Polycystic Ovarian Syndrome: a new perspective

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Introduction

Polycystic ovarian syndrome (PCOS) is sometimes called Stein-Leventhal Syndrome after the two doctors who first described it in 1935.[1] It is the most common ovarian disorder with typical features of obesity, anovulation, hyperandrogenism, hirsutism and infertility. The accepted nomenclature for the syndrome is PCOS, despite the fact that polycystic ovaries are non-specific findings and can occur in women with regular cycles and no hormonal derangements.[2] Other names such as hyperandrogenic chronic anovulation and functional ovarian hyperandrogenism have failed to gain wide acceptance.

rrence varies from 1-8% in genera! population depending upon the diagnostic criteria, as there is no universal definition for this syndrome.[3-5] Despite extensive research, the etiology and the mechanisms underlying PCOS are largely unknown, but there is considerable evidence suggesting that insulin plays the basic pathologic role along with a genetic component to the syndrome.

It is not purely an ovarian disease but an extremely heterogeneous clinical syndrome that should be recognized as a systemic endocrine and metabolic disorder. The first insight into the biochemical derangements came in the mid 1950s with the detection of elevated urinary luteinizing hormome (LH) levels; later increased androgen production was documented. Abnormalities in the hypothalamic-pituitary-ovarian axis with increased pulsatile LH release and increased pituitary gonadotroph sensitivity to gonadotropin releasing hormone were characterized in the early 1970s. In mid 1980s it was found that insulin resistance and hyperinsulinemia are also important components of PCOS that play a causal role in both hyperandrogenemia and chronic anovulation[6,7]

These metabolic derangements may predispose women with PCOS to a range of diseases such as type 2 diabetes mellitus, dyslipidemia, endometrial cancer and coronary artery syndrome.

Prevalence

Data on the prevalence of PCOS is variable mainly due
to the different sets of criteria for diagnosis. Studies in which polycystic ovaries were detected using ultrasonography report a prevalence of 2 1-22%, which does not give us an accurate estimate as many women with polycystic ovaries are endocrinologically normal.[2-8.9] Data from a cross-sectional study in Greece indicate 9% prevalence while defining PCOS as having oligomenorrhoea and hyperandrogenism.10 Another study by Knochenhauer and coworkers[11] assessed menstrual cycle characteristics and clinical androgen excess among 277 women undergoing a routine pre-employment history and physical examination in Alabama. The estimated prevalence was 4.6% with a possible range of 3.5-11.2%.

Spectrum of Clinical Presentation

1. Menstrual Dysfunction

Irregular and unpredictable uterine bleeding is the hallmark of PCOS, 85-90% women have oligomenorrhoea while 30-40% present with amennorhoea. These symptoms are clinical features of anovulation, but not all patients have anovulatory cycles as corpus luteum formation at the time of surgery has been found in approximately 16% of women with PCOS."

2. Androgen Excess

Approximately 80% of PCOS patients have excessive hair growth that usually has a male pattern.[12] Prolonged exposure to high levels of circulating androgens may even cause temporal balding. Acne is commonly seen but severe form of androgen excess such as clitoromegaly is absent.

3. Infertility

Infertility forms a part of the presenting complaint of significant number of patients.[12] Anovulation is thought to be the primary defect responsible.

4. Obesity

The onset of obesity has been correlated with the appearance of menstrual dysfunction. Patients usually have an android pattern of obesity.[13]

Predisposmg Factors

Studies have revealed that obesity is a predisposing factor for PCOS, weight reduction may cause improvement in menstrual irregularities.[14-16] Ethnicity may determine the risk for PCOS, women with certain racial background show increased incidence of the syndrome.[17] A study conducted in the U.S., France, Italy and Japan revealed that PCOS might manifest differently in different populations even when the biochemical androgen excess and insulin tolerance test values are comparable.'8 Available studies suggest that there is also a strong familial component to PCOS regardless of the diagnostic criteria used to ascertain probands and to assign affected status in kindreds.

Pathogenesis

Chronic anovulation along with hyperandrogenemia results in an increased number of atretic follicles (which become cysts) and increased interstitial tissue in the stroma of the ovaries[19-20] thus giving us the typical picture of the ovaries inPCOS.

