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Serum visfatin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome

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BACKGROUND: Visfatin, a protein secreted by adipose tissue, is suggested to play a role in pathogenesis of insulin resistance. In polycystic ovary syndrome (PCOS), insulin resistance might be involved in the development of endocrine and metabolic abnormalities. The aim of the study was to asses the relation between serum visfatin concentration and insulin sensitivity and markers of hyperandrogenism in lean and obese PCOS patients. METHODS: The study group consisted of 70 women with PCOS (23 lean and 47 obese) and 45 healthy women (25 lean and 20 obese). Euglycemic hyperinsulinemic clamp and the measurements of serum visfatin, sex hormones were performed. RESULTS: The PCOS group had lower insulin sensitivity ($P = 0.00049$) and higher serum visfatin ($P = 0.047$) in comparison to the control group. The decrease in insulin sensitivity was present in both the lean ($P = 0.019$) and obese ($P = 0.0077$) PCOS subjects, whereas increase in serum visfatin was observed only in lean PCOS subjects ($P = 0.012$). In the whole group, serum visfatin was negatively correlated with insulin sensitivity ($r = -0.27$, $P = 0.004$). This relationship was also observed in the subgroup of lean ($r = -0.30$, $P = 0.038$), but not obese women. Additionally, in lean women, visfatin was associated with serum testosterone ($r = 0.47$, $P = 0.002$) and free androgen index ($r = 0.48$, $P = 0.002$), independently of other potential confounding factors. CONCLUSIONS: Visfatin is associated with insulin resistance and markers of hyperandrogenism in lean PCOS patients.

Keywords: hyperandrogenism/insulin resistance/polycystic ovary syndrome/visfatin

Introduction

The adipose tissue is now considered an active organ, secreting substances, which may play a role in the pathogenesis of insulin resistance (Arner, 2005). In recent years numerous studies pointed out that so-called true adipokines (adiponectin, leptin), secreted only by fat cells, as well as other adipocytokines (tumor necrosis factor α, resistin, interleukin 6, interleukin 18), which can be secreted also by stromal cells in adipose tissue, play a significant role in regulation of insulin sensitivity (Zhang *et al.*, 1994; Hotamisligil *et al.*, 1995; Fernandez-Real *et al.*, 2001; Steppan *et al.*, 2001; Stefan *et al.*, 2002; Wood *et al.*, 2005). In 2005, visfatin, a new protein with potential insulin-mimetic action was discovered (Fukuhara *et al.*, 2005). According to the original paper, the expression of visfatin was found predominantly in visceral adipose tissue in humans and mice (Fukuhara *et al.*, 2005). *In vitro* studies showed an insulin-like effect of visfatin on regulation of glucose uptake in 3T3-L1 adipocytes and L6 myocytes, and also in adipocyte differentiation (Fukuhara *et al.*, 2005). There was also a strong correlation between plasma visfatin and visceral fat mass in humans (Fukuhara *et al.*, 2005). Subsequent observation in human population did not confirm the differences between visfatin expression in visceral and subcutaneous adipose tissue, and the link between plasma visfatin and visceral fat (Berndt *et al.*, 2005). Berndt *et al.* did not find the relation between visfatin and insulin sensitivity. They noticed a correlation of plasma visfatin and visceral visfatin expression with body mass index (BMI), or percentage body fat (Berndt *et al.*, 2005). Other authors observed increased visfatin concentration in type 2 diabetic patients in comparison to controls, and multivariate analysis showed that only waist to hip ratio was an independent predictor of plasma visfatin concentration (Chen *et al.*, 2006). Some observations revealed conflicting data regarding the role of visfatin in regulation of

insulin sensitivity in humans (Berndt *et al.*, 2005; Fukuhara *et al.*, 2005; Chen *et al.*, 2006).

Insulin resistance is a common feature in women with polycystic ovary syndrome (PCOS), affecting about 50% lean and obese PCOS patients (Dunaif *et al.*, 1989). The cause of insulin resistance in PCOS is not fully explained. It is suggested that the post-receptor defect in insulin signaling can be caused by a plasma derived factor, which could activate serine kinase of insulin receptor substrate and in that way inhibit insulin action (Li *et al.*, 2002).

The aim of the present study was to assess the relationship between serum visfatin and insulin sensitivity, and markers of hyperandrogenism in lean and obese PCOS patients.

