Reproductive BioMedicine Online (2010) **20**, 150–155

www.sciencedirect.com
www.rbmonline.com

ARTICLE

Visfatin and retinol-binding protein 4 concentrations in lean, glucose-tolerant women with PCOS

Bulent O Yildiz [a,*], Gurkan Bozdag [b], Umit Otegen [b], Ayla Harmanci [a], Kubra Boynukalin [b], Zehra Vural [b], Serafettin Kirazli [c], Hakan Yarali [b]

[a] *Endocrinology and Metabolism Unit, Department of Internal Medicine, Hacettepe University School of Medicine, Hacettepe, 06100 Ankara, Turkey;* [b] *Department of Obstetrics and Gynecology, Hacettepe University School of Medicine, Hacettepe, 06100 Ankara, Turkey;* [c] *Hematology Unit, Department of Internal Medicine, Hacettepe University School of Medicine, Hacettepe, 06100 Ankara, Turkey*

[*] Corresponding author. *E-mail address:* yildizbo@yahoo.com (BO Yildiz).

Bulent O Yildiz MD is Associate Professor of Medicine at Hacettepe University School of Medicine in Ankara, Turkey. He received his medical degree from Hacettepe University School of Medicine, and completed his residency in internal medicine and a fellowship in endocrinology and metabolism at the same institution. His postdoctoral training included a fellowship at the Center for Pharmacogenomics, David Geffen School of Medicine at UCLA, Los Angeles, California, where he also completed a National Institutes of Health K30 Graduate Training Program in Translational Investigation. Dr Yildiz's current research interests involve the study of polycystic ovary syndrome and neuroendocrine regulation of food intake and body weight.

Abstract Since insulin resistance is accepted to be a common feature of polycystic ovary syndrome (PCOS), the exact molecular mechanism(s) involved in glucose and lipid metabolism have been under investigation in the syndrome. Recently, two novel adipokines, namely visfatin and retinol-binding protein 4 (RBP4), have been suggested to play a role in insulin resistance and diabetes. This study sought to determine whether plasma concentrations of visfatin and RBP4 are altered in PCOS by comparing a total of 27 lean, normal glucose-tolerant PCOS patients with 19 age- and body mass index-matched healthy controls. The mean plasma visfatin concentrations were higher in PCOS patients than those in healthy subjects (37.9 ± 18.2 versus 19.8 ± 17.5, $P < 0.01$), while RBP4 concentrations were similar between the two. Both adipokines were correlated with each other in the whole ($r = 0.50$, $P < 0.01$) and in PCOS ($r = 0.52$, $P < 0.01$) groups but not in controls. The results suggest that lean, glucose-tolerant women with PCOS have increased circulating visfatin and unaltered RBP4 concentrations compared with healthy lean women. In order to clarify overlapping effects and their potential contribution to the pathophysiology of PCOS, further studies are needed.

KEYWORDS: insulin resistance, PCOS, retinol-binding protein 4, visfatin

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder of reproductive-age women, affecting approximately 5–10% of this population (Asuncion et al., 2000; Azziz et al., 2004; Diamanti-Kandarakis et al., 1999). Besides infertility and chronic anovulation, patients with PCOS may also suffer from cardiovascular disease (Dokras, 2008; Giallauria et al., 2008; Legro, 2003) which may be related to hyperandrogenism and increased risk of obesity,

1472-6483/$ - see front matter
doi:10.1016/j.rbmo.2009.10.016

dyslipidaemia, impaired glucose intolerance/type 2 diabetes mellitus, dysfibrinolysis and thrombophilic abnormalities (Diamanti-Kandarakis, 2008; Guzick, 1996; Orio et al., 2004; Shroff et al., 2007; Vural et al., 2005; Wang and Norman, 2004; Yildiz et al., 2002).

