RESEARCH ARTICLE

Impact of treatment with metformin on adipokines in patients with polycystic ovary syndrome

İlhan Tarkun[1], Emre Dikmen[2], Berrin Çetinarslan[1], Zeynep Cantürk[1]

[1] Department of Endocrinology and Metabolism
[2] Department of Internal Medicine, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey

Correspondence: İ. Tarkun. Kocaeli University, Faculty of Medicine, Department of Endocrinology and Metabolism, Kocaeli, Turkey
<ilhantarkun@superonline.com>

Accepted for publication July 30, 2010

ABSTRACT. *Background*. Adipose tissue synthesizes various adipokines such as resistin, adiponectin and visfatin, which have an effect on insulin resistance. This study was designed to show the effect of metformin, one of the most important drugs used to reduce insulin resistance in patients with polycystic ovary syndrome (PCOS), on these adipokines. *Methods*. The study group consisted of 24 women with PCOS and 25 healthy, age- and weight-matched, normally menstruating women. Hormone and lipid profiles, visfatin, adiponectin and resistin were measured in all cases, before and after metformin treatment. *Results*. Serum visfatin levels were found to be significantly higher in patients with PCOS, compared to controls. Following metformin treatment, a significant decrease was observed in visfatin levels compared to the baseline. A positive correlation was found between serum visfatin levels and BMI, waist circumference, HOMA, insulin and triglyceride levels. No statistically significant difference was observed in terms of serum adiponectin levels in women with PCOS before and after treatment, or in healthy controls. Serum resistin levels were significantly reduced by metformin treatment. *Conclusion*. These findings suggest that visfatin may be related to the obesity and insulin resistance that is frequently encountered in patients with PCOS. A reduction in serum visfatin and resistin levels was shown with metformin treatment, in patients with PCOS.

Keywords: adipokines, PCOS, insulin resistance, metformin

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder experienced by females of reproductive age. There are many aspects to the pathophysiology of PCOS. Among them is insulin resistance and its resultant hyperinsulinemia [1, 2]. Hyperinsulinemia has been shown to be one of the causes of the excess hyperandrogenemia seen in this condition. For this reason, insulin-sensitizing drugs have been used for many years in patients with PCOS, and they have significantly improved the metabolic state, ovulatory dysfunction and fertility rates in sufferers [3].

Adipose tissue not only has the capacity to store large quantities of fat as an energy source, it also synthesizes various adipokines including leptin, resistin, adiponectin and visfatin, which have effects on insulin resistance. In 2005, Fukaharo *et al.* defined visfatin as a pre-B colony enhancing factor (PBEF) [4]. Examination of visfatin's glucose-lowering effect was undertaken. A significant decrease in plasma glucose levels was observed when recombinant visfatin was administered to C57BL/6J mice. This effect was dose-dependent, although no change occurred in concurrent plasma insulin levels. In summary, a good deal of evidence now exists to show that visfatin and insulin share common *in vivo* and *in vitro* features.

Adiponectin is a protein hormone produced exclusively in adipose tissue where circulating levels correlate positively with levels of insulin sensitivity [5, 6]. Resistin is also a peptide secreted by adipose tissue and is assumed to contribute to peripheral insulin sensitivity [7].

The purpose of this study was to explore whether treatment of patients with PCOS with metformin has an impact on adipokines such as visfatin, adiponectin and resistin.

