Endocrine Journal 2011, **58** (4), 237-246

ORIGINAL

The effect of weight loss and treatment with metformin on serum vaspin levels in women with polycystic ovary syndrome

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Abstract. Many patients with polycystic ovary syndrome (PCOS) have insulin resistance, obesity (mostly visceral) and glucose intolerance, conditions associated with abnormalities in the production of vaspin, a novel adipokine that appears to preserve insulin sensitivity and glucose tolerance. The aim of the study was to assess serum vaspin levels in PCOS and the effects on vaspin levels of metformin or of weight loss. We studied 79 patients with PCOS and 50 healthy female volunteers. Normal weight patients with PCOS (n=25) were treated with metformin 850 mg bid for 6 months. Overweight/obese patients with PCOS (n=54) were prescribed a normal-protein, energy-restricted diet for 6 months; half of them were also given orlistat 120 mg tid and the rest were given sibutramine 10 mg qd. At baseline and after 6 months, serum vaspin levels and anthropometric, metabolic and hormonal features of PCOS were determined. Overall, patients with PCOS had higher vaspin levels than controls (p=0.021). Normal weight patients with PCOS had higher vaspin levels than normal weight controls (p=0.043). Vaspin levels were non-significantly higher in overweight/obese patients with PCOS than in overweight/obese controls. In normal weight patients with PCOS, metformin reduced vaspin levels non-significantly. In overweight/obese patients with PCOS, diet plus orlistat or sibutramine did not affect vaspin levels. Vaspin levels were independently correlated with body mass index in women with PCOS (p=0.001) and with waist circumference in controls (p=0.015). In conclusion, serum vaspin levels are elevated in PCOS but neither a small weight loss nor metformin affect vaspin levels significantly.

Key words: Polycystic ovary syndrome, Obesity, Vaspin, Metformin, Orlistat

POLYCYSTIC ovary syndrome (PCOS) is one of the commonest endocrine disorders in women of reproductive age, affecting 5-10% of them, and the leading cause of anovulatory infertility in developed countries [1, 2]. The most frequent signs of PCOS are irregular menstruation, due to oligo- or anovulation, as well as signs of androgen excess, including hirsutism, oily skin, acne and androgenic alopecia [3].

Patients with PCOS frequently have insulin resistance, pancreatic β-cell dysfunction, glucose intolerance, type 2 diabetes mellitus (T2DM) and dyslipidemia [1, 3-5]. Obesity, particularly visceral obesity, is another important feature of the syndrome and 38-88% of patients with PCOS are overweight or obese [6, 7]. It is well established that abdominal obesity increases cardiovascular risk significantly more than the increase in subcutaneous fat [8, 9]. The majority of women with PCOS suffer from a type of insulin resistance that is independent of obesity, is characteristic of this syndrome and its pathogenesis is unclear [10-12]. Insulin resistance is even more pronounced in obese women with PCOS [11].

Abdominal fat and particularly adipocytes cross-talk with almost all other tissues and systems. This cross-talk is mediated through the synthesis and secretion of a variety of bioactive peptides, collectively termed adipokines, and also through the presence of specific receptors on the membrane and nucleus of the adipocytes [13, 14]. Adipokines exert their actions in an autocrine, paracrine and endocrine manner. Accordingly, adipocytes receive and send signals to nearby tissues and also to remote organs [15]. These signals are in

Received Nov. 5, 2010; Accepted Jan. 25, 2011 as K10E-330
Released online in J-STAGE as advance publication Feb. 17, 2011

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turn modulated by a variety of metabolic factors [13].

Visceral adipose tissue-derived serine protease inhibitor (vaspin) is a novel adipokine that was recently identified in obese diabetic Otsuka Long-Evans Tokushima (OLETF) rats. Vaspin levels in the abdominal fat of OLETF rats are highest at 30 weeks; serum insulin levels are also highest at the same time period. However, vaspin levels decline at 50 weeks of age, when these rats become diabetic. Vaspin was also shown to improve glucose tolerance and insulin sensitivity in mice that develop obesity, hyperinsulinemia and hyperglycemia when fed a high-fat, high-sucrose diet [16]. Recent studies in humans showed a positive correlation between the expression of the vaspin gene in the abdominal fat with circulating vaspin levels, obesity and T2DM [17, 18]. The opposite change in vaspin levels when OLETF rats and humans become diabetic (decline and increase, respectively) suggest that the regulation and actions of vaspin differ across species.

Given that many patients with PCOS have insulin resistance, obesity (mostly visceral), glucose intolerance, T2DM and abnormalities in the secretion of steroid hormones from the ovaries and the adrenal glands, and all these conditions are associated with abnormalities in vaspin production, the present study was designed to assess 1) serum vaspin levels in normal weight, overweight and obese patients with PCOS, 2) the effects of low-calorie diet combined with orlistat or sibutramine for 6 months on vaspin levels in overweight and obese patients with PCOS, 3) the effects of treatment with metformin for 6 months on vaspin levels in normal weight patients with PCOS, and 4) the correlations between vaspin levels and the anthropometric, metabolic and ultrasonographic features of PCOS.

Patients and Methods

Patients

We studied 79 women with PCOS (mean age 23.9±5.8 years, mean body mass index (BMI) 30.1±6.7 kg/m^2) who were being followed-up in the Disorders of Menstruation Outpatient Clinic of the Second Department of Obstetrics and Gynecology of the Aristotle University of Thessaloniki. We also studied 50 healthy female volunteers with normal ovulating cycles (duration 28±2 days, serum progesterone levels >10 ng/mL in 2 consecutive cycles), without clinical or biochemical signs of hyperandrogenism and without polycystic ovaries on ultrasound (control group).

