

Insulin regulation of human ovarian androgens

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Hyperinsulinaemic insulin resistance is characteristic of many, if not all, women with polycystic ovary syndrome (PCOS). We will review evidence suggesting that hyperinsulinaemia promotes hyperandrogenism in PCOS by two distinct and independent mechanisms: (i) by increasing circulating ovarian androgens; and (ii) by directly reducing serum sex hormone-binding globulin concentrations. The net result of these actions is to increase circulating free testosterone concentrations. It appears likely that an inherent (probably genetically determined) ovarian defect need be present in women with PCOS, which makes the ovary susceptible to insulin stimulation of androgen production. Limited evidence suggests that hyperinsulinaemia might also promote ovarian androgen production by influencing pituitary release of gonadotrophins. This latter possibility, however, has not been critically evaluated. The clinical implication of these findings is that amelioration of hyperandrogenism in women with PCOS may be achieved by interventions which improve insulin sensitivity and reduce circulating insulin. Such measures might include, but are not limited to, weight loss, dietary modification, and insulin-sensitizing medications.

Key words: androgen/insulin/metabolism/ovary/steroid

Introduction

Polycystic ovary syndrome (PCOS) has been defined clinically by hyperandrogenism and anovulation. Recently, it has become apparent that

hyperinsulinaemic insulin resistance is also a prominent feature of both obese and non-obese women with PCOS, and increasing evidence suggests that hyperinsulinaemia plays a pivotal role in the pathogenesis of this disorder (Nestler and Strauss, 1991; Nestler, 1994).

We will review evidence indicating that insulin contributes to the hyperandrogenism of PCOS by both increasing serum concentrations of ovarian androgens and decreasing circulating sex hormone-binding globulin (SHBG) concentrations. Although evidence exists that insulin may also influence folliculogenesis directly, this area will not be reviewed here and has been discussed elsewhere (Nestler, 1994).

Insulin resistance: an integral feature of PCOS

The evidence is overwhelming that PCOS is a disorder characterized by insulin resistance and a compensatory hyperinsulinaemia. Both obese and non-obese women with PCOS have been shown to be more insulin-resistant and hyperinsulinaemic than age- and weight-matched normal women (Burghen *et al.*, 1980; Chang *et al.*, 1983; Pasquali *et al.*, 1983; Shoupe *et al.*, 1983; Geffner *et al.*, 1986; Stuart *et al.*, 1986; Dunaif *et al.*, 1987, 1989, 1990, 1992; Ciaraldi *et al.*, 1992; Dahlgren *et al.*, 1992; Rosenbaum *et al.*, 1993; Ehrmann *et al.*, 1995). It is noteworthy that insulin resistance and hyperinsulinaemia appear to be characteristic features of PCOS not only in the US, but in other societies as well. For example, women with PCOS from the US, Japan and Italy were compared with their respective normal counterparts (Carmina

et al., 1992). Clinical differences existed among these women, and women with PCOS from the US were significantly more obese than Japanese women with PCOS. Moreover, women with PCOS from the US and Italy were hirsute, whereas women with PCOS from Japan were not. Nonetheless, regardless of these differences, women with PCOS from all three countries manifested insulin resistance and hyperinsulinaemia. This common finding across multiple ethnic groups suggests that hyperinsulinaemic insulin resistance represents a universal feature of PCOS.

It is unlikely that the hyperinsulinaemic insulin resistance of PCOS occurs as a result of hyperandrogenism. Insulin resistance persists in women with PCOS who have undergone either subtotal (Imperato-McGinley *et al.*, 1978) or total (Nagamani *et al.*, 1986) removal of the ovaries, or in whom ovarian androgen production has been suppressed with the use of a long-acting gonadotrophin-releasing hormone (GnRH) agonist (Geffner *et al.*, 1986; Dunaif *et al.*, 1990; Nestler *et al.*, 1991a). Prepubertal women with acanthosis nigricans are hyperinsulinaemic, yet elevated serum androgen concentrations do not appear until several years following the diagnosis of insulin resistance (Richards *et al.*, 1985). Some women with point mutations in the insulin receptor gene causing hyperinsulinaemic insulin resistance have been shown to have PCOS (Moller and Flier, 1988; Yoshimasa *et al.*, 1988; Kim *et al.*, 1992). Finally, normal men have androgen concentrations 10–30-fold higher than women, yet they do not demonstrate insulin resistance. Collectively, these observations support the notion that the hyperinsulinaemia of PCOS is a causal factor in the accompanying hyperandrogenism.

