
PCOS and Insulin Resistance (IR): From Lifestyle to Insulin Sensitizers

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2.1 Introduction

According to the Rotterdam criteria, elaborated by the European Society of Human Reproduction and Embryology (ESHRE) and by the American Society for Reproductive Medicine (ASRM), polycystic ovary syndrome (PCOS) is defined by at least two of the three following criteria:

1. Clinical signs of hyperandrogenism or biochemical hyperandrogenemia
2. Oligo-/amenorrhea and chronic anovulation
3. Ultrasonographic evidence of at least one polycystic ovary and exclusion of other relevant diseases (i.e., hyperprolactinemia, thyroid diseases, androgen secretion tumors, and adrenal dysfunction hyperplasia) [1]

This evolution was relevant because it permitted the inclusion of women with PCOS who were excluded by previous NIH criteria [2]: those with polycystic ovaries affected by hyperandrogenism and ovulatory cycles or chronic anovulation and normal androgen levels.

Recently, the Androgen Excess and PCOS Society considered PCOS as an androgen excess disorder in biosynthesis, utilization, and/or metabolism of androgen in women [3].

PCOS occurs in as many as 8–10 % of women of reproductive age [4] with onset manifesting as early as puberty [5].

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The syndrome has strong association to infertility, hyperinsulinemia, insulin resistance (IR), impaired glucose tolerance, dyslipidemia, android fat distribution, and obesity. Approximately 60 % of women with PCOS are obese. IR is found in 40 % of normal weight women with PCOS and nearly all overweight women both with and without PCOS [6], but IR is not routinely assessed when diagnosing or treating PCOS. The IR can be treated by insulin sensitizers and/or with diet and exercise before and during fertility treatment and pregnancy. The IR is not currently diagnosed or treated routinely and the effect of treatment is undocumented. Treatment of IR in young women may reduce the risk of diabetes and CV complications later in life. Moreover, a detailed understanding of the importance of IR in female reproductive health and reproduction could lead to new treatment strategies and improved pregnancy and life birth rates for PCOS patients. The PCOS is a multigenic condition where the phenotype is modulated by external lifestyle factors. The risk of adults developing metabolic disease, particularly obesity, hypertension, diabetes, and CVD, is probably influenced by events during embryonic development and fetal and neonatal growth [7]. The risk of diseases during later life may be determined by events or conditions experienced by the mother even before pregnancy occurs.

The IR and hyperandrogenism are associated with altered coagulation and fibrinolysis leading to endothelial dysfunction, vascular chronic low-grade inflammation [8], and atherothrombosis [9]. In PCOS both normal and elevated concentrations of plasminogen activator inhibitor type-1 (PAI 1), an indicator of endothelial dysfunction and of a prothrombotic state, have been found [10]. Other biomarkers such as fibrinogen, coagulation factor VII, coagulation factor VIII, von Willebrand factor, tissue-type plasminogen activator, urokinase-type plasminogen activator (u-PA), and activated partial thromboplastin time have been studied in PCOS and the results are conflicting [11]. Increased high sensitivity C-reactive protein (hs-CRP) is associated with PCOS, cardiovascular disease, overweight, or lean PCOS [12] and IR [13].

In vitro fertilization (IVF) outcomes in PCOS patients have the same live birth rate as control IVF patients [14]. PCOS patients with IR, measured by homeostatic model assessment of insulin resistance (HOMA-IR), had a significantly lower maturation rate of their immature oocytes compared to those with normal IR [15].

Hyperandrogenism and hyperinsulinemia affect follicular microenvironment, resulting in reduced ovarian development of immature oocytes. Moreover, in PCOS patients, significant abnormalities are observed at the earliest stages of folliculogenesis. However, it is unclear which molecules are responsible for the abnormal regulation during early folliculogenesis. It has been reported that some growth factors and sex steroids may have a role in aberrant folliculogenesis in PCOS [16].