Subjects and Methods

Study group

The study group consisted of 70 women with PCOS (23 lean, BMI $<25\ kg\ m^{-2}$ and 47 overweight or obese, BMI $>25\ kg\ m^{-2}$) and 45 healthy, normally menstruating women (25 lean and 20 overweight or obese; control group). The patients with PCOS and obese control subjects were recruited from Outpatient Endocrinology and Gynaecology Clinics. The lean control subjects were recruited mainly from medical students and staff. The diagnosis of PCOS was established according to Rotterdam criteria (oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism and polycystic ovaries in ultrasonography (usg) scan as well as exclusion of other etiologies which mimics the PCOS phenotype). The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group 2004. Revised 2003 consensus on diagnostic criteria and long term health risk related to polycystic ovary syndrome (PCOS). A patient was considered to have PCOS if she fulfilled two out of three criteria mentioned above. We assumed that a patient had clinical hyperandrogensim or hyperandrogenemia if she presented the symptoms of hirsutism (more than eight points in Freeman-Gallway score) with or without acne. The menses status and ovulation was defined in the following way: a patient was considered to have oligo/amenorrhea and anovulation if she had fewer than six menses during the previous year. Exclusion of other disorders with similar clinical symptoms was done by performing the appropriate tests. The *trans*vaginal ultrasound scans were performed in 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 enlarged ovary ($>10\ cm^3$). The usg scan was performed on the same day when the oral glucose tolerance test (OGTT) was done and a blood sample is taken for estimation of visfatin, lipid parameters and sex-hormone levels.

To avoid the influence of disturbances in insulin secretion, and extreme obesity, all subjects with diabetes (fasting glucose >125 mg/dl or glucose in 120 min of OGTT >200 mg/dl) and with morbid obesity ($BMI > 40\ kg\ m^{-2}$) were excluded from the present study. None of the participants had cardiovascular disease, hypertension, infections or other serious medical problems. All women were non-smokers and they were not taking any anti-inflammatory drugs (within previous 3 months) or drugs known to affect carbohydrate and lipid metabolism. Before entering the study, a physical examination and appropriate laboratory tests were performed. The OGTT and estimation of insulin sensitivity by clamp studies were performed in all subjects (studied group and controls). Studies were performed in regularly cycling women during the early follicular phase (3–5 days) of their menstrual cycle and in the PCOS group, 3–5 days after a spontaneous menses, or independent of cycle phase in the presence of amenorrhea. The clamp study was performed in each patient 3–4 days after OGTT. All analyses were performed after an overnight fast. The study protocol was approved by Ethics Committee of Medical University of Bialystok, Poland. All subjects gave a written informed consent before entering the study.

Anthropometry

The BMI was calculated as body weight in kilograms divided by height in meters squared (kg/m^2). The waist circumference was measured at the widest circumference between the waist and thighs. The percentage of body fat was assessed by bioelectric impedance analysis using Tanita TBF-511 Body Fat Analyzer (Tanita Corp., Tokyo, Japan).

Insulin sensitivity

Insulin sensitivity was measured by the euglycemic hyperinsulinemic clamp technique according to DeFronzo *et al.* (1979), as previously described (Straczkowski *et al.*, 2004). On the morning of the study, two venous catheters were inserted into antecubital veins, one for the infusion of insulin and glucose and the other in the contralateral hand for blood sampling; the hand was heated to approximately 60°C. Insulin (Actrapid HM, Novo Nordisk, Copenhagen, Denmark) was given as a primed-continuous i.v. infusion for 2 h at $40\ mU\ m^{-2}\ min^{-1}$, resulting in constant hyperinsulinemia of approximately 75 μIU/ml. Arterialized blood glucose was obtained every 5 min and 20% dextrose (1.11 mol/l) infusion was adjusted to maintain plasma glucose levels at 90 mg/dl. The glucose infusion rate approached stable values during final 40 min of the study and the rate of whole-body glucose uptake (*M* value) was calculated as the mean glucose infusion rate from 80–120 min, corrected for glucose space and normalized per kilogram of fat-free mass (ffm).

Other laboratory analyses

Fasting blood samples were also taken from the antecubital vein before the beginning of the clamp to determine serum lipids and visfatin concentrations. To determine the serum visfatin concentration, the samples were frozen at −70°C and kept till analysed.