Insulin resistance is a common feature of the syndrome and the exact molecular mechanism(s) of insulin resistance in PCOS are yet to be fully elucidated. Although women with PCOS are more insulin resistant than would be expected from their body mass index (BMI) (Barber et al., 2006, 2008), the presence of central obesity may have a further deleterious effect on the current insulin resistance level. In this manner, the adipose tissue may have an active role in the formation or progression of available insulin resistance by secreting various adipokines (Aigner et al., 2009) and/or adipocytokines (Carmina et al., 2006; Haider et al., 2006).

Recently, two novel adipokines, namely visfatin and retinol-binding protein 4 (RBP4), have been suggested to play a role in insulin resistance, diabetes and obesity among patients with PCOS (Tan et al., 2006, 2007). Visfatin/pre-B cell colony-enhancing factor is abundantly expressed in visceral adipose tissue and exerts an insulin-mimetic effect by binding to the insulin receptor at a site distinct from insulin. Animal studies reported that an adipocyte-secreted protein, RBP4, may also contribute to insulin resistance (von Eynatten and Humpert, 2008). Although most of the available data suggest that both visfatin and RBP4 concentrations are increased in overweight PCOS patients (Barber et al., 2008; Ozkaya et al., 2008; Tan et al., 2006, 2007; Weiping et al., 2006), there have been few studies evaluating the serum concentrations of visfatin and RBP4 in lean individuals with PCOS (Chan et al., 2008; Hahn et al., 2007; Panidis et al., 2008).

As far as is known, there is no study assessing both visfatin and RBP4 in the same cohort of patients consisting of only lean, glucose-tolerant women suffering from PCOS. In the current study, the hypothesis was that circulating visfatin and RBP4 concentrations are increased in PCOS and that these two adipokines and their potential interaction contribute to the insulin resistance of the syndrome. The study specifically chose non-obese and normal glucose-tolerant women with PCOS in order to eliminate the confounding effects of obesity and glucose intolerance with regard to adipokine pathophysiology of PCOS.

Materials and methods

Subjects

This study prospectively recruited 27 lean, glucose-tolerant PCOS patients and 19 age- and BMI-matched healthy controls. The diagnosis of PCOS was made by the presence of any two of the following three criteria: (i) clinical and/or biochemical evidence of hyperandrogenism; (ii) chronic oligo-/anovulation; and/or (iii) polycystic ovaries on ultrasound (PCOS) (Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004). Hyperandrogenism and chronic oligo-anovulation were defined as previously described (Yarali et al., 2002; Yildiz et al., 2002). PCO was defined as the presence of 12 or more follicles in each ovary, each measuring 2–9 mm in diameter, and/or increased ovarian volume >10 ml (Balen et al., 2003; Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004). Cushing's syndrome, non-classical congenital adrenal hyperplasia, hyperprolactinaemia, thyroid dysfunction and androgen-secreting tumours were excluded as suggested (Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group, 2004). Subjects were not taking any medication for at least 3 months before the study. All the patients had clinical and/or biochemical hyperandrogenism and chronic anovulation, and 85% (23/27) of the patients had polycystic ovaries on ultrasound (PCO).

The control group consisted of healthy women who had regular menstrual cycles without clinical or biochemical hyperandrogenism or PCO. They did not have a history of any drug intake for at least 3 months. None of the participants in the study had impaired fasting glucose, impaired glucose tolerance or type 2 diabetes. Exclusion criteria included smoking and alcohol consumption in both groups.

Study protocol

The study protocol was approved by the institutional review board of Hacettepe University Medical School and informed consent was obtained from all participants.

Anthropometric measurements including (BMI) and waist circumference were determined. Subjects underwent a standard 2-h 75 g oral glucose tolerance test between 8 and 10 a.m. after an overnight fast during which fasting and 120 min glucose concentrations obtained. All sampling procedures were performed in the early follicular phase (days 2–5). Blood samples were drawn from large antecubital veins of the forearm and centrifuged, within 30 min of collection, at 4°C for 20 min at 1238 *g*. Fasting serum and plasma samples obtained were transferred into polypropylene tubes and stored for up to 3 months at −80°C until assayed for visfatin, RBP4 total testosterone, sex hormone binding globulin (SHBG), fasting insulin, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglycerides.

