Predictors of Insulin Resistance and Metabolic Complications in Polycystic Ovarian Syndrome in an Eastern Indian Population

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Abstract The purpose of this study was to assess the predictive values of central obesity and hyperandrogenemia in development of insulin resistance and dyslipidemia in the polycystic ovarian syndrome (PCOS) patients in our region. Differences of fasting blood glucose level, insulin resistance index HOMA-IR, lipid parameters, waist hip

ratio (WHR), body mass index, LH/FSH ratio and testosterone levels between 45 PCOS cases and 35 age matched controls were obtained. Strength of association between different parameters in the case group was assayed by Pearson's correlation analysis. Dependence of insulin resistance and WHR on different predictors was assessed by multiple linear regression assay. Total cholesterol, LDL cholesterol, LH, FSH, LH/FSH ratio, WHR and insulin resistance were significantly higher in the case group ($p < 0.05$). Serum testosterone showed strong correlation with insulin resistance and LH/FSH ratio ($r = 0.432$ and 0.747 , $p = 0.01$ and 0.001 respectively) in the PCOS patients while WHR and serum testosterone level stood out to be most significant predictors for the insulin resistance ($\beta = 0.361$ and 0.498 ; $p = 0.048$ and 0.049 respectively). Hyperandrogenemia and central obesity were the major factors predicting development of insulin resistance and its related metabolic and cardiovascular complications in our PCOS patients. We suggest early monitoring for androgen level and WHR in these patients for predicting an ensuing insulin resistance and modulating the treatment procedure accordingly to minimise future cardiovascular risks.

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Introduction

Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorders, affecting around 4–7 % of reproductive-aged women worldwide [1] and 4 to 11 % in India [2]. It is an endocrine disorder associated with female infertility due to chronic anovulation that is characterized

by hyperandrogenism with an increase in testosterone and dehydroepiandrosterone sulphate in up to 75 % patients [3]. Neuroendocrine dysfunction with exaggerated LH pulsatility and altered production of adrenal androgen are also reported [4]. Insulin resistance and hyperinsulinemia are closely linked to androgenic abnormalities found in this disease [5] that significantly predispose them to several metabolic and cardiovascular complications. Although, numerous studies have linked PCOS to obesity, type 2 diabetes mellitus (DM), dyslipidemia, hypertension, and heart disease [6–8], significant differences existed between them. As much as 64 % of PCOS patients were found to have insulin resistance that could not be predicted by weight alone [9]. Similarly, strong associations of insulin with various circulating androgens and waist hip ratio (WHR) in PCOS have been reported that were found to be independent of body mass index (BMI) [10–13].

Hyperinsulinemia consequent to obesity and insulin resistance places women with PCOS at far greater risk for developing type 2 diabetes, and 15–36 % of all type 2 DM reported in women, irrespective of age, is found in association with PCOS [14]. One third of women with PCOS fulfil criteria for the diagnosis of the metabolic syndrome [15]. These associated metabolic derangements greatly increase a woman's lifetime risk to develop type 2 DM and cardiovascular co-morbidities.

However, the link between PCOS and DM is multifaceted [16] and hence, although, measurement of parameters of glucose metabolism, lipid screening, and insulin concentrations have been suggested [17], no consensus recommendation for the assessment of DM risk factors exists till today. The link between PCOS and hyperinsulinemia is further complicated by the observations that suggest direct relationship between insulin resistance and androgen levels. Studies demonstrated positive correlations between insulin and androgen levels [5, 18] and such correlations existed in both obese and non-obese PCOS women [19]. Although, it is widely accepted that hyperinsulinemia due to insulin resistance causes hyperandrogenism, studies undertaken with reverse aim showed that testosterone administration caused insulin resistance in skeletal muscle of female rats and in adipocytes of women in vitro [20]. On the other hand, the results of some studies suggested that these two conditions might be two separate entities [18]. The absence of significant association between hyperandrogenemia and insulin resistance in some recent studies have further supported this view [21]. Multiple studies throughout the world indicated that several factors including ethnicity might contribute to these different inferences due to genetic polymorphisms [22–24] stressing on the fact that the association between metabolic and neuroendocrinological abnormalities with PCOS may vary between different population groups in different areas.