The plasma glucose was measured immediately by the enzymatic method using glucose analyser (YSI 2300 STAT Plus, Yellow Springs, OH, USA). The serum insulin was measured with the monoclonal immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium). The serum total and high-density lipoprotein (HDL)-cholesterol and triglycerides (TG) were assessed by enzymatic methods (Cormay, Warsaw, Poland). The serum low-density lipoprotein (LDL)-cholesterol was calculated according to the Friedewald's formula. The serum-free fatty acids (FFA) were measured using the colorimetric method (Duncombe, 1964).

The serum visfatin was measured with the radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, CA, USA) with a lowest detectable limit of 15.56 ng/ml and with intra-assay and inter-assay coefficients of variation (CVs) below 9% and 15%, respectively. Serum LH (sensitivity 0.07 mIU/ml; intraassay CV 4.7%, interassay CV 6.3%), FSH (sensitivity 0.3 mIU/ml; intrassay CV 2.8%, interassay CV 4.6%), testosterone (sensitivity 0.35 nmol/l; intraassay CV 7.8%, interassay CV 10.1%) and estradiol (sensitivity 10 pg/ml; intraassay CV 7.2%, interassay CV 7.5%) were measured by chemiluminescence method (ACS Chirone 180) and serum sex hormone-binding globulin (SHBG)—by immunoradiometric assay (ZenTech, Angleur, Belgium) with sensitivity below 0.3 nmol/l and intraassay and interassay CV 2.9%. Free androgen index was calculated as serum testosterone (nmol/l) $\times 100$/SHBG (nmol/l) ratio (Vermeulen *et al.*, 1999).

Statistical analysis

The statistics were performed with STATISTICA 7.0 program. The variables that did not have normal distribution (*M* value, visfatin, fasting and postload insulin, TG, FFA, SHBG, free androgen index) were log-transformed prior to analyses. For the purpose of data presentation, these variables were again transformed to absolute values in next section. The differences between the groups were evaluated with the factorial analysis of variance, with PCOS status and obesity as categorical variables, followed by the post-hoc Fischer's protected least-significant difference test. The relationships between serum visfatin and other variables were assessed using the Pearson product–moment correlation analysis and multiple regression analysis. The level of significance was accepted at $P < 0.05$.

Results

The clinical characteristics of the studied groups are shown in Table 1. The PCOS group had lower insulin sensitivity (7.87 ± 3.37 versus 10.61 ± 3.21 mg kg $_{\text{ffm}}^{-1}$ min^{-1}, $P = 0.00049$, data not shown) and higher concentrations of serum visfatin (67.14 ± 36.99 versus 53.56 ± 32.48 ng/ml, $P = 0.047$, data not shown) in comparison to the control group. The subgroup analysis revealed that insulin sensitivity was lower in both lean and obese PCOS women ($P = 0.019$ and $P = 0.0077$, respectively, Fig. 1a), whereas an increase in serum visfatin was attributable to the difference observed in the lean ($P = 0.012$), but not in obese subjects (Fig. 1b).

There was a significant effect of obesity on insulin sensitivity ($P = 0.003$), present both in the control and PCOS women ($P = 0.047$ and $P = 0.017$, respectively, Fig 1a). In contrast, the effect of obesity on serum visfatin was not significant ($P = 0.072$) and reached the level of significance only in the control subjects ($P = 0.021$, Fig. 1b).

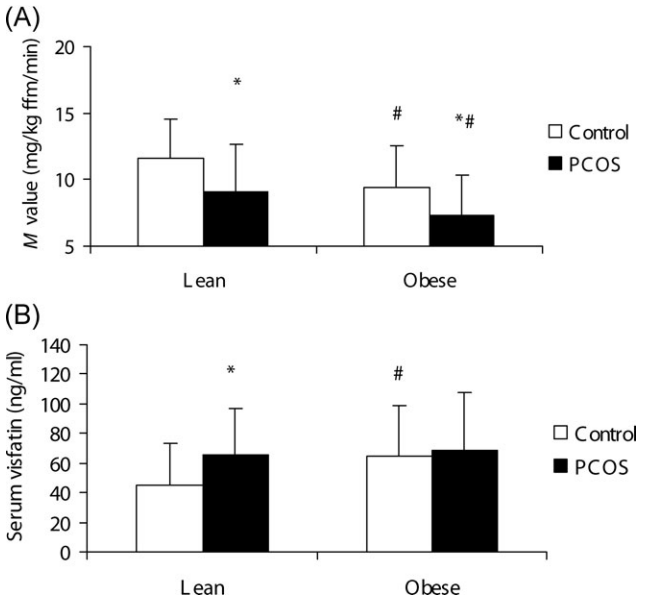

Figure 1: Insulin sensitivity (A) and serum visfatin (B) in control (25 lean and 20 obese) and polycystic ovary syndrome (PCOS, 23 lean and 47 obese) subjects $^{*}P < 0.05$ for the difference between respective PCOS and control groups. $^{\#}P < 0.05$ for the difference between respective lean and obese groups