Insulin and ovarian androgens

Effects of insulin on circulating ovarian androgens

Human ovaries possess insulin receptors (Poretsky *et al.*, 1984, 1985; Jarrett *et al.*, 1985) suggesting a role for this peptide in the regulation of ovarian function, and in-vitro studies have demonstrated that insulin can directly stimulate androgen production by ovarian stroma obtained from women with

PCOS (Barbieri *et al.*, 1986). Moreover, insulin and insulin-like growth factor I (IGF-I) augment luteinizing hormone (LH)-stimulated androgen biosynthesis in rat ovarian theca cells (Cara and Rosenfield, 1988; Cara *et al.*, 1990).

It has been substantially more difficult to demonstrate the effect of insulin on ovarian androgens *in vivo*. Multiple studies have been conducted where serum testosterone was monitored in women during an acute elevation of circulating insulin, and the results in women with PCOS appear to be confusing and conflicting. Serum testosterone either rose, did not change, or fell in the women with PCOS (Nestler *et al.*, 1987; Smith *et al.*, 1987; Stuart *et al.*, 1987; Micic *et al.*, 1988; Dunaif and Graf, 1989).

There are several problems associated with insulin infusion studies that may account for the conflicting results reported in the literature. First of all, the very nature of these studies dictates that the duration of insulin elevation is brief, lasting only a few hours. Furthermore, in many studies the degree of insulin elevation was far above the physiological range. Finally, several of these studies did not control for the volume of fluids infused, diurnal and day-to-day variations in steroid concentrations, or for unmeasured perturbations such as the well described increase in catecholamines that accompanies insulin infusions (Rowe *et al.*, 1981).

Perhaps the single situation in which investigators induced long-term hyperinsulinaemia while monitoring serum androgen concentrations was the case study reported by DeClue *et al.* (1991). These investigators cared for a young woman with PCOS who was diabetic and manifested a high degree of insulin resistance. High-dose insulin therapy was started, and marked hyperinsulinaemia was maintained over several months. During this period of insulin elevation, circulating testosterone concentrations progressively rose and ovarian volume (as measured by ultrasound) increased 2-fold. When the insulin infusion was discontinued and serum insulin concentrations began to fall, serum testosterone concentrations fell as well, eventually falling into the normal range. A clear concordance existed between serum insulin and testosterone concentrations, suggesting a cause-and-effect relationship.

These findings strongly suggested that insulin stimulated ovarian androgen production in this woman, and was directly responsible for the hyperandrogenism.

In order to study the role of physiological elevations of insulin in PCOS while avoiding problems associated with insulin infusions, we utilized the drug diazoxide (Proglycem, Medical Market Specialties, Boonton, NJ, USA) to suppress insulin release from the pancreas. When five obese women with PCOS were administered diazoxide for 10 days, the fasting serum insulin concentration was suppressed, serum glucose rose, and the insulin response to an oral glucose challenge decreased markedly (Nestler *et al.*, 1989). More importantly, serum total testosterone concentrations fell in all five women with PCOS during this period of insulin suppression by diazoxide. Mean serum total testosterone fell by 17% from 2.5 ± 0.4 nmol/l to 2.1 ± 0.3 nmol/l ($P < 0.007$). Moreover, serum SHBG levels rose during diazoxide administration from a mean value of 13.2 ± 1.0 nmol/l to 21.7 ± 4.1 nmol/l, but this elevation did not attain statistical significance ($P = 0.09$). Because of the concurrent fall in serum total testosterone and rise in SHBG concentrations, serum-free (i.e. non-SHBG-bound) testosterone concentrations fell by 28% from 0.19 ± 0.03 nmol/l to 0.14 ± 0.02 nmol/l ($P < 0.01$; Figure 1).

To verify that this decline in circulating testosterone was not due to diazoxide itself, as well as determining whether insulin regulates ovarian androgens in normal women, a control group of five non-obese healthy women was studied in an identical manner (Nestler *et al.*, 1990). In marked contrast with the obese women with PCOS, diazoxide treatment altered neither serum testosterone ($P = 0.71$) nor SHBG ($P = 0.24$) concentrations in the non-obese healthy women with normal amounts of circulating insulin.