Assays

Plasma visfatin C-terminal concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using a commercially available assay (Phoenix Europe, Karlsruhe, Germany). The intra- and inter-assay coefficients of variation (CV) for this assay were 4.3% and 5.8% respectively. The RBP4 concentrations were also analysed by ELISA using a commercially available assay (Phoenix Europe). The intra- and inter-assay CV for this assay were 5.1% and 6.9% respectively. Plasma glucose was measured by the glucose oxidase technique (Roche Molecular Biochemicals, Mannheim, Germany). Insulin was measured by radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX, USA). The intra- and inter-assay CV were 4.5% and 8.9% respectively. Total testosterone concentrations were measured by chemiluminescent enzyme immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA), with intra- and inter-assay CV of 6% and 7% respectively. SHBG was measured by immunoradiometric assay (Diagnostic

Systems Laboratories) with intra- and inter-assay CV of 3.7% and 9.4% respectively. Plasma total cholesterol, HDL-C and triglyceride concentrations were determined by enzymatic colourimetric method (Roche Molecular Biochemicals). The average intra- and inter-assay CV were 1.4% and 2.2%, respectively.

Free androgen index (FAI) was calculated from total testosterone and SHBG concentrations (FAI = total testosterone × 100/SHBG). Homeostasis model assessment-insulin resistance (HOMA-IR) (Araujo Penna et al., 2007) was applied by using the following formula: HOMA-IR = fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5. The quantitative insulin-sensitivity check index (QUICKI) was calculated as l/[log(fasting insulin) + log(fasting glucose)]. Homeostasis model assessment β-cell function (HOMA β-cell) was calculated as: HOMA β-cell = fasting insulin (μU/ml) × 20/fasting glucose (mmol/l) − 3.5.

Data analysis

The normality of the distribution of the variables was confirmed by Shapiro–Wilk test. Unpaired *t*- and Mann–Whitney *U* tests were used for normally and not normally distributed variables, respectively. Values were given as mean ± SD unless stated otherwise. Spearman test was used for correlation analyses. $P < 0.05$ was considered statistically significant. Data analysis was performed using the Statistics Package for Social Sciences version 13.0 (SPSS, Chicago, IL, USA).

Results

The baseline demographic, hormonal and biochemical features of the patients are presented in **Table 1**. While the mean age, BMI and waist circumference were comparable between the PCOS and control groups, both total testosterone and FAI were significantly higher in patients with PCOS, as expected ($P < 0.01$ for both). The median fasting insulin and HOMA-IR were also found to be higher in patients with PCOS, when compared with healthy controls ($P < 0.01$ for both). Although HOMA β-cell scores were higher in patients with PCOS, the result did not reach statistical significance). The fasting glucose and lipid profile were similar between the study groups.

The mean plasma visfatin concentrations were higher in PCOS patients than those in healthy subjects (37.9 ± 18.2 versus 19.8 ± 17.5, $P < 0.01$; **Figure 1**), while median RBP4 concentrations were similar between the two groups, as shown in **Table 1**.

Neither visfatin nor RBP4 were associated with BMI, FAI or lipids. There were correlations between visfatin and fasting insulin ($r = 0.42$ $P < 0.01$), HOMA-IR ($r = 0.36$, $P < 0.05$) and total testosterone ($r = 0.37$, $P < 0.05$) reaching statistical significance; but not with QUICKI ($r = -0.05$) or fasting glucose/insulin ratio ($r = -0.20$). Of interest, RBP4 concentrations were just associated with serum visfatin concentrations ($r = 0.50$, $P < 0.01$) as shown in **Figure 2**. Subgroup analyses showed that this correlation was significant in PCOS women ($r = 0.52$, $P < 0.01$) but not in healthy women ($r = 0.32$).

