Serum Pigment Epithelium-Derived Factor Is Elevated in Women with Polycystic Ovary Syndrome and Correlates with Insulin Resistance

Shumin Yang, Qifu Li, Li Zhong, Ying Song, Bo Tian, Qingfeng Cheng, Hua Qing, Wei Xia, Mei Luo, and Mei Mei

Department of Endocrinology, The First Affiliated Hospital of Chongqing Medical University, Chongqing 400016, China

Context: Serum pigment epithelium-derived factor (PEDF) is highly expressed in adipose tissue and plays an important role in insulin resistance (IR). However, there are no data on serum PEDF levels and their relationship with IR in polycystic ovary syndrome (PCOS) women.

Objective: To quantitate serum PEDF levels and examine their relationship with IR in women with PCOS.

Participants and Design: Ninety-six PCOS women and 63 healthy age-matched controls were recruited. Ninety-six PCOS women and 20 controls underwent hyperinsulinemic–euglycemic clamp to assess their insulin sensitivity, which was expressed as M value. IR was also estimated by homeostasis model assessment 2 (HOMA2-IR).

Setting: The study was performed at a clinical research center.

Results: PCOS women had lower M value and higher HOMA2-IR as compared with controls. Serum PEDF levels were much higher in PCOS women than in controls (5.45 ± 1.85 *vs.* $3.97 \pm 0.98 \mu$g/ml, $P < 0.01$). Spearman correlation analysis showed that in PCOS women, PEDF positively correlated with body mass index, waist circumference, HOMA2-IR, triglycerides, total cholesterol, low-density lipoprotein cholesterol, and systolic blood pressure and negatively correlated with M value and high-density lipoprotein cholesterol. Multiple linear regression analysis revealed that in PCOS women, after adjustment for body mass index, systolic blood pressure, and serum lipids (triglycerides, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol), PEDF was still associated with M value or HOMA2-IR.

Conclusions: The serum PEDF level is elevated in women with PCOS and is associated with IR. PEDF may play a role in the pathogenesis of IR in PCOS. **(*J Clin Endocrinol Metab* 96: 831–836, 2011)**

Polycystic ovary syndrome (PCOS) is one of the most common endocrinopathies in reproductive-age women, and it affects 5–7% of this group (1). It is characterized by disturbed menstrual cycle, ovulatory dysfunction, and hyperandrogenism (2–3). More than 40% of PCOS women might develop impaired glucose tolerance or type 2 diabetes mellitus (4–5). It has been confirmed that insulin resistance (IR) is a common feature in PCOS, given that both lean and obese women with PCOS have decreased insulin sensitivity when compared with body mass index (BMI)-matched control subjects (6–9).

It is now clear that adipocytes can secrete a large variety of proteins, such as leptin, adiponectin, and resistin (10). The production of adipokines that have been proposed to play roles in the pathogenesis of obesity, type 2 diabetes mellitus, and PCOS is of particular interest because these

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-2140 Received September 10, 2010. Accepted December 6, 2010.
First Published Online January 5, 2011

Abbreviations: BMI, Body mass index; DBP, diastolic blood pressure; HDL-c, high-density lipoprotein cholesterol; HOMA2-IR, homeostasis model assessment 2 of insulin resistance; IR, insulin resistance; LDL-c, low-density lipoprotein cholesterol; PCOS, polycystic ovary syndrome; PEDF, pigment epithelium-derived factor; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

adipokines have wide-ranging effects on carbohydrate and lipid metabolism (11–12). Pigment epithelium-derived factor (PEDF) is a recently discovered adipokine that plays an important role in IR: Crowe *et al.* (13) found serum PEDF level and adipocyte PEDF expression elevated in genetically obese (ob/ob) and high-fat diet–induced obese mice and reduced upon weight loss using either caloric restriction or ciliary neurotrophic factor administration; prolonged PEDF administration stimulates adipose tissue lipolysis, which results in ectopic lipid deposition and reduces insulin sensitivity, whereas neutralizing PEDF with PEDF antibody for 5 d in obese mice enhances insulin sensitivity. Clinical research has revealed that the serum PEDF level is associated with risk factors of metabolic syndrome and increased in proportion to the accumulation of the number of components of metabolic syndrome (14–15). Additionally, Nakamura *et al.* (16) found that PEDF is an independent determinant of IR in patients with essential hypertension.