Subsequent to these findings, the insulin-sensitizing drug metformin (Glucolab, Lab. Relab, Caracas, Venezuela) was administered to women with PCOS in three independent studies (Velazquez *et al.*, 1994; Crave *et al.*, 1995; Nestler and Jakubowicz, 1996). In the first report (Velazquez *et al.*, 1994), treatment of 26 PCOS women with metformin for 8 weeks resulted in decreases in

both total and free testosterone and a rise in SHBG. In the second report (Crave *et al.*, 1995), however, metformin therapy was combined with a hypocaloric diet, and this treatment regimen was not associated with any significant change in total or free testosterone or SHBG after a 4 month period. Interpretation of this latter study is difficult, however, because a parallel group of PCOS women who were treated with a placebo and diet did experience a significant reduction in free testosterone and rise in SHBG. One would have expected the PCOS women treated with metformin and diet to have responded at least similarly to, if not more dramatically than, the group treated with placebo and diet.

Most recently, we tested the hypothesis that two reported features of PCOS, namely, hyperinsulinaemic insulin resistance and increased ovarian cytochrome P450c17 α activity, are pathogenetically linked, and that hyperinsulinaemia stimulates P450c17 α activity in PCOS (Nestler and Jakubowicz, 1996). To accomplish this, 24 obese women with PCOS were enrolled into a randomized, single-blind, and placebo-controlled study. Oral glucose tolerance tests and 24 h GnRH agonist (leuprolide) stimulation tests were performed before and after oral administration of either metformin 500 mg ($n = 11$) or placebo ($n = 13$) three times daily for 4–8 weeks. The women were not screened for the presence of hyperinsulinaemia, insulin resistance or increased P450c17 α activity so that the results would be applicable to an unselected and general population of women with PCOS.

Metformin treatment reduced serum fasting and glucose-stimulated insulin. Amelioration of hyperinsulinaemia by metformin was also associated with decreased ovarian P450c17 α enzyme activity, as demonstrated by substantial reductions in the leuprolide-stimulated serum 17 α -hydroxyprogesterone response. Moreover, metformin treatment was associated with a decrease in basal and leuprolide-stimulated LH, a 44% decrease in serum-free testosterone, and a 3-fold rise in SHBG. None of these variables changed in the placebo group.

These findings suggest that hyperinsulinaemia increases P450c17 α activity in obese women with

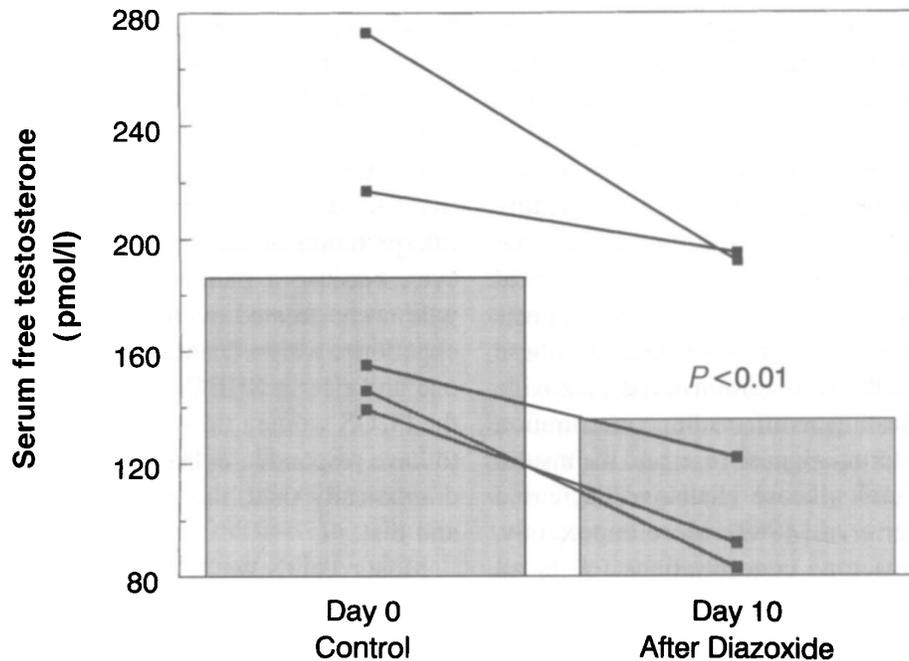


Figure 1. Serum-free testosterone concentrations in five obese women with polycystic ovary syndrome (PCOS) before (day 0) and after (day 10) administration of diazoxide (150 mg/day) for 10 days. Shaded bars represent mean values (adapted from Nestler *et al.*, 1989).

