RESEARCH ARTICLE

Evaluation of new adipocytokines and insulin resistance in adolescents with polycystic ovary syndrome

Ferhat Cekmez[1], Yasemin Cekmez[2], Özgür Pirgon[1], Fuat Emre Canpolat[1], Secil Aydinöz[3], Osman Metin Ipcioglu[4], Ferhan Karademir[3]

[1] Department of Pediatrics, GATA Medical Faculty, Ankara
[2] Gyneacology and Obstetrics Clinic, Goztepe Hospital, İstanbul
[3] Department of Pediatrics, GATA Medical Faculty, İstanbul
[4] Department of Biochemistry, GATA, Medical Faculty , Istanbul, Turkey

Correspondence: F. Cekmez, MD, GATA Medical Faculty, Department of Neonatology, Ankara, Turkey
<ferhat_cocuk@hotmail.com>

Accepted for publication February 2, 2011

To cite this article: Cekmez F, Cekmez Y, Pirgon Ö, Canpolat FE, Aydinöz S, Ipcioglu OM, Karademir F. Evaluation of new adipocytokines and insulin resistance in adolescents with polycystic ovary syndrome. *Eur. Cytokine Netw.* 2011; 22(1): 32-7 doi:10.1684/ecn.2011.0279

ABSTRACT. ***Aim.*** **The aim of this study was to investigate the relationship between four circulating adipocytokines (apelin, vaspin, visfatin, adiponectin) and markers of insulin sensitivity, in the context of polycystic ovary syndrome (PCOS) in adolescents.** ***Subjects and methods.*** **48, obese, adolescent girls (mean age: 15.6±3.4 years, mean body mass index standard deviation score (BMI-SDS): 2.31±0.1), and 37 control subjects (mean age: 16.2±3 years, mean BMI-SDS: 2.17±0.05) were enrolled the study. The diagnosis of PCOS was established according to the Rotterdam criteria. Hyperinsulinism and insulin resistance was evaluated using the homeostasis model assessment (HOMA-IR) from fasting samples. Plasma adiponectin and vaspin levels were determined by radioimmunoassay. Determination of visfatin and apelin levels was performed by enzyme immunoassay.** ***Results.*** **HOMA-IR, apelin and visfatin levels (4.9±2** ***versus*** **1.4±0.7, p<0.001; 2.2±1.1** ***versus*** **0.58±0.16, p<0.001; 31.3±11.1** ***versus*** **18.5±10.7, p<0.001; respectively) were significantly elevated, and adiponectin levels (2.01±1.02** ***versus*** **12.5±6.2, p<0.001) were significantly lower in the PCOS group. Vaspin levels were higher in the PCOS group than in the control group, but the differences were not significant. Apelin and visfatin correlated positively and adiponectin correlated negatively with BMI-SDS and HOMA-IR.** ***Conclusion.*** **Based on the findings of this study, apelin, visfatin and adiponectin levels can be used as specific markers for insulin sensitivity, and these adipocytokines might play a part in the pathogenesis of PCOS.**

Key words: adipocytokines, insulin resistance, polycystic ovary syndrome, adolescents

The etiology of polycystic ovary syndrome (PCOS) is complex and multifactorial. There is much evidence however, to suggest that adipose tissue plays an important role in the development and maintenance of PCOS pathology. There is a close correlation between adiposity and symptom severity in women with PCOS, and even modest reductions in weight generally translate into significant improvements in menstrual regularity, fertility and hyperandrogenic features. Insulin resistance is a common feature of PCOS, affecting about 50% of PCOS patients [1].

Adipose tissue, apart from storing fat, secretes a number of hormones called adipocytokines, four of which, visfatin, vaspin, apelin and adiponectin, appear to play an important role in metabolism and energy homeostasis [2, 3]. Initially it was suggested that most of the abnormalities observed in metabolic syndrome result from insulin resistance and/or hyperinsulinemia [4]. However, more recent studies have indicated an important role of adipose tissue hormones or "adipocytokines" in metabolic syndrome. Adiponectin is exclusively expressed and secreted by adipose tissue and is involved in glucose and lipid metabolism. Hypoadiponectinaemia has been shown to be associated with insulin resistance in animal and human studies [5]. 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 [6, 7]. Both tissue expression and plasma levels of visfatin increase in parallel with obesity. It has insulin-mimetic effects and lowers plasma glucose levels [8]. Apelin synthesis in adipocytes is stimulated by insulin, and plasma apelin level markedly increases in obesity associated with insulin resistance and hyperinsulinemia [9]. Vaspin was identified as an adipokine with insulin-sensitizing effects, and is predominantly secreted from visceral adipose tissue in a rat model of type 2 diabetes [10]. Youn *et al*. have recently shown that vaspin mRNA expression in adipose tissue is related to parameters of obesity and glucose metabolism in adults [11].

