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Insulin Resistance and the Polycystic Ovary Syndrome: Mechanism and Implications for Pathogenesis[1]

Andrea Dunaif

Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033

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I. Introduction

A. Background and historical perspective

POLYCYSTIC ovary syndrome (PCOS) is an exceptionally common disorder of premenopausal women characterized by hyperandrogenism and chronic anovulation (1, 2). Its etiology remains unknown. Although there have been no specific population-based studies, a 5–10% prevalence of this disorder in women of reproductive age is probably a reasonable conservative estimate. This is based as an upper limit on studies of the prevalence of polycystic ovaries, which found that ~20% of self-selected normal women had polycystic ovary morphology on ovarian ultrasound (3). Many of these women had subtle endocrine abnormalities (3). The lower estimate is based on the reported 3% prevalence rate of secondary amenorrhea for 3 or more months (4) and the fact that up to ~75% of women with secondary amenorrhea will fulfill diagnostic criteria for PCOS (5). PCOS women can also have less profound disturbances in menstrual function (1, 3, 6).

Since the report by Burghen *et al*. (7) in 1980 that PCOS was associated with hyperinsulinemia, it has become clear that the syndrome has major metabolic as well as reproductive morbidities. The recognition of this association has also instigated extensive investigation of the relationship between insulin and gonadal function (1, 8, 9, 10, 11). This review will summarize our current understanding of insulin action in PCOS, address areas of controversy, and propose several hypotheses for this association. Abnormalities of steroidogenesis and gonadotropin release will not be discussed in detail; these changes have been reviewed recently by Erhmann and colleagues (12) and by Crowley (13), respectively.

The association between a disorder of carbohydrate metabolism and hyperandrogenism was first described in 1921 by Achard and Thiers (14) and was called "the diabetes of bearded women (diabete des femmes a barbe)." The skin lesion, acanthosis nigricans, was reported to occur frequently in women with hyperandrogenism and diabetes mellitus by Kierland *et al*. (15) in 1947. Brown and Winkelmann (16) noted in 1968 that it was insulin-resistant diabetes mellitus, and a genetic basis was suggested by reports of

affected sisters (17), including a pair of identical twins who also had acromegaloid features (18). Several additional syndromes with distinctive phenotypic features, acanthosis nigricans, hyperandrogenism, and insulin-resistant diabetes mellitus have been identified (Table 1). These include the lipoatrophic (total and partial) diabetes syndromes, leprechaunism (intrauterine growth retardation, gonadal enlargement, elfin facies, and failure to thrive), and Rabson-Mendenhall syndrome (unusual facies, pineal hypertrophy, dental precocity, thickened nails, and ovarian enlargement) (8, 19, 20).

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Table 1. Syndromes of hyperandrogenism and hyperinsulinemia

Attention was focused on the association of hyperandrogenism, insulin resistance, and acanthosis nigricans in 1976 when Kahn and colleagues (21) described a distinct disorder affecting adolescent girls, which they designated the type A syndrome. These girls were virilized (*i.e.*, increased muscle bulk, clitoromegaly, temporal balding, deepening of the voice) and had extreme insulin resistance with diabetes mellitus as well as striking acanthosis nigricans. This group identified a second distinct extreme insulin resistance syndrome in postmenopausal women with acanthosis nigricans and features of autoimmune disease, which they termed the type B syndrome and determined that it was caused by endogenous antiinsulin receptor antibodies (22, 23). Subsequent studies have identified insulin receptor mutations as the cause of leprechaunism, Rabson-Mendenhall Syndrome, and some cases of type A syndrome (19, 23).

In 1980 Burghen and colleagues (7) reported that women with the common hyperandrogenic disorder, PCOS, had basal and glucose-stimulated hyperinsulinemia compared with weight-matched control women, suggesting the presence of insulin resistance. They noted significant positive linear correlations between insulin and androgen levels and suggested that this might have etiological significance. In the mid-1980s several groups noted that acanthosis nigricans occurred frequently in obese hyperandrogenic women (24, 25, 26, 27) (Fig. 1). These women had hyperinsulinemia basally and during an oral glucose tolerance test, compared with appropriately age- and weight-matched control women. The presence of hyperinsulinemia in PCOS women, independent of obesity, was confirmed by a number of groups worldwide (28, 29, 30).

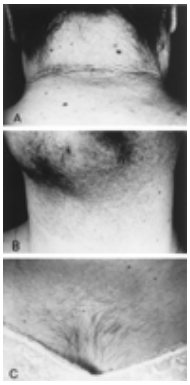

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Figure 1. A woman with PCOS who has acanthosis nigricans, a cutaneous marker of insulin resistance (panel A). She also has severe hirsutism on her face and chest (panels B and C). [Reproduced from A. **Dunaif** *et al.*: *Obstet Gynecol* 66:545–552, 1985 (25) with permission from The American College of Obstetricians and Gynecologists.]

Our study (25) suggested that these women had typical PCOS, except for increased ovarian stromal hyperthecosis, which is diagnosed by finding islands of luteinized theca cells within the ovarian stroma (25). When this is very extensive, it is called hyperthecosis and is associated with more profound hyperandrogenism (31). Hughesdon (32) reported, however, that upon careful examination of ovaries from PCOS women, small islands of hyperthecosis were usually present. This morphological change was more extensive in insulin-resistant PCOS women, suggesting that hyperinsulinemia had an impact on ovarian morphology as well as on function (25) (Fig. 2). This hypothesis has been further supported by the finding, in a subsequent study (33), of a positive correlation between hyperinsulinemia and ovarian stromal hyperthecosis.

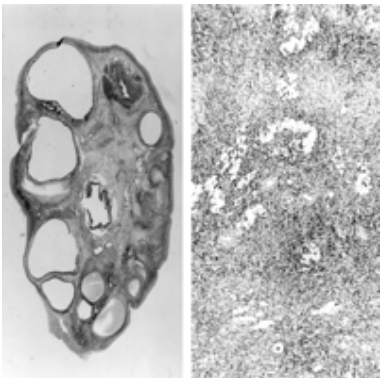

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Figure 2. Section of a polycystic ovary with multiple subscapular follicular cysts and stromal hypertrophy (*left panel*). At higher power (×100) islands of luteinized theca cells are visible in the stroma (*right panel*). This morphological change is called stromal hyperthecosis and appears to be directly correlated with circulating insulin levels. [Figure is used with permission from A. **Dunaif**.]

B. Definition of PCOS

The current recommended diagnostic criteria for PCOS are hyperandrogenism and ovulatory dysfunction with the exclusion of specific disorders, such as nonclassic adrenal 21-hydroxylase deficiency, hyperprolactinemia, or androgen-secreting neoplasms (1) (Table 2↓). The polycystic ovary morphology is consistent with, but not essential for, the diagnosis of the *syndrome* (1, 3). Polycystic ovaries are defined on ultrasound by the presence of eight or more subcapsular follicular cysts ≤10 mm and increased ovarian stroma (2, 3). These changes, however, can be present in women who are entirely endocrinologically normal (2, 3). Thus, the ovarian morphological change must be distinguished from the endocrine *syndrome* of hyperandrogenism and anovulation.