PCOS either by directly stimulating ovarian steroidogenesis and/or indirectly by stimulating gonadotrophin release. Importantly from a clinical standpoint, they also demonstrate that the hyperandrogenism of PCOS can be substantially ameliorated by reducing serum insulin with metformin.

Lack of effect of insulin on ovarian androgens in normal women: evidence for a PCOS gene?

In our studies performed with diazoxide, insulin suppression was associated with a reduction in free testosterone in women with PCOS (Nestler *et al.*, 1989) but not in normal women (Nestler *et al.*, 1990). At least two possible interpretations exist for these disparate results. First of all, insulin concentrations are not elevated in normal women. Serum insulin in normal women may simply not be sufficiently high to stimulate ovarian androgen biosynthesis, and hence insulin may not regulate ovarian androgens under physiological conditions.

However, an alternate and more attractive explanation is that normal women lack a genetic predisposition to the stimulatory action of insulin on ovarian androgens. That is, it seems likely

that there exists a PCOS gene or combination of genes, which makes the ovaries of a woman with PCOS susceptible to insulin stimulation of androgen production. This hypothesis would explain not only the experimental data from our laboratory, it is also consistent with the in-vitro studies of Barbieri *et al.* (1986), which demonstrated that insulin stimulates testosterone release by ovarian stroma of women with PCOS but not by ovarian stroma of normal women, and with the results of in-vivo insulin infusion studies which showed no effect of acute hyperinsulinaemia on circulating testosterone in normal women (Nestler *et al.*, 1987; Smith *et al.*, 1987; Stuart *et al.*, 1987; Dunaif and Graf, 1989). This hypothesis is further supported by the observation that there is familial clustering of PCOS (Givens, 1988), which suggests genetic inheritance, and by the possibility that premature male pattern baldness may be a clinical marker for the PCOS gene(s) in men (Carey *et al.*, 1994). Finally, this hypothesis would also explain why not every woman who is obese, and is therefore by definition hyperinsulinaemic, develops PCOS.

Mechanisms by which insulin could affect ovarian androgen production

Direct effects of insulin on ovarian androgen production

On the surface it may seem paradoxical that insulin should stimulate ovarian androgen production in a woman who is otherwise 'resistant' to insulin, but several theoretical mechanisms exist to explain how a woman resistant to the effects of insulin on glucose transport could nonetheless remain fully sensitive to insulin stimulation of ovarian androgenic pathways.

The most often cited possibilities are that insulin could either cross-react with the ovarian IGF-I receptor or bind to hybrid insulin receptors. We believe these explanations to be unlikely because: (i) the elevation in circulating insulin in PCOS women is usually modest and overlaps substantially with that observed in obese healthy women; and (ii) hybrid insulin receptors have not been identified on human ovaries.

It has also been suggested that insulin could act indirectly by reducing intrafollicular levels of IGF-binding protein 1 (IGFBP-1), thereby increasing intrafollicular concentrations of free IGF-I. IGF-I is a potent stimulator of LH-induced androgen synthesis by ovarian interstitial cells (Cara and Rosenfield, 1988; Adashi *et al.*, 1992), which may in part be due to an induction of LH receptors on these cells by IGF-I (Cara *et al.*, 1990). However, as reviewed elsewhere (Buyalos, 1994), this explanation also seems unlikely in view of evidence which suggests that total intrafollicular IGF-binding capacity in PCOS may be increased rather than reduced.

We believe that insulin probably stimulates ovarian androgen production in PCOS women by activating a signal transduction system which is distinct and separate from the system used to enhance glucose transport. Two studies, one conducted in human placental cytotrophoblasts (Nestler *et al.*, 1991b) and the other in swine ovarian granulosa cells (Romero *et al.*, 1993), support this idea by having shown that the inositol-phosphoglycan 'second messenger' system serves as the signal transduction pathway for the effects of insulin on steroidogenesis in these tissues. The importance of these observations is that the

inositolphosphoglycan signal transduction system may remain intact and fully functional in conditions characterized by insulin resistance in terms of a defective tyrosine kinase system and impaired glucose transport. Hence, by utilizing an alternate signal transduction pathway, the action of insulin on steroidogenesis would be preserved even in the face of glucose intolerance.