Cross-sectionally, serum visfatin was correlated with the whole-body insulin sensitivity, BMI, waist girth, postload glucose, serum FFA, HDL-cholesterol, serum FSH, LH/FSH ratio and SHBG and free androgen index (Table 2). In the subgroup analysis, the correlation between serum visfatin and insulin sensitivity was present in the lean ($r = -0.30$, $P = 0.038$), but not in obese subjects ($r = -0.18$, $P = 0.15$) and in the PCOS subjects ($r = -0.24$, $P = 0.047$), but not in the

Table 1: Clinical characteristics of the study groups

	Lean		Obese	
	Control ($n = 25$)	PCOS ($n = 23$)	Control ($n = 20$)	PCOS ($n = 47$)
Age (year)	26.24 ± 6.00	23.69 ± 3.46	28.00 ± 7.38	26.13 ± 6.19
BMI (kg m^{-2})	21.81 ± 2.00	21.39 ± 2.10	31.01 ± 4.37$^{\#}$	30.99 ± 3.99$^{\#}$
Waist girth (cm)	72.54 ± 5.49	72.83 ± 7.73	93.25 ± 11.71$^{\#}$	93.29 ± 11.75$^{\#}$
Percentage body fat	19.90 ± 6.24	21.56 ± 7.61	37.87 ± 9.62$^{\#}$	36.41 ± 14.82$^{\#}$
Fasting glucose (mg/dl)	81.74 ± 7.00	81.83 ± 7.32	87.76 ± 10.09$^{\#}$	86.98 ± 10.47$^{\#}$
Postload glucose[a] (mg/dl)	81.21 ± 16.28	82.60 ± 15.03	106.40 ± 27.39$^{\#}$	101.46 ± 31.34$^{\#}$
Fasting insulin (μIU/ml)	7.81 ± 3.85	9.29 ± 3.14	14.94 ± 10.29$^{\#}$	16.86 ± 9.33$^{\#}$
Postload insulin[a] (μIU/ml)	28.13 ± 15.23	36.98 ± 28.899	70.46 ± 71.38$^{\#}$	86.42 ± 98.75$^{\#}$
FFA (mmol/l)	0.53 ± 0.21	0.52 ± 0.20	0.62 ± 0.27	0.60 ± 0.21
Cholesterol (mg/dl)	178.28 ± 31.85	188.59 ± 44.89	179.64 ± 38.40	190.42 ± 36.05
Serum TG (mg/dl)	68.32 ± 22.24	69.91 ± 38.58	120.72 ± 94.25$^{\#}$	107.94 ± 55.98$^{\#}$
HDL-cholesterol (mg/dl)	58.73 ± 12.97	59.73 ± 11.82	52.78 ± 14.39	50.59 ± 12.84
LDL-cholesterol (mg/dl)	106.76 ± 33.72	103.45 ± 38.40	98.57 ± 28.94	117.87 ± 33.67*$^{\#}$
Serum LH (mIU/ml)	6.59 ± 4.54	11.65 ± 6.18*	4.73 ± 2.24	8.15 ± 3.58*$^{\#}$
Serum FSH (mIU/ml)	6.34 ± 1.52	5.85 ± 1.26	5.31 ± 1.85$^{\#}$	5.56 ± 1.43
Serum estradiol (pg/ml)	92.83 ± 82.06	93.75 ± 87.36	49.85 ± 35.71	60.15 ± 63.28
Serum testosterone (nmol/l)	1.75 ± 0.46	2.66 ± 0.85*	1.80 ± 0.30	2.87 ± 1.16*
Serum SHBG (nmol/l)	87.12 ± 47.97	69.33 ± 38.09	47.55 ± 28.72$^{\#}$	41.83 ± 33.62$^{\#}$
Free androgen index	2.65 ± 1.49	4.87 ± 2.65*	4.85 ± 1.93$^{\#}$	9.66 ± 1.83*$^{\#}$

Data are presented as mean ± SD.
*P < 0.05 for the difference between respective PCOS and control groups.
$^{\#}P < 0.05$ for the difference between respective lean and obese groups.
[a]Glucose and insulin concentration in 120 min Oral glucose tolerance test (OGTT).