This research article considers the various mechanisms that might underlie the link between excess adiposity and PCOS - including the effects of differential insulin sensitivity, abnormal steroid hormone metabolism and

doi: 10.1684/ecn.2011.0279

adipocytokine secretion. Greater attention to the therapeutic options available to reduce the impact of excess adiposity on ovarian and metabolic function is essential to the management of PCOS. We hypothesize that the four, circulating adipokines, mentioned above, are linked to markers of insulin sensitivity and PCOS in adolescents.

DONORS AND METHODS

Subjects

The Research Ethics Committee of Gulhane Military Academic Hospital approved the experimental protocol, and written consent was obtained from the parents. Signed, informed consent forms were obtained from the parents of the adolescents.

Forty eight, obese, adolescents (PCOS group; 48 girls, mean age: 15.6±3.4, mean body mass index standard deviation score (BMI-SDS): 2.31±0.1) were recruited from among the adolescents who attended the outpatient clinic of the Department of Pediatric Endocrinology between 2007 and 2008. Control subjects who were obese, healthy children (37 girls, mean age: 16.2±3, mean BMI-SDS: 2.17±0.05), were enrolled from patients who attended the hospital for minor illnesses such as the common cold, conjunctivitis etc.

Adolescents were excluded if they had had any prior major illness, including type 1 or type 2 diabetes, if they were taking medication, or if they 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. The family history for obesity and diabetes was obtained using questionnaires. None of the patients had a family history of diabetes.

Methods

Anthropometric measurements were taken for all patients. The height and weight were measured with an empty bladder in post-absorptive 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 metres) squared. Patients with a body mass index of $\geq 95^{th}$ percentile according to reference curves for Turkish children were accepted as obese [12]. The degree of obesity was quantified using Cole's least mean-square method, which normalizes BMI-skewed distribution and expresses BMI as an SD score (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. Obesity was defined as a BMS-SDS $\geq$1.64. Their pubertal development stages were assessed by a paediatric endocrinologist using the Tanner scale criteria. All subjects were within Tanner stage II-IV. After the child had rested for at least five minutes and was in a sitting position, diastolic and systolic pressure (mmHg) measurements were taken, using a mercury-gravity manometer and a cuff appropriate for body size. The appropriate-sized cuff for a child was long enough to completely encircle the circumference of the arm (with or without overlap), and wide enough to cover approximately 75% of the upper arm between the top of the shoulder and the olecranon, leaving sufficient room, both at the antecubital fossa to place the bell of the stethoscope comfortably, and at the upper edge of the cuff to prevent obstruction of the axilla. The onset of a clear tapping sound (Korotkoff sounds) defined as phase I, corresponded to systolic blood pressure. Phase IV was used as the diastolic blood pressure.

Definition of polycystic ovary syndrome

The diagnosis of PCOS was established according to the Rotterdam criteria (oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries on ultrasonography scan, as well as exclusion of other etiologies that mimic the PCOS phenotype) [13]. A patient was considered to have PCOS if she fulfilled two out of the three criteria mentioned above. We assumed that a patient had clinical hyperandrogenism or hyperandrogenemia if she presented symptoms of hirsutism (more than eight points for the Freeman-Gallway score), with or without acne. The menses status and ovulation were defined in the following way: a patient was considered to have oligo/amenorrhea and anovulation if she had had fewer than six menses during the previous year. Exclusion of other disorders with similar clinical symptoms was undertaken using the appropriate tests. Ultrasound scans were performed for all patients. The morphology of polycystic ovaries was considered if there were 12 or more follicles of 2-9 mm in diameter in each ovary and/or an enlarged ovary ($>10\,cm^3$). Studies were performed in regularly cycling girls during the early follicular phase (three-five days) of their menstrual cycle, and in the PCOS group, three-five days after a spontaneous menses, or independent of cycle phase in the presence of amenorrhea. All analyses were performed after an overnight fast.