Diagnosis of PCOS was based on the revised criteria of Rotterdam (2003) [19, 20]. None of the women studied had galactorrhea or any endocrine or systemic disease that could possibly affect reproductive physiology. A Synacthen test was performed with tetracosactide (Synacthen 0.25 mg/1 mL; Novartis Pharma, Rueil-Malmaison, France) on each woman with a basal 17α-hydroxyprogesterone (17α-OHP) plasma level >1.5 ng/mL to exclude congenital adrenal hyperplasia. No woman reported use of any medication that could interfere with the normal function of the hypothalamic-pituitary-gonadal axis during the last semester.

Study protocol

In all women, weight, height, and waist circumference (W) were measured. Body weight was measured with analog scales and in light clothing; height was measured barefoot with a stadiometer. BMI (kg/m^2) was calculated by dividing weight by height squared to assess obesity. *W* was obtained as the smallest circumference at the level of the umbilicus.

Baseline blood samples were collected between days 3 and 7 of the menstrual cycle in the control group and after a spontaneous bleeding episode in the PCOS group, after an overnight fast. The circulating levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), total testosterone (T), Δ4-androstenedione (Δ4-A), dehydroepiandrosterone-sulfate (DHEA-S), 17α-hydroxyprogesterone (17α-OHP), sex hormone-binding globulin (SHBG), glucose, insulin, thyroid stimulating hormone (TSH) and free thyroxin (FT4) were measured. Immediately after the baseline blood sampling an oral glucose tolerance test (OGTT) was performed; 75 g of glucose were administered orally and serum glucose levels were determined after 30, 60, 90 and 120 min. At the same day transvaginal ultrasonography was performed and the volume of each ovary was determined as well as the number of follicles in each ovary.

Patients with PCOS were divided according to BMI in Group 1 (normal BMI (<25 kg/m^2); n=25, mean age 19.9±3.0 years) and Group 2 (BMI >27 kg/m^2; n=54, mean age 25.9±5.9 years). Controls were also divided according to the BMI in Group 3 (normal BMI (<25 kg/m^2); n=25, mean age 31.3±4.5 years) and Group 4 (BMI>27 kg/m^2; n=25, mean age 33.9±4.6 years).

At baseline, the basal metabolic rate (in kcal/day) was calculated in Group 2 and adjusted for moderate daily physical activity as follows: In women 18-30

years of age: (0.0621 x weight in kg + 2.0357) x 240 x 1.3 and in women >31 years of age: (0.0342 x weight in kg + 3.5377) x 240 x 1.3.

Patients in Group 1 were treated with metformin 850 mg bid for 6 months. All baseline laboratory tests were repeated at the end of treatment with metformin (i.e. at 6 months).

Patients in Group 2 were prescribed a normal-protein, energy-restricted diet (basic metabolic rate - 600 kcal/day, consisting of 50% from carbohydrate, 30% from fat (10% saturated), and 20% from protein) for a period of 6 months. In 27 women of this Group, orlistat 120 mg tid before each meal was also given for 6 months (Xenical®, Roche (Hellas) S.A., Greece)[21]. In the remaining 27 patients of Group 2, sibutramine 10 mg qd was administered in the morning for 6 months (Reductil®, Abbot Hellas, Greece)[22]. All baseline laboratory tests, the OGTT and the transvaginal ultrasonography were repeated at the end of treatment with orlistat or sibutramine (i.e. at 6 months).

Informed consent was obtained from all women, and the study was approved by the institutional review board.

Methods

Serum levels of glucose, insulin, FSH, LH, PRL, androgens, 17α-OHP, TSH and FT4 were determined as described previously [23]. Serum vaspin levels were determined with an enzyme-linked immunosorbent assay (Antigenix America Inc. Human Vaspin Elisa kit, Product Number 501 CKX). Lower levels of detection was <0.2 ng/mL, the intra-assay coefficients of variation for low and high levels were 1.7 and 5.4%, respectively, and the inter-assay coefficients of variation for low and high vaspin levels were 2.5 and 6.15%, respectively. Free androgen index (FAI) was determined as follows: FAI = T (nmol/L) x 100 / SHBG (nmol/L) [24]. The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as follows: HOMA-IR = insulin (mIU/L) x glucose (mg/dL) / 405 [25]. The Quantitative insulin sensitivity check index (QUICKI) was calculated as follows: QUICKI = 1 / [logInsulin (mIU/L) + logGlucose (mg/dL)] [26].

Transvaginal ultrasonography

Transvaginal ultrasound scans of the ovaries were performed by an experienced sonographer (I. K.) in women who participated in the study. Ovarian volume was calculated by the formula $V = (\pi/6) \times D_{length} \times D_{width} \times D_{thickness}$, where D is dimension. The presence of polycystic ovaries was diagnosed by the presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter and/or increased ovarian volume (>10 cm^3).