However, there are no data on serum PEDF levels and their relationship to IR in PCOS women. The aims of this study were to compare serum PEDF levels between PCOS women and control subjects and to investigate the association of the serum PEDF level with IR assessed by hyperinsulinemic euglycemic clamp and by homeostasis model assessment 2 of IR (HOMA2-IR).

Materials and Methods

Study population

PCOS women (n = 96) were recruited from those who visited the Department of Endocrinology or the Department of Obstetrics and Gynecology of the First Affiliated Hospital of Chongqing Medical University from November 2007 to January 2010. The diagnosis of PCOS was based on the 2003 Rotterdam consensus (The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group) with at least two of the following features (17): 1) oligo-amenorrhea or chronic anovulation; 2) clinical and/or biochemical hyperandrogenism; 3) ultrasound appearance of polycystic ovaries, after exclusion of other known causes of hyperandrogenemia and ovulatory dysfunction, including 21-hydroxylase deficiency, congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumors, thyroid disease, and hyperprolactinemia. Sixty-three healthy age-matched volunteer women were recruited as control subjects. All control subjects had a normal menstrual cycle, and none had clinical and/or biochemical hyperandrogenism. Exclusion criteria for both groups also included the use of hormone medications (including oral contraceptives) within the past month and the use of medicines that affect insulin sensitivity (*e.g.*, metformin or thiazolidinediones) within the past three months. Informed consent was obtained from all participants, and the study was approved by the First Affiliated Hospital of Chongqing Medical University Ethical Committee.

Data collection

All patients underwent anthropometric measurements: height, weight, and waist circumference (WC). Blood pressure was measured with the subjects in a sitting position after a minimum 5-min rest. BMI was determined as the ratio between weight in kilograms and the square of height in meters.

Biochemical measurements

An oral glucose tolerance test with 75 g glucose was performed after overnight fasting on all subjects. All blood samples were centrifuged, and separated serum was kept frozen at −80 C until the time of the assay. Serum PEDF concentration was determined with PEDF sandwich ELISA kit (Millipore Corp, Billerica, MA). Intra- and interassay coefficients of variation were 5.6 and 6.5%, respectively. Fasting plasma glucose and 2-hour postload plasma glucose were measured by the Hexokinase-UV/NAD method (Olympus, Tokyo, Japan). Fasting serum insulin and total testosterone were determined by the electrochemiluminescence method (Roche, Basel, Switzerland). Serum lipids were measured as follows: total cholesterol (TC) using the cholesterol oxidase-HDAOS method (Wako, Osaka, Japan); triglycerides (TG) using the GPO-HDAOS Glycerol blanking method (Wako); high-density lipoprotein cholesterol (HDL-c) using the immunoinhibition (direct) method (Wako) and low-density lipoprotein cholesterol (LDL-c) using the selective protection enzymatic (direct) method (Wako).

IR index

HOMA2-IR: IR in all participants was estimated by HOMA2-IR, which was calculated by using the HOMA Calculator v2.2.2 downloaded from http://www.dtu.ox.ac.uk.