However, only a few studies are available from the South Asian countries and the Indian subcontinent that provided information regarding the differential relationship between the neuroendocrinological and metabolic profile among PCOS patients in these regions [25, 26]. Keeping these factors in mind, we aimed to analyze the neuroendocrinological abnormalities in the PCOS patients in our population group in eastern part of the country and find out their links with metabolic parameters like insulin resistance and lipid profile, and markers for body fat distribution like BMI and WHR.

Materials and Methods

Study Design and Settings

The present case control study was conducted in the Gynecology and Biochemistry departments of Burdwan Medical College and Hospital, Burdwan, West Bengal, India. This hospital is the only medical college and hospital in the Burdwan district of eastern India and serves a wide area in this region. The study period was 1 year that spanned the duration from February 2011 to January 2012.

Participants

Fifty two cases aged between 15 and 29 years were selected on convenience basis from the PCOS patients attending the Gynecology department. PCOS was diagnosed by the revised Rotterdam [27] criteria in the presence of at least two of the following: (1) oligomenorrhea and/or anovulation, (2) hyperandrogenism, clinical or biochemical, (3) polycystic ovaries with exclusion of other etiologies [27]. All subjects were previously screened to exclude other causes of hyperandrogenism, thyroid function abnormalities, diabetes mellitus, hyperprolactinemia, hypertension and other cardiovascular diseases. 40 age matched healthy volunteer women with normal menstrual cycle, no clinical or biochemical signs for hyperandrogenism, and ultrasound exclusion of any polycystic ovary were selected as control group. None of them had a history of taking oral contraceptives or any other drug during last 3 months that could affect or alter the lipid profile, insulin level, or carbohydrate metabolism. None of them had any history of any drug addiction including alcohol intake and smoking. None of the case or control subject was suffering from any clinical infection at the time of the study. Total study protocol strictly adhered to the guidelines of Helsinki declaration 1975 as revised in 2000 for human studies. Written consents were obtained from all participants and the study was approved by the properly constituted institutional ethical committee.

Measurement of Parameters

All parameters were assayed from 12 h fasting blood samples obtained from cases and control subjects. Plasma glucose, serum cholesterol along with its LDL and HDL fraction, and serum triglyceride were assayed by standard photometric methods in the autoanalyzer ERBA XL 600 obtained from Transasia. Total cholesterol and triglyceride were measured using enzymatic methods involving cholesterol ester hydrolase, cholesterol oxidase and peroxidase (CHOD-PAP method) and lipase–glycerokinase–oxidase (GPO-POD method) respectively. HDL cholesterol was measured by precipitation technique using the divalent cation Mn^{2+} and phosphotungstic acid. LDL cholesterol was assessed by using the formula adopted by Friedwald and colleagues [28]. FBG was measured using glucose oxidase peroxidase (GOD-POD) method. Hormonal assays were performed between second and fifth day of the last menstrual cycle. In cases with amenorrhea the cycle was induced by progestinic drugs. Serum LH, FSH and insulin levels were measured with ELISA kit AccuBind from Monobind Inc. USA. These methods has been reported to show high degree of correlation (correlation coefficient 0.975) with reference radioimmunoassay method. No cross reactivity was detected for C peptide in insulin assay. Separated serum were preserved at $-20^{\circ}C$ until the assay. All assays were done within 1 week of collection in fully automated ELISA reader from TECAN, Austria. Internal quality control for all tests were performed using Lypochek level 1 and 2 control materials obtained from Biorad, USA. Inter and intra-assay coefficient of variation (CV) for all techniques remained within 7.5 % during the test period.

Insulin resistance was assayed by homeostasis model assessment for insulin resistance (HOMA-IR). As a widely validated clinical and epidemiological tool for estimating

insulin resistance and β -cell function, HOMA is derived from a mathematical assessment of the balance between hepatic glucose output and insulin secretion from fasting levels of glucose and insulin. HOMA-IR is computed with the formula: fasting plasma glucose (mmol/l) times fasting serum insulin (mU/l) divided by 22.5.

Statistical Analysis

Statistical analysis was performed using SPSS version 16 for Windows. Significance in differences between mean values was assessed from independent *t* test. Strength of association between two variables was judged by Pearson's correlation analysis, whereas, significance of dependence and predictive values were analyzed by linear regression study. For all statistical calculations $p < 0.05$ was considered to be significant at a 95 % confidence level. There was no missing data.