DONORS AND METHODS

The study group consisted of 24 women with PCOS, and 25 healthy, age- and weight-matched, normally menstruating women. The Local Research Ethics Committee approved the study, and all patients involved gave their informed consent. Study groups were composed of patients between 17-40 years old, who had been admitted to endocrinology outpatient polyclinics with complaints of irregular menstrual cycles and/or increased hair growth. The diagnosis of PCOS was established according to the 2003 Rotterdam ESHR/ASRM endocrine criteria (oligo-ovulation and/or anovulation, clinical and/or biochemical hyperandrogenism and polycystic ovaries

doi: 10.1684/ecn.2010.0217

as defined by ultrasonography). PCOS can be diagnosed after the exclusion of other medical conditions and if two out of the three criteria mentioned above are met. Patients with systemic diseases (diabetes mellitus, thyroid disease, hypertension, cardiovascular diseases, chronic renal failure, and malignancy) were excluded from the study. Any patient with a history of taking any other medication such as lipid lowering drugs, oral contraceptive pills, ovulation induction products, anti-obesity drugs, corticosteroids, anti-diabetic and anti-hypertensive drugs within the previous six months were also excluded. Before entering the study, a physical examination and appropriate laboratory tests were performed. After overnight fasting, a 75 gram, oral glucose tolerance test was performed for all patients, and 120 min values obtained. Any patients with diabetes (glucose in 120 min of 75-gram OGTT ≥ 200 mg/dL) were excluded from study. Diseases that mimic PCOS, such as late-onset, congenital adrenal hyperplasia and Cushing's syndrome were ruled out by testing for 17-hydroxyprogesterone, and the 1 mg dexamethasone suppression screening test. All patients had normal thyroid function tests and normal prolactin levels. Hirsutism was graded using the Ferriman and Gallwey scoring system.

All of the women were treated with metformin, 850 mg twice a day, for six months. Patients were seen every eight weeks for verification of compliance, and assessment of side effects. After six months of treatment, the women were admitted to the clinical research center, where all clinical examination and laboratory evaluations were repeated. The control group was composed of healthy female volunteers who had regular menstrual cycles and no signs of clinical or biochemical hyperandrogenism. The PCOS and control groups were matched for body mass index (BMI) and age. The BMI was calculated as body weight in kilograms divided by height in meters squared (kg/m^2) at first admission. The waist circumference was measured at the widest circumference. Most of the women in the PCOS or control group were either obese or overweight, so both groups were proposed similar diets and exercise programs.

Serum samples were obtained from all women during the interval between the second to the fifth day of the early follicular phase of the menstrual cycle. The plasma levels of glucose, insulin, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), free and total testosterone, LH, FSH, prolactin, free T4, free T3, TSH, cortisol, dehydroepiandrosterone-sulfate (DHEA-SO4), androstenedion, 17-OH progesterone, estradiol (E2), sex-hormone binding globulin (SHBG), visfatin, adiponectin and resistin were measured after 8-12 hours fasting. Blood samples were taken from an antecubital vein. All parameters, apart from visfatin, adiponectin and resistin, were measured immediately. The blood samples were centrifuged at 4000 rpm for ten minutes, separated and stored at - 80°C until analyzed for visfatin, adiponectin and resistin.

Laboratory analyses

Visfatin-C concentration was measured using an enzyme-linked immunosorbent assay (ELİSA); a Visfatin C terminal (Human) EIA (Catalog No: EK-003-80 Phoenix Pharmaceuticals, Inc., California, USA) kit. Adiponectin was measured using an enzyme-linked immunosorbent assay (ELISA); an AssayMax Human Adiponectin (Acrp30) ELISA (Catalog EA2500-1 Lot 0201815 AssayPro, USA) kit. Resistin was measured using an AssayMax Human Resistin ELISA (Catalog ER 1001 Lot 0257822 AssayPro, USA) kit. Glucose, TC, HDL, TG, LDL were analyzed with an Aeroset analyzer using an Abbott Diagnostics, Wiesbaden, Germany kit. Insulin, free T4, free T3, TSH, cortisol, prolactin, FSH, LH, DHEA-SO4, E2, total testosterone levels measured using an electrochemiluminescent immunometric assay test method with a Cobas analyzer (Roche Diagnostics, Mannheim, Germany). SHBG levels were measured using a chemiluminescent immunometric assay test method with an Immulite 2000 analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, USA). Free testosterone, androstenedion levels were measured using an enzyme-linked immunosorbent assay (ELISA). The 17-OH progesterone level was measured using an enzyme-immune assay method with a Dynex-Dsx analyzer. Insulin resistance (IR) was determined using a number of different methods including fasting insulin, the homeostasis model assessment (HOMA), and the quantitative insulin sensitivity check index (QUICKI). The estimate of insulin resistance, the HOMA-IR score, was calculated using the formula: fasting serum insulin (μU) x fasting plasma glucose (mg/dL/405. QUICKI was derived by calculating the inverse sum of logarithmically expressed values of fasting insulin and glucose.

