# Insulin resistance, insulin sensitization and inflammation in polycystic ovarian syndrome

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## ABSTRACT

It is estimated that 5-10% of women of reproductive age have polycystic ovarian syndrome (PCOS). While insulin resistance is not part of the diagnostic criteria for PCOS, its importance in the pathogenesis of PCOS cannot be denied. PCOS is associated with insulin resistance independent of total or fat-free body mass. Post-receptor defects in the action of insulin have been described in PCOS which are similar to those found in obesity and type 2 diabetes. Treatment with insulin sensitizers, metformin and thiazolidinediones, improve both metabolic and hormonal patterns and also improve ovulation in PCOS. Recent studies have shown that PCOS women have higher circulating levels of inflammatory mediators like C-reactive protein, tumour necrosis factor-α, tissue plasminogen activator and plasminogen activator inhibitor-1 (PAI-1 ). It is possible that the beneficial effect of insulin sensitizers in PCOS may be partly due to a decrease in inflammation.

KEY WORDS: Polycystic ovarian syndrome, insulin sensitizers, inflammation, metformin, thiazolidinediones

Polycystic ovarian syndrome (PCOS) is characterised by chronic anovulation and hyperandrogenism (hyperandrogenism can exist in the absence of hyperandrogenemia e.g. enhanced tissue sensitivity to androgens) in premenopausal women. According to revised guidelines of PCOS Consensus Workshop Group,[1] to be diagnosed with PCOS, a woman must have 2 of the following 3 manifestations: irregular or absent ovulation, elevated levels of androgenic hormones and/or enlarged ovaries containing at least 12 follicles each.[1] Polycystic ovaries are defined as those found on ultrasound to contain 12 or more follicles measuring 2 to 9 mm in diameter and/or have an increased volume of 10 mL or greater. Only one ovary fulfilling these criteria is enough to meet the definition of polycystic ovaries. Other hyperandrogenic disorders such as non-classical congenital adrenal hyperplasia and androgen-secreting tumours have to be excluded for the diagnosis of PCOS. It should be stressed that polycystic ovaries are not a necessary feature of PCOS and that many women with polycystic ovaries do not have PCOS. Women found to have incidental polycystic ovaries on an ultrasound performed for another indication should not be considered to have PCOS unless there is corroborating clinical evidence of the syndrome.

Androgen excess may be with or without skin manifestations. It is estimated that 5-10% of women of reproductive age have PCOS.[2] Around 50% of PCOS women are obese and tend to have an android pattern of obesity.[3]

Chronic anovulation may present as irregular menstrual periods or amenorrhea. It is not essential to document anovulation by ultrasonography or progesterone measurements in the presence of a clear clinical history. In fact, PCOS occurs in 85 to 90% of women with oligomenorrhea and in 30-40% of women with amenorrhea.[4] Anovulation in PCOS women is associated with steady-state levels of gonadotropins and ovarian steroids. PCOS women are thus in a "chronic estrous state". Constant estrogen exposure leads to proliferation and hyperplasia of the endometrium and this can lead to unpredictable bleeding episodes. Unopposed estrogen exposure can be confirmed by progesterone withdrawal test (medroxyprogesterone acetate 10mg/day for 10 days), done after a negative urine pregnancy test.

Hyperandrogenism is usually suggested by the presence of hirsutism (occurs in approximately 80% of PCOS women) and can be documented by measuring androgen levels in the blood. Free testosterone is the most frequently elevated steroid in the blood in PCOS. Circulating levels of total testosterone, androstenedione and Dehydroepiandrosterone (DHEA) are also elevated. In obese PCOS women, sex hormone binding globulin (SHBG) levels are decreased (a well-known effect of obesity *per se*) and this leads to an increase in free testosterone levels. Furthermore, insulin is a negative regulator of the production of SHBG by the liver,[5] and SHBG levels are decreased in hyperinsulinemic conditions such as metabolic syndrome and visceral obesity.[6,7] Interestingly, concentrations of sulfated DHEA (DHEAS) are also increased in the blood. DHEAS is secreted exclusively by the adrenal glands. The mechanism of increased DHEAS production by the adrenals is not yet known, although insulin and IGF-1 have been shown to upregulate