Results and Analysis

Blood samples of seven patients and five control subjects were excluded from the result analysis due insufficient amount or occurrence of hemolysis or lipemia in their serum after separation. Thus, 45 cases and 35 controls were selected as subjects for the final data analysis. Significantly raised values of HOMA-IR, total cholesterol and LDL cholesterol without any such increase in the FBG (Table 1) revealed a state of insulin resistance and dyslipidemia in the PCOS group without any significant change in their glucose tolerance. Increased serum testosterone, LH and FSH values with markedly elevated LH/FSH ratio in the patient group suggested a hyperandrogenemic condition with an upregulated LH secretion that is known as the hall

Table 1 Results of independent *t* test showing differences between the mean values of selected parameters with their corresponding value of significance at 95 % confidence level

Parameter	Mean (SD) of cases (<i>N</i> = 45)	Mean (SD) of controls (<i>N</i> = 35)	<i>p</i> value
FBG (mmol/l)	4.99 (1.56)	4.52 (0.32)	0.199
HOMA-IR	1.93 (0.86)	1.1 (0.22)	0.001*
Serum cholesterol (mmol/l)	4.69 (0.71)	4.16 (0.29)	0.011*
LDL cholesterol (mmol/l)	2.92 (0.58)	2.57 (0.38)	0.021*
HDLc (mmol/l)	1.10 (0.06)	1.12 (0.11)	0.623
TG (mmol/l)	1.44 (0.54)	1.27 (0.13)	0.186
Serum LH (IU/l)	30.75 (5.6)	5.69 (1.99)	0.001*
Serum FSH (IU/l)	10.93 (2.23)	6.29 (2.06)	0.001*
Serum LH/FSH	2.90 (1.05)	1.00 (0.48)	0.001*
Serum testosterone (μ g/l)	1.20 (0.0.43)	0.41 (0.20)	0.001*
BMI	24.89 (3.23)	23.41 (1.62)	0.064
WHR	0.81 (0.07)	0.75 (0.04)	0.001*
Age (years)	22.54 (3.64)	23.15 (2.79)	0.528

Statistical test done by SPSS version 16 for windows

**p* value <0.05, significant at 95 % confidence level

Table 2 Pearson's correlation analysis showing strength of association between different parameters in the case group ($N = 45$)

		HOMA-IR	LH/FSH	Testosterone (ng/ml)	WHR	BMI
HOMA-IR	r	1.000	0.213	0.432*	0.368*	-0.110
	p value	–	0.233	0.012	0.035	0.541
Serum LH/FSH	r	0.213	1.000	0.747*	-0.192	-0.096
	p value	0.233	–	0.000	0.284	0.594
Serum testosterone ($\mu\text{g/l}$)	r	0.432*	0.747**	1.000	0.120	-0.018
	p value	0.012	0.000	–	0.507	0.919
WHR	r	0.368*	-0.192	0.120	1.000	0.360*
	p value	0.035	0.284	0.507	–	0.040
BMI	r	-0.110	-0.096	-0.018	0.360*	1.000
	p value	0.541	0.594	0.919	0.040	–

Statistical done by SPSS 16 for windows, r = Pearson's correlation coefficient

*Correlation is significant at the 0.05 level (2-tailed) at 95 % confidence level; **Correlation is significant at the 0.01 level (2-tailed) at 95 % confidence level

Table 3 Linear regression analysis showing the coefficients of predictive values of independent variables for the dependent variable HOMA-IR in the PCOS group ($N = 45$)

Coefficients ^a						
Model		Unstandardized coefficients		Standardized coefficients	t	Sig. (p value)
		B	Std. error	Beta		
1	(Constant)	-1.922	2.607	–	-0.737	0.467
	Serum LH/FSH ratio	-0.148	0.312	-0.117	-0.474	0.639
	Serum testosterone ($\mu\text{g/l}$)	1.511	0.736	0.498	2.054	0.049*
	WHR	6.249	3.026	0.361	2.065	0.048*
	BMI	-0.101	0.069	-0.246	-1.476	0.151

Statistical done by SPSS 16 for Windows

* p value <0.05, significant at 95 % confidence level

^a Dependent variable: HOMA-IR. Predictors of HOMA-IR: LH/FSH ratio, serum testosterone (ng/ml), WHR and BMI

mark of disease. A significantly raised WHR ratio in the case group without any such increase in the BMI indicated that central obesity is more commonly associated with the disease process without a major role of overall body fat distribution.