Ultrasonography

Transvaginal and/or transabdominal ultrasonography was performed in all patients. The morphology of the polycystic ovaries was considered if there were 12 or more follicles of 2-9 mm diameter in each ovary, and/or an enlarged ovary (> 10 cm^3).

Statistical analysis

The Statistical Package for the Social Science (SPSS version 13.0 for Windows, SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The person performing the data analysis was blind to diagnosis. Results were expressed as mean ± SD. The characteristics of distribution were tested using the Kolmogorov-Smirnof test. Because of skewed distribution insulin, testosterone and adiponectin levels, we used log-transformed values in the subsequent statistical analysis. The clinical and laboratory characteristics for the two groups were compared using Student's t-test for unpaired data in a normally distributed group. Undistributed groups were compared using the Mann-Whitney U test. For all analyses, a p-value less than < 0.05 was considered statistically significant. Bivariate correlation analysis (calculation of the Pearson coefficient) was used to assess the correlation of serum visfatin, adiponectin and resistin levels with each parameter.

RESULTS

Twenty-four female patients diagnosed with PCOS, and 25 healthy women of compatible age and BMI were

Table 1
Anthropometric, biochemical and hormonal data for women diagnosed with PCOS before and after treatment, and in healthy controls

	PCOS Before (n = 24)	**PCOS After (n = 24)**	**Controls (n = 25)**	**P1**	**P2**
Age	25.21 ± 5.99	25.21 ± 5.99	24.25 ± 3.76	NS	0.551
BMI (kg/m^2)	31.69 ± 6.52	30.7 ± 6.65	31.35 ± 6.31	0.777	0.864
Waist (cm)	100.21 ± 14.74	98.44 ± 15.26	100.9 ± 17.38	0.129	0.937
WHR	0.9 ± 0.07	0.91 ± 0.06	0.89 ± 0.08	0.659	0.458
Ferriman-Gallwey score	10.7 ± 1.7	8.6 ± 1.72	-	0.03	-
f-glucose (mg/dL)	92 ± 9.50	88.94 ± 6.72	88.45 ± 6.10	0.493	0.810
f-insulin (μIU/mL)	10.99 ± 4.57	7.75 ± 4.03	6.09 ± 2.09	0.002	0.001
HOMA-IR	2.94±1.76	1.61 ± 1.12	1.24 ± 1.27	0.03	0.02
QUICKI	0.35 ± 0.02	0.37 ± 0.02	0.34 ± 0.02	0.05	0.09
Cholesterol (mg/dL)	177 ± 32.31	181.31 ± 24.33	159.40 ± 18.80	0.407	0.002
TG (mg/dL)	120.73 ± 68.80	118.42 ± 65.12	79.65 ± 39.75	0.760	0.007
HDL-C (mg/dL)	52.66 ±10.40	53.36 ± 12.97	50 ± 9.93	0.572	0.390
LDL-C (mg/dL)	100.08 ± 27.66	102.26 ± 18.08	93.47 ± 16.69	0.629	0.123
LH (mIU/mL)	13.51 ± 11.01	10.06 ± 6.01	5.34 ± 1.82	0.344	0.002
FSH (mIU/mL)	5.61 ± 1.66	5.67 ± 1.28	6.48 ± 1.65	0.481	0.098
LH/FSH	2.4 ± 1.74	1.91 ± 1.27	0.85 ± 0.32	0.305	0.001
DHEA-S (mcg/dL)	288.57 ± 110.05	270.72 ± 113.64	169.48 ± 69.38	0.387	0.002
Estradiol (pg/mL)	97.71 ± 105.86	72.15 ± 58.26	73.26 ± 70.13	0.314	0.808
SHBG (nmol/L)	23.71 ± 12.32	25.77 ± 14.29	40.91 ± 21.21	0.273	0.013
Testosterone (ng/dL)	76.44 ± 29.36	61.43 ± 31.03	33.08 ± 13.57	0.038	0.000
Free testosterone (pg/mL)	3.55 ± 1.53	2.89 ± 2.69	2.39 ± 1.61	0.059	0.08
Androstenedion (ng/mL)	7.27 ± 5.56	3.58 ± 2.89	2.36 ± 0.93	0.05	0.05