adrenal 17-hydroxylase and 17,20-lyase activity.[8] It was believed in the past that LH/FSH ratio of more than 2 was part of the diagnostic criteria of PCOS. Women with PCOS have higher mean concentrations of LH, increased bioactivity of LH and low to low-normal levels of follicle stimulating hormone (FSH).[9,10] However, obese PCOS women do not have elevated LH levels; therefore a normal LH level or normal LH/FSH ratio does not rule out PCOS. LH/FSH ratio is now not included in the diagnostic criteria of PCOS.[11]

Under the influence of low but constant levels of FSH, multiple follicles of the ovary are stimulated but do not achieve maturation. The lifespan of the follicles may extend over several months, leading to multiple follicular cysts. Luteinized in response to constant and relatively high LH levels, these "arrested" follicles provide a constant supply of steroids. The atretic follicle becomes an androgenic follicle by default because atretic follicles are deficient in aromatase activity. Cultured follicular cells from the small follicles of polycystic ovaries produce small amounts of estradiol but show a dramatic increase in estrogen production when stimulated by FSH or IGF-1.[12] FSH therapy induces a larger cohort of follicles to develop in women with PCOS when compared with other infertile women.[13,14] A deficient *in vivo* ovarian response to FSH, possibly due to impaired interaction between signalling pathways associated with FSH and IGF-1, may be a key event in the pathogenesis of anovulation in PCOS.

## Insulin Resistance and PCOS

The association between hyperinsulinemia and PCOS was first noted by Burghen *et al* in 1980 who found a significant positive correlation between insulin, androstenedione and testosterone levels among PCOS women.[15] Subsequent studies confirmed insulin resistance as the cause of hyperinsulinemia. It is estimated that 20-40% of PCOS women have impaired glucose tolerance, a number approximately seven-fold higher than the rates in age and weight-matched women.[16,17] Prevalence of type 2 diabetes mellitus is also increased in PCOS women (15% versus 2.3% in normal women).[18] Lean PCOS women have lower rates of carbohydrate intolerance than obese PCOS women but even lean PCOS women have higher rates than age and weight-matched controls. Thus PCOS is associated with insulin resistance independent of total or fat-free body mass. Obese PCOS women are more insulin resistant than obese non-PCOS or non-obese PCOS women.[16,19] Ehrmann et al demonstrated pancreatic beta cell secretory dysfunction in a subset of PCOS women and this subset probably has the highest risk of developing carbohydrate intolerance and type 2 diabetes.[20,21] Rotterdam consensus panel recommends oral glucose tolerance tests for obese PCOS patients.[1]

Conversely, in a small study, Nestler et al found PCOS in 8 out 30 premenopausal women with type 2 diabetes.[22]

Insulin resistance is characterized by post-receptor defect in the action of insulin. The cause of this defect is still being elucidated. The first step in insulin's action involves binding to the cell-surface receptor.[23] Following insulin binding, the receptor undergoes auto-phosphorylation on specific tyrosine residues (accomplished by activation of insulin receptor tyrosine kinase). The activated receptor then activates insulin receptor substrates (such as IRS-1,2 and 3) which in turn bind to signalling molecules such as PI3 kinase and activate downstream signalling leading to insulin-mediated glucose transport.[24] Abnormalities in both insulin receptor tyrosine kinase (IRTK) activity and in mediators distal to the receptor are present in insulin resistance states.[25] Serine phosphorylation of insulin receptor decreases IRTK activity.[26,27]