The Pearson correlation analysis in the Table 2 showed that the strength of association of the insulin resistance marker HOMA-IR was considerably significant with the serum testosterone level and WHR values exclusively. As expected, a significant association also existed between the neuroendocrinological parameter LH/FSH ratio and the serum testosterone level that validated the strong link between the altered neuroendocrinological status and hyperandrogenemia found in the disease.

Most importantly, observations in multiple linear regression analysis in the Table 3 pointed out the major predictors of insulin resistance in the PCOS group. We compared the predictive values of LH/FSH ratio,

testosterone level, WHR and BMI for development of insulin resistance in these patients. The neuroendocrinological parameter LH/FSH ratio, and the overall body fat distribution marker BMI did not show any significant predictive value. On the other hand, marker of hyperandrogenemia i.e. the serum testosterone value, and WHR, a central obesity marker stood out as significant predictors of insulin resistance ($\beta = 0.498$ and 0.361 , $p = 0.049$ and 0.048 respectively).

Discussion

A significant increase in the HOMA-IR value was observed in our study group without any such elevation in their fasting blood glucose level ($p = 0.199$, Table 1) that complies with the fact that despite the PCOS women have postreceptor insulin abnormalities along with reduced

peripheral insulin receptor binding [29], they maintain normal glucose tolerance in contrast to that observed in classical type 2 DM [16]. Higher levels of total cholesterol and LDL cholesterol in them ($p = 0.011$ and 0.021 , Table 1) supported the observation of some recent studies that strongly suggested that young PCOS patients had a more potential risk to develop atherosclerotic vascular diseases in future due to metabolic, hormonal and inflammatory factors [30–32].

Testosterone levels in our PCOS patients were significantly higher ($p = 0.001$, Table 1) and showed marked association with the corresponding HOMA-IR values ($r = 0.432$, $p = 0.01$, Table 2). The relationship between hyperandrogenism and insulin resistance has been found to be significant so far in many human research studies [33, 34]. Studies demonstrated that women with elevated testosterone concentrations had a higher risk of developing type 2 diabetes mellitus [35]. Animal models have also shown that prenatal testosterone exposure can induce insulin resistance in early postnatal life [36]. Apter reported that adolescents with functional ovarian hyperandrogenism exhibit disproportionate, early pubertal increases in mean serum insulin levels [37] and Carbould showed that testosterone administration to female rats caused insulin resistance in the skeletal muscles and the adipocytes of women in vitro [20]. Importantly, results of our study keep in track with above observations by showing a significant dependence of the HOMA-IR values on the serum testosterone levels in the PCOS patients ($\beta = 0.498$, $p = 0.049$), which imply that hyperinsulinemia plays a critical intermediary role in androgen induced follicular abnormalities that are the hallmark of the disease. Effect of exaggerated insulin signalling on ovarian cells is highlighted in some recent studies involving Zucker fatty (fa/fa) rats that are well-understood models of obesity and hyperinsulinemia. Multiple cyst development and atresia in ovarian follicles due to presence of continuous insulin resistance were found in their ovaries even in presence of lower androgen levels [38].

Nevertheless, for most of the time androgen levels remain increased in the PCOS patients that associate them with an increased risk of metabolic syndrome [39] along with other neuroendocrinological abnormalities. Centrally, androgen excess may reduce hypothalamic feedback inhibition, resulting in increased GnRH pulsatility, particularly during puberty [40]. GnRH stimulation results in an increase in LH/FSH ratio in women with PCOS and recent studies have revealed that women with PCOS have higher baseline and GnRH-stimulated LH concentrations [41]. In congruency with these views, the LH/FSH ratio showed significant higher values in our cases ($p = 0.001$, Table 1) and showed a strong association with the serum testosterone level in them ($r = 0.747$, $p < 0.001$, Table 2). We propose that increased LH/FSH ratio caused a further

increase in testosterone level promoting insulin resistance that played a crucial role in development of cysts and follicular atresia in these patients.