Plasma androgen is produced directly by secretion and indirectly by peripheral metabolism of secreted precursors. Both the ovaries and the adrenals in response to their trophic hormones LH and adrenocorticotropic hormone (ACTH), secrete these androgens and their precursors. Androgens in women are not specifically under negative feedback control by these pituitary hormones because they are by-products of estradiol and cortisol secretion. The rate-limiting step in steroidogenesis is the formation of pregnenolone from cholesterol, which is regulated by trophic hormones. The rate-limiting step in androgen formation is regulation of cytochrome P450c17, a bifunctional enzyme with both 17,20-lyase and 17-hydroxylase activities. Cytochrome P450c17 converts progesterone to 1 7a-hydroxyprogesterone via its 17a-hydroxylase activity, and then converts l7ot-hydroxyprogesterone to androstenedione by virtue of its[17,20] -lyase activity in the ovarian theca cells. The expression of cytochrome P450c 17 is dependant on the concentration of trophic hormones, LH in the ovary[21-23] and ACTH in the adrenal cortex.[24-25] Thus, dysregulation of the cytochrome P450c[17] gene may result in functional ovarian hyperandrogenism alone, functional adrenal hyperandrogenism alone, or both together.

Other subtle generalized disturbances of steroid metabolism, including tendencies toward excessive estrogen and cortisol secretion is frequently present. The exact cause of dysregulation of steroidogenesis is unknown.

Ovarian Dysfunction

Functional ovarian hyperandrogenism is found in 70% of the patients with PCOS.[26] The presence of enlarged polycystic ovaries suggests that the ovaries are the primary sites of abnormality in PCOS. Theca cells from polycystic ovaries secrete abnormal amounts of steroids in culture both before and after LH stimulation.[27] But we know that LH has a stimulatory effect on theca cells, hence other factors also come into play. However, when patients with PCOS are given LH analogue human chorionic gonadotropin (HCG), they show 1 7-hydroxyprogesterone and androstenedione hyperresponsiveness.[28.29] Recent evidence suggests that insulin is capable of acting through its own receptor in polycystic ovaries and causes theca cell proliferation.[30,31] Studies reveal that cytochrome P450c[17] activity is increased due to stimulation by insulin.[32.33] Insulin also stimulates ovarian androgen production[34-38], lowers serum sex-hormone binding globulin (SSBG)[39,40] and also enhances endogenous and exogenously induced release of LH mediated by gonadotropin releasing hormone (GnRH). Insulin like growth factors (IGFs) may also contribute to the theca cell proliferation of polycystic ovaries.[41]

Adrenal Dysfunction

Functional adrenal hyperandrogenism, glucocorticoidsuppressible ACTH-dependant 17-ketosteroid excess is found in approximately one half of hyperandrogenic women and patients with functional ovarian hyperandrogenism.[26] There is evidence suggesting that patients with PCOS sometimes have mild increases in plasma ACTH and cortisol responsiveness to corticotropin-releasing hormone, spontaneous ACTH and cortisol secretions[42], cortisol responsiveness to ACTH and urinary free cortisol excretion.[43]

Peripheral Steroid Metabolism

Peripheral steroid metabolism is altered in PCOS. Rodin and co-workers reported evidence supporting the dysregulation of 11 ß-3-hydroxy steroid dehydrogenase in PCOS.[44] Marginally increased activity of 5a-reductase has also been reported[44.45] which may attribute to androgen excess.[46] Adipose tissue converts inactive precursors to testosterone and estrone[47] and has an important role in the pathophysiology. In some cases of chronic hyperandrogenic anovulation, weight reduction normalizes androgen levels[48-52], this may also be due to the lowered insulin levels.[29.24]

Insulin Resistance

In 1980, it was first reported that patients with PCOS had high insulin levels suggesting that they were insulin resistant.[54] It was also observed that such patients had subtle acanthosis nigricans, which was a cutaneous marker for insulin resistance.[55] Studies have confirmed that insulin resistance is limited to women with polycystic ovary morphology and chronic anovulation[56] Insulin-sensitizing drugs have restored ovulation in PCOS[57,58] proving that insulin-resistance is a unique feature of the syndrome of chronic anovulation and hyperandrogenemia.