Blood samples

Fasting blood samples (at 8:00 AM) were obtained by venipuncture after an overnight fast (at least 12 h), to measure serum glucose and insulin levels, and other parameters. After clotting, the serum was separated and analysed immediately. Plasma glucose was determined by the glucose oxidase method. Plasma insulin was measured using the IMMULITE immunoassay (IMMULITE Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma concentrations of total cholesterol, triglycerides and high-density lipoprotein-cholesterol (HDL-cholesterol) were measured using routine enzymatic methods with the Olympus 2700 Analyzer. Low density lipoprotein cholesterol levels (LDL-cholesterol) were calculated using the Friedewald formula. Plasma adiponectin and vaspin levels were determined by radioimmunoassay (Linco Research, St. Charles, MO, USA). Determination of visfatin, apelin levels was performed by enzyme immunoassay (visfatin C-terminal [human] EIA; Phoenix Pharmaceuticals, Belmont, CA, USA). LH (sensitivity 0.07 mIU/mL; intra-assay CV 4.7%, inter-assay CV 6.3%), FSH (sensitivity 0.3 mIU/ml; intra-ssay CV 2.8%, inter-assay CV 4.6%), testosterone (sensitivity 0.35 nmol/L; intra-assay CV 7.8%, inter-assay CV 10.1%) and estradiol (sensitivity 10 pg/mL; intra-assay CV 7.2%, inter-assay CV 7.5%) were measured using a chemiluminescence method (ACS Chirone 180)

and serum sex hormone-binding globulin (SHBG) – by immunoradiometric assay (ZenTech, Angleur, Belgium), with a sensitivity below 0.3 nmol/L, and intra-assay and inter-assay CV 2.9%. The free androgen index was calculated as the serum testosterone (nmol/L) x 100/SHBG (nmol/L) ratio.

Insulin sensitivity indices

The "gold standard" for measuring insulin sensitivity is the euglycemic-hyperinsulinemic clamp technique. Another common method is to use a frequently sampled intravenous glucose tolerance test, with the minimal model assessment of insulin sensitivity. Unfortunately, neither of these two methods is feasible in the pediatric age group. Previous studies have evaluated simple indices for assessing insulin sensitivity in a wide range of conditions associated with insulin resistance. In a study of prepubertal and pubertal obese children and adolescents, HOMA-IR and QUICKI correlated significantly with indices derived from the glycemic and insulinemic responses to an OGTT [14]. Vaccaro *et al*. recently reported that QUICKI performed no better than HOMA-IR and fasting insulin as surrogate measures of insulin resistance in the adult population [15]. We have previously reported that HOMA-IR was more reliable than FGIR and QUICKI as a measure of insulin resistance among children and adolescents [16].

The insulin sensitivity index was derived from fasting blood samples. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting insulin concentration (μU/mL) × fasting glucose concentration (mmol/L)/22.5 [12]. Insulin resistance in adolescents is defined as levels of the homeostasis model assessment for insulin resistance (HOMA-IR) greater than 3.16 [17].

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 10.1 (SPSS, Chicago, IL, USA) was used for analysis.

RESULTS

The characteristics of the 48 PCOS adolescents and 37 control subjects are summarized in *table 1*. The PCOS group included 48 obese patients and the control group included 37 sex-, age- and pubertal stage-matched, obese adolescents without PCO. Both the PCOS group and control group showed no significant differences in terms of age, total cholesterol or LDL-cholesterol.

Table 1
Clinical and laboratory characteristics of the study population.

	Adolescents with PCOS	**Controls**	**p**
N	48	37	
Age (years)	16.9 ± 0.3	17.2 ± 0.2	NS
Weight (kg)	85 ± 19.8	75.6 ± 15.8	<0.001
Height (cm)	155.2 ± 11.3	152.3 ± 13.1	NS
BMI (kg/m^2)	35.1 ± 4.3	30.7 ± 2.2	<0.001
BMI-SDS	2.31 ± 0.14	2.17 ± 0.05	<0.001
Systolic blood pressure (mmHg)	120.9 ± 14	108.7 ± 11	<0.001
Diastolic blood pressure (mmHg)	78.1 ± 11.9	74.3 ± 9.7	NS
Lipids			
Total cholesterol (mg/dL)	175 ± 36	166 ± 25	0.17
Triglycerides (mg/dL)	129 ± 70	82 ± 41	0.001
LDL-cholesterol (mg/dL)	105 ± 32	93 ± 24	0.059
HDL-cholesterol (mg/dL)	45 ± 9	55 ± 13	<0.001
Fasting glucose (mg/dL)	89.8 ± 12.9	89.20 ± 7.47	NS
Fasting insulin (μU/mL)	32.2 ± 23.0	11.05 ± 2.9	<0.001
LH (mIU/mL)	11.65 ± 6.1	6.59 ± 4.5	p<0.05
Testesterone (nmol/L)	2.66 ± 0.8 .	1.75 ± 0.4	p<0.05
SHBG (nmol/L)	47.1 ± 28.1	69.2 ± 33.7	NS
Free androgen index	4.8 ± 2.6	2.6 ± 1.4	p<0.05
HOMA-IR	4.99 ± 2.08	1.47 ± 0.7	<0.001
Adipocytokines			
Vaspin (μg/L)	0.75 ± 1.04	0.56 ± 0.33	NS
Apelin (ng/mL)	2.22 ± 1.15	0.58 ± 0.16	<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 means±SD. difference at p<0.05 level.
HOMA-IR: homeostasis model assessment for insulin resistance (fasting insulin (μU/mL) X fasting glucose (mg/dL)/405. BMI-SDS: body mass index-standard deviation score.