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Table 2. Diagnostic criteria for PCOS—% participants agreeing at 1990 NICHD PCOS Conference (1)

Gonadotropin-secretory changes, with a characteristic increase in LH relative to FSH release, have long been appreciated in PCOS (34, 35). Frequent (*e.g.*, every 10 min), prolonged (12–24 h) serial blood sampling studies have revealed that there is a significant increase in the frequency and the amplitude of LH release with normal FSH release in PCOS (36, 37). The increased LH pulse frequency reflects an increase in GnRH release and suggests the presence of a hypothalamic defect in PCOS (13, 37). Other causes of hyperandrogenism, however, can result in similar gonadotropin-secretory changes, such as androgen-secreting neoplasms (38) or adrenal hyperandrogenism resulting from nonclassic 21-hydroxylase deficiency (39). Ovulatory women with the polycystic ovary morphology can have increased LH/FSH ratios (2). Because of the pulsatile nature of gonadotropin release, a single blood sample can fail to detect an increased LH/FSH ratio (40). This, as well as its lack of specificity, has led to the recommendation that LH/FSH ratios not be included in the diagnostic criteria for PCOS (1).

Other nomenclature has been proposed for the *syndrome*, *e.g.*, chronic hyperandrogenic anovulation (CHA) (1). Many hyperandrogenic anovulatory women have significantly increased ovarian steroidogenic responses to stimulation with GnRH analogs that Rosenfield and colleagues (41) have termed functional ovarian hyperandrogenism (FOH). They have proposed this as an alternative name for PCOS (12). The majority of

women who have hyperandrogenemia and chronic anovulation will have polycystic ovary (PCO) on ultrasound and will have responses to GnRH analogs consistent with FOH (1, 2, 12) (Fig. 3). Thus, the terms PCOS, FOH, and CHA define similar groups of women (Fig. 3).

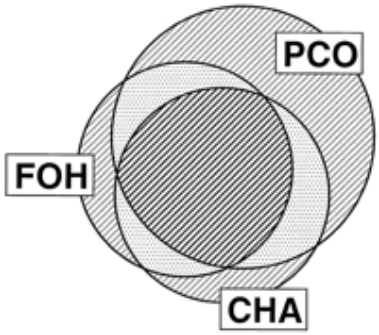

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Figure 3. The majority of women with CHA will also have polycystic ovary morphology (PCO) and responses to GnRH analogs consistent with FOH. [Figure is used with permission from A. **Dunaif**.]

PCOS often has a menarchal age of onset characterized by a failure to establish a regular pattern of menses (42). Hirsutism may develop peripubertally or during adolescence (42) or it may be absent until the third decade of life (43). Seborrhea, acne, and alopecia are other common clinical signs of hyperandrogenism (44, 45). Some women never develop signs of androgen excess because of genetic differences in target tissue number and/or sensitivity to androgens (46). The clinical consequence of chronic anovulation is some form of menstrual irregularity ranging from oligomenorrhea (menses every 6 weeks to 6 months), amenorrhea, or dysfunctional uterine bleeding (2, 5, 6). Infertility may be the presenting symptom of the anovulation. Depending on the population studied, 16–80% of PCOS women are obese (47, 48, 49). Mild to moderate acanthosis nigricans is commonly present in obese PCOS women (25, 26, 27, 49, 50). A rapid progression of androgenic symptoms and/or true virilization (increased muscle bulk, clitoromegaly, temporal balding, and/or deepening of the voice) are rare in PCOS (2, 6, 42). PCOS women can occasionally have acromegaloid features (44).

It is important to recognize that there is an inherent bias of ascertainment in studies of PCOS that constrains the assessment of the frequency of associated clinical and biochemical findings. Obviously, all women will have polycystic ovaries when this feature is an essential diagnostic criterion. Studies that use an increased LH/FSH ratio as a selection criterion will be biased toward finding increased pulsatile LH release when gonadotropin secretion is examined. The appropriate study would be a population-based one in which clinical and biochemical features were systematically examined in a defined population of women. Until such a study is performed, the prevalence of PCOS and frequency of associated findings will remain subject to debate.

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II. Insulin Action in PCOS

A. Glucose tolerance

Insulin resistance is an important defect in the pathogenesis of noninsulin-dependent diabetes mellitus (NIDDM) (51). Despite the fact that hyperinsulinemia, reflecting some degree of peripheral insulin resistance, was well recognized in PCOS by the mid-1980s (Fig. 4), glucose tolerance was not systematically investigated until our study in 1987 (49). We found that obese PCOS women had significantly increased glucose levels during an oral glucose tolerance test compared with age- and weight-matched ovulatory hyperandrogenic (*i.e.*, elevated plasma androgen levels) and control women (Fig. 4). Twenty percent of the obese PCOS women had impaired glucose tolerance or frank NIDDM by National Diabetes Data Group Criteria (49, 52) (Fig. 4). The women studied ranged in age from 18–36 yr with a mean age of 27 yr for the obese PCOS women. There were no significant differences, however, in glucose levels during the oral glucose tolerance test in the nonobese PCOS women compared with age- and weight-matched control women (Fig. 4).

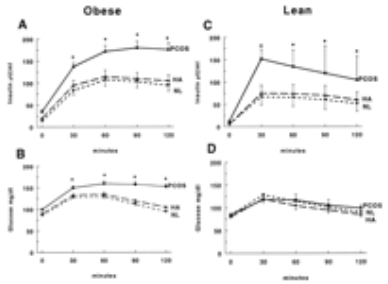

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Figure 4. Insulin (panels A and C) and glucose (panels B and D) responses basally and after a 40 g/m^2 oral glucose load in obese and lean PCOS women, ovulatory hyperandrogenic women (HA) women, and age- and weight-matched ovulatory control women. Insulin responses are significantly increased only in PCOS women, suggesting that hyperinsulinemia is a unique feature of PCOS and not hyperandrogenic states in general (panels A and B). Glucose responses are

significantly increased only in obese PCOS women (C), and ~20% of obese PCOS women have impaired glucose tolerance or NIDDM using National Diabetes Data Group Criteria (52). [Derived from Ref. 49.]

A subsequent study in postmenopausal women with a history of PCOS found a significantly increased prevalence of NIDDM as well as of hypertension (see below) (53). We have continued to find prevalence rates of glucose intolerance as high as ~40% in obese PCOS women when the less stringent World Health Organization (WHO) criteria are used (49, 52, 54, 55, 56, 57). The majority of affected women are in their third and fourth decade of life, but we and others (58) have encountered PCOS adolescents with impaired glucose tolerance or NIDDM. These prevalence rates of 20–40% are substantially above prevalence rates for glucose intolerance reported in population-based studies in women of this age (5.3% by National Diabetes Data Group criteria and 10.3% by WHO criteria in women aged 20–44 yr) (59). We have found that the prevalence of glucose intolerance is significantly higher in obese PCOS women (~30%) than in concurrently studied age-, ethnicity-, and weight-matched ovulatory control women (~10%) (48). In contrast, we have found that nonobese PCOS women have impaired glucose tolerance only occasionally, consistent with the synergistic negative effect of obesity and PCOS on glucose tolerance (54, 55). Finally, based on the prevalence of glucose intolerance in women (59), the prevalence of glucose intolerance in PCOS (49), and on a conservative estimate of the prevalence of PCOS (~5%), it can be extrapolated that PCOS-related insulin resistance contributes to approximately 10% of cases of glucose intolerance in premenopausal women. The study in postmenopausal women with a history of PCOS found a 15% prevalence of NIDDM (53), consistent with our extrapolated prevalence estimates. It is thus clear that PCOS is a major risk factor for NIDDM in women, regardless of age.