Conclusion

The data indicate that lean, normal glucose-tolerant women with PCOS may have increased circulating visfatin concentrations without any alteration in RBP4 concentrations. Of interest, a correlation between visfatin and RBP4 is presented among lean women with PCOS, which has not been reported elsewhere.

In the available literature, serum concentrations of visfatin and RBP4 are widely examined among overweight PCOS

Table 1 Clinical, hormonal and biochemical characteristics of polycystic ovary syndrome (PCOS) women and controls.

Variable	*PCOS (n = 27)*	*Control (n = 19)*	*P-value*
Age (years)	25.4 ± 4.4	27.0 ± 4.4	NS
Body mass index (kg/m^2)	23.2 ± 2.9	22.5 ± 3.2	NS
Waist (cm)	74.9 ± 9.1	74.8 ± 15.9	NS
Total testosterone (ng/dl)	103.7 ± 25.5	51.2 ± 18.1	< 0.01
Free androgen index	5.9 (4.3–7.4)	1.6 (1.1–3.3)	< 0.01
Fasting glucose (mg/dl)	84.1 ± 7.2	81.9 ± 6.4	NS
Fasting insulin (μU/ml)	12.9 (10.9–16.3)	9.2 (6.7–10.7)	< 0.01
Fasting glucose:insulin ratio	6.4 (5.5–7.5)	8.6 (7.9–12.7)	< 0.01
HOMA-IR	2.7 (2.4–3.3)	1.7 (1.3–2.1)	< 0.01
HOMA β-cell	240.8 (176.9–304.8)	181.5 (128.9–181.5)	NS
QUICKI	0.33 (0.32–0.34)	0.35 (0.34–0.37)	< 0.01
Total cholesterol (mg/dl)	164.4 ± 28.2	169.7 ± 29.8	NS
HDL-C (mg/dl)	62.4 ± 13.8	65.1 ± 12.1	NS
Triglycerides (mg/dl)	68.0 (53.0–90.0)	73.1 (57.2–99.0)	NS
Visfatin (ng/ml)	37.9 ± 18.2	19.8 ± 17.5	< 0.01
RBP4 (mg/dl)	91.5 (39.9–148.8)	44.8 (33.2–148.8)	NS

Values are mean ± SD or, for not normally distributed variables, median (interquartile range).
HDL-C, high-density lipoprotein cholesterol; HOMA β-cell, homeostasis model assessment – β-cell function; HOMA-IR, homeostasis model assessment – insulin resistance; NS, not statistically significant; QUICKI, quantitative insulin-sensitivity check index; RBP4, retinol-binding protein 4.

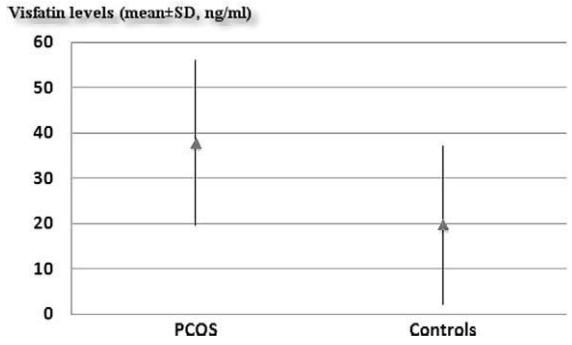

Figure 1 Visfatin concentrations of patients with polycystic ovary syndrome (PCOS) and controls.