A recent study by Legro and colleagues revealed that approximately 40% of women with PCOS had glucose intolerance, 31% had impaired glucose tolerance and 7.5% had Type 2 diabetes mellitus59, these rates were significantly higher than those of the controls. In the presence of peripheral insulin resistance, pancreatic ß-ceIls insulin secretion increases in a compensatory fashion and type 2 diabetes mellitus develops when the

compensation is no longer sufficient to maintain euglycemia.[60] Now there is evidence suggesting that B-cell dysfunction is, in addition to insulin resistance, a feature of PCOS.[61-63] It has been suggested that protein kinase C-mediated serine phosphorylation of the insulin receptor is important in the pathogenesis of hyperglycemia-induced insulin resistance.M

Cause of high insulin in PCOS

Hyperinsulinemia is probably the result of both increased insulin secretion and decrease in insulin clearance. A study in women with PCOS found decreased hepatic insulin extraction.[63] Molar ratios of circulating insulin to C-peptide are increased in PCOS, suggesting decreased hepatic extraction of insulin, but such ratios also reflect insulin secretion.[65]

Effect of Androgens on Insulin Resistance

Androgens produce mild insulin resistance. Prolonged testosterone administration to female-to-male transexuals to produce circulating testosterone levels in the normal male range resulted in modest but significant decreases in insulin-medicated glucose uptake in euglycemic clamp studies.[66] Modest improvements in insulin sensitivity in PCOS during androgen suppression or anti-androgen therapy have been found when less insulin-resistant, less obese or non-obese women with PCOS have been studied.[67.68] However suppressing androgen level does not completely restore insulin sensitivity to normal and administering androgens does not produce insulin-resistance of the same magnitude as that seen in PCOS.

Diagnosis and Evaluation

Setting the diagnostic criteria for PCOS is a challenge for practitioners since there is no one universal definition for the syndrome. As researchers unveil new pathophysiologic mechanisms underlying the manifestations of PCOS, the diagnostic criteria are correspondingly modified. The consensus towards a definition was reached in 1992.[69] The diagnosis is based mainly on clinical history and physical examination. Though laboratory examinations may be performed, they are mainly for excluding disorders of hormone production, enzyme deficiencies and other metabolic disorders.

Differential Diagnosis

As PCOS has a very wide clinical spectrum resulting from hyperandrogenemia and anovulation it becomes necessary to exclude conditions with similar presentations. These include functional and neoplastic processes. More detail about differentiating these conditions from PCOS is described in the following table:

Functional disorders	Neoplastic orders
Ovarian hyperthecosis	Androgen producing tumor of the ovaries
Congenital adrenal hyperplasia (CAH)	
Cushing's Syndrome	

Symptomatology

Though the clinical presentation of PCOS may resemble several other disorders, they most frequently occur in PCOS. Identification of the following three features is important when diagnosing PCOS. However, it must be kept in mind that these symptoms may not be present in all the patients.

1. Development of hirsutism.

2.	Episodes of irregular menstrual bleeding.

3.	The gradual onset of symptoms.

The development of hirsutism in PCOS is gradual and must be distinguished from that due to an androgen-producing neoplasm. Patients with PCOS usually have irregular menses and most of them have less than 6 episodes a year. The most striking feature in these patients is the lack of the premenstrual symptoms. Thus the episodes are unpredictable and anovulation is suggestive along with a history of failure to conceive. Symptoms are mostly recognized at the time or soon after puberty. These include hirsutism, obesity and irregular bleeding. Though a regular bleeding pattern is usually attained by one and a half years after menarche, if this period exceeds three years, PCOS must be suspected.

Physical Examination

The original description of PCOS can be applied to most patients; these features may vary considerably in different ethnic settings, as it is now believed that they may have a possible genetic link. The following factors should be assessed in the physical examination.