B. Insulin action in vivo in PCOS

Although insulin has a number of actions, in addition to those regulating glucose metabolism, such as inhibition of lipolysis and stimulation of amino acid transport (51), the effects of insulin on glucose metabolism are usually examined in studies of insulin resistance (60). This can be studied quantitatively in humans with the euglycemic glucose clamp technique: a desired dose of insulin is administered and euglycemia is maintained by a simultaneous variable glucose infusion whose rate is adjusted based on frequent arterialized blood glucose determinations and a negative feedback principle (60, 61, 62). At steady state, the amount of glucose that is infused equals the amount of glucose taken up by the peripheral tissues and can be used as a measure of peripheral sensitivity to insulin, known as insulin-mediated glucose disposal (IMGD) or M (61, 62). The suppression of hepatic glucose production by insulin can be assessed by the use of a simultaneous infusion of isotopically labeled glucose. Insulin-mediated glucose disposal occurs only in muscle (skeletal and cardiac) and in fat; muscle accounts for about 85% of this (60).

Euglycemic glucose clamp studies have demonstrated significant and substantial decreases in insulin-mediated glucose disposal in PCOS (54, 55) (Fig. 5). This decrease (~35–40%) is of a similar magnitude to that seen in NIDDM (Fig. 5). Obesity (fat mass *per se*), body fat location (upper *vs.* lower body, *e.g.*, waist to hip girth ratio), and muscle mass all have important independent effects on insulin sensitivity (63, 64, 65, 66). Alterations in any of these parameters could potentially contribute to insulin

resistance in PCOS. PCOS women have an increased prevalence of obesity (6, 47), and women with upper, as opposed to lower body, obesity have an increased frequency of hyperandrogenism (66). Since muscle is the major site of insulin-mediated glucose use (60) and androgens can increase muscle mass (67), potential androgen-mediated changes in lean body (primarily muscle) mass must also be controlled for in PCOS (54, 55). Studies in which body composition, assessed by the most precise available method (hydrostatic weighing), has been matched to normal control women, and in which lean PCOS women, who had body composition and waist to hip girth ratios similar to controls, were studied, have confirmed that PCOS women are insulin resistant, independent of those potentially confounding parameters (1, 55, 68). The impact of hyperandrogenism on insulin sensitivity is discussed below, but studies in cultured cells have confirmed the impression from these *in vivo* studies that an intrinsic defect in insulin action is present in PCOS (69).

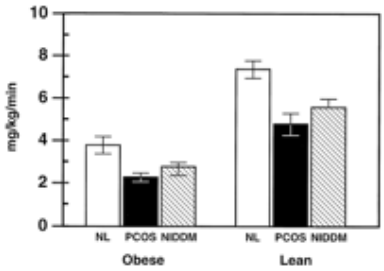

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Figure 5. Insulin-mediated glucose disposal at steady-state insulin levels of ~600 pmol/liter (~100 μU/ml) is decreased by 35–40% in PCOS women compared with age- and weight-matched control women. This decrease is similar in magnitude to that seen in NIDDM. [Figure is used with permission from A. **Dunaif**.]

Basal hepatic glucose production and the ED_{50} value of insulin for suppression of hepatic glucose production are significantly increased only in obese PCOS women (54, 55) (Fig. 6). This synergistic negative effect of obesity and PCOS on hepatic glucose production is an important factor in the pathogenesis of glucose intolerance (49, 54, 55, 70). This is analogous to NIDDM in general where defects in insulin action, presumably genetic, synergize with environmentally induced insulin resistance, primarily obesity-related, to produce glucose intolerance (51, 60). Sequential multiple-insulin-dose euglycemic clamp studies have indicated that the ED_{50} insulin for glucose uptake is significantly increased, and that maximal rates of glucose disposal are significantly decreased in lean and in obese PCOS women (55) (Fig. 6). It appears, however, that body fat has a more pronounced negative effect on insulin sensitivity in women with PCOS (68, 71).

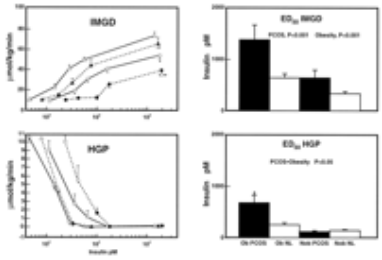

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Figure 6. Parameters of *in vivo* insulin action during sequential multiple-dose euglycemic glucose clamp studies in nonobese PCOS women (▲, Nob PCOS); nonobese normal women (△, Nob NL); obese PCOS women (•, Ob PCOS); and obese normal women (○, Ob NL). The maximal response in the dose-response curves (*top left*) for insulin-mediated glucose disposal (IMGD) is significantly decreased in obesity (**P* < 0.001) and in PCOS (***P* < 0.01). The ED_{50} insulin IMGD is significantly increased in PCOS women (*P* < 0.001) and in obese women (*P* < 0.001) (*top right*). Basal rates of hepatic glucose production (HGP) are not significantly different in the four groups (*bottom left*). The statistical interaction is significant between PCOS and obesity on the ED_{50} insulin for suppression of HGP (*bottom right*), which is increased significantly in obese PCOS women (**P* < 0.001) indicating a synergistic deleterious effect of obesity and PCOS on hepatic insulin sensitivity. [Reproduced with permission by A. **Dunaif** *et al. Diabetes* 41:1257–1266, 1992 (55).]

C. Insulin secretion in PCOS

In the presence of peripheral insulin resistance, pancreatic ß-cell insulin secretion increases in a compensatory fashion. NIDDM develops when the compensatory increase in insulin levels is no longer sufficient to maintain euglycemia (72, 73). It is essential, therefore, to examine ß-cell function in the context of peripheral insulin sensitivity. Under normal circumstances, this relationship is constant (72, 74) (Fig. 7). ß-Cell dysfunction is felt to be present for values falling below this hyperbolic curve (73, 74). This relationship can be quantitated as the product of insulin sensitivity and first-phase insulin release known as the disposition index (72).

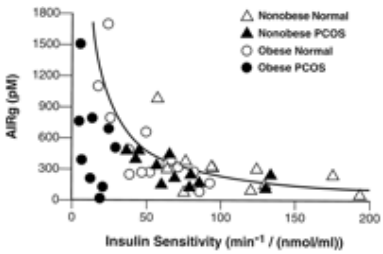

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Figure 7. The relationship between insulin sensitivity (SI) determined by frequently sampled intravenous glucose tolerance test and first-phase insulin secretion to an intravenous glucose load (AIRg). The majority of PCOS women fall below the normal curve determined in concurrently studied age- and weight-matched control women as well as normative data in the literature. [Derived from Ref. 57.]

Fasting hyperinsulinemia is present in obese PCOS women and this is, in part, secondary to increased basal insulin secretion rates (Fig. 4♠ and Ref.75). Insulin responses to an oral glucose load are increased in lean and obese PCOS women (Fig. 4♠), but acute insulin responses to an intravenous glucose load (AIRg), first-phase insulin secretion, are similar to weight-matched control women (49, 57). When the relationship between insulin secretion and sensitivity is examined, lean and obese PCOS women fall below the relationship in weight-matched control women, and the disposition index is significantly decreased by PCOS as well as by obesity (57) (Fig. 7♠). Further evidence for ß-cell dysfunction in PCOS is provided by the elegant studies of Erhmann *et al*. (76), who have demonstrated defects in ß-cell entrainment to an oscillatory glucose infusion and decreased meal-related insulin secretory responses (75). These defects are much more pronounced in PCOS women who have a first-degree relative with NIDDM, suggesting that such women may be at particularly high risk to develop glucose intolerance (76). There are reports of increased insulin secretion in PCOS, but these studies have not examined insulin secretion in the context of insulin sensitivity and/or have included women in whom the diagnosis was made on the basis of ovarian morphological changes rather than endocrine criteria (71, 77). In summary, the most compelling evidence suggests that ß-cell dysfunction, in addition to insulin resistance, is a feature of PCOS. The ability to diagnose PCOS at the time of puberty will make possible prospective longitudinal studies of the ontogeny of these defects.