A number of growth factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (BFGF), insulin-like growth factor I (IGF 1), and epidermal growth factor (EGF), localize within growing follicles and regulate important aspects of folliculogenesis [17].

The hepatocyte growth factor (HGF) and c-Met-mediated epithelial mesenchymal mechanisms are important for follicle development [18]. HGF was reported as a growth factor that controls several key functions, including the regulation of growth

and differentiation of ovarian follicles. It has been reported that the HGF/c-Met signalling modulates every major component of folliculogenesis, including steroidogenesis, the growth of theca and granulosa cells, and apoptosis of granulosa cells [19]. Nevertheless, it is currently unknown whether the HGF/c-Met signalling is involved in the development of immature oocytes in PCOS.

In one study [20], the levels of HGF in serum and in follicular fluid and the mRNA expression of c-Met in granulosa cells were slightly higher in patients with PCOS than in normal patients taken as control, and the same study showed that the increase in serum HGF levels after hCG treatment was similar in the PCOS patient and in control group with no PCOS assessment. Higher levels of HGF in serum and in follicular fluid and the increased c-Met expression in granulosa cells were important for the maturation and fertilization of oocytes, and the increased c-Met expression in granulosa cells might be a marker for the fertilization of oocytes [20].

In relation to PCOS and pregnancy, several studies indicate that women with PCOS have increased risk of adverse obstetric outcomes [21], with an increased risk of preeclampsia, pregnancy-induced hypertension, gestational diabetes, and preterm birth. Whether an increased risk of pregnancy complications has been related to PCOS, an increased BMI or fertility treatment is not fully understood.

Despite the diagnostic criteria, PCOS is still an unclear disease in terms of pathogenesis; both genetic and environmental factors may contribute to the onset of PCOS features [22]. On such genetic predisposition, environmental factors may play a key role, such as peculiar lifestyle, type of food, living condition, and also the impact during the intrauterine growth [23].

2.2 The Impact of Obesity on the Features of PCOS Patients

Although the pathogenesis of PCOS is complex and fully understood, insulin resistance and obesity are proposed to be key metabolic defects in its etiology [24].

Obesity and abdominal obesity are common in PCOS with 10–50 % of women with PCOS having a BMI above the acceptable range of 19–25 kg/m² [25]. Both these enhance the features of insulin resistance and are associated with reproductive dysfunction including menstrual irregularity, increased serum androgens and luteinizing hormone (LH), infertility, and complications during pregnancy and childbirth [26, 27].

There is considerable evidence that a key etiologic feature of PCOS is the presence of insulin resistance, which is present in the majority of women with PCOS, although its severity varies between lean and obese women [28].

Insulin resistance is defined as the inability of insulin to exert its physiological effect. It is manifested peripherally (at the tissues level) or centrally (at the liver level) through a reduction in the ability of insulin to lower plasma glucose. This can be demonstrated as impaired insulin-stimulated glucose uptake and suppression of lipolysis at the muscles or adipose tissue, hepatic glucose overproduction, and suppression of glycogen synthesis. The mechanism implicated in the etiology of insulin resistance includes elevated levels of plasma free fatty acid, cytokines such as tumor

necrosis factor alpha and interleukin 6, leptin, resistin, and the peroxisome proliferator-activated receptor gamma (PPAR gamma) [29].

The gold standard for detecting insulin resistance is the euglycemic clamp technique, where a constant infusion of insulin is maintained and glucose infusion is adjusted to maintain euglycemia [30].

At steady state, the insulin-mediated glucose disposal rate can be calculated by the amount of infused glucose representing that taken up by tissues. Using the euglycemic clamp technique, both lean and obese women with PCOS had significantly lower rates of insulin-mediated glucose uptake than weight-matched controls of comparable age, thus indicating greater insulin resistance [31]. Obesity and PCOS had additive and deleterious effects on insulin sensitivity. Other studies of insulin resistance in PCOS support the additive role of obesity on the level of insulin resistance seen [32].