women (Barber et al., 2008; Ozkaya et al., 2008; Tan et al., 2006, 2007; Weiping et al., 2006). Initially, Tan et al. (2006) demonstrated both increased visfatin mRNA and protein concentrations in adipose tissue and adipocytes in women with PCOS having a BMI as high as >28 kg/m^2, with a parallel increase in plasma concentration. Subsequently, Ozkaya et al. (2008) reported that serum visfatin concentrations were significantly higher in overweight women with PCOS than in controls and it may be decreased by administration of two daily doses of 850 mg metformin for 3 months. In concordance, when compared with healthy controls, the plasma concentration of visfatin was also found to be significantly higher in patients with PCOS, even if only lean/normal-weight subjects are examined (Kowalska et al., 2007; Panidis et al., 2008; Zwirska-Korczala et al., 2008). Kowalska et al. (2007) reported a positive correlation between serum visfatin concentrations and FAI ($r = 0.48$, $P = 0.002$) and testosterone ($r = 0.47$, $P = 0.002$) whereas a negative relationship was observed with insulin sensitivity ($r = -0.30$, $P = 0.038$) in 23 lean PCOS women. In addition, Panidis et al. (2008) reported a positive correlation with LH ($r = 0.298$, $P < 0.05$) as well as with FAI ($r = 0.359$, $P < 0.005$) but negative association with SHBG ($r = -0.266$, $P < 0.05$) in 25 normal-weight women with PCOS. In this study of 27 PCOS women, positive associations were detected between visfatin and total testosterone, fasting insulin and HOMA-IR, whereas visfatin did not show a correlation with any of the other clinical, hormonal or biochemical variables including BMI, FAI, fasting insulin, fasting glucose/insulin ratio, QUICKI and lipids. This study has also failed to show an association between visfatin and HOMA β-cell in contrast with a previous study reporting an association between serum visfatin concentrations and β-cell deterioration in patients with type 1 and type 2 diabetes (Lopez-Bermejo et al., 2006).

RBP4 is a new adipokine which appears to modulate insulin sensitivity in mice (Muoio and Newgard, 2005; Yang et al., 2005). In addition, altered RBP4 concentrations have also been presented among human subjects with obesity and type 2 diabetes mellitus (Abahusain et al., 1999), even before the overt appearance of diabetes itself (Graham et al., 2006). Weiping et al. (2006) reported that RBP4 concentrations were higher in 39 patients with PCOS when compared with 45 healthy controls. Of interest, the only variable correlated with the RBP4 concentration was found to be glucose disposal rate. Similarly, Tan et al. (2007) revealed significant up-regulation of RBP4 mRNA in both subcutaneous and omental adipose tissue as well as in isolated subcutaneous adipocytes of PCOS women, in addition to elevated serum RBP4 concentrations when compared with healthy controls. In contrast, Barber et al. (2008) failed to establish a significant difference between serum concentrations of RBP4 of patients with PCOS and controls. However, cross-sectional area of abdominal visceral fat depots, but not subcutaneous, derived from axial magnetic resonance images at the level of the mid-L4 vertebral body was correlated with serum concentrations. When only lean PCOS cases are included, the available data is more inconclusive. In 58 lean PCOS patients, Hahn et al. (2007) did not observe any significant difference with regard to RBP4 concentrations when compared with 64 control cases. In contrast, Chan et al. (2008) observed higher concentrations of RBP4 among 37 lean patients when checked against with 37 age- and BMI-matched controls. The present study failed to demonstrate any significant difference of RBP4 concentrations between the PCOS and control groups or any correlations of serum RBP4 concentrations with clinical, hormonal or biochemical variables. It is difficult to comment on whether the lack of statistical significance is due to a genuine absence of a correlation or due to the limited sample size of this study. Factors such as limited sample sizes of previously published studies on the subject and lack of relevant data required for a priori assumptions of an appropriate sample size analysis prevent a definitive conclusion. Contradictory results of multiple small-sized studies may be merely a reflection of random pre-analytical or analytical variation. Nevertheless, a post hoc power analysis of this study indicated that at least 200 individuals in each group would be needed to attain a power of 80%, suggesting that a difference of circulating RBP4 concentrations between PCOS and control women, if present, is quite modest.