D. Insulin clearance in PCOS

Hyperinsulinemia can result from decreases in insulin clearance as well as from increased insulin secretion. Indeed, decreased insulin clearance is usually present in insulin-resistant states since insulin clearance is receptor-mediated, and acquired decreases in receptor number and/or function are often present in insulin resistance secondary to hyperinsulinemia and/or hyperglycemia (78, 79). Thus, PCOS would be expected to be associated with decreases in insulin clearance; however, relatively few studies have examined this question. Direct measurement of posthepatic insulin clearance during euglycemic clamp studies has not been abnormal in PCOS (54, 56). Circulating insulin to C-peptide molar ratios are increased in PCOS, suggesting decreased hepatic extraction of insulin, but such ratios also reflect insulin secretion (28, 80). Direct measurement of hepatic insulin clearance in non-PCOS hyperandrogenic women has found it to be decreased (81). The one study of this question in PCOS found decreased hepatic insulin extraction by model analysis of C-peptide levels (75). Therefore, in PCOS, hyperinsulinemia is probably the result of a combination of increased basal insulin secretion and decreased hepatic insulin clearance.

E. Cellular and molecular mechanisms of insulin resistance

1. Molecular mechanisms of insulin action (Figs. 8♠ and 9♠). Insulin acts on cells by binding to its cell surface receptor (51, 82, 83). The insulin receptor is a heterotetramer

made up of two α,ß- dimers linked by disulfide bonds (84) (Fig. 8✦). Each α,ß-dimer is the product of one gene (85, 86). The α-subunit is extracellular and contains the ligand-binding domain whereas the ß-subunit spans the membrane, and the cytoplasmic portion contains intrinsic protein tyrosine kinase activity, which is activated further by ligand-mediated autophosphorylation on specific tyrosine residues (87) (Fig. 8✦). The insulin receptor belongs to a family of protein tyrosine kinase receptors that includes the insulin-like growth factor-I (IGF-I) receptor, with which it shares substantial sequence and structural homology, as well as the epidermal growth factor (EGF), fibroblast growth factor, platelet-derived growth factor, and colony-stimulating factor-1 receptors (88). A number of oncogene products are also protein tyrosine kinases (85, 89).

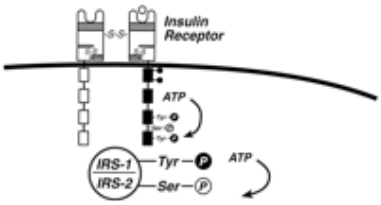

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Figure 8. The insulin receptor is a heterotetramer consisting of two α,ß-dimers linked by disulfide bonds. The α-subunit contains the ligand-binding site, and the ß-subunit contains a ligand-activated tyrosine kinase. Tyrosine autophosphorylation increases the receptor's tyrosine kinase activity whereas serine phosphorylation inhibits it. [Adapted with permission from C. R. Kahn: *Diabetes* 43:1066–1084, 1994 (51).]

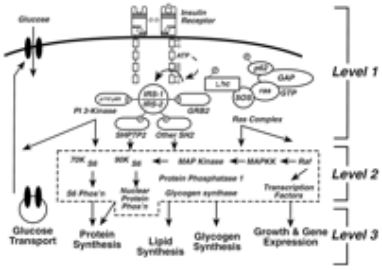

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Figure 9. The tyrosine-phosphorylated insulin receptor phosphorylates intracellular substrates, such as insulin receptor substrate (IRS)-1 and IRS-2, initiating signal transduction and the plieotropic actions of insulin. The activation of PI3-K (PI3-kinase) by tyrosine-phosphorylated IRS-1 appears to be the pathway for insulin-mediated glucose transport. The Ras-MAP kinase pathway appears to regulate cell

growth and glycogen synthesis. [Adapted with permission from C. R. Kahn: *Diabetes* 43:1066–1084, 1994 (51).]

Ligand binding induces, probably via conformational changes, autophosphorylation of the insulin receptor on specific tyrosine residues and further activation of its intrinsic kinase activity (Fig. 8♠) (90, 91, 92). The activated insulin receptor then tyrosine phosphorylates intracellular substrates to initiate signal transduction (Fig. 9♠) (82). Over the last few years a number of these substrates have been characterized. The first was insulin receptor substrate-1 (IRS-1), which serves as a docking molecule for signaling and adaptor molecules (93, 94). The tyrosine-phosphorylated insulin receptor tyrosine phosphorylates IRS-1 on specific motifs, and these phosphorylated sites then bind signaling molecules, such as the SH2 domain of phosphatidylinositol 3-kinase (PI3-K), or the adaptor molecule, Nck (51, 82, 94). This leads to activation of downstream signaling pathways, such as that leading to insulin-mediated glucose transport, which appears to be modulated through the PI3-K signal cascade (82). More recently, insulin receptor substrate-2 (IRS-2), another substrate for the insulin receptor, has been identified (95, 96). Shc (an adaptor molecule) can also bind directly to the insulin receptor initiating signal transduction (82, 97).

Insulin has numerous target tissue actions, such as stimulation of glucose uptake, gene regulation, DNA synthesis, and amino acid uptake (51, 82). The mechanisms of insulin receptor signal specificity are currently a subject of intense investigation. It now appears that the Ras-Raf-MEK pathway is involved in the regulation of cell growth and metabolism whereas the PI3-K pathway is involved in glucose uptake (98, 99, 100, 101). The mechanisms by which the insulin signal is terminated remain incompletely understood. Receptor-mediated endocytosis and recycling are well known to occur and may be important to signal termination (83, 102). Serine phosphorylation has been shown to terminate signaling by the EGF receptor (103, 104), another tyrosine kinase growth factor receptor, and it can be shown under a variety of experimental conditions that insulin receptor serine phosphorylation decreases its tyrosine kinase activity (105, 106, 107, 108). It has been postulated that protein kinase C (PKC)-mediated serine phosphorylation of the insulin receptor is important in the pathogenesis of hyperglycemia-induced insulin resistance (102, 109). Recent evidence suggests that tumor necrosis factor-α (TNF-α)-mediated serine phosphorylation of IRS-1 inhibits insulin receptor signaling and is the mechanism of TNF-α-induced insulin resistance (110). Studies addressing this important question have been constrained by a lack of sensitive anti-phosphoserine antibodies. Identification of phosphoserine residues usually requires painstaking phosphoamino acid analysis of ^{32}P-labeled receptors (111). The use of fluorophore labeling of phosphoserine promises to provide a sensitive methodology for examining *in vivo* serine phosphorylation events (112).

In summary, insulin action is mediated through a ligand-activated tyrosine kinase receptor, similar to a number of other growth factors. A variety of phosphorylation-dephosphorylation signaling cascades are then activated, leading to the pleiotropic actions of insulin. The mechanisms of signal specificity and termination require further investigation.

2. Molecular insulin action defects in PCOS. Studies in adipocytes, a classic insulin target tissue, have failed to confirm earlier reports in blood cells of decreases in insulin receptor number and/or receptor affinity in PCOS (25, 26, 27, 113) when appropriately

weight-matched controls have been included. The one adipocyte study reporting a decrease in insulin receptor number used a control group consisting primarily of lean individuals (114). Studies of insulin action in isolated PCOS adipocytes have revealed marked decreases in insulin sensitivity together with less striking, but significant, decreases in maximal rates of insulin-stimulated glucose transport (55, 115) (Fig. 10✦). There is evidence for decreases in adipocyte levels of adenosine in PCOS (116), but whether this is a primary defect or secondary to hyperinsulinemia is unclear. The decrease in maximal rates of adipocyte glucose uptake is secondary to a significant decrease in the abundance of GLUT4 glucose transporters (117). Similar defects are present in NIDDM and in obesity but are ameliorated by control of hyperglycemia and hyperinsulinemia as well as by weight reduction, suggesting acquired rather than intrinsic defects (65, 118, 119, 120). In contrast, in PCOS such defects can occur in the absence of obesity, glucose intolerance, or changes in waist to hip girth ratios (55, 117). Moreover, these abnormalities are not significantly correlated with sex hormone levels, suggesting that abnormalities of insulin action in PCOS may be intrinsic (55, 117).