Statistical analysis

According to previous studies, at least 25 women should be included in each group in order to have an 80% statistical power to identify a 25% difference in serum vaspin levels between groups at the 5% significance level. Data analysis was performed with the statistical package SPSS (version 17.0; SPSS Inc., Chicago, IL). All tested parameters followed normal distribution as assessed with the Kolmogorov-Smirnov test and are reported as mean±SD. Because women with PCOS were younger and had greater BMI than controls, comparisons between patients and controls were performed with analysis of covariance adjusting for age and BMI. Changes between baseline and end-of-treatment were assessed with the paired samples *t*-test. Bivariate correlations between vaspin levels and other parameters were assessed with Pearson correlation. Independent correlations between vaspin levels and other parameters were assessed with stepwise linear regression analysis including parameters that were significantly correlated with vaspin levels in univariate analysis. In all cases, a p value <0.05 was considered significant.

Results

Comparisons between patients with PCOS (Groups 1 and 2, n=79) and controls (Groups 3 and 4, n=50) are shown in Table 1. Patients with PCOS had higher serum vaspin levels than controls (p=0.021; Figure). Patients with PCOS had lower FSH levels (p=0.026) and higher LH and PRL levels (p<0.001 and p=0.019, respectively). In addition, T, Δ4-A, DHEA-S levels and FAI were higher in patients with PCOS than in controls (p<0.001 for all comparisons). Serum levels of 17α-OHP were also higher in patients with PCOS (p=0.007), whereas SHBG levels were lower (p<0.001). Serum glucose and insulin levels, the area under the OGTT curve and the HOMA-IR index did not differ between groups. However, the glucose/insulin ratio and the QUICKI were lower in patients with PCOS than in controls (p=0.014 and p=0.015, respectively). The patients with PCOS had more follicles and larger ovaries than controls (p<0.001 for both comparisons).

Table 1 Differences between all patients with polycystic ovary syndrome (PCOS) and all controls.

	Patients with PCOS (n=79)	Controls (n=50)	*p* (adjusted for BMI and age)
Age (years)	23.9±5.8	32.6±4.7	NA
BMI (kg/m^2)	30.1±6.7	25.1±4.0	NA
Waist (cm)	90.8±15.6	80.8±10.1	0.048
FSH (mIU/mL)	6.1±1.7	7.9±2.8	0.026
LH (mIU/mL)	8.3±5.8	5.9±2.8	0.001
Prolactin (ng/mL)	14.7±7.2	12.2±4.3	0.019
Testosterone (ng/dL)	81.9±27.6	32.9±14.4	<0.001
Δ4-A (ng/mL)	2.8±0.8	1.7±0.5	<0.001
DHEA-S (ng/mL)	3313.9±1405.4	1944.6±811.8	<0.001
FAI	10.68±7.28	1.98±1.16	0.001
17α-OHP (ng/mL)	1.1±0.5	0.7±0.3	0.007
SHBG (nmol/L)	33.4±15.9	69.2±33.7	0.001
Glucose (mg/dL)	101.7±13.4	97.0±9.8	NS
Insulin (μIU/mL)	17.0±17.9	9.2±6.8	NS
Glucose/insulin	8.29±4.23	14.86±9.03	0.014
HOMA-IR	4.25±3.93	2.24±1.71	NS
QUICKI	0.32±0.03	0.35±0.03	0.015
Area under the OGTT curve	16177.6±2879.9	14565.6±3352.5	NS
Ovarian volume (cm^3)	8.2±3.0	5.3±1.8	<0.001
Ovarian follicles	11.6±4.5	6.2±1.9	<0.001
Vaspin (ng/mL)	3.04±3.09	0.58±0.73	0.021

NA, not applicable; NS, not significant; BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; Δ4-A, Δ4-androstenedione; DHEAS, dehydroepiandrosterone sulfate; FAI, free androgen index; 17α-OHP, 17α-hydroxyprogesterone; SHBG, sex hormone-binding globulin; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index; OGTT, oral glucose tolerance test

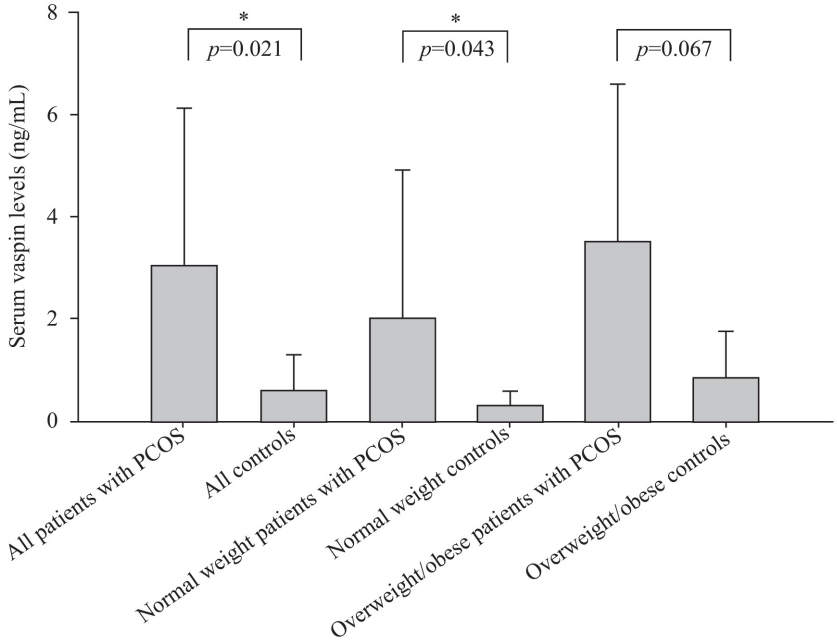

Figure Serum vaspin levels in patients with polycystic ovary syndrome (PCOS) and controls. *, indicates a significant difference between groups (p<0.05)

Table 2 Differences between normal weight patients with polycystic ovary syndrome (PCOS) and normal weight controls at baseline and changes after 6 months of metformin treatment in normal weight patients with PCOS.