Hyperinsulinemic–euglycemic clamp

Hyperinsulinemic–euglycemic clamps performed 6–8 d after oral glucose tolerance test were used to assess insulin sensitivity in 96 PCOS women and 20 controls. Clamps were carried out according to our previous report with some modifications (18): after fasting overnight, subjects were admitted to the hospital, where iv catheters were placed in both arms for insulin and glucose infusion and for blood sampling. The insulin infusion was performed with a 10-min priming dose of short-acting human insulin (Humulin, Lilly) and then maintained at a rate of 120 mU/(m^2 × min) for the next 170 min, for a total of 180 min. A variable infusion of 20% glucose was started at the fourth minute to maintain the plasma glucose concentration at 5.2 mmol/liter (4.9–5.5 mmol/liter), and blood samples for the measurement of plasma glucose were obtained at 5-min intervals throughout the clamp. Plasma glucose was measured by the glucose oxidase method using the Biosen 5030 Glucose Analyzer (EKF Industrie, Elektronik GmbH, Barleben, Germany). M value (mg/min × kg^{-1}) was calculated from the glucose infusion rates during the last 60 min of the hyperinsulinemic–euglycemic clamp.

Diagnostic criteria for overweight/obesity

Diagnosis of overweight/obesity was based on the Guidelines for Prevention and Control of Overweight and Obesity in Chinese Adults: 24.0 kg/m^2 ≤ BMI ≤ 27.9 kg/m^2 was overweight, BMI ≥ 28.0 kg/m^2 was obese, and BMI < 24.0 kg/m^2 was lean (19).

TABLE 1. Physical and biochemical characteristics of PCOS women and control subjects

Variables	Controls (n = 63)	PCOS (n = 96)	*P* value
Age, years	25.41 ± 2.47	25.48 ± 4.33	0.91
BMI, kg/m^2	21.12 ± 2.18	23.14 ± 4.21	<0.05
WC, cm	69.79 ± 6.46	78.18 ± 11.68	<0.05
SBP, mm Hg	110.00 ± 8.28	109.67 ± 8.14	0.32
DBP, mm Hg	70.44 ± 8.19	69.49 ± 6.40	0.44
Fasting plasma glucose, mmol/liter	4.87 ± 0.38	5.19 ± 0.61	<0.05
2-h postload plasma glucose, mmol/liter	5.46 ± 1.10	7.09 ± 1.96	<0.05
TC, mmol/liter	4.26 ± 0.87	4.06 ± 0.84	0.17
TG, mmol/liter	0.76 ± 0.41	1.42 ± 1.59	<0.05
HDL-c, mmol/liter	1.37 ± 0.27	1.04 ± 0.23	<0.05
LDL-c, mmol/liter	2.06 ± 0.53	1.94 ± 0.65	0.22
Fasting serum insulin, mU/liter	7.49 ± 8.52	10.20 ± 8.42	<0.05
Total testosterone, ng/dl	46.00 ± 21.20	59.76 ± 29.74	<0.05
PEDF, μg/ml	3.97 ± 0.98	5.45 ± 1.85	<0.05
M value, mg/min × kg^{-1}[a]	12.17 ± 1.16	8.38 ± 2.92	<0.05
HOMA2-IR	0.98 ± 0.40	1.53 ± 0.95	<0.05

Data are presented as mean ± SD and analyzed by independent sample *t* test; HOMA2-IR, TG, fasting serum insulin, fasting plasma glucose, and 2-h postload plasma glucose were $\log_2$-transformed before comparison.

[a] Ninety-six PCOS women and 20 control subjects underwent hyperinsulinemic–euglycemic clamp.

Statistical analysis

All statistical analysis was performed using the statistical software SPSS 13.0. Results are expressed as mean ± SD. Fasting serum insulin, TG, fasting plasma glucose, 2-hour postload plasma glucose and HOMA2-IR were $\log_2$-transformed before analysis due to nonnormal distribution. Data involving more than two groups were assessed by one-way ANOVA [with Games Howell test (in case of heterogeneity of variance) and Student-Newman-Keuls test (in case of homogeneity of variance) for *post hoc* analysis]. Independent sample *t* test was used in comparisons between two groups. Spearman correlation and multiple linear regression analysis were used to evaluate the relationship of PEDF with IR and other covariates. *P* values <0.05 (two-tailed) were considered statistically significant.