Data are presented as mean ± SD.
P1: for the difference between respective PCOS patients before and after treatment.
P2: for the difference between respective PCOS patients and control group.

included in the study. *Table 1* shows anthropometric, biochemical and hormonal data for the women diagnosed with PCOS before and after treatment, and for the healthy controls. As expected, serum insulin, HOMA, total testosterone, free testosterone and androstenedione levels in the PCOS group were found to be significantly higher compared to the controls. Following a six-month treatment with metformin in patients with PCOS, the hirsutism score and the serum fasting insulin level decreased significantly. Also, statistically significant decreases were demonstrated in total testosterone and androstenedione levels.
Table 2 shows serum visfatin, adiponectin and resistin levels in women diagnosed with PCOS before and after treatment, and in healthy controls. Serum visfatin levels were found to be significantly higher in patients with PCOS, compared to controls (129.49 ± 152.97 ng/mL *vs* 25.70 ± 14.06 ng/mL). Following metformin treatment, a significant decrease was observed in visfatin levels compared to the baseline (129.49 ± 152.97 ng/mL *vs* 48.22 ± 33.49). A positive correlation was found between serum visfatin levels, BMI, waist circumference, HOMA, insulin and triglyceride levels *(table 3)*. There was a negative correlation with QUICKI.
No statistically significant difference was observed in terms of serum adiponectin levels in women with PCOS before and after treatment, or in healthy controls (23.01 ± 13.78 ng/mL, 28.06 ± 11.43 ng/mL and 28.00 ± 14.25 ng/mL, respectively). Serum-resistin levels were significantly reduced by metformin treatment. A negative correlation was found between serum adiponectin levels, fasting insulin, triglyceride levels and HOMA-IR. A positive correlation was observed between resistin, BMI, and waist circumference, and a negative correlation with HDL-cholesterol levels.

DISCUSSION

PCOS is a syndrome, the etiology of which remains controversial. It is frequently observed in reproductive-age

Table 2
Serum visfatin, adiponectin and resistin levels in women diagnosed with PCOS before and after treatment, and in healthy controls

	PCOS Before (n = 24)	**PCOS After (n = 24)**	**Controls (n = 25)**	**P1**	**P2**
Visfatin (ng/mL)	129.49 ± 152.97	48.22 ± 33.49	25.70 ± 14.06	**0.014**	**0.023**
Adiponectin (ng/mL)	23.01 ± 13.78	28.06 ± 11.43	28.00 ± 14.25	0.057	0.087
Resistin (ng/mL)	1.479 ± 0.474	1.277 ± 0.32	1.61 ± 0.79	**0.007**	0.134

Data are presented as mean ± SD.
P1: for the difference between respective PCOS patients before and after treatment.
P2: for the difference between respective PCOS patients and control group.

Table 3
Relationship between serum adiponectin, resistin and visfatin and anthropometric biochemical and hormonal parameters in patients with PCOS.