The impact of insulin in the presence of obesity in PCOS is particularly notable in high rates of glucose intolerance and diabetes seen in obese women with PCOS [33]. Glucose abnormalities may be detected by measurement of the fasting glucose; however, this method may be less sensitive in obese women with PCOS. A significant number of women with PCOS demonstrating glucose intolerance on a 2 h glucose challenge will have normal fasting glucose level according to the American Diabetes Association (ADA) criteria [33, 34].

Evidence exists that an android body fat distribution (i.e., a central distribution of body fat) induces higher risk of cardiovascular disease and diabetes than does a gluteofemoral fat distribution [34, 35]. In particular, visceral adipose tissue exhibits functional differences in comparison to subcutaneous adipose tissue when studied in vitro [36]. Production of adipocyte-associated cytokines, such as adiponectin and leptin, as well as inflammatory markers such as IL6, varies between the two adipocyte compartments [37]. Under conditions of visceral fat accumulation, these compartmental differences may contribute to the association between visceral fat and insulin resistance [38].

Women with PCOS tend to accumulate fat in a central distribution as demonstrated by increased waist-to-hip ratio [39]. In PCOS, this increase in visceral fat is associated with a worse metabolic profile, with higher fasting insulin levels, dyslipidemia, and higher serum androgen concentrations [40]. Central fat mass correlates with serum levels of inflammatory markers such as C-reactive protein, independently of age and total fat mass as reported in a recent study on PCOS and BMI-matched controls [41]. Modest alterations, therefore, in the visceral fat compartment may produce a significant impact on metabolic parameters.

The predominant underlying risk factor for development of metabolic syndrome is abdominal obesity and likely represents a consequence of insulin resistance. Patients with PCOS has been reported to have an increased risk of metabolic syndrome (MS), which refers to a clustering within the same individual of hyperinsulinemia, mild to severe glucose intolerance, dyslipidemia, and hypertension, and an increased risk for cardiovascular disease and diabetes [42]. The prevalence of metabolic syndrome in PCOS ranges from 33.4 to 47.3 % in published trials [43].

In 2006, the International Diabetes Federation defined the features of the MS and defined central obesity as present when the waist circumference is above 80 cm; in European women, this was considered as a necessary prerequisite risk factor for the diagnosis of MS [44].

The risk factors for MS are as follows:

- Waist circumference >88 cm
- Elevated triglycerides >150 mg/dL (>1.7 mmol/l)
- Reduced HDL cholesterol <50 mg/dL (<1.29 mmol/l)
- Elevated blood pressure untreated >130 mmHg systolic or >85 mmHg diastolic
- Fasting plasma glucose >100 mg/dL (over 5.6 mmol/l) or previous diagnosis of type 2 diabetes mellitus

Women with PCOS have lower HDL levels, higher LDL to HDL ratio, and higher triglyceride levels than healthy eumenorrheic women. All these are inductors of subclinical atherosclerosis as demonstrated by the increased thickness of the carotid intima media and by the higher endothelial dysfunction observed in PCOS patients [9], probably related to the insulin resistance and/or to the higher free testosterone plasma level [45].

The impact of metabolic syndrome on the presence of cardiovascular morbidity in PCOS has not been studied; as metabolic syndrome is recognized as a significant risk factor for cardiovascular disease in the general population, obese women with PCOS with metabolic syndrome would appear to be at increased risk as well [46]. Such risk has also been demonstrated to be higher in postmenopausal women, previously demonstrated to be PCOS during fertile life [47].

2.3 Endocrine Profile of PCOS Patients

PCOS is characterized by increased ovarian and adrenal androgens, increased luteinizing hormone (LH) levels, high estrogen levels (mainly estrone) due to extraglandular conversion from androgens, lower levels of sex hormone binding protein (SHBG), and higher levels of insulin, the latter often present in overweight or obesity. Hyperandrogenism is a key feature of the syndrome, although it is not constant [48]. It is mainly of ovarian origin with an adrenal contribution, since a certain percentage of PCOS women might show a mild steroidogenic defect in adrenal glands (such as for 21-hydroxylase) or just a higher adrenal hyperactivation due to stress [49].