	Baseline			Month 6	
	Normal weight patients with PCOS (n=25)	Normal weight controls (n=25)	*p* (adjusted for age)	Normal weight patients with PCOS (n=25)	*p* (vs. baseline)
Age (years)	19.9±3.0	31.3±4.5	NA	19.9±3.0	NS
BMI (kg/m^2)	23.2±4.4	21.9±1.6	NS	22.9±4.9	0.041
Waist (cm)	74.5±10.1	73.7±5.6	NS	NM	NA
Waist/hip	0.75±0.05	0.76±0.05	NS	NM	NA
FSH (mIU/mL)	5.9±1.8	8.1±2.5	0.038	6.1±3.2	NS
LH (mIU/mL)	10.9±6.8	7.1±3.3	NS	12.6±13.5	NS
Prolactin (ng/mL)	15.1±6.7	12.1±3.8	NS	15.0±7.2	NS
Testosterone (ng/dL)	81.7±17.4	36.4±14.5	<0.001	72.1±16.5	0.032
Δ4-A (ng/mL)	2.9±0.9	1.8±0.4	0.004	2.8±0.8	NS
DHEA-S (ng/mL)	3230.9±828.9	2138.9±788.6	NS	3520.8±1110.9	NS
FAI	8.6±3.9	1.9±0.9	0.006	6.7±2.6	0.023
17α-OHP (ng/mL)	1.2±0.5	0.7±0.3	NS	1.4±0.8	NS
SHBG (nmol/L)	38.9±18.6	76.4±30.8	NS	41.9±15.5	NS
Glucose (mg/dL)	99.8±16.3	95.1±7.8	NS	87.0±7.3	<0.001
Insulin (μIU/mL)	18.4±30.4	8.2±7.0	NS	10.8±9.3	NS
Glucose/insulin	9.9±5.2	15.5±7.3	NS	12.0±6.7	NS
HOMA-IR	4.4±6.4	1.9±1.8	NS	2.3±1.9	NS
QUICKI	0.33±0.03	0.36±0.03	NS	0.35±0.03	0.007
Area under the OGTT curve	16273.8±2993.6	13009.8±1680.8	NS	NM	NA
Ovarian volume (cm^3)	7.5±2.3	5.3±1.9	0.017	NM	NA
Ovarian follicles	11.9±4.9	6.0±1.9	<0.001	NM	NA
Vaspin (ng/mL)	2.02±2.89	0.29±0.28	0.043	1.85±3.38	NS

NS, not significant; NA, not applicable; NM, not measured. Other abbreviations are defined in Table 1.

Comparisons between normal weight patients with PCOS (Group 1, n=25) and normal weight controls (Group 3, n=25) are shown in Table 2. Normal weight patients with PCOS had higher serum vaspin levels than normal weight controls (p=0.043; Figure). Serum glucose and insulin levels, the area under the OGTT curve, the glucose/insulin ratio and the HOMA-IR and QUICKI indices did not differ between groups.

Comparisons between overweight/obese patients with PCOS (Group 2, n=54) and overweight/obese controls (Group 4, n=25) are shown in Table 3. Serum vaspin levels were higher in patients with PCOS compared with controls (3.51±3.09 *vs*. 0.85±0.91 ng/mL, respectively) but the difference did not reach significance (p=0.067). Serum glucose and insulin levels, the area under the OGTT curve and the HOMA-IR index also did not differ between groups. However, the glucose/insulin ratio and the QUICKI were lower in overweight/obese patients with PCOS than in overweight/obese controls (p=0.011 and p=0.016, respectively).

Overweight/obese patients with PCOS (Group 2, n=54) had also higher serum vaspin levels compared with normal weight patients with PCOS (Group 1, n=25)(3.51±3.09 *vs*. 2.02±2.89 ng/mL, respectively; p=0.041). In addition, serum vaspin levels were higher in overweight/obese controls (Group 4, n=25) than in normal weight controls (Group 3, n=25) (0.85±0.91 *vs*. 0.29±0.28 ng/mL, respectively; p=0.009).

In normal weight patients with PCOS (Group 1, n=25), treatment with metformin for 6 months resulted in a reduction in BMI (p=0.041; Table 2). A reduction in serum vaspin levels was also observed (from 2.02±2.89 to 1.85±3.38 ng/mL) but was not significant (p=0.714). Regarding the changes in hormones, only the reduction in T levels and FAI were significant (p=0.032 and p=0.023, respectively). Among meta-

Table 3 Differences between overweight/obese patients with polycystic ovary syndrome (PCOS) and overweight/obese controls at baseline and changes after 6 months of low-calorie diet combined with either sibutramine or orlistat in overweight/obese patients with PCOS.

