laboratory measurement methods may also play a role [1, 24–26].

In the present study, the relations between fasting plasma insulin, fasting glucose concentration and insulin resistance were studied in obese children. Although no significant correlation was found between visfatin and fasting glucose, similarly to the study of Araki et al. [14], our results show significant correlations between visfatin and fasting insulin, and between visfatin and HOMA. However, a positive relationship between visfatin and HOMA disappeared when multivariate analysis was performed. In a population-based study of adult women, no relationship was found between visfatin and metabolic parameters including fasting serum glucose, insulin and HOMA [26]. Moreover, Berndt et al. [13] reported no significant correlation between visfatin plasma concentrations, fasting plasma insulin and glucose concentrations in adults. There are conflicting data on the association of visfatin with obesity and insulin resistance parameters. It has been suggested that the inconsistencies in clinical studies may be attributed to differences in the specificity of the immunoassays used [24]. In our obese children the positive correlations between plasma visfatin and fasting insulin or HOMA, taken together with higher plasma visfatin, fasting glucose and insulin levels of obese children, may indicate that visfatin has an insulin-like activity in an insulin-resistant milieu of obesity. Previously, visfatin had been reported to imitate the effects of insulin through a binding site on the insulin receptor [5]. Since it has been suggested that visfatin is a marker of visceral adiposity rather than obesity, our results may suggest a possible relationship between visceral adiposity and insulin resistance in obese children.

In our study, significantly higher TC, LDL-C, VLDL-C and triglycerides (except HDL-C) were found in the obese children (table 2). A low LDL-C concentration had been reported as a characteristic finding in healthy Turkish children [27]. We were unable to demonstrate a correlation between plasma concentrations of visfatin and levels of lipids in obese children. Similarly, Haider et al. [7] did not observe a correlation between serum concentrations of visfatin and lipids in obese children, except for HDL-C. However, in two other studies [1, 26], the authors reported a positive correlation between plasma concentrations of visfatin and HDL-cholesterol levels in adolescent children and in female adults. Human visfatin gene is located at 7q22.3, which has been reported to be a linkage region for insulin resistance syndrome [28]. Jian et al. [29] reported that a single nucleotide polymorphism at different loci of visfatin gene was associated with triglyceride and total cholesterol levels. These reports suggest that visfatin may play a role in lipid homeostasis. However, the underlying mechanism is currently unknown [26]. Because inhibition of cholesteryl ester transfer-protein increases HDL-C level and decreases LDL-C level [30], one explanation for the role of visfatin in cholesterol homeostasis may be via inhibition of cholesteryl ester transfer pro-

tein [26]. In contrast to previous studies, the absence of a relationship between visfatin and HDL-C in our study may be related to characteristic low HDL-C concentrations in Turkish children overall [27].

In conclusion, we found higher visfatin, fasting glucose, fasting insulin, HOMA and lipid parameters (TC, LDL-C, triglycerides) in obese children. Significant positive correlations were found between plasma visfatin and anthropometric indices (weight, BMI, waist and hip circumferences) and between visfatin, insulin and HOMA in obese children with univariate analysis. Multivariate regression analysis showed positive relationships of visfatin levels with BMI and bodyweight in obese children.

Since our results derived from cross-sectional data and the relatively small sample size of the cohort of children, we suggest that our findings may lay a foundation for further hypotheses on this topic. Additional prospective studies with larger patient numbers are needed to clarify the role of visfatin on insulin resistance in obese children.