Androstenedione and testosterone are the markers of androgen secretion from the ovary, whereas dehydroepiandrosterone sulfate (DHEAS) is the best markers of adrenal secretion. The great part of testosterone is derived from peripheral conversion of androstenedione and from direct ovarian production. Dysregulation of cytochrome p450c17, the androgen-forming enzyme in both the adrenal glands and the ovaries, is the central pathogenic mechanism underlying hyperandrogenism in PCOS patients [50].

Hyperandrogenism correlates positively with insulin resistance, and obesity worsens insulin resistance. Additionally, obesity is associated with lower concentrations of SHBG that is the major binding protein for testosterone. Normally, less than 3 % of testosterone circulates as unbound in the serum. The presence of hyperandrogenism reduces the hepatic synthesis of SHBG and leads to a relative excess of free circulating androgens. In PCOS, hirsutism usually occurs with decreased SHBG levels and obesity [51].

Moreover, estrone plasma levels, a weak estrogen with biological activity 100 times less than estradiol, are increased as the result of peripheral conversion of androstenedione by aromatase activity. Excess of estrone leads to a hyperestrogenic state, and this might predispose patient to endometrial proliferation and to a higher risk for endometrial cancer [52].

Compared to normal women, women with PCOS had an accelerated LH pulse pattern. However, when obese women with PCOS were studied in comparison to lean PCOS women, an attenuated pattern of LH secretion was noted in the obese women. Twenty four hours mean LH concentrations have also been noted to be lower with increasing BMI in obese women [53].

Overall obesity appears to exert a significant, although modest, impact on hyperandrogenism in PCOS, mediated primarily via impact on free hormone concentration. There is also evidence of attenuation of LH secretion with obesity resulting in a lower LH to FSH ratio. Hirsutism is reported more frequently in obese women with PCOS, consistent with the impact of higher free androgen concentration.

A great percentage of PCOS patients are overweight to severely obese, and any excess of weight can induce a reduction of peripheral tissue sensitivity to insulin, thus inducing the compensatory hyperinsulinism. Hyperinsulinemia might be central in the pathogenesis of the syndrome because it can induce higher ovarian androgen production and anovulation [54], sustained also by the abnormal LH secretion, with a higher frequency of menstrual abnormalities than in normoinsulinemic PCOS patients [55]. Insulin resistance and compensatory hyperinsulinemia are metabolic disturbances easily observable in at least 45–65 % of PCOS patients and frequently appear to be related to excessive serine phosphorylation of the insulin receptor [56].

2.4 Lifestyle Modification in PCOS Treatment

One of the primary goals of treatment in PCOS is the normalization of serum androgens and restoration of reproductive function; however, the presence of metabolic disturbance has become a prominent feature of the disease that may interfere with long-term health.

In non-PCOS subjects, weight loss and exercise reduce insulin resistance, plasma lipids, and blood pressure. In PCOS, therapeutic use of insulin sensitizing agents normalizes hyperinsulinemia and hyperandrogenism. However, management of lifestyle factors, where possible, is preferable to pharmacological management because of potential costs, side effects, and long-term maintenance. Metabolic abnormalities are significantly increased by obesity in PCOS, with high prevalence rates of impaired glucose tolerance and diabetes as well as dyslipidemia.

In PCOS, caloric restriction improves insulin sensitivity measured through euglycemic clamps, fasting glucose/insulin ratios, homeostasis model assessment (HOMA), OGTT stimulated insulin, and fasting insulin. Weight loss in obese women with PCOS has been demonstrated to reduce hyperlipidemia, in particular total cholesterol and triglycerides levels and plasminogen activator inhibitor-1 (PAI-1) activity, a marker of impaired fibrinolysis and atherothrombosis [57]. Weight loss also decreases ovarian cytochrome P450 c17 alpha activity and reduces basal adipocyte lipolysis.