There was no significant difference between the PCOS group and the control group as regards BMI-SDS (2.31±0.14 *versus* 2.17 ±0.05). However; triglyceride levels (129±70 *versus* 82±41 mg/dL, p: 0.001) were significantly elevated, and HDL-cholesterol levels (45±9 *versus* 55±13) were significantly lower in the PCOS group.

Serum LH, testosterone levels and the free androgen index (11.65±6.1 *versus* 6.59±4.5 mIU/mL, 2.66±0.8 *versus* 1.75±0.4 nmol/L and 4.8±2.6 *versus* 2.6±1.4, p<0.05; respectively) were found to be significantly higher in the PCOS group than in the control group.

HOMA-IR, apelin and visfatin levels (4.9±2 *versus* 1.4±0.7, p<0.001; 2.2±1.1 *versus* 0.58±0.1 ng/mL, p<0.001; 31.3±11.1 *versus* 18.5±10.7 ng/mL, p<0.001; respectively) were significantly elevated, and adiponectin levels (2.01±1.02 *versus* 12.5±6.2 μg/mL, p<0.001) were significantly lower in the PCOS group. Vaspin levels (0.75±1.04 *versus* 0.56±0.33 μg/L) were higher in the PCOS group than in the control group, although the difference was not significant.

Table 2 shows a correlation between adipocytokines and BMI-SDS and HOMA-IR. Apelin correlated positively with BMI-SDS (r: 0.34, p: 0.02) and HOMA-IR and r: 0.86, p<0.001).Visfatin also correlated positively with BMI-SDS (r: 0.61, p<0.001) and HOMA-IR (r: 0.63, p<0.001), and adiponectin correlated negatively with BMI-SDS (r: -0.46, p: 0.002) and HOMA-IR (r:-0.44, p: 0.004).

DISCUSSION

Studies performed during the last decade have indicated that adipose tissue is not only a site for triglyceride storage, but also a source of several biologically active mediators, including leptin, tumor necrosis factor-α, adiponectin, apelin, visfatin, vaspin, acylation-stimulating protein, resistin, interleukin-6, plasminogen activator inhibitor-1, and transforming growth factor-b [18, 19].

In this study, we assessed the role of the adipcytokines in PCOS, and compared the levels of cytokines derived from adipose tissue in adolescents with PCOS and control adolescents without PCOS. Thus, we found significantly higher visfatin, apelin and HOMA-IR levels and lower adiponectin levels in the PCOS group than in the control group. Therefore, these data support the idea that the high levels of adipocytokines related to insulin resistance, may have a role in the early development of PCOS in obese adolescents.

Adipocytokines are an exciting new link between PCOS and insulin resistance, but also obesity and cardiovascular disease, hypertension, as well as hyperlipidemia [20]. As in our report, Toulis *et al*. and Yılmaz *et al*. reported that adiponectin levels seem to be lower in women with PCOS compared with non-PCOS controls, and low levels of adiponectin in PCOS are probably related to insulin resistance [21, 22]. In contrast to other reports, Arikan *et al*. found higher adiponectin levels in non-obese, young women with PCOS, however, they also reported no significant changes in circulating resistin levels in non-obese young women with PCOS [23].

Berndt *et al*. showed that plasma visfatin correlates significantly with percentage body fat, body mass index, and the 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 non-diabetic subjects [24]. In two recent studies [25, 26], plasma visfatin levels were higher in patients with type 2 diabetes mellitus than in normoglycemic controls. However, it was unclear if the higher visfatin levels were associated with the diabetes itself or with the greater amount of visceral adipose tissue in diabetic subjects [26]. Our findings showed that there were higher visfatin levels and lower adiponectin levels in the PCOS group than in the control group. Taken together, these findings suggest that visfatin might play a role in the pathogenesis of PCOS, reflecting the study by Kowalska *et al.* [27].