1.	Obesity

2.	Signs and symptoms of hyperandrogenism

3.	Acanthosis nigricans

4.	Ovaries

Bilaterally enlarged ovaries are found in PCOS on pelvic examination, which may be facilitated by ultrasound imaging. In most cases of PCOS, hyperandrogenism is manifested only as hirsutism. Some degree of temporal balding and acne formation is usually seen, however extreme expressions of androgen excess such as virilization and clitoromegaly are not typical findings. Fifty percent of the patients with PCOS are obese.[70] This obesity is characterized by an increased waist-to-hip ratio or an android appearance as opposed to truncal obesity.[13] It is important to distinguish this obesity from that of Cushing's syndrome or that due to excess cortisol production in patients who are massively overweight. Despite their obesity patients with PCOS do not display moon facies or abdominal stnae. Acanthosis nigricans is usually seen in obese patients with PCOS at the nape of the neck, the axilla, the area beneath the breasts and other intertrigenous areas.

Investigations

Laboratory tests performed while suspecting PCOS are usually done for exclusion of other disorders. Clinical suspicion of PCOS warrants patients to undergo a minimum endocnnological evaluation. If the patients exhibit mild to severe hirsutism associated with rapid onset of symptoms, assays for serum total testosterone and dehydroepiandrosterone sulfate (DHEA sulfate) should be done to exclude the possibility of an androgen-producing tumor of the ovary and the adrenal glands respectively. Levels of 17-hydroxyprogesterone may be determined to detect congenital adrenal hyperplasia (CAH) owing to 21-hydroxylase deficiency. A circulating level greater than 3ng/mL warrants further evaluation by an ACT!-! stimulation test.

Though LH levels are increased and so is the LH to follicle stimulating hormone (FSH) ratio in PCOS, determination of the circulating levels of these glycoproteins has not proven to be useful as a diagnostic tool. Similarly, levels of circulating serum testosterone are increased but not used for diagnosis; however, such an assay may be a

part of the initial screening hormone panel for PCOS and a utility to determine the efficacy of treatment of hirsutism.[71]

Long Term Disease Risks

The role of insulin in the pathogenesis of PCOS is far more than what health practitioners ever believed. Almost 15% of the patients with PCOS are diabetic. The high insulin levels cause a disbalance between the androgen and estrogen ratio with higher levels of circulating androgens, which reduces the protective effect of estrogens on the cardiovascular system. Dyslipidemias occur with relatively high levels of total cholesterol, low density lipoproteins (LDL) and triglycerides, and lower levels of high density lipoproteins (HDL)[72], predisposing to faster atherosclerosis and putting the patient at a greater risk for hypercholestrolemia and cardiovascular diseases.[73] Studies reveal that the risk for coronary heart disease is elevated threefold in women having type II diabetes mellitus[74] and twofold in women with hypertension.[73] Obesity[75] and increased waist-to-hip ratio[76] are also risk factors for cardiovascular disease in women. All these risk factors are usually present in patients with PCOS and the calculated risk for myocardial infarction in such women is seven times more than that in a normal healthy woman.[77]

Treatment

Women with PCOS have both abnormally elevated LH secretion[78,79] and hyperinsulinemia as a result of insulin resistance.[80] The combination of hypersecretion of LH and insulin causes ovarian androgen overproduction.[81] In turn, ovarian androgen overproduction causes hirsutism and prevents normal ovarian follicle growth, preventing regular ovulation. Lowering LH hypersecretion (oral contraceptive pills or GnRH agonist analogues) can treat PCOS or by reversing the hyperinsulinemia that is caused by insulin resistance (weight loss or metformin). An intriguing idea is to use oral contraceptives plus metformin in combination to simultaneously attack the 2 principle causes of PCOS: hypersecretion of LH and insulin.

In one recently reported clinical trial, Elter and colleagues82 randomized 40 non-obese women with PCOS to treatment with either an oral contraceptive alone (ethinyl estradiol 35 micrograms daily plus cyproterone acetate 2 mg daily) or the oral contraceptive plus metformin 500 mg 3 times daily for 4 months. In both groups, circulating androgens, such as androstenedione and testosterone, were significantly suppressed from baseline by 4 months of treatment. However, androstenedione levels were more suppressed by the combination therapy of the oral contraceptive plus metformin than by the oral contraceptive alone. In addition, weight loss occurred in the combination therapy group, but weight loss did not occur in the group treated with the oral contraceptive alone. An objective measure of hirsutism, the Ferriman-Galiwey score, was similarly improved in both treatment groups. This study suggests that the combination of metformin plus an oral contraceptive may be especially useful when an important objective of treatment is weight loss. For the treatment of hirsutism, at least over 4 months, the oral contraceptive performed as well as the combination of metformin plus the oral contraceptive.