Studies in adipocytes from women with PCOS reveal adipocyte insensitivity to inhibition of lipolysis by insulin as well as a decrease in maximal rates of adipocyte glucose uptake.[28,29] While these defects are also present in obesity and type 2 diabetes, they can occur in PCOS in the absence of obesity. Dunaif et al reported decreased insulin receptor auto-phosphorylation in 50% of fibroblasts removed from PCOS women[30] and this was due to increased receptor serine phosphorylation. Serine phosphorylation, as noted above, has been associated with decreased insulin receptor tyrosine auto-phosphorylation. In fact, this is the probable mechanism of TNF-$\alpha$ induced insulin resistance.[31] Since serine phosphorylation of P450c17 (the key regulatory enzyme of androgen biosynthesis) increases enzyme activity leading to androgen biosynthesis,[32]it is possible that a single defect (serine phosphorylation) can produce both insulin resistance and hyperandrogenism in a subgroup of PCOS women.[33] Lin et al showed reduced insulin stimulated lactate production in granulosa-lutein cells obtained from women with PCOS, whereas the same cells obtained from normal ovulatory subjects responded with increased lactate production after insulin exposure.[34]

*In vitro* human theca cell studies have shown that insulin has direct stimulatory effects on ovarian steroidogenesis.[35-37]Nestler et al showed that insulin produced a greater increase in androgen production by theca cells isolated from women with PCOS than in cells obtained from subjects without PCOS,[35] and that this effect is mediated specifically through insulin receptor rather than through IGF receptor "cross-talk". There is some data to suggest that insulin enhances the effect of LH on preovulatory ovarian follicles causing premature activation and subsequent follicle arrest.[38] It is possible that hyperinsulinemia (due to insulin resistance) drives the LH effect on ovarian theca cells to cause androgen excess which are intrinsically programmed to produce more androgen.[39] Excess androgens are known to interfere with the process of follicular maturation,[40] thus inhibiting ovulation and producing more arrested follicles. It has been postulated that the PCOS ovaries are more resistant to the metabolic effects of insulin than to the steroidogenic effects of insulin.[2,41] Further studies are needed to clarify the "selective insulin resistance" phenomenon.

## Insulin Sensitization in PCOS

In the last decade many trials have been held showing the efficacy of insulin sensitizers biguanides and thiazolidinediones in improving many aspects of the multifactorial PCOS. Trials have been done with metformin and two thiazolidinediones,

Troglitazone and Rosiglitazone. Metformin is a biguanide which reduces plasma glucose concentrations in type 2 diabetes patients. Metformin in type 2 diabetics does not lead to weight gain and can induce weight loss in some patients. Metformin predominantly works by reducing hepatic glucose production, inhibiting gluconeogenesis both directly and indirectly (by decreasing free fatty acid concentrations).[42,43] There is some data to suggest that it may slightly improve peripheral insulin sensitivity.[44,45] Studies with metformin in PCOS revealed reductions in androgen levels and improvements in ovulation when metformin was given for a duration of 10-24 weeks (in various studies). However, only in some of these studies was the effect independent of the weight loss induced by metformin.[46-49] Metformin has also been found to reduce the high rates of gestational diabetes in PCOS.[50]

Thiazolidinediones (TZDs) represent a novel class of drugs that decrease peripheral insulin resistance by enhancing insulin action in the skeletal muscle, liver and adipose tissue.[51] These agents are believed to work through binding and modulating the activity of a family of nuclear transcription factors termed peroxisome proliferator-activated receptors (PPARS). Studies with TZD in PCOS subjects have shown an improvement of the androgen levels and ovulation rate and enhanced insulin sensitivity without any reduction in the weight of subjects.[52-54] Troglitazone (withdrawn from the market in 2000 due to hepatotoxicity) was the first drug of this class to be studied. Studies have now been done with Rosiglitazone showing a decrease in testosterone, androstenedione and DHEA levels and an increase in SHBG (thereby causing a decrease in free testosterone levels) along with an improvement in insulin sensitivity.[55,56] In a recent study by Ghazeeri et al, Rosiglitazone improved both spontaneous and clomiphene-induced ovulation rates.[55] It is interesting that Troglitazone has recently been shown to have independent effects on ovarian steroidogenesis[57] and thus a direct effect of TZD apart from improvement of insulin resistance cannot be ruled out.