Table 2: Relationships of serum visfatin with anthropometric, biochemical and hormonal parameters in the whole study group ($n = 115$)

Parameter	*R*	*P*
BMI	0.23	0.013
Waist girth	0.24	0.011
Insulin sensitivity	−0.27	0.004
Postload glucose[a]	0.20	0.035
Serum FFA	0.20	0.045
HDL-cholesterol	−0.27	0.004
Serum FSH	−0.27	0.006
LH/FSH ratio	0.24	0.02
SHBG	−0.24	0.012
Testosterone	0.12	0.24
Free androgen index	0.29	0.004

[a]Glucose concentration in 120 min OGTT.

control group ($r = -0.15$, $P = 0.31$). Additionally, in lean women serum visfatin was related to serum testosterone and free androgen index ($r = 0.47$, $P = 0.002$ and $r = 0.48$, $P = 0.002$, respectively; Fig. 2). These associations were also present within the subgroup of lean PCOS subjects ($r = 0.46$, $P = 0.027$ and $r = 0.42$, $P = 0.048$, respectively). In the obese group, serum visfatin was associated with FFA ($r = 0.28$, $P = 0.027$) and HDL-cholesterol ($r = -0.36$, $P = 0.003$).

In the multiple regression analysis we observed that the association of visfatin with insulin sensitivity lost its significance after adjustment for BMI and FFA in the whole group ($\beta = 0.09$, $P = 0.26$) and for FFA in the subgroup of lean women ($\beta = -0.24$, $P = 0.14$). By contrast, visfatin remained a significant predictor of serum testosterone ($\beta = 0.77$, $P = 0.0009$) and free androgen index ($\beta = 0.41$, $P = 0.00096$) in lean subjects, after adjustment for possible confounding factors, including age, BMI, fasting and post-load glucose and insulin, *M* value, FFA, cholesterol, TG, LH/FSH ratio and SHBG.

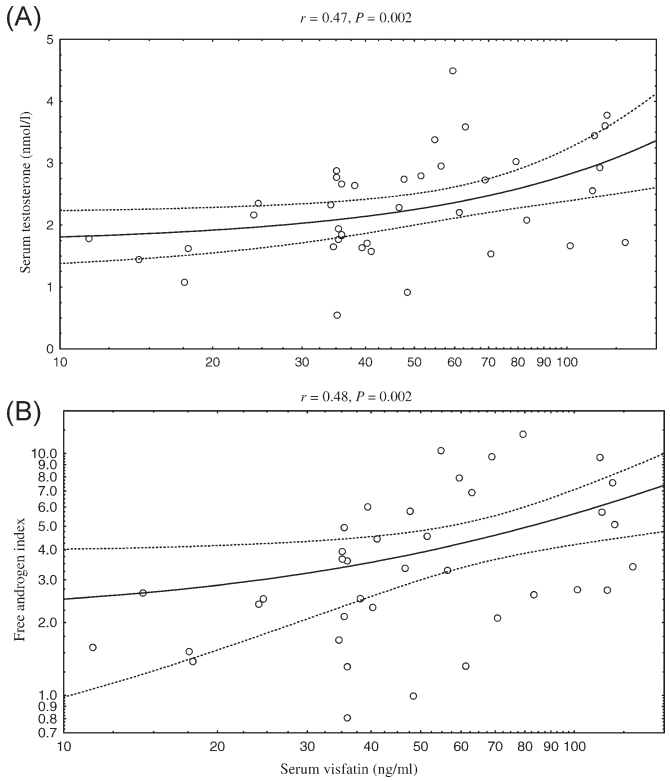

Figure 2: Correlation between serum visfatin and serum testosterone (A) and free androgen index (B) in lean subjects. Individual values of serum visfatin and free androgen index are shown on log-transformed scale.