Recently, apelin mRNA and protein were identified in adipocytes and vascular stromal fractions isolated from mouse and human subcutaneous adipose tissue [28].

Table 2
Correlation of adipocytokines and lipids with BMI-SDS and HOMA-IR.

	BMI-SDS		**HOMA-IR**	
	r	**p**	**r**	**p**
Lipids				
Total cholesterol (mg/dL)	0.57	0.54	0.1	0.64
Triglycerides (mg/dL)	0.04	0.63	0.16	0.22
LDL-cholesterol (mg/dL)	0.52	0.36	0.13	0.4
HDL-cholesterol (mg/dL)	-0.06	0.69	-0.11	0.48
Fasting glucose (mg/dL)	0.42	<0.001	0.44	<0.001
Fasting insulin (μU/mL)	0.61	<0.001	0.58	<0.001
LH (mIU/mL)	0.25	0.4	0.13	0.58
SHBG (nmol/L)	0.23	0.3	0.19	0.34
Free androgen index	0.52	<0.001	0.49	<0.001
HOMA-IR	0.86	<0.001		
Adipocytokines				
Vaspin (μg/L)	0.28	0.3	0.03	0.61
Apelin (ng/mL)	0.34	0.02	0.86	<0.001
Adiponectin (μg/mL)	-0.46	0.002	-0.44	0.004
Visfatin (ng/mL)	0.61	<0.001	0.63	<0.001

Apelin transcripts and immunoreactivity are expressed in the central nervous system and in various peripheral tissues, including the heart, lung, and mammary gland [29, 30]. Widespread expression of apelin in peripheral tissues is associated with its synthesis by endothelial cells [31]. Hosoya *et al*. showed that plasma apelin levels markedly increase in insulin resistance and hyperinsulinemia [9]. In this study, there were higher apelin levels in the PCOS group than in the control group. Additionally, apelin and visfatin levels correlated positively and adiponectin levels negatively with HOMA-IR levels.

Vaspin is a newly described adipocytokine expressed predominantly in visceral, white adipose tissues. In several studies, serum vaspin levels were shown to decrease with a worsening of diabetes and body weight loss, whereas levels could be normalized by insulin or pioglitazone treatment [32]. In the present study, there were no significant differences in vaspin levels between controls and PCOS patients. Several studies involving vaspin have found no significant relationship with the pathophysiology of PCOS and insulin resistance. However, the role of various adipokines as connectors between PCOS and insulin resistance has been better elucidated in recent years.

Based on the findings of this study, apelin, visfatin and adiponectin levels could be used as specific markers for insulin sensitivity, and these adipokines may contribute to the pathogenesis of PCOS. We know that PCOS is a complex and heterogeneous disorder, and it is suggested that the post-receptor defect in insulin signaling might be caused by a plasma-derived factor that could activate the serine kinase of the insulin receptor substrate and, in that way, inhibit insulin action.

Disclosure. *None of the authors has any conflict of interest or financial support to disclose.*

REFERENCES

1. Dunaif A, Segal KR, Futterweit W, *et al*. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* 1989; 38: 1165-74.

2. Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. *Trends in Endocrinology and Metabolism* 2002; 13: 84-9.

3. Gil-Campos M, Canete R, Gil A. Adiponectin, the missing link in insulin resistance and obesity. *Clin Nutr* 2004; 23: 963-74.

4. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; 37: 1595-607.

5. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. *Obesity Reviews* 2005; 6: 13-21.

6. Weyer C, Funahashi T, Tanaka S, *et al*. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hypersulinemia. *Journal of Clinical Endocrinology and Metabolism* 2003; 86: 1930-5.

7. Punthakee Z, Delvin EE, O'Loughlin J, *et al*. Adiponectin, adiposity and insulin resistance in children and adolescents. *J Clin Endocrinol Metab* 2006; 91: 2119-25.

8. Gil-Campos M, Canete R, Gil A. Adiponectin, the missing link in insulin resistance and obesity. *Clin Nutr* 2004; 23: 963-74.

9. Hosoya M, Kawamata Y, Fukusumi S, *et al*. Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J Biol Chem* 2000; 275: 21061-7.