Insulin resistance, on the other hand, did not seem to influence the LH/FSH ratio as evident from the insignificant association between them in the correlation studies ($r = 0.233$, $p = 0.233$, Table 2) as well as an inconsequential predictive value of the LH/FSH ratio on insulin resistance ($\beta = -0.117$, $p = 0.639$, Table 3). These are areas of unresolved understanding with regard to PCOS. Although, proposed mechanisms for insulin induced reproductive abnormalities include inappropriate ovarian steroidogenesis, excessive LH secretion and abnormalities in glucose uptake [18], in effect increased insulin does not appear to govern gonadotropin secretion directly. PCOS women treated with pioglitazone demonstrated improved insulin sensitivity without alterations in LH pulse frequency or amplitude, or gonadotropin responses to GnRH [37].

From the regression analysis (Table 3) it was also clear that serum testosterone level and WHR were the only significant predictors for an increased insulin resistance in our PCOS group. BMI, on the other hand, was not a significant predictor for insulin resistance in them. It was also not found to be significantly higher in the case population, whereas, WHR showed a significantly increased value ($p = 0.064$ and 0.001 respectively, Table 1). Dependence of insulin resistance on WHR in multiple linear regression analysis further suggested that abdominal obesity is a better predictor for insulin resistance than the overall body fat distribution in our PCOS population. Recent studies have revealed that women with PCOS matched for BMI have greater WHR and insulin resistance [13]. Since WHR is more directly related to the visceral adipose tissue that, in turn is associated with an increased production of a large number of functional molecules, it is implicated, at least in part, in both insulin resistance and low-grade inflammation. However, ethnic variations considerably influence the association of BMI and WHR with PCOS. It has been found that in the Iranian population, both BMI and WHR are significantly higher in PCOS patients with metabolic syndrome [42] that does not match with our study findings. Thus, ethnicity along with other variables have significantly contributed to incongruent relationships between the BMI and WHR with PCOS from place to place.

In conclusion, the results of our study attempt to throw considerable light on identification of early risk factors and thereby modulating the treatment process in PCOS patients accordingly to achieve maximum therapeutic benefit in our region. Despite the many reasons women seek medical care for PCOS, the greatest long term risk for these women is coronary artery disease (CAD). No single blood test can predict or quantify this CAD risk. Rather, precursor states of insulin resistance likely predict long term CAD risk well

before glucose abnormalities that has been corroborated in our study also where we observed a significant increase in insulin resistance without any such alteration in the fasting blood glucose level (Table 1). Animal models have shown that insulin resistance alone damages myocardial cells [43], whereas, human data link HOMA-IR to left ventricular dysfunction [44]. As insulin resistance is often the first step in a progression to DM and CAD, an early treatment based on this finding is advocated now for reduction in androgen and insulin levels, prevention of impaired glucose tolerance and DM, a better potential for improved ovulation and prevention of metabolic syndrome in the PCOS patients [45]. In our study WHR in comparison to BMI, was found to be a better predictor for insulin resistance in the PCOS group that indicate the need for adoption of appropriate treatment regimen accordingly. No single drug therapy has been proved to be effective in reducing the insulin resistance and other abnormalities in PCOS patients due to multifaceted derangements. Although, long term metformin therapy (2 years, 1,600 mg per day) in young, obese PCOS women reduced fasting insulin, hyperandrogenism and produced borderline reductions in HOMA-IR ($p = 0.05$) [46], it had the least reduction in HOMA-IR ($<20\%$) when compared to orlistat and pioglitazone over a 4 month treatment course [47]. The pioglitazone is a thiazolidinedione that acts as PPAR γ agonist and stimulate gene transcription that alters lipid and glucose metabolism, decreases lipolysis and decreases fat deposition [48]. It also decrease fatty acid release, suppress gluconeogenesis and reduce tumor necrosis factor α disruption of insulin activity [49]. Along with a reduction in insulin resistance, these agents also significantly reduce the central obesity in DM, a common trait found in PCOS also [50, 51]. Although, effective in induction of ovulation and regularising the menstruation in PCOS patients [52, 53], it has its limitations in women seeking pregnancy, where metformin stands out to be a better and safer alternative [52]. Our findings thus may provide significant help in formulating the therapeutic protocol to minimize the neuroendocrinological and metabolic complications during the reproductive period in PCOS women in this region.