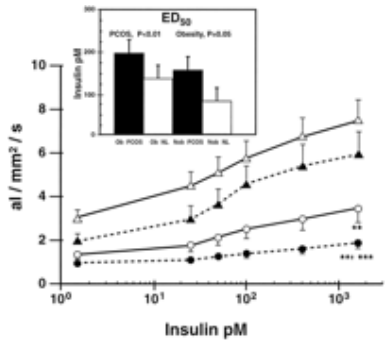

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Figure 10. Insulin-stimulated adipocyte U-[^{14}C]glucose transport in nonobese PCOS women (▲, Nob PCOS); nonobese normal women (△), Nob NL); obese PCOS women (•, Ob PCOS); and obese normal women (○, Ob NL). Basal rates of glucose transport are decreased significantly (*) in PCOS *vs.* normal women ($P < 0.01$) and in nonobese *vs.* obese women ($P < 0.001$). Maximal insulin-stimulated increments above basal are significantly decreased in PCOS *vs.* normal women (***, $P < 0.01$) and in obese *vs.* nonobese women (**, $P < 0.001$). The ED_{50} insulin is increased significantly in PCOS *vs.* normal and in obese *vs.* nonobese women (*inset*). [Reproduced with permission from A. **Dunaif** *et al.*: *Diabetes* 41:1257–1266, 1992 (55).]

To further evaluate the postbinding defect in insulin action in PCOS, we examined insulin receptor function in receptors isolated from cultured skin fibroblasts. Because fibroblasts are removed from the *in vivo* environment for several generations, they provide a constant source of insulin receptors that are not influenced by the hormonal imbalance of PCOS. Consistent with our earlier results from the adipocyte studies, fibroblasts from PCOS women showed no change in insulin binding or receptor affinity

(69). However, in approximately 50% of PCOS fibroblasts (PCOS-ser), we observed decreased insulin receptor autophosphorylation (69). This was secondary to markedly increased basal autophosphorylation with minimal further insulin-stimulated autophosphorylation (Fig. 11). Phosphoamino acid analysis revealed decreased insulin-dependent receptor tyrosine phosphorylation and increased insulin-independent receptor serine phosphorylation (69) (Fig. 11). The ability of the PCOS-ser insulin receptors to phosphorylate an artificial substrate was also significantly reduced (Fig. 12).

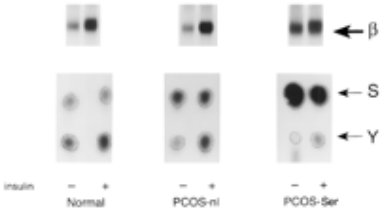

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Figure 11. Representative autoradiograms of autophosphorylated skin fibroblast insulin receptor ß-subunits (*top*) and phosphoamino acid analysis (*bottom*) ± 1 μM insulin from a normal (control), a PCOS woman with normal insulin-stimulated tyrosine phosphorylation (PCOS-nl) and a PCOS woman with high basal autophosphorylation on serine residues (PCOS-ser); S-serine, Y-tyrosine. Basal autophosphorylation is increased and there is minimal further insulin-stimulated phosphorylation in the PCOS-ser ß-subunits. The high basal phosphorylation represents phosphoserine, and phosphotyrosine content does not increase in response to insulin in the PCOS-ser ß-subunits. [Reproduced from A. **Dunaif** *et al.*: *J Clin Invest* 96:801–810, 1995 (69) by copyright permission of The American Society for Clinical Investigation.]

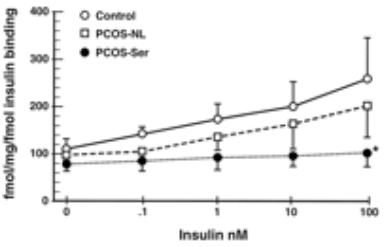

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Figure 12. Phosphorylation of poly GLU4:TYR1 by partially purified skin fibroblast insulin receptors. Skin fibroblast insulin receptors were directly extracted from confluent cell cultures, partially purified, and incubated in the presence of 0–100 nM, and assays of the phosphorylation of poly GLU4:TYR1 were performed.

One-way ANOVA, $P < 0.005$; PCOS-ser < control and PCOS-nl, $P < 0.05$ Tukey's test. The values are the mean ± SEM from five PCOS-ser (•), four PCOS-nl (■), and four control (○) subjects. [Reproduced from A. **Dunaif** *et al.*: *J Clin Invest* 96:801–810, 1995 (69) by copyright permission of The American Society for Clinical Investigation.]

Serine phosphorylation of the insulin receptor has been shown in cell-free systems and *in vivo* to inhibit the receptor's tyrosine kinase activity, analogous to our findings in the PCOS-ser insulin receptors (69, 105, 106, 107, 108). Thus, this defect in the early steps of the insulin-signaling pathway may cause the insulin resistance in PCOS-ser women. Increased insulin-independent serine phosphorylation in PCOS-ser insulin receptors appears to be a unique disorder of insulin action since other insulin-resistant states, such as obesity, NIDDM, type A syndrome, and leprechaunism, do not exhibit this abnormality (1, 51, 65, 69) (Table 1♠). The PCOS-ser phosphorylation abnormality appears to be physiologically relevant because it is present in insulin receptors partially purified from skeletal muscle, a classic insulin target tissue, and because the same pattern of abnormal phosphorylation occurs in insulin receptors phosphorylated in intact cells (69).

Fibroblasts from approximately 50% of PCOS women (PCOS-nl) have no detectable abnormality in insulin receptor phosphorylation (69) (Figs. 11♠ and 12♠). Although these women demonstrate the same PCOS phenotype and the same degree of insulin resistance as the PCOS-ser women with abnormal phosphorylation, insulin receptor phosphorylation in fibroblasts and skeletal muscle from these women is similar to that of control women (69). This observation suggests that a defect downstream of insulin receptor signaling, such as phosphorylation of IRS-1 or activation of PI3-K, is responsible for insulin resistance in PCOS-nl women (51, 69, 102). Indeed, our recent human studies demonstrate a significant decrease in muscle PI3-K activation during insulin infusion in PCOS women (121), consistent with a physiologically relevant defect in the early steps of insulin receptor signaling.

We found no insulin receptor mutations in two PCOS-ser women by direct sequencing of genomic DNA (120), and sequence analysis of the tyrosine kinase domain in the ß-subunit of an additional eight PCOS-ser women also revealed no mutations (69). This finding has recently been confirmed by other investigators (122). Immunoprecipitation and mixing experiments suggest that a factor extrinsic to the insulin receptor is responsible for the excessive serine phosphorylation (69). PCOS-ser insulin receptors autophosphorylate normally, if they are first immunoprecipitated from wheat-germ agglutinin (WGA) lectin eluates. Furthermore, mixing control human insulin receptors and WGA eluates from PCOS-ser fibroblasts results in increased insulin-independent serine phosphorylation and decreased insulin-stimulated tyrosine phosphorylation of the normal receptors (69) (Fig. 13♠). Both experiments suggest that a factor present in WGA eluates is responsible for the abnormal phosphorylation.