The idea that insulin stimulates ovarian androgen production by directly activating its own receptor is further bolstered by the report from Franks' group that steroidogenic effects of insulin are mediated by the insulin receptor itself and not by the IGF-I receptor in primary cultures of human ovarian granulosa cells (Willis and Franks, 1995). Furthermore, using human theca cells, we recently provided evidence that the inositolglycan system serves as the signal transduction system for insulin stimulation of testosterone production by human ovarian theca cells (Nestler *et al.*, 1997).

Indirect effects of insulin on ovarian androgen production

PCOS is often characterized by abnormalities in LH secretion by the pituitary. Some studies have found that LH pulse frequency is increased in PCOS (Burger *et al.*, 1985; Waldstreicher *et al.*, 1988; Imse *et al.*, 1992; Berga *et al.*, 1993), while other studies have found no difference in LH pulse frequency between PCOS women and eumenorrhoeic women (Kazer *et al.*, 1987; Dunaif *et al.*, 1988; Couzinet *et al.*, 1989). In general, however, LH pulse amplitude appears to be increased in women with PCOS in comparison with healthy age- and weight-matched control women (Berga *et al.*, 1993). It is possible that some of the defects in LH dynamics are caused or aggravated by hyperinsulinaemia.

Insulin receptors have been identified in the human pituitary (Unger *et al.*, 1991), and insulin has been shown to modulate anterior pituitary function *in vitro* (Yamashita and Melmed, 1986). In fact, insulin has been shown to specifically augment pituitary release of gonadotrophins *in vitro* (Adashi *et al.*, 1981). Hence, a potential mechanism whereby insulin could enhance ovarian androgen production would be by altering LH release by the pituitary. Theoretically, insulin-induced increases in either LH pulse frequency or amplitude might

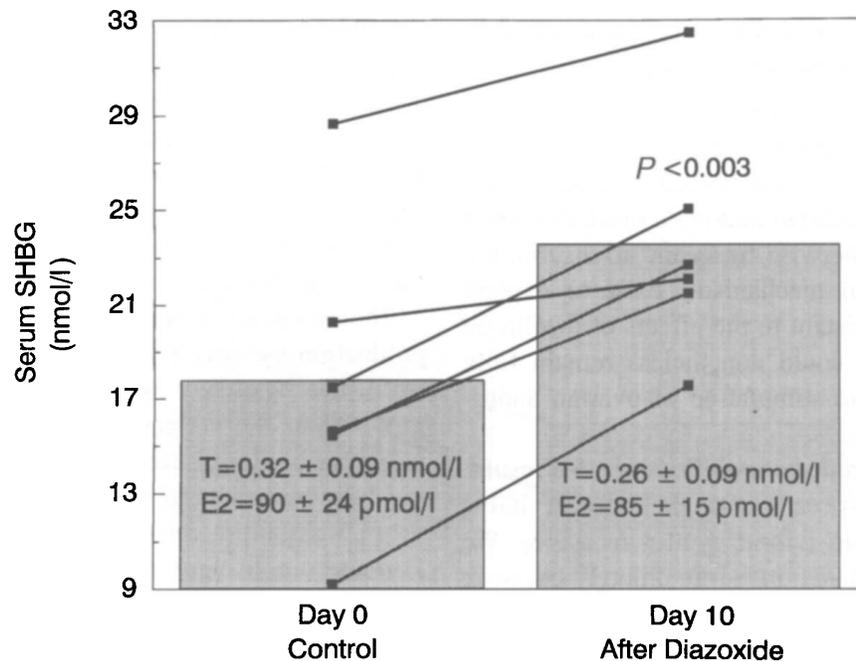


Figure 2. Serum SHBG concentrations in six obese women with polycystic ovary syndrome (PCOS) before (day 0) and after (day 10) administration of diazoxide (150 mg/day) for 10 days. Serum androgens and oestrogens were clamped at low and constant values throughout the study period via administration of the gonadotrophin-releasing hormone (GnRH) agonist, leuprolide. Shaded bars represent mean values. T = testosterone and E2 = oestradiol (adapted from Nestler *et al.*, 1991).

result in enhanced ovarian androgen production. The idea that insulin enhances LH release in women with PCOS is supported by our recent finding that reducing circulating insulin with metformin leads to a decrease in basal and GnRH-stimulated LH release (Nestler and Jakubowicz, 1996). Finally, our preliminary studies tend to suggest that insulin enhances LH pulse amplitude but does not alter LH pulse frequency in obese women with PCOS (J.E.Nestler, unpublished data).