Discussion

In the present study, we showed an increased serum visfatin concentration in PCOS women and its relationships with indices of insulin resistance and hyperandrogenism. These findings might suggest that visfatin could play a role in pathogenesis of PCOS. Surprisingly, the above observations were found only in the lean subjects. In previous studies, authors did not find a relation between circulating visfatin concentration and insulin action (Berndt *et al.*, 2005; Pagano *et al.*, 2006). Therefore, one might suppose that the correlation observed in our study might be due to the inclusion of this specific clinical group of insulin resistance i.e. patients with PCOS. The observed negative relation between the whole body glucose uptake and circulating visfatin might be in contrast with the suggested insulin-mimetic action of visfatin (Fukahara *et al.*, 2005). There is only one study regarding visfatin in PCOS and the researchers reported an increased adipose tissue visfatin expression and plasma visfatin levels (Tan *et al.*, 2006). This study was performed on a small group ($n = 8$) of overweight or obese PCOS subjects and no data on lean PCOS women was reported (Tan *et al.*, 2006). Other researchers reported an increase in serum visfatin concentration in different insulin resistant conditions, such as type 2 diabetes (Chen *et al.*, 2006), morbid obesity (Haider *et al.*, 2006c) or gestational diabetes (Krzyżanowska *et al.*, 2006), although opposing observations were also reported (Chan *et al.*, 2006; Pagano *et al.*, 2006). The correlation observed in our study indicates a possibility that in certain conditions visfatin cannot exert its potential beneficial metabolic actions or its increase is a secondary event in order to prevent further development of insulin resistance. On the other hand, insulin was shown to inhibit visfatin release from adipocytes (Haider *et al.*, 2006b), so the observed correlation might simply reflect insulin inability to suppress visfatin production in insulin resistance. However, this issue cannot be resolved on the basis of our cross-sectional study. Also, there is still a possibility that the observed association between visfatin and insulin sensitivity can be sex-specific.

Additionally, in agreement with the results obtained here, there is data related to FFA, HDL and post-load glucose concentration in the studied population. The contribution of FFA to development of insulin resistance is widely accepted (Roden *et al.*, 1996). The correlation between plasma visfatin and FFA was observed in both the obese subjects and in the whole study group. Studies in humans showed no effect of a 5-h intralipid infusion on circulating visfatin and adipose tissue mRNA expression (Pagano *et al.*, 2006a). In another study, it was demonstrated that lipid infusion decreased rosiglitazone-induced visfatin release, although there was no

effect of FFA elevation on plasma visfatin in the control subjects (Heider *et al.*, 2006a).

Serum visfatin was related to BMI and waist circumference, but not to percentage of body fat or fat mass estimated by bioelectric impedance method. Therefore, our data does not fully support the hypothesis that adipose tissue could be the most important source of circulating visfatin. Also Berndt at al. did not show a relationship between plasma visfatin and visceral fat mass assessed by computed tomography scans (Berndt *et al.*, 2006). Other authors suggest that macrophages of adipose tissue can be a major source of visfatin (Curat *et al.*, 2006).

An interesting observation is the lack of increase in serum visfatin in the obese PCOS subjects and absence of its association with insulin action in the whole obese group. PCOS is a complex and heterogeneous disorder and it is likely that different factors can contribute to its pathogenesis and characteristic features in different group of patients. Probably, obesity represents a state of dysregulation of visfatin secretion and thus can mask the effect of other factors, genetic or environmental, on this process. Our study indicates the importance of analyzing lean subjects in attempt to understand the metabolic role of visfatin *in vivo*. Our results on the lack of association of visfatin with insulin action in the obese group are in accordance with those reported by other researchers (Pagano *et al.*, 2006).

The novel result of our study is the correlation between visfatin and markers of hyperandrogenism in the whole group and especially in the lean subjects. Furthermore, visfatin remains an independent predictor of serum testosterone and free androgen index in the lean subjects. It should be pointed out that the relation between visfatin and testosterone is independent of insulin sensitivity and other confounding factors. This may indicate that visfatin could influence ovarian androgen secretion, however, we cannot rule out the influence of other unknown factors, which might regulate the release of both substances. The explanation of the association between visfatin and androgens is at present unknown. In the original study performed by Fukahara *et al.* (2005) visfatin was shown to exert insulin-mimetic properties through stimulation of insulin receptor. In view of the fact that hyperinsulinemia might stimulate ovarian androgen synthesis and secretion and thus contribute to the pathogenesis of PCOS, it is possible that relationships observed in our study might be due to insulin-like visfatin action. The above observation could add some new information to our understanding of this complex disease.

In conclusion, our data indicates that visfatin is associated with insulin resistance and markers of hyperandrogenism in lean PCOS patients.

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