## PCOS, Infertility and Early Miscarriages

PCOS is one of the commonest causes of infertility in females. It is known that anovulation or decreased ovulation is the primary cause of this infertility and as mentioned above, both metformin and TZDs increase the rates of ovulation. Metformin also improves the number of oocytes collected in PCOS women for *in vitro* fertilization after FSH stimulation.[58] Metformin also improves pregnancy rates. Studies with TZDs on pregnancy rates are currently on.

PCOS women also suffer from early miscarriages.[59] It is possible that PCOS women have a hostile uterine environmental milieu which causes decreased conception and/or early miscarriages. Elevated PAI-1 (plasminogen activator inhibitor-1, an endogenous inhibitor of fibrinolysis) levels have been independently associated with recurrent miscarriages in PCOS women.[60] Hypofibrinolysis due to elevated PAI-1 levels may lead to placental microthrombi and therefore infertility. Metformin enhances luteal phase uterine vascularity and blood flow[61] and has been shown to reduce the rate of first trimester spontaneous abortions.[62,63] It is also possible that the reduced rate of pregnancy loss achieved with metformin may be because of better egg quality.

## PCOS, Inflammation and Cardiovascular Disease

Insulin resistance has been associated with an increased incidence of cardiovascular disease and atherosclerosis is now considered to be an inflammatory disorder.[64,65] Insulin resistance has recently been associated with increased levels of inflammatory mediators in the blood.[66,67] Studies have therefore been conducted to look at inflammation in PCOS. Gonzelez et al noted increased levels of tumour necrosis factor alpha (cytokine which causes insulin resistance and is secreted by the adipose tissue) in PCOS women as compared to controls.[68] Interestingly, lean PCOS women had higher TNF-α levels than normal lean women while the levels were similar in obese PCOS and obese controls. Kelly et al noted increased C-reactive protein levels and tissue plasminogen activator (t-PA) levels in PCOS women as compared to healthy weight-matched controls.[69,70] However when adjusted for insulin sensitivity, C-RP was no longer significantly different between groups but t-PA levels remained significantly different. Women with PCOS also have higher PAI-1 activity and higher fibrinogen levels than controls.[71] However, in another study, PAI-1 levels were not significantly different from controls when adjusted for BMI.[72] Glueck et al demonstrated that PAI-1 activity was an independent risk factor for miscarriages in PCOS.[60]

While the above studies suggest that PCOS is associated with a state of increased inflammation, clinical studies have yet to definitively demonstrate an increased rate of cardiovascular disease in PCOS.[73]

Thiazolidinediones have been shown to decrease inflammation in obese and diabetic subjects.[74-76] TZDs have also been shown to reduce carotid intimal medial thickness, normalize vascular endothelial function and improve fibrinolytic and coagulation parameters.[77] Rosiglitazone therapy for 26 weeks reduced MMP-9 (a matrix metalloproteinase, implicated in atherosclerotic plaque rupture) and C-RP levels in type 2 diabetics.[78] In studies in PCOS women, Troglitazone reduced PAI-1 levels[53] and improved endothelial-dependent vasodilation.[79] It is possible that the beneficial effect of TZDs in PCOS may be partly due to the decrease in inflammation. Metformin has also been shown to decrease PAI-1 and C-RP levels in PCOS women.[80,81]

## Conclusion

While insulin resistance is not a part of the diagnostic criteria for PCOS, its importance in the pathogenesis of PCOS cannot be denied. The treatment of PCOS in the past has largely centred on anti-androgen therapy for symptomatic control, cyclic hormones for regular menses and ovulation induction for infertility. While weight loss is helpful in the therapy of PCOS, it may be difficult to achieve. Furthermore, a significant percentage of PCOS women are lean but insulin resistant. Insulin sensitizers are unique in PCOS because they offer

both metabolic and gynaecologic benefit.[82] Although the use of insulin sensitizers in PCOS has not been approved by the FDA (Food and Drug Administration, USA) yet, it is probable that PCOS will be a recognized indication for TZDs and metformin in future.

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