	Baseline			Month 6	
	Overweight/obese patients with PCOS (n=54)	Overweight/obese controls (n=25)	*p* (adjusted for BMI and age)	Overweight/obese patients with PCOS (n=54)	*p* (vs. baseline)
Age (years)	25.9±5.9	33.9±4.6	NA	25.9±5.9	NS
BMI (kg/m^2)	33.2±5.1	28.3±3.0	NA	29.2±5.4	<0.001
Waist (cm)	98.4±11.3	87.9±8.5	NS	88.3±11.2	<0.001
Waist/hip	0.84±0.07	0.81±0.06	NS	0.82±0.05	<0.001
FSH (mIU/mL)	6.2±1.7	7.8±3.2	NS	5.9±2.7	NS
LH (mIU/mL)	7.1±4.8	4.8±1.5	0.001	11.6±12.5	0.011
Prolactin (ng/mL)	14.6±7.5	12.4±4.7	NS	13.5±5.9	NS
Testosterone (ng/dL)	81.9±31.4	29.4±13.8	<0.001	72.5±37.4	0.030
Δ4-A (ng/mL)	2.8±0.8	1.7±0.6	<0.001	7.7±36.9	NS
DHEA-S (ng/mL)	3352.3±1609.6	1750.4±803.1	0.002	3154.4±1498.4	NS
FAI	11.62±8.26	2.07±1.31	0.012	8.06±6.5	0.001
17α-OHP (ng/mL)	1.1±0.5	0.7±0.3	0.034	1.5±1.0	0.013
SHBG (nmol/L)	30.8±14.1	61.9±35.5	0.004	41.6±22.4	<0.001
Glucose (mg/dL)	102.5±11.9	98.9±11.2	NS	95.8±11.3	0.001
Insulin (μIU/mL)	16.4±7.2	10.2±6.6	NS	11.2±8.3	<0.001
Glucose/insulin	7.56±3.54	14.23±10.62	0.011	11.93±7.14	<0.001
HOMA-IR	4.19±2.01	2.53±1.63	NS	2.71±2.17	<0.001
QUICKI	0.31±0.02	0.35±0.04	0.016	0.34±0.03	<0.001
Area under the OGTT curve	16133.1±2853.4	16121.4±3883.3	NS	14966.1±2688.9	0.028
Ovarian volume (cm^3)	8.6±3.2	5.3±1.8	<0.001	9.4±6.6	NS
Ovarian follicles	11.5±4.3	6.4±1.9	<0.001	10.4±4.2	NS
Vaspin (ng/mL)	3.51±3.09	0.85±0.91	NS	4.16±3.08	NS

NS, not significant; NA, not applicable. Other abbreviations are defined in Table 1.

bolic parameters, there was a reduction in serum glucose levels ($p<0.001$) and an increase in the QUICKI index ($p=0.007$) whereas serum insulin levels, the glucose/insulin ratio and the HOMA-IR index did not change significantly.

In overweight/obese patients with PCOS (Group 2, n=54), low-calorie diet combined with either orlistat (n=27) or sibutramine (n=27) resulted in a) a decrease in BMI and W ($p<0.001$ for both changes *vs*. baseline; Table 3), b) an increase in serum LH and SHBG levels ($p=0.011$ and $p<0.001$, respectively) and a decline in serum T levels and FAI ($p=0.03$ and $p=0.001$, respectively), c) a decrease in serum glucose and insulin levels, in the area under the OGTT curve and in the HOMA-IR index ($p=0.001$, $p<0.001$, $p=0.028$ and $p<0.001$, respectively) and a rise in the glucose/insulin ratio and in the QUICKI ($p<0.001$ for both changes). An increase in serum vaspin levels was also observed (from 3.51±3.09 to 4.16±3.08 ng/mL) but was not significant ($p=0.110$ *vs.* baseline).

Regarding the correlations between serum vaspin levels and other parameters in the total study population (n=129), in univariate analysis, vaspin positively correlated with BMI ($r=0.460$, $p<0.001$), W ($r=0.407$, $p<0.001$), waist/hip ratio (WHR, $r=0.269$, $p=0.002$), serum T levels ($r=0.309$, $p<0.001$), FAI ($r=0.333$, $p<0.001$), mean volume of the two ovaries ($r=0.287$, $p=0.001$) and mean number of follicles in the two ovaries ($r=0.374$, $p<0.001$). In univariate analysis, vaspin negatively correlated with age ($r=-0.239$, $p=0.007$), serum FSH levels ($r=-0.262$, $p=0.003$), serum SHBG levels ($r=-0.351$, $p<0.001$), the glucose/insulin ratio ($r=-0.217$, $p=0.014$) and the QUICKI ($r=-0.239$, $p=0.007$). In stepwise linear regression analysis, vaspin levels were independently correlated only with BMI and mean number of follicles in the two ovaries

(p<0.001 for both correlations).

In women with PCOS (Groups 1 and 2, n=79), in univariate analysis, vaspin levels at baseline positively correlated with BMI (r=0.376, p=0.001) and with W (r=0.320, p=0.004). In stepwise linear regression analysis, vaspin levels were independently correlated only with BMI (p=0.001).

In controls (Groups 3 and 4, n=50), in univariate analysis, vaspin levels at baseline positively correlated with W (r=0.346, p=0.015), WHR (r=0.311, p=0.030), FAI (r=0.314, p=0.028) and the area under the OGTT curve (r=0.320, p=0.025). In stepwise linear regression analysis, vaspin levels were independently correlated only with W (p=0.015).