Pasquali et al. studied obese PCOS women undergoing a hypocaloric diet with or without metformin administration [58]. A 12.3 % reduction in visceral fat was noted in those treated with diet alone versus 2.5 % reduction in subcutaneous fat. This suggests that there is a great sensitivity to reduction in the more metabolically active visceral component of adipose tissue with energy restriction.

Weight reduction in PCOS women improves insulin sensitivity as measured by euglycemic clamp studies [59]. Both insulin values of a 2 h oral glucose tolerance test [60] and fasting insulin parameters [61] have been shown to improve with weight reduction in PCOS patients.

Hyperandrogenism is one of the defining characteristics of PCOS and it is a relevant target for treatment in PCOS women. Hirsutism, a common clinical sign of PCOS, has been noted to be worsened in obese PCOS patients [62]. Serum testosterone and free androgen index are also increased in these types of patients [63]. Pasquali et al., after 3 months of treatment, did not show any change in sex steroid concentration in obese PCOS women [64].

In contrast, other studies, have demonstrated improvements in serum androgens or in SHBG levels, with decrease in free testosterone levels [65], and improvement in hirsutism [66]. Although altered gonadotropin secretion has been demonstrated in PCOS, studies evaluating weight loss have not demonstrated restoration of normal gonadotropin secretion. No improvements in LH pulsatility were found after weight loss as shown by Guzick et al. [61].

Van Dam et al. [67] studied women with PCOS after short-term dietary restriction with 24 h frequent sampling. They demonstrated an increased LH basal and pulsatile secretion, although they did demonstrate a 23 % reduction in serum testosterone. Overall these data indicate that reductions in androgens observed after weight loss may not be mediated by changes of pulsatile gonadotropin secretion but perhaps through other mechanisms such as changes in insulin sensitivity at the pituitary level.

Modest weight loss has been shown to have a significant effect in improving menstrual cycles and ovulation in PCOS [61]. There are no randomized data on the impact that modest weight reduction has on the live birth rate, either with pregnancies resulting from spontaneous ovulation or in response to fertility treatment. Lifestyle modification also reduces the long-term risk of diabetes, heart disease, and possible endometrial cancer in PCOS women.

Useful changes include the following: dietary modification with reduction in calories by limiting daily intake to 1,400 kcal with low daily intake of simple and complex sugar and increased in protein intake, low intake of sugary drinks, avoid snacking between meals, and increase intake of low glycemic index fruits and

vegetables. Other important lifestyle modifications are smoking cessation, moderate alcohol/caffeine intake, and regular moderate daily exercise (at least 30 min a day at the very least).

Some studies [68] have shown that lifestyle changes (in this case, intensive exercise with a goal of over 150 min/week of activity) resulting in weight loss reduced the risk of type 2 diabetes [69]. The same studies found lifestyle changes to be superior to metformin administration. Thus, all women with PCOS should be encouraged to follow a healthy diet to engage in regular exercise. Their lifestyle changes to achieve pregnancy will improve and the risks during pregnancy will be reduced. A healthier lifestyle will also reduce their long-term risk of diabetes, hypertension, dyslipidemia, and cardiovascular diseases. It is important for all primary care providers to identify patients who may have PCOS. These patients need to undergo the appropriate screening tests and should be counselled about diet and exercise. Pharmacological intervention could be combined with this approach as appropriate, but the abovementioned studies suggest that lifestyle modification is the first-line treatment.

2.5 Use of Insulin Sensitizer Agents and Inositol in PCOS Treatment

The logic for use of insulin sensitizer drugs, such as metformin, to treat PCOS patients is the fact that about 45–65 % of these patients have been demonstrated to have insulin resistance and a compensatory hyperinsulinemia that negatively affect ovarian function in terms of steroidogenesis and follicular recruitment and maturation [21].

Excess insulin increases androgen concentrations blocking follicular maturation and increasing cytochrome P450 c17a activity, a key enzyme in the synthesis of both ovarian and adrenal androgens [21]. This situation typically increases 17-hydroxyprogesterone (17OHP), androstenedione, and testosterone plasma levels. The excess of intraovarian androgens negatively modulates follicular function and ovarian activity, thus inducing the typical stromal hypertrophy and maintaining ovarian atresia and anovulation [21].