Results

General characteristics

PCOS women and control subjects were well matched in terms of age (25.48 ± 4.33 *vs.* 25.41 ± 2.47 yr, $P = 0.91$). Compared with control subjects, PCOS women had higher levels of BMI, WC, fasting plasma glucose, 2-hour post-load plasma glucose, TG, fasting serum insulin and total testosterone and lower HDL-c ($P < 0.05$); there were no significant differences between the two groups in terms of systolic blood pressure (SBP), diastolic blood pressure (DBP), TC, or LDL-c ($P > 0.05$). As expected, compared with control subjects, PCOS women had a lower M value (8.38 ± 2.92 *vs.* 12.17 ± 1.16 mg/min × kg^{-1}, $P < 0.05$) and higher HOMA2-IR (1.53 ± 0.95 *vs.* 0.98 ± 0.40, $P < 0.05$) (Table 1). Serum PEDF levels normally distributed in the two groups, ranging from 2.68 to 5.36 μg/ml in control subjects and ranging from 3.68 to 7.48 μg/ml in PCOS women (Fig. 1). Serum PEDF levels averaged 3.97 ± 0.98 μg/ml in control subjects and increased 1.37-fold in PCOS women ($P < 0.05$, Fig. 1) (Table 1).

Subgroup analysis

According to BMI, PCOS women and controls were further divided into lean (BMI < 24 kg/m^2) and overweight/obese subgroups (BMI ≥ 24 kg/m^2). Lean PCOS women had a higher PEDF level (5.29 ± 1.63 *vs.* 3.95 ± 0.97 μg/ml, $P < 0.05$, Fig. 1) and lower M value as compared with lean controls (12.17 ± 1.16 *vs.* 9.53 ± 2.67 mg/min × kg^{-1}, $P < 0.05$). Overweight/obese PCOS women had increased HOMA2-IR (2.11 ± 1.27 *vs.* 1.08 ± 0.44, $P < 0.05$) and a tendency toward elevated

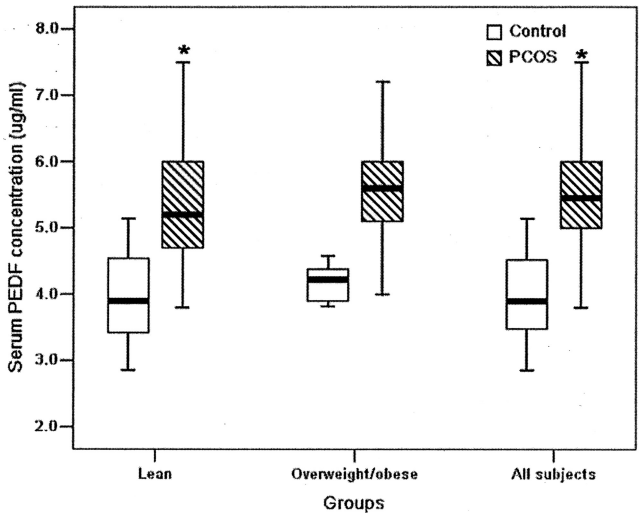

FIG. 1. Box plots showing serum PEDF concentration in different groups. *, $P < 0.05$ compared with corresponding control group.

TABLE 2. Subgroup analysis

Variables	Lean		Overweight/obese	
	Controls (n = 58)	PCOS (n = 62)	Controls (n = 5)	PCOS (n = 34)
Age, years	25.34 ± 2.53	25.15 ± 4.30	26.20 ± 1.64	26.09 ± 4.39
BMI, kg/m^2	20.70 ± 1.64	20.56 ± 1.79	25.98 ± 1.86	27.84 ± 3.15
PEDF, μg /ml	3.95 ± 0.97	5.29 ± 1.63*	4.20 ± 1.20	5.75 ± 2.19
HOMA2-IR	0.97 ± 0.40	1.21 ± 0.48	1.08 ± 0.44	2.11 ± 1.27*
M value[a]	12.17 ± 1.16	9.53 ± 2.67*	—	6.29 ± 2.11

Data are presented as mean ± SD and were analyzed by one-way ANOVA with the Games Howell test (in case of heterogeneity of variance) or SNK test (in case of homogeneity of variance) for *post hoc* analysis. —, failed to recruit any overweight/obese control subjects to undergo clamp.