Discussion

Vaspin (**V**isceral **A**dipose tissue-derived **S**er**PIN**), a novel adipokine isolated from abdominal fat in 2000, is a 45.2 kD protein [16] that is also present in serum [27]. Vaspin is a member of the superfamily of serpins (**SER**in **P**roteinase **IN**hibitor**S**) [28, 29]. The gene that encodes for vaspin is termed OL-64 and is present at the long arm of chromosome 14 (14q32.1) [30] and the cDNA consists of 1,245 bases and encodes for 415 aminoacids [16].

In the present study, serum vaspin levels in patients with PCOS were higher than in healthy controls, after adjusting for age and BMI (Table 1 and Figure). Moreover, normal weight patients with PCOS had higher serum vaspin levels than normal weight controls (p=0.043; Figure), whereas overweight/obese patients with PCOS also had higher vaspin levels compared with overweight/obese controls even though the difference between the latter two groups was marginally non-significant (p=0.067). In addition, overweight/obese patients with PCOS had higher serum vaspin levels than normal weight patients with PCOS (p=0.041) and overweight/obese controls had higher serum vaspin levels than normal weight controls (p=0.009). We also observed that in the total study population (n=129) serum vaspin levels were independently correlated in stepwise linear regression analysis with BMI and mean number of follicles in the two ovaries (p<0.001 for both correlations). In addition, the glucose/insulin ratio and the QUICKI were lower in patients with PCOS compared with controls (p=0.014 and p=0.015, respectively; Table 1). These findings suggest that obesity and insulin resistance induce an increase in serum vaspin levels.

It is of interest that serum vaspin levels are elevated in women with PCOS (i.e. a condition with a high prevalence of insulin resistance, glucose intolerance and T2DM) given the recent observation by Youn *et al* [18] that insulin-resistant obese patients also have increased serum vaspin levels. In the former study [18], a significant correlation between serum vaspin levels and BMI was observed, in accordance with our findings.

It has been reported that obesity and insulin resistance increase vaspin expression in abdominal fat as well as its concentration in the serum. This increase in vaspin levels might represent a compensatory response against the obesity- and insulin resistance-stimulated expression of yet to be identified proteases. The former proteases are synthesized in abdominal fat but also in other tissues and appear to suppress the action of insulin. Therefore, the induction of vaspin expression might represent a defense mechanism against insulin resistance. In animal models, administration of recombinant vaspin suppressed the expression of insulin resistance-promoting lipokines (including resistin, tumor necrosis factor-α and leptin) and stimulated the synthesis of insulin sensitizing lipokines (e.g. adiponectin and glucose transporter 4). These findings support the notion that vaspin down-regulates the expression of genes associated with insulin resistance and this action is more prominent in the abdominal fat [16]. Since vaspin appears to exert insulin-sensitizing effects, our finding that serum vaspin levels are increased in women with PCOS (who were more insulin resistant than controls in our study, since they had lower glucose/insulin ratio and QUICKI index than the latter) suggests that this increase represents a compensatory mechanism to preserve insulin sensitivity.

There are only three studies that assessed vaspin levels in patients with PCOS yielding conflicting results [31-33]. Tan *et al* [31] studied 12 patients with PCOS and 12 controls and determined serum vaspin levels, vaspin mRNA levels in the subcutaneous and visceral fat, as well as the effects of glucose, insulin and steroid hormones on serum vaspin levels and on vaspin mRNA levels in the abdominal fat. Serum vaspin levels were higher in patients with PCOS compared with controls (p<0.05), vaspin mRNA levels were higher in the abdominal fat (p<0.05) and adding glucose resulted in a rise in vaspin mRNA levels in the abdominal fat and vaspin secretion in the culture media (p<0.001). In the second study, Escobar-Morreale *et al* [32] measured

serum vaspin levels in a larger sample of patients with PCOS and in obese patients without hyperandrogenemia (n=42 in both groups). PCOS and obesity did not affect serum vaspin levels. In addition, serum vaspin levels did not differ between patients with normal or impaired glucose tolerance. In the third study, Cakal *et al* [33] determined serum vaspin and C-reactive protein (CRP) levels in 24 patients with PCOS, in 23 women with polycystic ovaries (PCO) and in 24 controls. Patients with PCOS or PCO had higher serum vaspin and CRP levels than controls. Accordingly, Cakal *et al* [32] suggested that patients with PCOS or PCO might have increased risk for T2DM and atherosclerosis. Our results are in accordance with the findings of Tan *et al* [31] and Cakal *et al* [33], i.e. patients with PCOS overall had higher serum vaspin levels than controls overall (Table 1, Figure), normal weight patients with PCOS had higher vaspin levels than normal weight controls (Figure) and overweight/obese patients with PCOS and overweight/obese controls had higher vaspin levels than normal weight patients with PCOS and normal weight controls, respectively (p=0.041 and p=0.009, respectively).

In our study, treatment with metformin 850 mg bid for 6 months resulted in a small but significant decrease in BMI in normal weight patients with PCOS. In addition, serum T and glucose levels were reduced, the QUICKI increased, whereas the HOMA-IR index was not affected. Regarding serum vaspin levels, a small fall was observed but was not significant (Table 2). Our findings are in agreement with those of Escobar-Morreale *et al* [32] who also reported a small decrease in serum vaspin levels after treatment with the same metformin dose and for the same duration in a smaller sample of patients with PCOS (n=19). In contrast, Tan *et al* [31] reported a significant reduction in serum vaspin levels after treatment with the same metformin dose and for the same duration in 12 overweight/obese patients with PCOS. The difference in the change in serum vaspin levels between ours and the study by Tan *et al* might be attributed to the inclusion of overweight/obese patients with PCOS and to the reduction in the HOMA-IR index in the study by Tan *et al*, whereas we administered metformin to normal weight patients with PCOS and we did not observe a change in HOMA-IR.