However, the present study has some limitations. Although, there is not much ethnic variations among the eastern Indian population, inclusion of a larger number of subjects from different areas of the region could have increased the power of the study. We could not carry out any genetic polymorphic study on these patients which could have linked their anthropometric characteristic to their metabolic abnormalities. Neither did we get any detailed study that could analyse the genetic polymorphism and any pharmacogenetic characteristics in the PCOS patients in this region. Under these contexts of limitations, we propose that the results of our present study are only

preliminary and suggestive; and merit verification and validation by larger scale studies involving variant groups of people in bigger areas in future along with comparing their genetic polymorphic characteristics.

Conflict of interest There is no conflict of interest regarding any type of personal, administrative or financial matter with the present study.

References

1. Ehrmann DA. Polycystic ovary syndrome. *N Engl J Med*. 2005; 352(12):1223–36.
2. Norman RJ, Mahabeer S, Masters S. Ethnic differences in insulin and glucose response to glucose between white and Indian women with polycystic ovary syndrome. *Fertil Steril*. 1995;63(1): 58–62.
3. Huang A, Brennan K, Azziz R. Prevalence of hyperandrogenemia in the polycystic ovary syndrome diagnosed by the National Institutes of Health 1990 criteria. *Fertil Steril*. 2010;93(6): 1938–41.
4. Soule SG. Neuroendocrinology of the polycystic ovary syndrome. *Baillieres Clin Endocrinol Metab*. 1996;10(2):205–19.
5. Burghen GA, Givens JR, Kitabchi AE. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. *J Clin Endocrinol Metab*. 1980;50(1):113–6.
6. Barber TM, McCarthy MI, Franks S, Wass JA. Metabolic syndrome in polycystic ovary syndrome. *Endokrynol Pol*. 2007;58(1): 34–41.
7. Beydoun HA, Stadtmauer L, Beydoun MA, Russell H, Zhao Y, Oehninger S. Polycystic ovary syndrome, body mass index and outcomes of assisted reproductive technologies. *Reprod Biomed Online*. 2009;18(6):856–63.
8. Legro RS, Kusanman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab*. 1999;84(1):165–9.
9. DeUgarte CM, Bartolucci AA, Azziz R. Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. *Fertil Steril*. 2005;83(5):1454–60.
10. Dunaif A, Segal KR, Futterweit W, Dobrjansky A. Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes*. 1989;38(9):1165–74.
11. Chang RJ, Nakamura RM, Judd HL, Kaplan SA. Insulin resistance in nonobese patients with polycystic ovarian disease. *J Clin Endocrinol Metab*. 1983;57(2):356–9.
12. Dunaif A, Hoffman AR, Scully RE, Flier JS, Longcope C, Levy LJ, et al. Clinical, biochemical, and ovarian morphologic features in women with acanthosis nigricans and masculinization. *Obstet Gynecol*. 1985;66(4):545–52.
13. O'Connor A, Phelan N, Tun TK, Boran G, Gibney J, Roche HM. High-molecular-weight adiponectin is selectively reduced in women with polycystic ovary syndrome independent of body mass index and severity of insulin resistance. *J Clin Endocrinol Metab*. 2010;95(3):1378–85.
14. Azziz R. Controversy in clinical endocrinology: diagnosis of polycystic ovarian syndrome: the Rotterdam criteria are premature. *J Clin Endocrinol Metab*. 2006;91(3):781–5.
15. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghaziz MN. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2006;91(1):48–53.