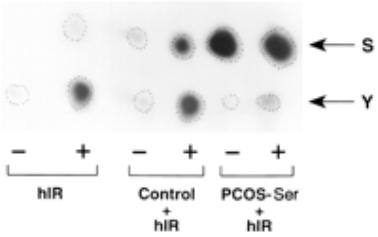

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Figure 13. Phosphoamino acid analysis of immunopurified human insulin receptors (hIR) ß-subunits basally and mixed with WGA-Sepharose eluates from control or PCOS-ser fibroblasts. hIRs were immunopurified from WGA-Sepharose eluates, mixed in a ratio of 10 fmol hIR:1 fmol PCOS-ser or control lectin eluate insulin-binding activity, and autophosphorylation ± 1 μM insulin was examined. Phosphoamino acid analysis revealed a striking increase in phosphoserine content and a marked decrease in insulin-stimulated phosphotyrosine content after mixing hIR with PCOS-ser lectin eluates as compared with mixing hIR with control lectin eluates or in the absence of mixing. [Reproduced from A. **Dunaif** *et al.*: *J Clin Invest* 96: 801–810, 1995 (69) by copyright permission of The American Society for Clinical Investigation.]

The serine/threonine kinase, PKC, is a candidate for the putative serine phosphorylation factor (108). However, evidence against this possibility includes the observation that no phosphothreonine is detected in the PCOS-ser insulin receptors, and PKC has been shown to phosphorylate threonine 1336 of the insulin receptor (123). Furthermore, the IGF-I receptor, which is a known substrate of PKC under certain conditions, phosphorylates normally in PCOS-ser women (69, 124). Finally, preliminary Western blot analyses showed no significant differences in the abundance of PKC isoforms in PCOS-ser fibroblasts compared with controls (A. **Dunaif**, unpublished observations).

Other serine/threonine kinases that might cause the increased serine phosphorylation of PCOS-ser insulin receptors include a casein kinase I-like enzyme and cAMP-dependent protein kinase (125, 126). However, the casein kinase I-like enzyme has been shown to phosphorylate insulin-stimulated insulin receptors twice as well as unstimulated insulin receptors (125). This phosphorylation pattern differs from what we observe with PCOS-ser insulin receptors, namely excessive serine phosphorylation in the absence of insulin. cAMP-dependent protein kinase is a candidate because increases in cAMP cause serine phosphorylation of insulin receptors in cultured lymphocytes (127). However, insulin receptor phosphorylation by cAMP-dependent protein kinase is probably indirect because the human insulin receptor ß-subunit does not contain the amino acid sequences classically recognized by this kinase (128).

Alternatively, a novel serine/threonine kinase or an inhibitor of a serine/threonine phosphatase may be responsible for the abnormal phosphorylation of PCOS-ser insulin receptors (69, 129). Because it is present in WGA eluates, the PCOS-ser factor is either a membrane glycoprotein or a protein associated with a glycoprotein. In some respects, our putative serine phosphorylation factor is similar to a recently identified inhibitor of insulin receptor tyrosine kinase, the membrane glycoprotein PC-1 (130) (Fig. 14✦). Both

factors are extrinsic to the insulin receptor, both are present in WGA eluates from human skin fibroblasts, and both appear to inhibit insulin receptor tyrosine kinase activity. This represents an important new mechanism for human insulin resistance related to factors that modulate the tyrosine kinase activity of the insulin receptor (51) (Fig. 14). The major difference between the two factors is that PC-1 is not associated with increased insulin-independent serine phosphorylation characteristic of the PCOS-ser insulin receptors (69, 130, 131). Recent studies suggest that TNF-α produces insulin resistance by a related mechanism: serine phosphorylation of IRS-1, which then inhibits insulin receptor tyrosine kinase activity (Fig. 7). Isolation and characterization of the factor in PCOS-ser fibroblasts are now in progress, as is the mapping of phosphorylated serine residues in PCOS-ser insulin receptors.

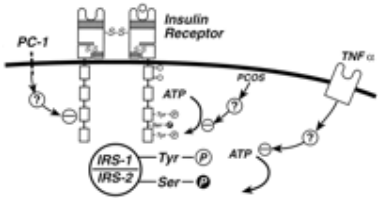

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Figure 14. Insulin resistance in ~50% of PCOS women appears to be secondary to a cell membrane-associated factor, presumably a serine/threonine kinase, that serine-phosphorylates the insulin receptor-inhibiting signaling. Serine phosphorylation of IRS-1 appears to be the mechanism for TNFα-mediated insulin resistance. The membrane glycoprotein PC-1 also inhibits insulin receptor kinase activity, but it does not cause serine phosphorylation of the receptor. These are examples of a recently appreciated mechanism for insulin resistance secondary to factors regulating the receptor's tyrosine kinase activity. [Figure used with permission from A. **Dunaif**.]

Although fibroblasts are not classic insulin target cells, defects identified in insulin receptor number and/or kinase activity in them have reflected insulin receptor mutations (19). Thus, the presence of the putative serine phosphorylation factor in cultured cells of PCOS-ser women suggests that the abnormal insulin receptor phosphorylation is genetically programmed. In addition, we have found that some first degree relatives of PCOS women are insulin resistant, including brothers, consistent with a genetic defect (132). Recent twin (133) and family studies (134) have also suggested that insulin resistance is a genetic defect in PCOS. Our putative serine phosphorylation factor is a candidate gene for a mutation producing the insulin resistance associated with PCOS (see below).

F. Constraints of insulin action studies in PCOS

There is general consensus in the literature that obese PCOS women are insulin resistant. Controversy remains as to the pathogenesis of the insulin resistance, and there are studies that suggest that obesity *per se* or increased central adiposity are responsible

for the associated defects in insulin action (135, 136). Many of the conflicting studies can be explained by differing diagnostic criteria for PCOS and by the inclusion of both lean and obese women in the experimental sample. Our studies (49) and those in the United Kingdom (137, 138) strongly suggest that anovulation is associated with insulin resistance. We found insulin resistance only in women with hyperandrogenism and anovulation (Fig. 4). Studies using ovarian morphology to ascertain women have found that only anovulatory women with PCO morphology are insulin resistant (137, 138). Women with regular ovulatory menses and hyperandrogenism [elevated plasma androgen levels (49)] (Fig. 4) or with PCO detected by ovarian ultrasound (137, 138) are not insulin resistant. Therefore, studies that have defined PCOS by PCO morphology without further assessment of ovulation could have included women who were not insulin resistant. Similarly, studies that have included ovulatory hyperandrogenic women will bias the sample with insulin-sensitive subjects.

One reason for the general acceptance of the diagnostic criteria for PCOS of hyperandrogenism and anovulation (1) (Table 2, see above) is that they define the insulin-resistant subset. Even with subjects so identified, not all are insulin resistant, despite using the relatively lenient criterion of 1 SD below the control mean value for insulin action. Moreover, the occasional PCOS woman can have insulin sensitivity more than 2 SDs (95% confidence interval) above the control mean (117). There is clearly heterogeneity in this feature of the syndrome. Obesity is another important factor, and it appears that it has a more pronounced effect on insulin action in PCOS than in control women (71). Ideally, lean and obese PCOS women should be studied separately (30, 49, 54, 55, 68). If groups are pooled, PCOS women should be matched to controls so that the spectrum of body weights are equally represented. This is often not the case so that, although mean body mass may be similar, the PCOS group often contains more obese individuals, thereby skewing the results (114). Moreover, there are very few studies in the literature in which lean PCOS woman have been separately studied (30, 54, 55, 68, 135). There are also major ethnic variations in insulin sensitivity, and this is another less well appreciated potential confounding factor (56). Recent studies from Denmark suggest that adiposity accounts for insulin resistance in their PCOS population in contrast to our US population (135, 136).