The reduction in serum vaspin and glucose levels, the improvement in insulin sensitivity and the reduction of insulin resistance indices after metformin treatment in overweight/obese patients in the study by Tan *et al* [31] lead these authors to hypothesize that the elevated vaspin levels in the serum and in the abdominal fat are compensatory against insulin resistance and glucose intolerance. The reduction in serum vaspin levels during metformin treatement might be due to the suppression of hepatic glucose production by the latter agent [34-36].

In our study, treatment of overweight/obese patients with PCOS for 6 months with a low-calorie diet in combination with either orlistat (n=27) or sibutramine (n=27) did not affect serum vaspin levels despite the significant improvement in several anthropometric, hormonal, and metabolic parameters. There are no other studies on the effect of a low-calorie diet with or without antiobesity agents on serum vaspin levels in overweight/obese patients with PCOS. Escobar-Morreale *et al* [32] reported a significant (p=0.025) fall in serum vaspin levels 6 months after bariatric surgery in 26 female patients with morbid obesity (16 had PCOS). However, it should be emphasized that the mean weight loss in the former study was 39.0 kg and the W was reduced by a mean of 22.0 cm. The non-significant reduction in serum vaspin levels in our study might be because we enrolled overweight or obese patients with PCOS and we observed smaller body weight and W reductions (11.2 kg and 10.1 cm, respectively).

Finally, we observed a reduction in serum T levels and in the FAI in both normal weight women with PCOS after treatment with metformin and in overweight/obese women with PCOS after administration of a low-calorie diet combined with orlistat or sibutramine. The main mechanism underlying this reduction in circulating androgen levels appears to be the improvement in insulin resistance. Insulin resistance is associated with an increase in circulating androgen levels, since insulin stimulates theca cell androgen synthesis and suppresses SHBG synthesis in the liver, further increasing the free portion of circulating androgens [37-39]. In our study, metformin and weight loss induced an increase in the QUICKI in normal weight and overweight/obese women with PCOS, respectively, whereas the latter also showed significant improvements in other markers of insulin resistance, including a decrease in serum insulin levels, the area under the OGTT curve and the HOMA-IR index and an increase in the glucose/insulin ratio.

Women with PCOS were significantly younger and had greater BMI than controls and this represents a limitation of our study. However, all comparisons between

women with PCOS and controls were performed with analysis of covariance adjusting for age and BMI and therefore we believe that our results are valid.

In conclusion, our findings suggest that PCOS and obesity are associated with elevated serum vaspin levels. Treatment with metformin of normal weight patients with PCOS lowers serum vaspin levels but not significantly. In addition, a small weight loss does not appear to affect serum vaspin levels. The increased serum vaspin levels in patients with PCOS and particularly in overweight or obese women might represent a compensatory mechanism to preserve insulin sensitivity and glucose tolerance.

References

1. Dunaif A (1997) Insulin resistance and the polycystic ovary syndrome: mechanism and implication for pathogenesis. *Endocr Rev* 18: 774-800.
2. Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, Zapanti ED, Bartzis MI (1999) A Survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. *J Clin Endocrinol Metab* 84: 4006-4011.
3. Nestler JE (2008) Metformin for the treatment of the polycystic ovary syndrome. *N Engl J Med* 358: 47-54.
4. Wild RA, Painter PC, Coulson PB, Carruth KB, Ranney GB (1985) Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 61: 946-951.
5. Diamanti-Kandarakis E, Kandaraki E, Christakou C, Panidis D (2009) The effect of pharmaceutical intervention on lipid profile in polycystic ovary syndrome. *Obes Rev* 10: 431-441.
6. Balen AH, Conway GS, Kaltsas G, Techatrasak K, Manning PJ, West C, Jacobs HS (1995) Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. *Hum Reprod* 10: 2107-2111.
7. Legro RS (2000) The genetics of obesity. Lessons for polycystic ovary syndrome. *Ann NY Acad Sci* 900: 193-202.
8. Chang RJ, Nakamura RM, Judd HL, Kaplan SA (1983) Insulin resistance in nonobese patients with polycystic ovarian disease. *J Clin Endocrinol Metab* 57: 356-359.
9. Dunaif A, Graf M, Mandeli J, Laumas V, Dobrjansky A (1987) Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperisulinemia. *J Clin Endocrinol Metab* 65: 499-507.
10. Dunaif A, Segal KR, Futterweit W, Dobrjansky A (1989) Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes* 38: 1165-1174.
11. Trujillo ME, Scherer PE (2006) Adipose tissue-derived factors: impact on health and disease. *Endocr Rev* 27: 762-778.
12. Vona-Davis L, Howard-McNatt M, Rose DP (2007) Adiposity, type 2 diabetes and the metabolic syndrome in breast cancer (Complication of Obesity). *Obes Rev* 8: 395-408.
13. Kershaw EE, Flier JS (2004) Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 89: 2548-2556.
14. Wajchenberg BL (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* 21: 697-738.
15. Thörne A, Lönnqvist F, Apelman J, Hellers G, Arner P (2002) A pilot study of long-term effects of a novel obesity treatment: omentectomy in connection with adjustable gastric banding. *Int J Obes Relat Metab Disord* 26: 193-199.
16. Hida K, Wada J, Eguchi J, Zhang H, Baba M, Seida A, Hashimoto I, Okada T, Yasuhara A, Nakatsuka A, Shikata K, Hourai S, Futami J, Watanabe E, Matsuki Y, Hiramatsu R, Akagi S, Makino H, Kanwar YS (2005) Visceral adipose tissue-derived serine protease inhibitor: a unique insulin-sensitizing adipocytokine in obesity. *Proc Natl Acad Sci* 102: 10610-10615.
17. Kloting N, Berndt J, Kralisch S, Kovacs P, Fasshauer M, Schon MR, Stumvoll M, Blüher M (2006) Vaspin gene expression in human adipose tissue: association with obesity and type 2 diabetes. *Biochem Biophys Res Commun* 339: 430-436.
18. Youn BS, Klöting N, Kratzsch J, Park JW, Lee N, Song ES, Ruschke K, Oberbach A, Fasshauer M, Stumvoll M, Blüher M (2008) Serum vaspin concentrations in human obesity and type 2 diabetes. *Diabetes* 57: 372-377.
19. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 19: 41-47.
20. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group (2004) Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 81: 19-25.
21. Panidis D, Farmakiotis D, Rousso D, Kourtis A, Katsikis I, Krassas G (2008) Obesity, weight loss, and the polycystic ovary syndrome: effect of treatment with diet and orlistat for 24 weeks on insulin resistance and androgen