References

1.Stein IF] Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol 1935;29:181.

2.Clayton RN, Ogden V. Hodgkinson J. et al. How common are polycystic ovaries in normal women and what is their significance for the fertility of the population? Clin Endocrinol 1992;37:127-34.

3.Franks S. Polycystic ovary syndrome: a changing perspective. Clin Endocrinol 1989;31 :87-120.

4.Futterweit W, Mechanick JI. Polycystic ovarian disease etiology, diagnosis. and treatment. Compr Ther 1988;l 4:12-20.

5.Franks S. Polycystic ovary syndrome. N Engl J Med 1995;333:853-61.

6.Burghen GA, Givens JR, Kitabchi AE. Correlation of hyperandrogenism with hyperinsulism in polycystic ovarian disease. J Clin Endocrinol Metab 1980:50:113-16.

7.Dunaif A, Graf M, Mandeli J, et al. Characterizatin of groups of hyperandrogenic women with acanthosis nigricans. impaired glucose tolerance and/or hyperinsulinemia. J Clin Endocrinol Metab 1987:65:499-507.

8.Farquar CM, Birdsall M, Manning P. et al. The prevalence of polycystic ovaries on ultrasound scanning in a population of randomly selected women. Aust NZ Obstet Gynecol 1994:34:67-72.

9.PoIson DW, Adams J, Wadsworth J, et al. Polycystic ovaries- a common finding in normal women. Lancet 1988:1:870-2.

10.Diamanti-Kandarakis E, Kouli C, Tsianateli T, et al. A survey of PCOS in Greek population. 79th Annual Meeting of the Endocrine Society, Minneapolis, MN 1997.

11.Knochenhauer ES, Key TJ, Kahsar-Miller M, et al. Prevalence of the polycystic ovary syndrome in unselected black and white women of the Southeastern United States: a prospective study. J Clin Endocrinol Metab 1 998:83:3078-82.

12.Goldzieher JW, Green JA. The polycystic ovaiy: clinical and histologic features. J Clin Endocrinol Metab 1962:22:325.

l3.Wild R. Consequences and treatment of polycystic ovary syndrome. In: Dunaif A, Givens JR. Haseltine FP, et al Eds. Polycystic Ovary Syndrome. Cambridge MA: Blackwell Scientific; 1992, p. 311.

14.Dunaif A, GrafM, Mandeli J, et al. Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance and/or hyperinsulinemia. J Clin Endocrinol Metab 1987:65:499-507.

15.Lesser KB, Garcia FAR. Association between polycystic ovary syndrome and glucose intolerance during pregnancy. J Maternal Fetal Med 1 997;6:303-7.

16.Harlass FE, Plymate SR. Fariss BL, et al. Weight loss is associated with correction of gonadotropin and sex steroid abnormalities in the obese anuvolatory female. Fertil Steril 1984;42:649-52.

17.Dunaif A, Sorbara L, Delson R, et al. Ethnicity and polycystic ovary syndrome are associated with independent and additive decreases in insulin action in Caribbean Hispanic women. Diabetes 1993:42:1462-8.

18.Carmina E, Koyama T, Chang L et al. Does ethnicity influence the prevalence of adrenal hyperandroenism and insulin resistance in polysystic ovary syndrome? Am J Obstet Gynecol 1992;167:1807-12.

19.Goldzieher JW, Green JA. The polycystic ovary. Clinical and histologic features. Clin Endocrinol Metab 1962:22:325-38.

20.Orsini LF, Venturoli S. Lorusso R, et al. Ultrasonic findings in polycystic ovarian disease. Fertil Steril 1985:43:709-14.

21. Anakwe 0, Payne A. Noncoordinate regulation of de novo synthesis of cytochrome P-450 cholestrolside-chain cleavage and cytochrome P-450 l7alpha-hydroxylase/C17.20 lyase in mouse Leydig cell cultures: relation to steroid production. Mol Endocrinol l987;1:595.