The use of metformin might be suggested when abnormal insulin sensitivity is diagnosed [70]. Metformin reduces hepatic glucose production from 9 to 30 % on peripheral tissues, such as muscle cells and adipocytes, and acts by increasing glucose uptake through the glucose transport system.

Metformin positively acts on hormonal PCOS abnormalities through a direct and/or indirect action on steroidogenesis [21]; the recovery of normal ovulatory function is probably due to the direct action of metformin on the ovarian tissues and to the metformin-induced normalization of the ovarian steroidogenesis with normal feedback on the pituitary gland, lowering LH secretion and restoring LH pulse secretion. Metformin improves ovarian and adrenal steroidogenesis; in fact, insulin plays specific modulatory roles on these two glands that have the same enzymatic pathways [71].

The use of insulin sensitizers do not reduce hyperandrogenism better than oral contraceptive [72], but as recently reported, the typology of PCOS to be treated is of great relevance, since only when insulin sensitivity is abnormal metformin shows a greater efficacy on all the PCOS features including hyperandrogenism [73]. Other metabolically active hormones such as leptin, resistin, adiponectin, and ghrelin are positively activated by metformin administration and thus participate in the improvement of the reproductive function at the hypothalamus-pituitary-ovarian level.

In the last years, a higher attention has been given to the role of inositol-phosphoglycan (IPG) mediators of insulin action [74], and growing evidences suggest that a deficiency of D-chiro-inositol (DCI) containing IPG might be at the basis of insulin resistance, frequent in PCOS patients. PCOS patients have high urinary clearance of DCI [75] and that metformin administration in obese PCOS patients improves the release of DCI-IPG mediator [76].

DCI is synthesized by an epimerase that converts myo-inositol into DCI and, depending on the specific needs of the two molecules, each tissue has a typical conversion rate [77]. Considering that ovaries never become insulin resistant and being MYO administration able to induce regular menses in both lean and obese hyperinsulinemic PCOS patients [74], a possible modulatory role of MYO on the insulin-mediated endocrine effects has been hypothesized [74]. Recent studies suggest that some abnormal action of insulin might be dependent from IPG mediators of insulin action and suggest that a deficiency in a specific DCI-containing IPG may underlie insulin resistance, similarly to type 2 diabetes. DCI administration has been demonstrated to reduce insulin resistance both in lean and obese patients with PCOS improving ovarian function and decreasing hyperandrogenism [78]. Such studies suggested the putative presence of a defect in the insulin signalling pathway in which DCI-PG is the mediator of insulin action, thus contributing to the pathophysiology of the insulin resistance of PCOS [75]. Besides DCI, MYO has been reported to be greatly correlated to ovarian function [79] and oocyte quality in patients undergoing IVF procedures, independently from circulating plasma levels [80]. Such data support a specific role also for MYO on gonadotropin-induced ovarian function [81] though not confirmed by others [75].

MYO administration has been demonstrated to modulate insulin sensitivity in overweight PCOS patients improving all hormonal parameters and improving insulin sensitivity [74, 81]. The daily dosage of 2 g in the morning has been reported to be effective in hyperinsulinemic obese PCOS patients with fasting insulin levels above 12 mU/ml [81]. Such insulin level seems to be a putative cutoff that suggests when MYO administration might give higher chances of success not only on hormonal parameters but also on hyperinsulinemia and insulin sensitivity [81].

In conclusion, PCOS is a complex syndrome, with hormonal and metabolic aspects, and the therapeutical approach for PCOS patients needs to consider these two aspects together. Metformin as well as inositol integrative administration might be easily used to solve the metabolic aspects of PCOS impairments. Lifestyle as well as hormonal treatments has to be considered relevant therapeutic tools to be used together with insulin sensitizer drugs.

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