[a] Sixty-two PCOS lean women, 34 overweight/obese PCOS women, and 20 lean controls underwent hyperinsulinemic–euglycemic clamp.

*, $P < 0.01$ compared with BMI-matched control group.

PEDF levels (5.75 ± 2.19 *vs.* 4.20 ± 1.20 μg/ml, $P = 0.32$, Fig. 1) (Table 2).

Bivariate analysis

Spearman correlation analysis showed that in women with PCOS, PEDF positively correlated with HOMA2-IR (Fig. 2A), BMI, WC, SBP, TC, TG, and LDL-c ($r = 0.30$, $r = 0.23$, $r = 0.22$, $r = 0.30$, $r = 0.21$, $r = 0.49$ and $r = 0.24$, respectively, $P < 0.05$) and negatively correlated with M value ($r = -0.29$, $P < 0.05$, Fig. 2B) and HDL-c ($r = -0.23$, $P < 0.05$). PEDF did not significantly correlate with age, DBP, fasting plasma glucose, 2-hour postload plasma glucose, or total testosterone ($P > 0.05$).

Multivariate analysis

Multiple linear regression analysis with a model including the serum PEDF level as the dependent variable showed that after adjustment for BMI, SBP, TC, TG, HDL-c, and LDL-c, the M value was still associated with the serum PEDF level ($R^2 = 0.27$, standardized coefficient $\beta = -0.24$, $P < 0.05$) in PCOS women. When M value was replaced by HOMA2-IR, similar results were obtained after adjustment for BMI, SBP, TC, TG, HDL-c, and LDL-c, HOMA2-IR was still associated with the serum PEDF level ($R^2 = 0.26$, standardized coefficient $\beta = 0.27$, $P < 0.05$).

Discussion

Our data show that compared with age-matched control subjects, women with PCOS had decreased insulin sensitivity (*i.e.*, IR) and increased serum PEDF levels. Spearman correlation analysis revealed that in PCOS women, the serum PEDF level correlated with BMI, WC, SBP, M value, HOMA2-IR, and serum lipids (TG, TC, LDL-c, and HDL-c). Subgroup analysis showed that compared with age- and BMI-matched controls, PCOS women still had decreased insulin sensitivity and increased PEDF levels, indicating that changes in serum PEDF levels in PCOS women probably independently correlate with insulin sensitivity, in addition to obesity. Multiple linear regression analysis further demonstrated that after controlling for BMI, SBP, and serum lipids (TG, TC, LDL-c, and HDL-c), PEDF was still associated with M value and HOMA2-IR