10. Klöting N, Berndt J, Kralisch S, *et al*. Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. *Biochem Biophys Res Commun* 2006; 339: 430-6.

11. Youn BS, Klöting N, Kratzsch J, *et al*. Serum vaspin concentrations in human obesity and type 2 diabetes. *Diabetes* 2008; 57: 372-7.

12. Ozturk A, Mazicioglu MM, Hatipoglu N, *et al*. Reference body mass index curves for Turkish children 6 to 18 years of age. *J Pediatr Endocrinol Metab* 2008; 9: 827-36.

13. Kauffman RP, Baker TE, Baker VM, DiMarino P, Castracane VD. Endocrine and metabolic differences among phenotypic expressions of polycystic ovary syndrome according to the 2003 Rotterdam consensus criteria. *Am J Obstet Gynecol* 2008; 198: 670.e1-7.

14. Guzzaloni G, Grugni G, Mazzilli G, Moro D, Morabito F. Comparison between beta-cell function and insulin resistance indexes in prepubertal and pubertal obese children. *Metabolism* 2002; 51: 1011-6.

15. Vaccaro O, Masulli M, Cuomo V, *et al*. Comparative evaluation of simple indices of insulin resistance. *Metabolism* 2004; 53: 1522-6.

16. Atabek ME, Pirgon O. Assessment of insulin sensitivity from measurements in fasting state and during an oral glucose tolerance test in obese children. *J Pediatr Endocrinol Metab* 2007; 20: 187-95.

17. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in men. *Diabetologia* 1985; 28: 412-9.

18. Rajala MW, Scherer PE, Minireview: PE. The adipocyte—at the crossroads of energy homeostasis, infl ammation, and atherosclerosis. *Endocrinology* 2003; 144: 3765-73.

19. Peterson MC. Circulating transforming growth factor beta-1: a partial molecular explanation for associations between hypertension, diabetes, obesity, smoking and human disease involving fibrosis. *Med Sci Monit* 2005; 11: RA229-32.

20. Chen MP, Chung FM, Chang DM, *et al*. Elevated plasma level of visfatin/pre-B cell colonyenhancing factor in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2006; 91: 295-9.

21. Toulis KA, Goulis DG, Farmakiotis D, *et al*. Adiponectin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. *Hum Reprod Update* 2009; 15: 297-307.

22. Yilmaz M, Bukan N, Demirci H, *et al*. Serum resistin and adiponectin levels in women with polycystic ovary syndrome. *Gynecol Endocrinol* 2009; 25: 246-52.

23. Arikan S, Bahceci M, Tuzcu A, Kale E, Gökalp D. Serum resistin and adiponectin levels in young non-obese women with polycystic ovary syndrome. *Gynecol Endocrinol* 2010; 26: 161-6.

24. Berndt J, Kloting N, Kralisch S, *et al*. Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes* 2005; 54: 2911-6.

25. Hammarstedt A, Pihlajamaki J, Sopasakis VR, *et al*. Visfatin is an adipokine but it is not regulated by thiazolidinediones. *J Clin Endocrinol Metab* 2006; 91: 1181-4.

26. Chen MP, Chung FM, Chang DM, *et al*. Elevated plasma level of visfatin/pre-B cell colonyenhancing factor in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2006; 91: 295-9.

27. Kowalska I, Straczkowski M, Nikolajuk A, *et al*. Serum visfatin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome. *Human Reproduction* 2007; 22: 1824-9.

28. Boucher J, Masri B, Daviaud D, *et al*. Apelin, a newly identifi ed adipokine up-regulated by insulin and obesity. *Endocrinology* 2005; 146: 1764-71.

29. Habata Y, Fujii R, Hosoya M, *et al*. Apelin, the natural ligand of the orphan receptor APJ, is abundantly secreted in the colostrum. *Biochim Biophys Acta* 1999; 1452: 25-35.

30. O'Carroll AM, Selby TL, Palkovits M, Lolait SJ. Distribution of mRNA encoding B78/apj, the rat homologue of the human APJ receptor, and its endogenous ligand apelin in brain and peripheral tissues. *Biochim Biophys Acta* 2000; 1492: 72-80.

31. Kleinz MJ, Davenport AP. Immunocytochemical localization of the endogenous vasoactive peptide apelin to human vascular and endocardial endothelial cells. *Regul Pept* 2004; 118: 119-25.

32. K. Hida, J. Wada, J. Eguchi, H. et al. Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. *Proc Natl Acad Sci USA* 2005; 102: 10610-5.