22. Magoffin D. Evidence that luteinizing hormone-stimulated differentiation of purified ovarian thecal-interstitial cells is mediated by both type I and type II adenosine 3',5'-monophosphate-dependent protein kinases. Endocrinology 1989:125:1464.

23. McAllister J. Kerin J. Trant J. et al. Regulation of cholestrol side-chain cleavage and I 7alpha-hydroxylasellyase activities in proliferating human theca intema cells in long term monolayer culture. Endocrinology 1989;125:1959.

24. DiBlasio A, Voutilainen R, Jaffe R, et al. Hormonal regulation of messenger ribonucleic acids for P450scc (cholestrol side-chain cleavage enzyme) and P450c 7 (1 7alpha-hydroxylase/l 7,20 lyase) in cultured human fecal adrenal cells. J Clin Endocrinol Metab 1987;65:170.

25. John M, John M, Boggaram V, et al. Transcriptional regulation of steriod hydroxylase genes by ACTH. Proc NatI Acad Sci USA 1986:83:47 15.

26. Ehrmann DA, Rosenfield RL. Barnes RB, et al. Detection of functional ovarian hyperandrogenism in women with androgen excess. N Engi J Med 1 992;327: 157.

27. Gilling-Smith C, Wills DS, Beard RW, et al. Hypersection of androstenedione by isolated theca cells from polycystic ovaries. J Clin Endocrinol Metab 1994:79:1158.

28. lbanez L, Hall J, Potau N, et al. Ovarian 17-hydroxyprogesterone hyper responsiveness to gonadotropin-releasing hormone (GnRH) agonist challenge in women with polycystic ovary symdroms is not mediated by luteinizing hormone hypersection: Evidence from GnRH agonist and human chronic gonadotropin stimulation testing. J Clin Endocrinol Metab 1996:81:4103.

29. Jakubowicz D, Nestler J. I 7alpha-Hydroxyprogesterone responses to leuprolide and serum androgens in obese women with and without polycystic ovary syndrome after dietary weight loss. J Clin Endocrinol Metab 1997:82:556.

30. Nestler J, Jakubowicz D, Falcon A, et al. Insulin stimulaes testosterone biosynthesis by human thecal cells from women with polycystic ovary syndrome by activating its own receptor and using inositolglycan mediators as the signal transduction system. J Clin Endocrinol Metab 1998:83:2001.

31. Willis D, Franks S. Insulin action in human granulosa cells from normal and polycystic ovaries is mediated by the insulin receptor and not the type-I insulin-like growth factor receptor. J Clin Endocrinol Metab 1995:80:3788.

32. Franks S. Polycystic ovary syndrome. N EngI J Med 1995;333:853-61. ~rratum, N EngI J Med 1995;333:1435.J

33. Moghetti P. Castello R, Negri C, et al. Insulin infusion amplifies 17 alfa-hydroxy corticosteriod intermediates response to adrenocorticotropin in hyperandrogenic women; apparent relative impairment of 17,20-lyase activity. J Clin Endocrinol Metab 1996:81:881-6.

34. Barbieri RL. Makris A, Randall RW. et al. Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. J Clin Endocrin Metab 1986:62:904-10.

35. Cars iF, Rosenfield RL. Insulin-like growth factor I and insulin polenhiate luteinizing hormone-induced androgen synthesis by rat ovarian thecal-interstitial cells.

Endocrinology 1988; 123:733-9.

36. Bergh C, Carlsson B, Olsson JH, et al. Regulation of androgen production in cultured human thecal cells by insulin-like growth factor I and insulin. Fertil Steril 1993:59:323-31.

37. Nahun R, Thong KJ, Hillier SG. Metabolic regulation of androgen production by human thecal cells in vitro. Hum Reprod 1995:10:75-81.

38. Nestler JE. Barlascini CO. Matt DW, et al. Suppression of serum insulin by diazoxide reduces serum testosterone levels in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 1989:68:1027-32.

39. Plymate SR. Matej LA. Jones RE, et al. Inhibition of sex hormone-binding globulin production in the human hepatoma (HEP-G2) cell line by insulin and prolactin. J Clin Endocnnol Metab I 988:67:460-4.