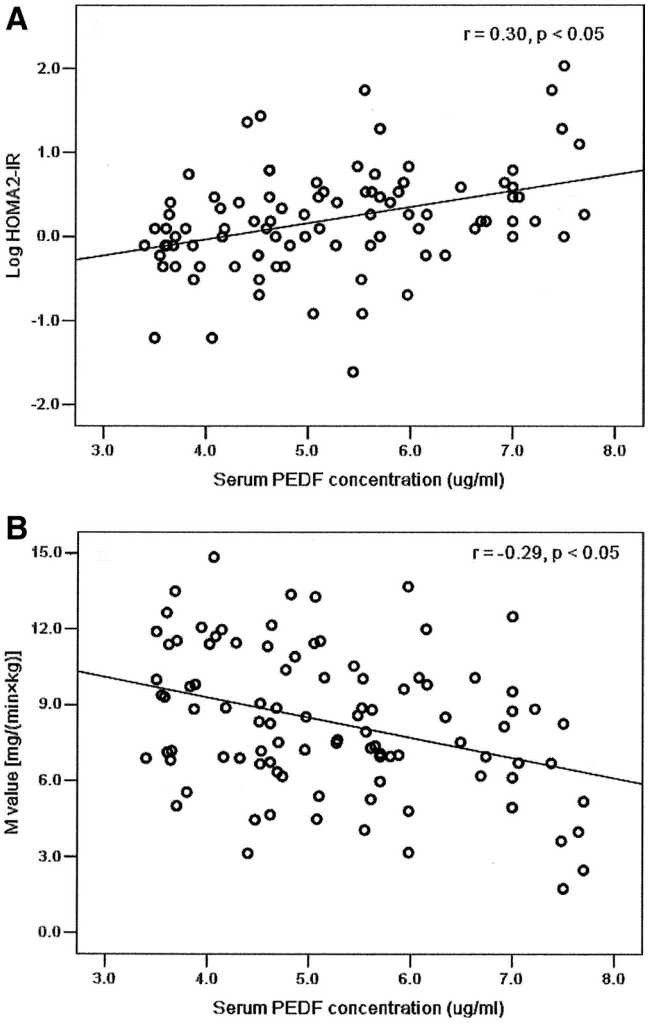

FIG. 2. Scatter plots showing the correlation of serum PEDF levels with IR in PCOS women. A, The serum PEDF level positively correlated with HOMA2-IR in PCOS women. B, The serum PEDF level negatively correlated with M value in PCOS women.

in PCOS women. To our knowledge, this report is the first suggesting that serum PEDF is elevated in women with PCOS and is associated with IR.

In the present study, we chose two methods to assess insulin sensitivity: hyperinsulinemic–euglycemic clamp and HOMA2-IR. Hyperinsulinemic–euglycemic clamp is generally accepted as the gold standard for insulin sensitivity evaluation (20–21). HOMA2-IR is also a well-recognized IR index (22–23). Both methods revealed decreased insulin sensitivity in PCOS women, although the changes of HOMA2-IR in the lean subgroup were not significant. This lack of significance could be explained by the fact that HOMA2-IR is not as precise as hyperinsulinemic–euglycemic clamp, especially in nonobese subjects (1, 24).

There have been no reports on the association of serum PEDF levels with IR in PCOS women. However, studies on other populations may provide clues as the relationship between these two parameters. A study on 196 subjects from the general Japanese population (aged 65.7 ± 9.3 yr) reported that serum PEDF levels were associated with WC and TG (14). Jenkins *et al.* (25–26) found that compared with control subjects, type 2 diabetic patients had an elevated PEDF level (3.2 ± 2.0 *vs.*5.3 ± 2.8 μg/ml) and the PEDF level correlated with BMI and LDL-c; they also found that an elevated PEDF level correlated with BMI, SBP, and TG in type 1 diabetic patients. A potential explanation for the relationship between PEDF and adiposity/serum lipids could be PEDF production by adipocytes and the function of PEDF as a regulator of adipogenesis and lipid metabolism (13, 25). However, our study found that compared with BMI-matched controls, PCOS women still had increased PEDF levels (the lack of a significant elevation of PEDF in overweight/obese PCOS women may have been due to the small number of overweight/obese control subjects), indicating that changes of PEDF levels in PCOS women were probably related to other features of PCOS (*e.g.*, IR), in addition to obesity. Because the majority of PCOS women also exhibit IR (6–9), it is conceivable that PEDF plays a role in the pathogenesis of IR in PCOS. A study on 125 Caucasian men reported that the serum PEDF level positively correlated with BMI and TG and negatively correlated with insulin sensitivity index, and after controlling for BMI and TG, the serum PEDF level was still associated with insulin sensitivity (27). Additionally, Nakamura *et al.* (16) found PEDF was an independent determinant of IR in patients with essential hypertension. Similarly, we found that PEDF was an independent determinant of IR in women with PCOS.