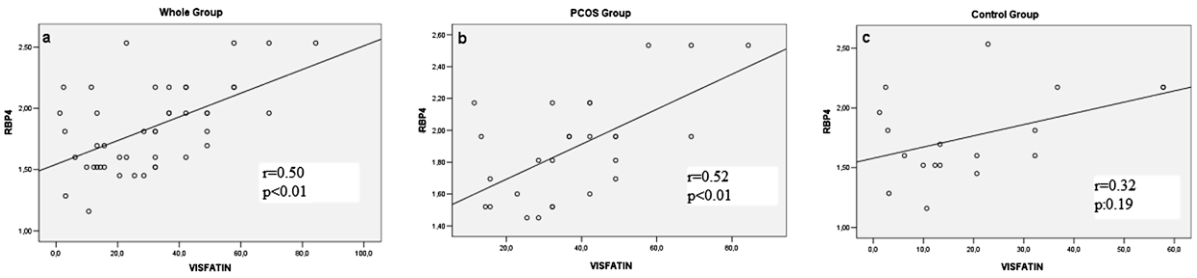

Figure 2 Scatter plot histograms showing correlations between retinol-binding protein 4 (RBP4) and visfatin concentrations within (**a**) the whole group, (**b**) the polycystic ovary syndrome group, and (**c**) the control group.

Both visfatin and RBP4 seem to play similar roles in the pathogenesis of PCOS. Although it is not clear, both of them may be responsible from the increased insulin resistance and presence of androgenic phenotype of the syndrome to an extent. However, it is hard to explain the whole mechanism responsible for the impaired glucose metabolism and hyperandrogenaemia with a few cytokines, adipokines or adipocytokines. One may postulate that different molecules may be the leading subject responsible for each phenotypic expression of the syndrome in different subgroups. In fact, while some authors claimed that RBP4 is responsible for higher androgen concentrations and hirsutism scores (Aigner et al., 2009) rather than glucose metabolism, others reported a close correlation with lipid (Chan et al., 2008) or glucose metabolism (Barber et al., 2006; Weiping et al., 2006). These conflicting results may be based on not only the BMI but geographical divergence within the patients with PCOS. The variations in the commercial immunoassays (Graham et al., 2007) might also have played a role in differences between the earlier reports and the current data.

Although the exact role that visfatin and RBP4 play in the pathophysiology of PCOS remains to be determined, it is possible that both the alterations in the circulating concentrations of these adipokines and their potential close interaction might have an impact on the phenotypic features of the syndrome including hyperandrogenism, insulin resistance, glucose intolerance and adiposity. Regarding an alteration in the circulating concentrations of an adipokine, the finding of increased visfatin concentrations in association with fasting insulin and total testosterone concentrations, in the absence of obesity and glucose intolerance, suggests that dysfunctional production and/or action of this adipokine might play a role in the insulin resistance and hyperandrogenism of PCOS. On the other hand, a close correlation between circulating visfatin and RBP4 concentrations is also reported among the same cohort of patients with PCOS. Of interest, there is no correlation when only control subjects are undertaken, which may suggest that an interaction between visfatin and RBP4 might have a specific impact on the pathophysiology of PCOS. This finding may also point to the fact that overlapping effects of such molecules may be responsible for the conflicting results in cases with PCOS.

The main limitations of the current study are the small sample size and cross-sectional nature. However, it is worthy to note that obesity and diabetes are not confounding factors for any of the groups in the current study. The results suggest that lean normal glucose-tolerant women with PCOS have increased circulating visfatin and unaltered RBP4 concentrations compared with healthy lean women. A more detailed understanding of the individual roles of adipokines and apparently complex interrelations between them merit further consideration, in order to clarify overlapping effects and their potential contribution to the pathophysiology of PCOS.

References

Abahusain, M.A., Wright, J., Dickerson, J.W., et al., 1999. Retinol, alpha-tocopherol and carotenoids in diabetes. Eur. J. Clin. Nutr. 53, 630—635.