16. Dunaif A, Finegood DT. Beta-cell dysfunction independent of obesity and glucose intolerance in the polycystic ovary syndrome. *J Clin Endocrinol Metab*. 1996;81(3):942–7.
17. Traub ML. Assessing and treating insulin resistance in women with polycystic ovarian syndrome. *World J Diabetes*. 2011;2(3):33–40.
18. Baptiste CG, Battista MC, Trottier A, Baillargeon JP. Insulin and hyperandrogenism in women with polycystic ovary syndrome. *J Steroid Biochem Mol Biol*. 2010;122(1–3):42–52.
19. Rittmaster RS, Deshwal N, Lehman L. The role of adrenal hyperandrogenism, insulin resistance, and obesity in the pathogenesis of polycystic ovarian syndrome. *J Clin Endocrinol Metab*. 1993;76(5):1295–300.
20. Corbould A. Effects of androgens on insulin action in women: is androgen excess a component of female metabolic syndrome? *Diabetes Metab Res Rev*. 2008;24(7):520–32.
21. Wongwananuruk T, Rattanachaiyanont M, Indhavivadhana S, Leerassiri P, Techatrasak K, Tanmahasamut P, et al. Prevalence and clinical predictors of insulin resistance in reproductive-aged Thai women with polycystic ovary syndrome. *Int J Endocrinol*. 2012;2012:529184.
22. Schweighofer N, Lerchbaum E, Trummer O, Schwetz V, Pilz S, Pieber TR, et al. Androgen levels and metabolic parameters are associated with a genetic variant of F13A1 in women with polycystic ovary syndrome. *Gene*. 2012;. doi:10.1016/j.gene.2012.04.050.
23. Liu N, Ma Y, Wang S, Zhang X, Zhang Q, Fu L, et al. Association of the genetic variants of luteinizing hormone, luteinizing hormone receptor and polycystic ovary syndrome. *Reprod Biol Endocrinol*. 2012;10(1):36.
24. Dasgupta S, Sirisha P, Neelaveni K, Anuradha K, Sudhakar G, Reddy BM. Polymorphisms in the IRS-1 and PPAR-gamma genes and their association with polycystic ovary syndrome among South Indian women. *Gene*. 2012;. doi:10.1016/j.gene.2012.04.050.
25. Wijeyaratne CN, Seneviratne Rde A, Dahanayake S, Kumarpeli V, Palipane E, Kuruppu N, et al. Phenotype and metabolic profile of South Asian women with polycystic ovary syndrome (PCOS): results of a large database from a specialist endocrine clinic. *Hum Reprod*. 2011;26(1):202–13.
26. Sundararaman PG, Manomani R, Sridhar GR, Sridhar V, Sundaravalli A, Umachander M. Risk of atherosclerosis in women with polycystic ovary syndrome: a study from south India. *Metab Syndr Relat Disord*. 2003;1(4):271–5.
27. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004;81(1):19–25.
28. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18(6):499–502.
29. Marsden PJ, Murdoch A, Taylor R. Severe impairment of insulin action in adipocytes from amenorrheic subjects with polycystic ovary syndrome. *Metabolism*. 1994;43(12):1536–42.
30. Shroff R, Kerchner A, Maifeld M, Van Beek EJ, Jagasia D, Dokras A. Young obese women with polycystic ovary syndrome have evidence of early coronary atherosclerosis. *J Clin Endocrinol Metab*. 2007;92(12):4609–14.
31. Wild S, Pierpoint T, Jacobs H, McKeigue P. Long-term consequences of polycystic ovary syndrome: results of a 31 year follow-up study. *Hum Fertil*. 2000;3(2):101–5.
32. Fulghesu AM, Sanna F, Uda S, Magnini R, Portoghese E, Batetta B. IL-6 serum levels and production is related to an altered immune response in polycystic ovary syndrome girls with insulin resistance. *Mediators Inflamm*. 2011;. doi:10.1155/2011/389317.
33. Acien P, Quereda F, Matallin P, Villarroja E, Lopez-Fernandez JA, Acien M, et al. Insulin, androgens, and obesity in women with and without polycystic ovary syndrome: a heterogeneous group of disorders. *Fertil Steril*. 1999;72(1):32–40.
34. Luque-Ramirez M, Alpanes M, Escobar-Morreale HF. The determinants of insulin sensitivity, beta-cell function, and glucose tolerance are different in patients with polycystic ovary syndrome than in women who do not have hyperandrogenism. *Fertil Steril*. 2010;94(6):2214–21.
35. Ding EL, Song Y, Malik VS, Liu S. Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA*. 2006;295(11):1288–99.