levels. *Fertil Steril* 89: 899-906.
22. Florakis D, Diamanti-Kandarakis E, Katsikis I, Nassis GP, Karkanaki A, Georgopoulos N, Panidis D (2008) Effect of hypocaloric diet plus sibutramine treatment on hormonal and metabolic features in overweight and obese women with polycystic ovary syndrome: a randomized, 24-week study. *Int J Obesity* 32: 692-699.
23. Piouka A, Farmakiotis D, Katsikis I, Macut D, Gerou S, Panidis D (2009) Anti-Müllerian hormone levels reflect severity of PCOS but are negatively influenced by obesity: relationship with increased luteinizing hormone levels. *Am J Physiol Endocrinol Metab* 296: E238-243.
24. Morley JE, Patrick P, Perry HM (2002) Evaluation of assays available to measure free testosterone. *Metabolism* 51: 554-559.
25. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28: 12-19.
26. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ (2000) Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 85: 2402-2410.
27. Carrell R, Travis J (1985) α1-Antitrypsin and the serpins: variation and countervariation. *Trends Bioch Sci* 10: 20-24.
28. Gettins PG (2002) Serpin structure, mechanism, and function. *Chem Rev* 102: 4751-4804.
29. Baxter EW, Cummings WJ, Fournier REK (2005) Formation of a large, complex domain of histone hyperacetylation at human 14q32.1 requires the serpin locus control region. *Nucleic Acids Res* 33: 3313-3322.
30. Hida K, Wada J, Zhang H, Hiragushi K, Tsuchiyama Y, Shikata K, Makino H (2000) Identification of genes specifically expressed in the accumulated visceral adipose tissue of OLETF rats. *J Lipid Res* 41: 1615-1622.
31. Tan BK, Heutling D, Chen J, Farhatullah S, Adya R, Keay SD, Kennedy CR, Lehnert H, Randeva HS (2008) Metformin decreases the adipokine Vaspin in overweight women with polycystic ovary concomitant with improvement in insulin sensitivity and a decrease in insulin resistance. *Diabetes* 57: 1501-1507.
32. Escobar-Morreale HF, Luque-Ramirez M, San-Millan JL (2009) Serum visceral adipose tissue-derived serine protease inhibitor concentrations in human obesity and polycystic ovary syndrome. *Diabetes Care* 32: e6.
33. Cakal E, Ustun Y, Engin-Ustun Y, Ozkaya M, Kilinc M (2010) Serum vaspin and C-reactive protein levels in women with polycystic ovaries and polycystic ovary syndrome. *Gynecol Endocrinol* epub ahead of print.
34. Johnson AB, Webster JM, Sum CF, Heseltine L, Argyraki M, Cooper BG, Taylor R (1993) The impact of metformin therapy on hepatic glucose production and skeletal muscle glycogen synthase activity in overweight type II diabetic patients. *Metabolism* 42: 1217-1222.
35. Perriello G, Misericordia P, Volpi E, Santucci A, Santucci C, Ferrannini E, Ventura MM, Santeusanio F, Brunetti P, Bolli GB (1994) Acute antihyperglycemic mechanisms of metformin in NIDDM. Evidence for suppression of lipid oxidation and hepatic glucose production. *Diabetes* 43: 920-928.
36. Inzucchi SE, Maggs DG, Spollett GR, Page SL, Rife FS, Walton V, Shulman GI (1998) Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. *N Engl J Med* 338: 867-873.
37. Nestler JE, Jakubowicz DJ (1997) Lean women with polycystic ovary syndrome respond to insulin reduction with decreases in ovarian P450c17 alpha activity and serum androgens. *J Clin Endocrinol Metab* 82: 4075-4079.
38. Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, Clore JN, Blackard WG (1991) A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. *J Clin Endocrinol Metab* 72: 83-89.
39. Nestler JE (2008) Metformin for the treatment of the polycystic ovary syndrome. *N Engl J Med* 358: 47-54.