We have consistently found significant decreases in insulin-mediated glucose disposal in both lean and obese PCOS women (54, 55, 56). Similarly, our group (57) as well as Yen's group (68) have found significant decreases in insulin sensitivity (SI) determined by modified frequently sampled intravenous glucose tolerance test with minimal model analysis in such PCOS women (57). Insulin resistance has been found in PCOS women of many racial and ethnic groups including Japanese, Caribbean and Mexican Hispanics, non-Hispanic Whites, and African Americans (55, 56, 139, 140).

G. PCOS as a unique NIDDM subphenotype (Table 3)

Our studies in premenopausal women, extrapolated data based on prevalence estimates of PCOS and glucose intolerance, and studies in postmenopausal women with a history of PCOS all suggest that PCOS-related insulin resistance confers a significantly increased risk for NIDDM (see above). Familial clustering of affected individuals as well as studies in monozygotic twins indicate that NIDDM has an important genetic component (51, 102, 141, 142, 143, 144). Insulin resistance is a major inherited abnormality, but studies in which insulin secretion has been examined in the context of insulin sensitivity demonstrate that ß-cell dysfunction may also be an important contributing factor to the ultimate development of the NIDDM phenotype (51, 145,

146). There is clearly genetic heterogeneity with insulin resistance being absent in some affected individuals (146, 147).

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Table 3. Adult NIDDM syndromes

The underlying genetic defects have been identified in fewer than 5% of NIDDM individuals and consist of mutations in genes such as the insulin receptor gene, mitochondrial DNA, or the glucokinase gene (Table 3✦) (19, 51, 102, 144, 148, 149). Defects in a number of candidate genes, such as GLUT4, GLUT2, and hexokinase, have been excluded (102, 150). The major cause of insulin resistance in typical NIDDM is reduced insulin-stimulated muscle glycogen synthesis. Defects found in NIDDM in insulin receptor number and/or phosphorylation or glucose transport, however, are reversible with the control of hyperglycemia (51, 65, 102, 151), elevated free fatty acid levels (152), and/or hyperinsulinemia (119). Only one study has shown an intrinsic abnormality in NIDDM-cultured cells (153): decreased insulin-stimulated glycogen synthesis. Studies in NIDDM first-degree relatives, who are normoglycemic but insulin resistant, suggest that there is an inherited decrease in both insulin-stimulated muscle glucose transport/phosphorylation and glycogen synthase activity that results in the reduced glycogen synthesis (154, 155, 156). In contrast, in PCOS, intrinsic abnormalities in the early steps of insulin receptor signaling are present, making this the first common NIDDM subphenotype in which such defects have been identified (69, 102, 151). Moreover, the defective pattern of insulin receptor phosphorylation is unique, suggesting it should be possible to distinguish PCOS-related insulin resistance from that related to other NIDDM genotypes. This should make it possible to assign affected status accurately for linkage studies of the genetics of PCOS-related insulin resistance (157).

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III. Hypotheses Explaining the Association of Insulin Resistance and PCOS

A. Causal association

1. Do androgens cause insulin resistance? If glucose utilization is expressed as a function of muscle mass rather than total body mass, women do appear to be more insulin sensitive than men (158, 159). Moreover, when isolated fat cells are compared, female adipocytes are more sensitive than male adipocytes to insulin-mediated glucose uptake (160). These are subtle differences, however, and do not approach the degree of impairment in insulin sensitivity observed in PCOS (54, 55). Finally, in the rare syndromes of extreme insulin resistance and hyperandrogenism, specific molecular defects in insulin action have been clearly identified as the cause of insulin resistance (19, 161).

It is possible, however, that androgens may produce mild insulin resistance. Women receiving oral contraceptives containing "androgenic" progestins can experience decompensations in glucose tolerance, as can individuals receiving synthetic anabolic steroids (162, 163). Prolonged testosterone administration to female-to-male transsexuals, which produced circulating testosterone levels in the normal male range, resulted in significant decreases in insulin-mediated glucoses uptake in euglycemic clamp studies (164). These decreases were largest at lower doses of insulin (~25% at ~ 300 pM steady-state levels), not significant at moderate insulin doses (~1,000 pM steady-state levels), and minimal at higher doses (~7% at ~5,000 pM steady-state levels) (164) (Fig. 15). Studies in testosterone-treated castrated female rats have suggested that androgen-mediated insulin resistance may be the result of an increase in the number of less insulin-sensitive type II b skeletal muscle fibers (165) and an inhibition of muscle glycogen synthase activity (166).

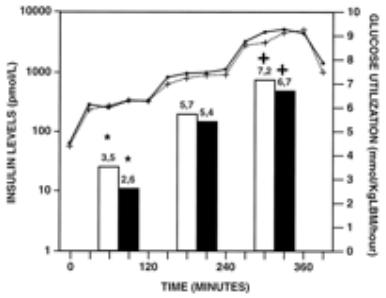

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Figure 15. Hyperinsulinemic euglycemic clamp studies basally and during treatment with virilizing doses of testosterone in 13 female-to-male transsexuals. Insulin-mediated glucose disposal decreased significantly at low and at high doses of insulin. [Reproduced with permission from K. H. Polderman *et al.*: *J Clin Endocrinol Metab* 79:265–271, 1994 (164). © The Endocrine Society.]

It has been more difficult to demonstrate that decreasing androgen levels improve insulin sensitivity in PCOS. We found no significant changes in peripheral or hepatic

insulin action in profoundly insulin-resistant obese PCOS women by single-insulin dose (steady-state insulin levels ~600 pM) glucose clamp studies after prolonged androgen suppression produced by the administration of an agonist analog of GnRH (167). Diamanti-Kandarakis and colleagues (168) reported that antiandrogen therapy did not alter insulin sensitivity in PCOS. Other investigators have found modest improvements in insulin sensitivity in PCOS during androgen suppression or antiandrogen therapy (169, 170) (Fig. 16✦). Such changes were apparent in less insulin-resistant, less obese, or nonobese PCOS women (169, 170). Moreover, insulin resistance was improved but not abolished (170) (Fig. 16✦). It is of considerable interest that the effects of sex steroids on insulin sensitivity appear to be sexually dimorphic. Testosterone administration to obese males improves insulin sensitivity (171), and synthetic estrogen administration to male-to-female transsexuals produces insulin resistance (164).

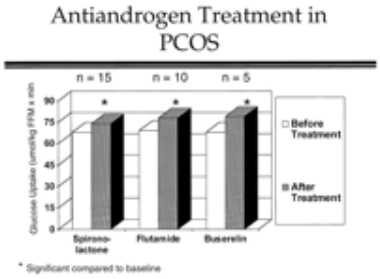

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Figure 16. Basal and insulin-mediated glucose disposal in 43 hyperandrogenic women and 12 control women. The hyperandrogenic women were studied before and after 3–4 months of antiandrogen therapy with spironolactone, flutamide, or Buserelin. Insulin-mediated glucose disposal increased significantly during treatment ($P < 0.01$). [Adapted with permission from P. Moghetti *et al.*: *J Clin Endocrinol Metab* 81:952–960, 1996 (170). © The Endocrine Society.]