	Adiponectin		Resistin		Visfatin	
	r	p	r	p	r	p
Age (years)	0.025	0.887	0.105	0.267	0.034	0.769
BMI (kg/m^2)	- 0.217	0.098	0.288	**0.034**	0.499	**0.001**
Waist circumference	- 0.145	0.199	0.305	**0.024**	0.405	**0.003**
İnsulin (μU/mL)	- 0.345	**0.008**	0.185	0.177	0.264	**0.039**
HOMA-IR	- 0.338	**0.012**	0.218	0.110	0.289	**0.034**
QUICKI	0.317	**0.015**	- 0.297	0.106	- 0.290	**0.032**
Total cholesterol (mg/dL)	- 0.156	0.310	0.029	0.836	- 0.065	0.603
LDL-C (mg/dL)	- 0.167	0.098	0.185	0.177	- 0.139	0.312
HDL-C (mg/dL)	0.126	0.312	- 0.453	**0.001**	- 0.124	0.342
Triglyceride (mg/dL)	- 0.276	**0.041**	0.216	0.113	0.340	**0.02**
LH (mIU/mL)	0.015	0.887	- 0.020	0.885	- 0.140	0.298
FSH (mIU/mL)	- 0.066	0.603	- 0.104	0.449	0.189	0.254
Total testosterone(ng/dL)	- 0.163	0.256	- 0.133	0.333	0.043	0.765
SHBG (nmol/L)	0.308	**0.022**	- 0.234	0.086	- 0.139	0.287

r: correlation coefficient.

women, accompanied by hyperandrogenism, insulin resistance and increased type 2 DM. Following the discovery of the hormone leptin in 1994, it has been confirmed that fatty tissue not only regulates energy metabolism in our body, it also releases many biological molecules collectively referred as adipo(cyto)kines, which contribute to peripheral insulin sensitivity. The role of adipokines in the pathogenesis of the important features of PCOS, such as insulin resistance and central obesity, has attracted much attention. Studies are proceeding currently on the effect and benefit of drugs used in the treatment of this syndrome with regard to reducing insulin resistance. This study was designed to show the effect of metformin, one of the most important drugs used to reduce insulin resistance in patients with PCOS, on these adipokines.

Recent studies have demonstrated that visfatin, an adipokine secreted from visceral fatty tissue, could influence insulin sensitivity. In fact in several studies, high visfatin levels were found in patients with type 2 DM and gestational DM [8, 9]. The effects of visfatin in PCOS have attracted attention as a result of the identification of its insulin-like effects. In our study, serum visfatin levels were found to be significantly higher in patients with PCOS compared to controls. In addition, in six case-control studies previously conducted in patients with PCOS, it was found that plasma/serum visfatin levels were significantly higher than in a BMI-matched, healthy control group [10-15].

The actual reason for the increased visfatin levels in patients with PCOS has not been clearly established. In several previous studies, plasma visfatin levels in patients with normal weight and PCOS were found to be higher than those in the control group with normal weight [11, 13, 14]. Tan *et al.* found results similar to those of our study, that plasma visfatin levels in overweight and obese patients with PCOS were higher than in the control group [10]. In the same study, in parallel with plasma visfatin levels, visfatin mRNA expression in subcutaneous and omental fatty tissue was demonstrated to be significantly higher than that found in the control group.

Controversial results have been obtained in studies investigating the relationship between obesity and the plasma visfatin level. Haider *et al.* found significantly higher plasma visfatin levels in obese subjects relative to individuals of normal weight, however, Pagano *et al.* demonstrated lower levels [16, 17]. Similarly, the relationship between visfatin and BMI, waist circumference and waist/hip ratio is also controversial [11, 12, 14, 17, 18]. In our study, there was a positive correlation between visfatin levels in patients with PCOS, and BMI, waist circumference, insulin levels and the HOMA index. Similarly, Chan *et al.* demonstrated a positive correlation between plasma visfatin levels in PCOS patients and BMI [12].