There were some limitations to this study. First, we recruited only five overweight/obese control subjects, and the number of cases was far fewer than BMI-matched PCOS women. This might lead to some insignificant statistical results: compared with BMI-matched overweight/obese controls, PCOS women showed a trend of having an increased serum PEDF level, which was in line with the changes in lean control subjects. However, the difference was not statistically significant in overweight/obese subgroups. Second, none of the overweight/obese control subjects underwent hyperinsulinemic–euglycemic clamp, so we could not evaluate their insulin sensitivity via M value. However, we used HOMA2-IR to compare the two groups and found that overweight/obese control subjects had decreased insulin sensitivity. Third, this study was cross-sectional and thus could not address the question of whether elevation of serum PEDF was a cause or consequence of IR in women with PCOS.

In conclusion, our study demonstrates that increased serum PEDF levels are associated with IR in women with PCOS. Further longitudinal and interventional studies are needed to clarify the clinical and pathophysiological significance of an elevated serum PEDF level in women with PCOS.

Acknowledgments

The present study was completed successfully with the help of the Chongqing Key Laboratory of Neurology.

Address all correspondence and requests for reprints to: Qifu Li, M.D., Department of Endocrinology, The First Affiliated Hospital of Chongqing Medical University, 1 Youyi Street, Chongqing 400016, China. E-mail: liqifu@yeah.net.

This work was supported by National Natural Science Foundation of China Grants 81070668 (to Q.F.L.) and 30570876 (to Q.F.C.).

Disclosure Summary: The authors have nothing to declare.

References

1. **Ehnnann DA** 2005 Polycystic ovary syndrome. N Engl J Med 352: 1223–1236
2. **Norman RJ, Dewailly D, Legro RS, Hickey TE** 2007 Polycystic ovary syndrome. Lancet 370:685–697
3. **Acien P, Quereda F, Matallin P, Villarroya E, Lopez-Fernandez JA, Acien M, Mauri M, Alfayate R** 1999 Insulin, androgens, and obesity in women with and without polycystic ovary syndrome: a heterogeneous group of disorders. Fertil Steril 72:32–40
4. **Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J** 1999 Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 22:141–146
5. **Lakhani K, Prelevic GM, Seifalian AM, Atiomo WU, Hardiman P** 2004 Polycystic ovary syndrome, diabetes and cardiovascular disease: risks and risk factors. J Obstet Gynaecol 24:613–621
6. **Wang Y, Qu J, WuX, Hou L, Erkkola R** 2010 Different phenotypes of polycystic ovary syndrome by Rotterdam criteria are differently steroidogenic but similarly insulin resistant. Fertil Steril 93:1362–1365