40. Nestler JE, Powers LP, Matt DW. et al. A direct effect of hyperinsulinemia on serum sex hormone-binding globulin level in obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 1991:72:83-9.

41. Duleba AJ, Spaczynski RZ, Olive DL. Insulin and insulin-like growth factor I stimulate the proliferation of human ovarian theca-ititerstitial cells. Fertil Steril 1998:69:235.

42. Invitti C, De Martin M, Delitala, et al. Altered morning and nighttime pulsatile corticotropin and cortisol release in polycystic ovary syndrome. Metabolism 1998:47: 143.

43. Luppa P. Muller B, Jacob K. et al. Variations of steroid hormone metabolities in serum and urine in polycystic ovary syndrome after nafarelin stimulation: Evidence for an altered corticoid excretion. J Clin Endocrinol Metab 1995:80:280.

44. Rodin A, Thakkar H, Taylor N, et al. Hyperandrogenism in polycystic ovary syndrome: Evidence of dysregulation of 11 Beta-hydroxysteroid dehydrogenase. N Engl J Med 1994:330:460.

45. Stewart P, Beastall G, Shackleton C. et al. 5alpha-Reductase activity in polycystic ovary syndrome. Lancet 1990:1:431.

46. Mowszowicz I, Melanitou E. Kirchhoffer M, et al. Dihydrotestosterone stimulates 5alpa-reductase activity in pubic skin fibroblasts. J Clin Endocrinol Metab l983;53:320.

47. Bleau G. Roberts K, Chapdelaine A. The in vitro and in vivo uptake and metabolism of steroids in human adipose tissue. J Clin Endocrinol Metab 1974:39:236.

48. Bates G, Withworth N. Effect of body weight reduction on plasma androgens in obese, infertile women. Fertil Steril l982;38:406.

49. Crave JC, Fimbel S. Lejeune H, et al. Effects of diet and metformin administration on sex hormone-binding globulin, androgens, and insulin in hirsute and obese women. J Clin Endocnnol Metab 1995;80:2057.

50. Kiddy DS, Hamilton-Fairly D. Bush A, et al. Improvement in endocrine and ovarian function during dietary treatment of obese women with polycystic ovary syndrome. Clin Endocrinol 1992:36:105.

51. Kim M, Friedman C, Barrows H, et al. Serum androgen concentration in the massively obese reproductive woman: The response to weight loss. Trans Am Assoc Gynecol Obstet Soc 1982:1:26.

52. Kopelman PG. White N. Pilkington F, et al. The effect of weight loss on sex steroid secretion and binding in massively obese women. Clin Endocrinol 1981:14:113.

53. Nestler JE. Errors in the measurement of plasma free testosterone-author's response. J Clin Endocrinol Metab 1997:82:2015.

54. Burghen GA, Givens JR. Kitabchi AE. Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease. J Clin Endocrinol Metab 1980:50:113-16.

55. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanisms and implications for pathogenesis. Endocr Rev 1997: 8:774-800.

56. Robinson S. Kiddy J, Gelding SV. et al.The relationship of insulin insensitivity to menstrual pattern in women with hyperandrogenism and polycystic ovaries. Clin Endocrinol 1 993;39:35 1-5.

57. Dunaif A, Scott D, Finegood D, et al. The insulin sensitizing agent troglitazone: A novel therapy for the polycystic ovary syndrome. J Clin Endocrinol Metab 996:81 :3299-306.

58. Nestler JE, Jakubowicz DJ, Evans WS, et al. Effects of metformin on spontaneous and clomiphene-induced ovulation in the polycystic ovary syndrome. N Engi J Med 1998:338:1876-80.

59. Legro RS. Kunselman A, Dodson WC. eta]. Prevalence and predictors of nsk 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 1999:84:165-9.

60. Bergman RN. Toward physiological tinderstanding of glucose tolerance: Minimal model approach. Diabetes 1989:38:1512-27.

61. Dunaif A. Finegood DB. Beta-cell dysfunction independent of obesity and glucose intolerance in the polycystic ovaiy syndrome. J Clin Endocrinol Metab 1996:81:942-7.