Aigner, E., Bachofner, N., Klein, K., et al., 2009. Retinol-binding protein 4 (RBP4) in polycystic ovary syndrome—association with steroid hormones and response to pioglitazone treatment. J. Clin. Endocrinol. Metabol. 94, 1229—1235.

Araujo Penna, I., Canella, P.R., Vieira, C.S., et al., 2007. Cardiovascular risk factors are reduced with a low dose of acarbose in obese patients with polycystic ovary syndrome. Fertil. Steril. 88, 519—522.

Asuncion, M., Calvo, R.M., San Millan, J.L., et al., 2000. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J. Clin. Endocrinol. Metabol. 85, 2434—2438.

Azziz, R., Woods, K.S., Reyna, R., et al., 2004. The prevalence and features of the polycystic ovary syndrome in an unselected population. J. Clin. Endocrinol. Metabol. 89, 2745—2749.

Balen, A.H., Laven, J.S., Tan, S.L., et al., 2003. Ultrasound assessment of the polycystic ovary: international consensus definitions. Hum. Reprod. Update 9, 505—514.

Barber, T.M., Hazell, M., Christodoulides, C., et al., 2008. Serum levels of retinol-binding protein 4 and adiponectin in women with polycystic ovary syndrome: associations with visceral fat but no evidence for fat mass-independent effects on pathogenesis in this condition. J. Clin. Endocrinol. Metabol. 93, 2859—2865.

Barber, T.M., McCarthy, M.I., Wass, J.A., et al., 2006. Obesity and polycystic ovary syndrome. Clin. Endocrinol. (Oxf.) 65, 137—145.

Carmina, E., Orio, F., Palomba, S., et al., 2006. Endothelial dysfunction in PCOS: role of obesity and adipose hormones. Am. J. Med. 119 (356), e1—e6.

Chan, T.F., Tsai, Y.C., Chiu, P.R., et al., 2008. Serum retinol-binding protein 4 levels in nonobese women with polycystic ovary syndrome. Fertil. Steril. [e-publication ahead of print November 22].

Diamanti-Kandarakis, E., 2008. Polycystic ovarian syndrome: pathophysiology, molecular aspects and clinical implications. Expert Rev. Mol. Med. 10, e3.

Diamanti-Kandarakis, E., Kouli, C.R., Bergiele, A.T., et al., 1999. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J. Clin. Endocrinol. Metabol. 84, 4006—4011.

Dokras, A., 2008. Cardiovascular disease risk factors in polycystic ovary syndrome. Semin. Reprod. Med. 26, 39—44.

Giallauria, F., Orio, F., Palomba, S., et al., 2008. Cardiovascular risk in women with polycystic ovary syndrome. J. Cardiovasc. Med. (Hagerstown) 9, 987—992.

Graham, T.E., Wason, C.J., Bluher, M., et al., 2007. Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. Diabetologia 50, 814—823.

Graham, T.E., Yang, Q., Bluher, M., et al., 2006. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N. Engl. J. Med. 354, 2552—2563.

Guzick, D.S., 1996. Cardiovascular risk in women with polycystic ovarian syndrome. Semin. Reprod. Endocrinol. 14, 45—49.

Hahn, S., Backhaus, M., Broecker-Preuss, M., et al., 2007. Retinol-binding protein 4 levels are elevated in polycystic ovary syndrome women with obesity and impaired glucose metabolism. Eur. J. Endocrinol. 157, 201—207.

Haider, D.G., Schaller, G., Kapiotis, S., et al., 2006. The release of the adipocytokine visfatin is regulated by glucose and insulin. Diabetologia 49, 1909—1914.

Kowalska, I., Straczkowski, M., Nikolajuk, A., et al., 2007. Serum visfatin in relation to insulin resistance and markers of hyperandrogenism in lean and obese women with polycystic ovary syndrome. Hum. Reprod. 22, 1824—1829.