Metformin has been used for more than 10 years in the treatment of PCOS. Metformin regulates insulin resistance in peripheral tissues and reduces insulin release. Özkaya *et al.* conducted a study, investigating the relationship between metformin treatment and serum visfatin levels in patients with PCOS [19]. In this study, a significant decrease was observed in visfatin levels following a three-month treatment of patients with PCOS, similar to that observed in our study. Conversely, Steiner *et al.* showed that serum visfatin levels in PCOS patients were increased after metformin treatment [20]. However, there are several inconsistencies in this study. For example, insulin resistance as measured by HOMA-IR did not improve with metformin, in contrast to a number of other studies, and interestingly, visfatin levels did not change in the rosiglitazone treatment arm. Heider *et al.* showed that insulin and glucose levels over phosphotidyl inositol 3 kinase and protein kinase B pathways directly affected visfatin levels [21]. The decrease in visfatin observed in patients with PCOS may be the result of a reduction in insulin resistance and insulin levels. The effect of a reduction in body weight and fatty tissue in these results cannot be overlooked.

Adiponectin is possibly the most important adipokine. The reason for this is that it is the only cytokine synthesized in and released from fatty tissue, and it has well established, anti-atherogenic, anti-inflammatory and insulin-sensitizer properties. It is known that adiponectin levels are decreased significantly in obese subjects as compared to subjects of normal weight [22]. Moreover, it has been found that serum adiponectin concentrations are inversely related to the severity of insulin resistance [23]. The relationship between PCOS, obesity and insulin resistance, the role of adiponectin in the pathogenesis of PCOS, and whether or not there is any relationship to the treatment of insulin resistance in PCOS, have attracted our attention.

Studies on adiponectin levels in PCOS patients have produced some controversial results. Women with PCOS generally show hypoadiponectemia. Authors have suggested that obesity, insulin resistance or hyperandrogenemia may be the cause of this hypoadiponectemia in this patient group. In our study, adiponectin levels were found to be lower in the PCOS group than in the control group. However, this decrease did not reach the statistical significance. In several previous studies, serum adiponectin levels were found to be similar in patients with PCOS and a BMI-matched control group, which further supported the results of our study [24-28]. The lack of any significant difference in serum adiponectin levels between PCOS patients and control groups with similar BMI has led to the suggestion that adiponectin has no direct role in the pathogenesis of PCOS [24-27]. Studies on adiponectin levels in PCOS patients following metformin treatment have also had controversial results. In some studies, serum adiponectin levels increased significantly after metformin treatment in PCOS patients [28, 29]. In other studies, an increase in serum adiponectin was observed with metformin treatment, but this increase was not statistically significant, as in our study [30, 31]. In the present study, we found a negative correlation between adiponectin levels and serum insulin and HOMA, and a positive correlation with QUICKI.

Resistin is an adipokine thought to be released in large amounts from macrophages, as well as from human fatty tissue: the relationship between resistin and obesity and insulin resistance has yet to be clarified. This molecule is regarded as a hormone that facilitates insulin resistance as it has been demonstrated that while serum resistin level increase in obese mice, the anti-diabetic agent rosiglitasone decreased levels, and that administration of recombinant resistin to mice caused impairment in glucose tolerance and the insulin effect. Therefore, Steppan *et al.* named this new hormone resist+in[sulin] [7]. Although there are studies supporting the relationship between resistin, obesity and insulin resistance, there are also studies reporting contrary views [32-35].

As in many other studies, our study could not determine any difference between BMI-matched PCOS patients and control groups in terms of serum resistin levels. In our study, a linear relationship was determined in the group of patients with PCOS between serum resistin levels and BMI and waist circumference, and this finding was compatible with those in the literature. Seow *et al.*, found not only that serum resistin levels in patients with PCOS were similar to that of a control group, they also determined that resistin mRNA expression in adipocytes was two-fold higher in the group of patients with PCOS than the control group. In the light of this finding, they suggested that resistin might exert a local paracrine effect in obesity and insulin resistance in PCOS [36].