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References

1 Jin H, Jiang B, Tang J, et al. Serum visfatin concentrations in obese adolescents and its correlation with age and high-density lipoprotein cholesterol. Diabetes Res Clin Pract. 2008;79(3): 412–8.
2 Juge-Aubry CE, Henrichot E, Meier CA. Adipose tissue: a regulator of inflammation. Best Pract Res Clin Endocrinol Metab. 2005;19(4):547–66.
3 Zahorska-Markiewicz B, Janowska J, Olszanecka-Glinianowicz M, Zurakowski A. Serum concentrations of TNF-alpha and soluble TNF-alpha receptors in obesity. Int J Obes. 2000;24 (11):1329–95.
4 Olszanecka-Glinianowicz M, Zahorska-Markiewicz B, Janowska J, Zurakowski A. Serum concentrations of nitric oxide, TNF-alpha and TNF soluble receptors in women with overweight and obesity. Metabolism. 2004;53(10):1268–73.
5 Fukuhara A, Matsuda M, Nishizawa M, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science. 2005;307(5708):426–30.
6 Despres JP, Moorjani S, Ferland M, et al. Adipose tissue distribution and plasma lipoprotein levels in obese women. Importance of intraabdominal fat. Arteriosclerosis. 1989;9(2):203–10.
7 Haider DG, Holzer G, Schaller G, et al. The adipokine visfatin is markedly elevated in obese children. J Pediatr Gastroenterol Nutr. 2006;43(4):548–9.
8 Ibanez L, Lopez-Bermejo A, Suarez L, Marcos MV, Diaz M, de Zegher F. Visceral adiposity without overweight in children born small-for-gestational age. J Clin Endocrin Metab. 2008 Mar 11 (Epub).
9 Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest. 2000;106(4):473–81.
10 Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev. 2000;21 (6):697–738.
11 Varma V, Yao-Borengasser A, Rasouli N, Bodles AM, Phanavanh B, Lee MJ, et al. Human visfatin expression: relationship to insulin sensitivity, intramyocellular lipids, and inflammation. J Clin Endocrinol Metab. 2007;92(2):666–72.
12 Chen MP, Chung FM, Chang DM, Tsai JC, Huang HF, Shin SJ, et al. Elevated plasma level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2006;91(1):295–9.
13 Berndt J, Klöting N, Kralisch S, Kovacs P, Fasshauer M, Schön MR, et al. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. Diabetes. 2005;54(10):2911–6.
14 Araki S, Dobashi K, Kubo K, Kawagoe R, Yamamoto Y, Kawada Y, et al. Plasma visfatin concentration as a surrogate marker for visceral fat accumulation in obese children. Obesity. 2008;16(2):384–8.
15 Pagano C, Pilon C, Olivieri M, Mason P, Fabris R, Serra R, et al. Reduced plasma visfatin/pre-B cell colony-enhancing factor in obesity is not related to insulin resistance in humans. J Clin Endocrinol Metab. 2006;91(8):3165–70.
16 Jian WX, Luo TH, Gu YY, Zhang HL, Zheng S, Dai M, et al. The visfatin gene is associated with glucose and lipid metabolism in a Chinese population. Diabet Med. 2006;23(9):967–73.
17 Neyzi O, Binyıldız P, Alp H. Türk çocuklarında büyümegelişme normları 1.Tartı ve boy değerleri [Growth charts for Turkish children 1. Body weight and height values]. İstanbul Tıp Fak Mec. [Journal of Istanbul Medical Faculty] 1978; Suppl. 41:74–7.
18 Tanner JM. Growth and maturation during adolescence. Nutr Rev. 1981;39(2):43–55.
19 Cole TJ, Bellizzi MC, Flegal KM, Deitz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000;320:1240–6.
20 Friedwald WT, Levy RI, Frederickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499–502.
21 Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. Endoc Rev. 1985;6(1):45–85.
22 Bouhours-Nouet N, Gatelais F, Boux de Casson F, Rouleau S, Coutant R. The insulin-like growth factor-I response to growth hormone is increased in prepubertal children with obesity and tall stature.J Clin Endocrinol Metab. 2007;92(2): 629–35.
23 Zahorska-Markiewicz B, Olszanecka-Glinianowicz M, Janowska J, Kocełak P, Semik-Grabarczyk E, Holecki M, et al. Serum concentration of visfatin in obese women. Metabolism. 2007;56:(8)1131–4.
24 Nusken KD, Nusken E, Petrasch M, Rauh M, Dötsch J. Preanalytical influences on the measurement of visfatin by enzyme immunoassay. Clin Chim Acta. 2007;382(1-2):154–6.
25 Körner A, Garten A, Blüher M, Tauscher R, Kratzsch J, Kiess W. Molecular characteristics of serum visfatin and differential detection by immunoassays. J Clin Endocrinol Metab. 2007;92 (12):4783–91.
26 Chen CC, Li TC, Li CI, Liu CS, Lin WY, Wu MT, et al. The relationship between visfatin levels and anthropometric and metabolic parameters: association with cholesterol levels in women. Metabol Clin Exp. 2007;56(9):1216–20.
27 Iscan A, Yigitoglu R, Vurgun N, Uyanik BS, Akyildiz M. Low levels of high density lipoprotein cholesterol in Turkish children: an important risk factor. Acta Paediatr Jpn. 1998;40(1): 41–6.
28 Arya R, Blangero J, Williams K, et al. Factors of insulin resistance syndrome-related phenotypes are linked to genetic locations on chromosomes 6 and 7 in nondiabetic Mexican-Americans. Diabetes 2002;51:841–7.
29 Jian WX, Luo TH, Gu YY, et al. The visfatin gene is associated with glucose and lipid metabolism in a Chinese population. Diabet Med. 2006;23:967–73.
30 Brousseau ME, Schaefer EJ,Wolfe ML, et al. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. N Engl J Med. 2004;350:1505–15.