62. Ehrniann DA. Studs J, Byme NM, et al. Insulin secretory defects in polycystic ovary syndrome: Relationship to insulin sensitivity and family history of non-insulin-dependent diabetes mellitus. J Clin Invest 1995:96:520-7.

63. O'Meara NM, Blackman JD. Ehrmann DA, es al. Defects in beta-cell function in functional ovarian hyperandrogenism. J Clin Endocrinol Metab 1993:76:1241-7.

64. Pillay TS, Xiao S. Olefsky JM. Glucose-induced phosphorylation of the insulin rcceptor. J Clin Invest 1996:97:613-20.

65. Bufflngton CK, Kitabchi AE. Fvidence for a defect in insulin metabolism in hyperandrogenic women with po]ycystic ovary syndrome. Metabolism 1994:43:1367-72.

66. Polderman KH, Gooren JG, Asscherman H, et al. Induction of insulin resistance by androgens and estrogens. J Clin Endocrinol Metab 1994:79:265-71.

67. Elkind-Hirsh KE, Vales CT Malinak LR. Insulin resistance improves in hyperandrogenic womets treated with Lupron. Fertil Steril 1993:60:634-41.

68. Moghetti P. Tosi F, Castello R, et al. The insulin resistance in women with hyperandrogenisni is partially reversed by antiandrogen treatment: evidence that androgens impair insulin action in women. J Clin Endocrinol Metab 1996:81:952-60.

69. Zawadzki JK. Dunaif A. Diagnostic criteria for polycystic ovary syndrome: Towards a rational approach. In: Dunaif A. Givens JR. Haseltine FP. et al Eds. Polycystic Ovary Syndrome. Oxford: Blackwell. 1992. p. 377.

70. Franks S. Polycystic ovary syndrome. N EngI J Med I 995:333:853.

71. Chang RI, Katz SE. Diagnosis of polycystic ovary syndrome. Endocrinol Metab Clin North Am 1999: 28:397-408.

72. Talbott E, Guzick D, Clerici A. et al. Coronary heart disease risk factors in women

with polycystic ovary syndrome. ArteriosclerThromb Vase Biol 1995:15:821-6.

73.Kannel WB. Metabolic risk factors for coronary heart disease in women: Perspective from the Framingham study. Am Heart J 1987:114:413-19.

74.Manson JE. Colditz GA. Stampfer MJ. et al. A prospective study of matunsyonset diabetes mellitus and coronary heart disease and stroke in women. Arch Intem Med 1991:151:1141-7.

75.Hubert HB. Feinleib M. McNamara PM. et al. Obesity as an independent risk factor for cardiovascular disease: A 26 year follow-up of participants in the Framingham Heart Study. Circulation 1983;67:968-77

76.Lapidus L. Bengtsson E, Larsson B, et al. Distribution of adipose tissue and risk of cardiovascular disease and death in the population study of women in Gothenburg, Sweden. BMJ 1984:289:1259-61.

77.Dablgren E. Jansen P0, Johansson S. et a]. Polycystic ovary syndrome and risk for myocardial infarction: Evaluated from a risk factor model based on a prospective population study of women. Acta Obstet Gynecol Scand 1992:71:599-694.

78.Yen SSC, Vela P, Rankin J. Inappropriate secretion of follicle stimulating hormone and luteinizing hormone in polycystic ovarian disease. J Clin Endocrinol Metab 1970:30:435-42.

79.Rebar R. Judd HL, Yen SSC. et a]. Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. J Clin Invest 1976:57:1320-9.

80.Barbieri RL. Ryan KJ. Hyperandrogenism, insulits resistance and acanthosis nigricans syndrome: a common endocrinopathy with distinct pathophysiologic features. Am J Obstet Gynecol 1983:147:90-101.

81.Barbieri RL. Makris A, Randall RW, et al. Insulin stimulates androgen accumulation in incubations of ovarian stroma obtained from women with hyperandrogenism. J Clin Endocrinol Metab 1986:62:904-10.

82.Elter K, Imir G, Durmusoglu F. Clinical endocrine and metabolic effects of metformin added to ethinyl estradiol-cyproterone acetate in non-obese women with polycystic ovary syndrome: a randomized controlled study. Human Reprod 2002; 17: 1729-37.