Steiner *et al.* investigated the effect of metformin and rosiglitazone treatment on resistin levels in patients with PCOS [20]. They showed no changes in resistin levels at the end point in any of the treatment arms. However, in this study, resistin levels were very low at baseline and insulin resistance did not improve after treatment. This study is the first to show that treatment with metformin significantly reduces resistin levels. This reduction may be associated with a reduction in insulin resistance or weight loss. However, this subject warrants further study.

Despite the limitation due to the number of subjects included, our study evaluates the response of adipokines, such as serum visfatin, adiponectin and resistin, which are directly associated with insulin resistance, to treatment with metformin in patients with PCOS. A reduction was shown in serum visfatin and resistin levels with metformin treatment in patients with PCOS. Detailed retrospective studies with long-term follow-up and larger populations are required to confirm the clinical significance of these findings.

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

REFERENCES

1. Dunaif A. Insulin action in the polycystic ovary syndrome. *Endocrinol Metab Clin North Am* 1999; 28: 341-59.
2. Holte J. Disturbances in insulin secretion and sensitivity in women with polycystic ovary syndrome. *Bailleries Clin Endocrinol Metab* 1996; 10: 221-47.
3. Katsiki N, Georgiadou E, Hatzitolios AI. The role of insulin sensitizing agents in the treatment of poly cystic ovary syndrome. *Drugs* 2009; 69: 1417-31.
4. Fukuhara A, Matsuda M, Nishizawa M, *et al.* Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science* 2005; 307: 426-30.
5. Daimon M, Oizumi T, Saitoh T, *et al.* Decreased serum levels of adiponectin are a risk factor for the progression to type 2 diabetes in Japanese population: the Funagata study. *Diabetes Care* 2003; 26: 2015-20.
6. Snehalatha C, Mukesh B, Simon M, Viswanathan V, Haffner SM, Ramachandran A. Plasma adiponectin is an independent predictor of type 2 diabetes in Asian Indians. *Diabetes Care* 2003; 26: 3226-9.
7. Steppan CM, Bailey ST, Bhat S, *et al.* The hormone resistin links obesity to diabetes. *Nature* 2001; 409: 307-12.
8. Chen MP, Chung FM, Chang DM, *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: 295-9.
9. Krzyzanowska K, Krugluger W, Mittermayer F, *et al.* Increased visfatin concentrations in women with gestational diabetes mellitus. *Clin Sci (Lond)* 2006; 110: 605-9.

10. Tan BK, Chen J, Digby JE, Keay SD, Kennedy CR, Randeva HS. Increased visfatin mRNA and protein levels in adipose tissue and adipocytes in women with polycystic ovary syndrome (PCOS): paralel increase in plasma visfatin. *J Clin Endocrinol Metab* 2006; 91: 5022-8.

11. Kowalska I, Strackowski M, Nikalajuk A, *et al.* Serum visfatin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome. *Hum Reprod* 2007; 22: 1824-9.

12. Chan TF, Chen YL, Chen HH, Lee CH, Jong SB, Tsai EM. Increased plasma visfatin concentrations in women with polycystic ovary syndrome. *Fertil Steril* 2007; 88: 401-5.

13. Panidis D, Farmakiotis D, Rousso D, *et al.* Plasma visfatin levels in normal weight women with polycystic ovary syndrome. *Eur J Intern Med* 2008; 19: 406-12.

14. Gen R, Akbay E, Muşlu N, Sezer K, Çayan F. Plasma visfatin level in lean women with PCOS: relation to proinflammatory markers and insulin resistance. *Gynecol Endocrinol* 2009; 25: 241-5.

15. Jongwutiwes T, Lertvikool S, Leelaphiwat S, Rattanasiri S, Jultanmas R, Weerakiet S. Serum visfatin in Asian women with polycystic ovary syndrome. *Gynecol Endocrinol* 2009; 25: 536-42.