Insulin and SHBG

Insulin influences the clinical androgenic state not only by directly affecting the metabolism of ovarian androgens, but also indirectly by regulating circulating concentrations of SHBG. SHBG binds testosterone with high affinity, and it is commonly held that it is the unbound fraction of testosterone, and not the SHBG-bound fraction, that is bioavailable to tissues. Regulation of circulating SHBG by insulin constitutes an important additional mechanism by which insulin promotes hyperandrogenism. By reducing circulating SHBG, insulin increases the delivery of testosterone to tissues because more testosterone is unbound and bioavailable.

To determine whether insulin can directly influence SHBG metabolism *in vivo*, the effect of insulin suppression by diazoxide on serum SHBG concentrations was examined under conditions where serum androgen and oestrogen concentrations remained unchanged (Nestler *et al.*, 1991a). Ovarian steroidogenesis in six obese women with PCOS was suppressed for 2 months by the administration of a long-acting GnRH agonist. Despite substantial reductions in both serum androgens and oestrogens (serum testosterone concentrations fell by 82%), serum SHBG concentrations did not change. In contrast, when diazoxide was then administered for 10 days to inhibit insulin release (while concurrently continuing GnRH treatment), serum SHBG concentrations rose significantly from a mean value of 17.8 ± 2.6 nmol/l to 23.5 ± 2.0 nmol/l ($P < 0.003$; Figure 2). Because ovarian steroidogenesis was suppressed in these women, diazoxide treatment did not alter serum androgen or oestrogen concentrations. Diazoxide does not affect SHBG production by cultured HepG2 cells (S.R.Plymate, personal communication, 1990), nor does it alter serum SHBG values of non-

obese healthy women with normal concentrations of circulating insulin (Nestler *et al.*, 1990). Thus, these observations suggest that the rise in serum SHBG concentrations following the administration of diazoxide was due to suppression of insulin release, and that hyperinsulinaemia can reduce serum SHBG values in obese women with PCOS, independently of any effect on serum sex steroids.

More recently, results of in-vivo studies suggest that insulin regulates SHBG not only in obese women with PCOS but in normal men and women as well (Peiris *et al.*, 1993; Preziosi *et al.*, 1993; Strain *et al.*, 1994). The results of these studies suggest that regulation of SHBG metabolism by insulin may be a generalized physiological phenomenon, and that SHBG may serve as a biological marker for hyperinsulinaemic insulin resistance in humans (Nestler, 1993).

Therapeutic implications

The causal role of insulin in the hyperandrogenism of PCOS has important clinical therapeutic implications. It predicts that free testosterone concentrations in women with PCOS could be reduced by lowering circulating insulin through weight loss, accomplished either through diet or weight reduction surgery. This idea is supported by two reports (Kiddy *et al.*, 1992; Crave *et al.*, 1995), which showed that serum SHBG rose and free testosterone fell in PCOS women who experienced modest weight loss by dieting. These findings constitute strong evidence that dietary therapy is overlooked as a viable treatment option for women with PCOS. It is also possible that weight loss would not be required to improve reproductive function, and that dietary modification designed to decrease overall insulinaemia might suffice. For example, by ingesting more complex carbohydrates in smaller amounts rather than intermittent large binges of food.

Medications which reduce circulating insulin levels, such as metformin (Velazquez *et al.*, 1994; Nestler and Jakubowicz, 1996) or troglitazone (Dunaif *et al.*, 1996), might also prove to be effective therapies for the hyperandrogenism of PCOS.

Conclusions

Collectively, experimental evidence suggests that hyperinsulinaemia can produce hyperandrogenism in women with PCOS via two distinct and independent mechanisms: (i) by increasing circulating ovarian androgens; and (ii) by directly reducing serum SHBG concentrations. The net result of these actions is to increase circulating free testosterone concentrations. It appears likely that an inherent (probably genetically determined) ovarian defect needs to be present in women with PCOS, which makes the ovary susceptible to insulin stimulation of androgen production. Limited evidence suggests that hyperinsulinaemia might also promote ovarian androgen production by influencing pituitary release of gonadotrophins. This latter possibility, however, has not been critically evaluated.

The clinical implication of these findings is that amelioration of hyperandrogenism in women with PCOS may be achieved by interventions which improve insulin sensitivity and reduce circulating insulin. Such measures might include, but are not limited to, weight loss, dietary modification, and insulin-sensitizing medications.

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