7. **Svendsen PF, Madsbad S, Nilas L** 2010 The insulin-resistant phenotype of polycystic ovary syndrome. Fertil Steril 94:1052–1058
8. **Shumak SL** 2009 Even lean women with PCOS are insulin resistant. BMJ 338:b954
9. **Galluzzo A, Amato MC, Giordano C** 2008 Insulin resistance and polycystic ovary syndrome. Nutr Metab Cardiovasc Dis 18:511–518
10. **Ronti T, Lupattelli G, Mannarino E** 2006 The endocrine function of adipose tissue: an update. Clin Endocrinol 64:355–365
11. **Carmina E, Orio F, Palomba S, Longo RA, Cascella T, Colao A, Lombardi G, Rini GB, Lobo RA** 2006 Endothelial dysfunction in PCOS: role of obesity and adipose hormones. Am J Med 119: 356.e1–356.e6.
12. **Orio Jr F, Palomba S, Cascella T, Milan G, Mioni R, Pagano C, Zullo F, Colao A, Lombardi G, Vettor R** 2003 Adiponectin levels in women with polycystic ovary syndrome. J Clin Endocrin Metab 88:2619–2623
13. **Crowe S, Wu LE, Economou C, Turpin SM, Matzaris M, Hoehn KL, Hevener AL, James DE, Duh EJ, Watt MJ** 2009 Pigment epithelium–derived factor contributes to insulin resistance in obesity. Cell Metab 10:40–47
14. **Yamagishi S, Adachi H, Abe A, Yashiro T, Enomoto M, Furuki K, Hino A, Jinnouchi Y, Takenaka K, Matsui T, Nakamura K, Imaizumi T** 2006 Elevated serum levels of pigment epithelium–derived factor (PEDF) in the metabolic syndrome. J Clin Endocrin Metab 91:2447–2450
15. **Stejskal D, Karpísek M, Svesták M, Hejduk P, Sporová L, Kotolová H** 2010 Pigment epithelium–derived factor as a new marker of metabolic syndrome in Caucasian population. J Clin Lab Anal 24:17–19
16. **Nakamura K, Yamagishi S, Adachi H, Matsui T, Kurita Y, Imaizumi T** 2010 Serum levels of pigment epithelium–derived factor (PEDF) are an independent determinant of insulin resistance in patients with essential hypertension. Int J Cardiol 143:96–98
17. **Rotterdam ESHRE/ASRM–Sponsored PCOS Consensus Workshop Group** 2004 Revised 2003 consensus on diagnostic criteria and longterm health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19:41–47
18. **Weiping L, Qingfeng C, Shikun M, Xiurong L, Hua Q, Xiaoshu B, Suhua Z, Qifu L** 2006 Elevated serum RBP4 is associated with insulin resistance in women with polycystic ovary syndrome. Endocrine 30:283–287.
19. **Chen C, Lu FC** 2004 Guidelines for prevention and control of overweight and obesity in chinese adults. Biomed Environ Sci 17(Suppl): 1–36
20. **Lewanczuk RZ, Paty BW, Toth EL** 2004 Comparison of the [13C] glucose breath test to the hyperinsulinemic–euglycemic clamp when determining insulin resistance. Diabetes Care 27:441–447
21. **Monzillo LU, Hamdy O** 2003 Evaluation of insulin sensitivity in clinical practice and in research settings. Nutr Rev 61:397–412
22. **Caumo A, Perseghin G, Brunani A, Luzi L** 2006 New insights on the simultaneous assessment of insulin sensitivity and beta–cell function with the HOMA2 method. Diabetes Care 29:2733–2734
23. **Mojiminiyi OA, Abdella NA** 2010 Effect of homeostasis model assessment computational method on the definition and associations of insulin resistance. Clin Chem Lab Med 48:1629–1623
24. **Diamanti–Kandarakis E, Kouli C, Alexandraki K, Spina G** 2004 Failure of mathematical indices to accurately assess insulin resistance in lean, overweight, or obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 89:1273–1276
25. **Jenkins A, Zhang SX, Gosmanova A, Aston C, Dashti A, Baker MZ, Lyons T, Ma JX** 2008 Increased serum pigment epithelium derived factor levels in Type 2 diabetes patients. Diabetes Res Clin Pract 81:e4–e7
26. **Katakami N, Kaneto H, Yamasaki Y, Matsuhisa M** 2008 Increased serum pigment epithelium–derived factor levels in type 1 diabetic patients with diabetic retinopathy. Diabetes Res Clin Pract 82:e5–e7
27. **Sabater M, Moreno–Navarrete JM, Ortega FJ, Pardo G, Salvador J, Ricart W, Frühbeck G, Fernández–Real JM** 2010 Circulating Pigment Epithelium–Derived Factor Levels Are Associated with Insulin Resistance and Decrease after Weight Loss. J Clin Endocrinol Metab 95:4720–4728