16. Haider DG, Schindler K, Schaller G, Prager G, Wolzt M, Ludvik B. Increased plasma visfatin concentrations in morbidly obese subjects are reduced after gastric banding. *J Clin Endocrinol Metab* 2006; 91: 1578-81.

17. Pagano C, Pilon C, Olivieri M, Mason P, Fabris R, Serra R. 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: 3165-70.

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

19. Ozkaya M, Cakal E, Ustun Y, Ustun YE. Effect of metformin on serum visfatin levels in patients with polycystic ovary syndrome. *Fertil Steril* 2010; 93: 880-4.

20. Steiner CA, Janez A, Jensterle M, Reisinger K, Forst T, Pfützner A. Impact of treatment with rosiglitazone or metformin on biomarkers for insulin resistance and metabolic syndrome in patients with polycystic ovary syndrome. *J Diabetes Sci Technol* 2007; 1: 211-7.

21. Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M. The release of the adipocytokine visfatin is regulated by glucose and insulin. *Diabetologia* 2006; 46: 1909-14.

22. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; 257: 79-83.

23. Weyer C, Funahashi T, Tanaka S, *et al.* Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001; 86: 1930-5.

24. Orio F, Palomba S, Cascella T, *et al.* Adiponectin levels in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2003; 88: 2619-23.

25. Panidis D, Kourtis A, Farmakiotis D, Mouslech T, Rousso D, Koliakos G. Serum adiponectin levels in women with polycystic ovary syndrome. *Hum Reprod* 2003; 18: 1790-6.

26. Spranger J, Möhlig M, Wegewitz U, *et al.* Adiponectin is independently associated with insulin sensitivity in women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2004; 61: 738-46.

27. Gulcelik N, Aral Y, Serter R, Demir Y, Çulha C. Adiponectin is an independent determinant of insulin resistance in women with polycystic ovary syndrome. *Gynecol Endocrinol* 2006; 22: 511-5.

28. Jakubowska J, Bohdanowicz-Pawlak B, Mılewicz A, *et al.* Plasma cytokines in obese women with polycystic ovary syndrome, before and after metformin treatment. *Gynecol Endocrinol* 2008; 24: 378-84.

29. Agarwal N, Rice PL, Bolusani H, *et al.* Metformin reduces arterial stifness and improves endothelial function in young women with polycystic ovary syndrome: A randomized placebo-controlled, crossover trial. *J Clin Endocrinol Metab* 2010; 95: 722-30.

30. Moran LJ, Meyer C, Hutchison SK, Zoungas S, Teede HJ. Novel inflammatory markers in overweight women with and without polycystic ovary syndrome and following pharmocological intervention. *J Endocrinol Invest* 2010; 33: 258-65.

31. Trolle B, Lauszus FF, Frystyk J, Flyvbjerg A. Adiponectin levels in women with polycystic ovary syndrome: impact of metformin treatment in a randomized controlled study. *Fertil Steril*, 2010 [Epub ahead of print].

32. Li J, Yu X, Pan W, Unger RH. Gene expression profile of rat adipose tissue at the onset of high diet obesity. *Am J Physiol Endocrinol Metab* 2002; 282: E1334-41.

33. McTernan PG, McTernan CL, Chetty R, *et al.* Increased resistin gene and protein expression in human abdominal adipose tissue. *J Clin Endocrinol Metab* 2002; 87: 2407-10.

34. Way JM, Gorgun CZ, Tong Q, *et al.* Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. *J Biol Chem* 2001; 276: 25651-3.

35. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Resistin gene expression in human adipocytes is not related to insulin resistance. *Obes Res* 2002; 10: 1-5.

36. Seow KM, Juan CC, Wu LY, *et al.* Serum and adipocyte resistin in polycystic ovary syndrome with insulin resistance. *Hum Reprod* 2005; 19: 48-53.