36. Padmanabhan V, Veiga-Lopez A, Abbott DH, Recabarren SE, Herkimer C. Developmental programming: impact of prenatal testosterone excess and postnatal weight gain on insulin sensitivity index and transfer of traits to offspring of overweight females. *Endocrinology*. 2010;151(2):595–605.
37. Mehta RV, Patel KS, Coffler MS, Dahan MH, Yoo RY, Archer JS, et al. Luteinizing hormone secretion is not influenced by insulin infusion in women with polycystic ovary syndrome despite improved insulin sensitivity during pioglitazone treatment. *J Clin Endocrinol Metab*. 2005;90(4):2136–41.
38. Honnma H, Endo T, Kiya T, Shimizu A, Nagasawa K, Baba T, et al. Remarkable features of ovarian morphology and reproductive hormones in insulin-resistant Zucker fatty (fa/fa) rats. *Reprod Biol Endocrinol*. 2010;8:73.
39. Coviello AD, Legro RS, Dunaif A. Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. *J Clin Endocrinol Metab*. 2006;91(2):492–7.
40. Chhabra S, McCartney CR, Yoo RY, Eagleson CA, Chang RJ, Marshall JC. Progesterone inhibition of the hypothalamic gonadotropin-releasing hormone pulse generator: evidence for varied effects in hyperandrogenic adolescent girls. *J Clin Endocrinol Metab*. 2005;90(5):2810–5.
41. Lewandowski KC, Cajdler-Luba A, Salata I, Bienkiewicz M, Lewinski A. The utility of the gonadotrophin releasing hormone (GnRH) test in the diagnosis of polycystic ovary syndrome (PCOS). *Endokrynol Pol*. 2011;62(2):120–8.
42. Mehrabian F, Khani B, Kelishadi R, Kermani N. The prevalence of metabolic syndrome and insulin resistance according to the phenotypic subgroups of polycystic ovary syndrome in a representative sample of Iranian females. *J Res Med Sci*. 2011;16(6):763–9.
43. Yang ZH, Peng XD. Insulin resistance and heart injury in rats with insulin resistance or type 2 diabetes mellitus. *Acta Cardiol*. 2010;65(3):329–35.
44. Basyigit F, Temizhan A, Malcok O, Kahraman E, Cakal E, Selcuk MT, et al. The relationship between insulin resistance and left ventricular systolic and diastolic functions and functional capacity in patients with chronic heart failure and metabolic syndrome. *Turk Kardiyol Dern Ars*. 2010;38(3):173–81.
45. Pasquali R, Gambineri A. Targeting insulin sensitivity in the treatment of polycystic ovary syndrome. *Expert Opin Ther Target*. 2009;13(10):1205–26.
46. Oepelt PG, Mueller A, Jentsch K, Kronawitter D, Reissmann C, Dittrich R, et al. The effect of metformin treatment for 2 years without caloric restriction on endocrine and metabolic parameters in women with polycystic ovary syndrome. *Exp Clin Endocrinol Diabetes*. 2010;118(9):633–7.
47. Cho LW, Kilpatrick ES, Keevil BG, Coady AM, Atkin SL. Effect of metformin, orlistat and pioglitazone treatment on mean insulin resistance and its biological variability in polycystic ovary syndrome. *Clin Endocrinol*. 2009;70(2):233–7.
48. Glintborg D, Andersen M. Thiazolidinedione treatment in PCOS—an update. *Gynecol Endocrinol*. 2010;26(11):791–803.

49. De Leo V, Musacchio MC, Palermo V, Di Sabatino A, Morgante G, Petraglia F. Polycystic ovary syndrome and metabolic comorbidities: therapeutic options. *Drugs Today*. 2009;45(10):763–75.
50. Asadipooya K, Kalantar-Hormozi M, Nabipour I. Pioglitazone reduces central obesity in polycystic ovary syndrome women. *Gynecol Endocrinol*. 2012;28(1):16–9.
51. Du Q, Wang YJ, Yang S, Wu B, Han P, Zhao YY. A systematic review and meta-analysis of randomized controlled trials comparing pioglitazone versus metformin in the treatment of polycystic ovary syndrome. *Curr Med Res Opin*. 2012;28(5):723–30.
52. Goldenberg N, Glueck C. Medical therapy in women with polycystic ovarian syndrome before and during pregnancy and lactation. *Minerva Ginecol*. 2008;60(1):63–75.
53. Koo YA, Shin SY, Yoon BK, Choi D. Pioglitazone for treating polycystic ovary syndrome in non-obese women of reproductive age with different clinical presentations. *Gynecol Endocrinol*. 2007;23(8):461–7.