Legro, R.S., 2003. Polycystic ovary syndrome and cardiovascular disease: a premature association? Endocr. Rev. 24, 302—312.

Lopez-Bermejo, A., Chico-Julia, B., Fernandez-Balsells, M., et al., 2006. Serum visfatin increases with progressive beta-cell deterioration. Diabetes 55, 2871—2875.

Muoio, D.M., Newgard, C.B., 2005. Metabolism: A is for adipokine. Nature 436, 337—338.

Orio Jr., F., Palomba, S., Cascella, T., et al., 2004. Is plasminogen activator inhibitor-1 a cardiovascular risk factor in young women with polycystic ovary syndrome? Reprod. BioMed. Online 9, 505—510.

Ozkaya, M., Cakal, E., Ustun, Y., et al., 2008. Effect of metformin on serum visfatin levels in patients with polycystic ovary syndrome. Fertil. Steril. [e-publication ahead of print 24 December].

Panidis, D., Farmakiotis, D., Rousso, D., et al., 2008. Plasma visfatin levels in normal weight women with polycystic ovary syndrome. Eur. J. Intern. Med. 19, 406—412.

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.

Shroff, R., Kerchner, A., Maifeld, M., et al., 2007. Young obese women with polycystic ovary syndrome have evidence of early coronary atherosclerosis. J. Clin. Endocrinol. Metabol. 92, 4609—4614.

Tan, B.K., Chen, J., Digby, J.E., et al., 2006. Increased visfatin messenger ribonucleic acid and protein levels in adipose tissue and adipocytes in women with polycystic ovary syndrome: parallel increase in plasma visfatin. J. Clin. Endocrinol. Metabol. 91, 5022—5028.

Tan, B.K., Chen, J., Lehnert, H., et al., 2007. Raised serum, adipocyte, and adipose tissue retinol-binding protein 4 in overweight women with polycystic ovary syndrome: effects of gonadal and adrenal steroids. J. Clin. Endocrinol. Metabol. 92, 2764—2772.

von Eynatten, M., Humpert, P.M., 2008. Retinol-binding protein-4 in experimental and clinical metabolic disease. Expert Rev. Mol. Diagn. 8, 289—299.

Vural, B., Caliskan, E., Turkoz, E., et al., 2005. Evaluation of metabolic syndrome frequency and premature carotid atherosclerosis in young women with polycystic ovary syndrome. Hum. Reprod. 20, 2409—2413.

Wang, J.X., Norman, R.J., 2004. Risk factors for the deterioration of glucose metabolism in polycystic ovary syndrome. Reprod. BioMed. Online 9, 201—204.

Weiping, L., Qingfeng, C., Shikun, M., et al., 2006. Elevated serum RBP4 is associated with insulin resistance in women with polycystic ovary syndrome. Endocrine 30, 283—287.

Yang, Q., Graham, T.E., Mody, N., et al., 2005. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 436, 356—362.

Yarali, H., Yildiz, B.O., Demirol, A., et al., 2002. Co-administration of metformin during rFSH treatment in patients with clomiphene citrate-resistant polycystic ovarian syndrome: a prospective randomized trial. Hum. Reprod. 17, 289—294.

Yildiz, B.O., Haznedaroglu, I.C., Kirazli, S., et al., 2002. Global fibrinolytic capacity is decreased in polycystic ovary syndrome, suggesting a prothrombotic state. J. Clin. Endocrinol. Metabol. 87, 3871—3875.

Zwirska-Korczala, K., Sodowski, K., Konturek, S.J., et al., 2008. Postprandial response of ghrelin and PYY and indices of low-grade chronic inflammation in lean young women with polycystic ovary syndrome. J. Physiol. Pharmacol. 59 (Suppl. 2), 161—178.

Declaration: The authors report no financial or commercial conflicts of interest.

Received 1 May 2009; refereed 12 May 2009; accepted 29 September 2009.