Givens and colleagues (172) have proposed that androgens have differential effects on insulin action, with testosterone worsening insulin sensitivity and the adrenal androgen, dehydroepiandrosterone (DHEA), improving it. This hypothesis is based on differing correlations of these steroids with insulin-binding studies in blood cells and on their observation that women with elevated dehydroepiandrosterone sulfate (DHEAS) levels have normal insulin sensitivity (172). The one direct *in vitro* study supporting this hypothesis was constrained by a small sample size (n = 3), and the examination of testosterone and DHEA effects on insulin binding using blood cells rather than a more relevant insulin target tissue (172). Studies in which DHEA or DHEAS have been administered to humans have failed to support this hypothesis. Administration of supraphysiological amounts of DHEA (which also result in testosterone elevations since DHEA is a testosterone prehormone) has produced mild hyperinsulinemia in women, but had no effects on insulin sensitivity in men, as would be expected given the sexually dimorphic effects of androgens on insulin action (173, 174). Moreover, PCOS women with elevated DHEAS levels similar to those in ovulatory hyperandrogenic women are

significantly more insulin resistant, arguing against an insulin-sensitizing action of DHEA (49, 175).

In summary, the modest hyperandrogenism characteristic of PCOS may contribute to the associated insulin resistance. Additional factors are necessary to explain the insulin resistance, since suppressing androgen levels does not completely restore normal insulin sensitivity (167, 170). Further, androgen administration does not produce insulin resistance of the same magnitude as that seen in PCOS (54, 55, 164). Finally, there are clearly defects in insulin action that persist in cultured PCOS skin fibroblasts removed from the hormonal milieu for generations (see above) (69).

2. Does hyperinsulinemia cause hyperandrogenism? The syndromes of extreme insulin resistance are commonly associated with hyperandrogenism when they occur in premenopausal women (19, 20) (Table 1). The cellular mechanisms of insulin resistance in these conditions range from antibodies that block insulin binding to its receptor (type B syndrome) to genetic defects in the receptor resulting in decreased numbers and/or depressed function of the receptor (type A syndrome, leprechaunism); the common biochemical feature is profound hyperinsulinemia (19, 20) (Table 1). Accordingly, it has been proposed that hyperinsulinemia causes hyperandrogenism. Insulin can be shown experimentally to have a variety of direct actions on steroidogenesis in humans (1, 9, 20). Insulin can stimulate ovarian estrogen, androgen, and progesterone secretion *in vitro* (1, 20, 176). Although some of these actions have been observed at physiological insulin concentrations, most actions have been observed at higher concentrations (1, 20).

The presence of insulin receptors in crude ovarian membranes does not necessarily indicate a physiological role for insulin in the regulation of steroidogenesis since such receptors are widely distributed through the body (51, 83). Insulin is present in human follicular fluid but in concentrations most likely representing an ultrafiltrate of plasma rather than local production (177). In contrast, IGF-I is produced by human ovarian tissue, and IGF-I receptors are present in the ovary (178, 179). IGF-I and its receptor share considerable sequence, structural, and functional homology with insulin and its receptor, respectively (180). The IGF-I receptor is a heterotetramer with two α,ß-dimers assembled analogous to the insulin receptor (85, 88, 181, 182, 183) (see above). Insulin can bind to the ligand-binding domain of the IGF-I receptor and activate the tyrosine kinase activity of the ß-subunit and the intracellular events normally mediated by IGF-I (85, 88, 180, 181). IGF-I can bind to and activate the insulin receptor, resulting in rapid effects on glucose metabolism (85, 88, 181). In general, the affinity of the IGF-I receptor for insulin is considerably less than it is for IGF-I and *vice versa* (181). However, this varies by tissue; thus data on receptor affinity cannot be extrapolated from one tissue to another. There are also so-called "atypical" IGF-I receptors that bind IGF-I and insulin with similar affinity (184, 185). α,ß-Dimers of the insulin and IGF-I receptor can assemble together to form hybrid heterotetramers (11, 182, 186, 187).

Insulin-like growth factor-binding proteins (IGFBPs) are major regulators of IGF action. IGFBPs can specifically bind IGF-I and modulate its cellular actions by altering its bioavailability (182, 188). Insulin decreases hepatic production of IGFBP-1 and may, thus, make IGF-I more biologically available (182). Growth factor regulation of ovarian steroidogenesis appears to be primarily a paracrine system with locally produced IGF-I and IGFBPs acting on neighboring cells in concert with gonadotropins (1, 178, 179, 189). A number of other growth factors, including IGF-II, EGF, and transforming growth factor-α and -ß, appear to have a role in the regulation (both stimulatory and

inhibitory) of ovarian steroidogenesis (1, 188, 190). Insulin cannot interact directly with the receptors for these hormones (84, 88, 181, 182). However, the receptors for some of these growth factors, such as the EGF receptor (which binds both EGF and transforming growth factor-α), are also protein kinases (1, 84, 88). Thus the potential exists for communication between the insulin-IGF-I system and the other protein kinase growth factor systems through receptor "cross-talk" and/or by shared kinases or phosphatases that may regulate all of these receptors (51, 191). For example, serine phosphorylation of the EGF receptor also decreases its tyrosine kinase activity (103, 104). In rodents, hyperinsulinemia can result in up-regulation of ovarian IGF-I-binding sites, and this may provide yet another mechanism by which insulin can modulate growth factor action (192).

Insulin in high concentrations can mimic IGF-I actions by occupancy of the IGF-I receptor (1, 181, 182), and this has been a proposed mechanism for insulin-mediated hyperandrogenism (8, 9, 10). However, it has recently been shown that insulin has specific actions on steroidogenesis acting through its own receptor (193). Moreover, these actions appear to be preserved in insulin-resistant states (193, 194), presumably because of differences in receptor sensitivity to this insulin action or because of differential regulation of the receptor in this tissue. Our studies in cultured skin fibroblasts suggest that a mechanism for this may be selective defects in insulin action. Both insulin- and IGF-I-stimulated glycogen synthesis are significantly decreased in PCOS fibroblasts whereas thymidine incorporation is similar to control fibroblasts (Fig. 17♦) (195). Thus only the signaling pathways regulating carbohydrate metabolism may be impaired in PCOS, while those involved in steroidogenesis are preserved. This would explain the paradox of persistent insulin-stimulated androgen production in insulin-resistant PCOS women. Insulin decreases hepatic IGFBP-1 production, the major circulating IGF-I-binding protein (183). Thus, bioavailable IGF-I levels are increased in insulin-resistant PCOS women, and this may contribute to the ovarian steroidogenic abnormalities via activation of the IGF-I receptor (68). In lean PCOS women, increases in GH release may also affect ovarian steroidogenesis (68).

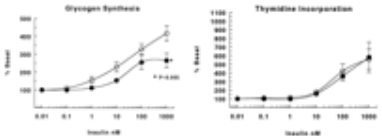

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Figure 17. Dose-response curves for insulin-stimulated glycogen synthesis (*left panel*) and thymidine incorporation (*right panel*) in confluent skin fibroblasts from PCOS (•) and control (○, NL) women. Maximal responses for insulin-stimulated glycogen synthesis were significantly decreased ($P < 0.001$). There were no significant differences in thymidine incorporation in the PCOS fibroblasts (*right panel*). The dose-response curves for IGF-I were similar to those for insulin (data not shown). [Reproduced with permission from A. **Dunaif** (195).]

It has been more difficult to demonstrate insulin actions on steroidogenesis in humans *in vivo* because it is not feasible to administer insulin to nondiabetics for prolonged periods (1, 196, 197, 198). Relatively physiological levels of insulin (100 μU/ml or 600 pM), when infused over approximately 2 h, can slightly increase plasma androstenedione levels in normal women (1). However, these increases are minor and are not in the range seen in women with hyperandrogenism. Moreover, it is arguable whether insulin contributes to androgen production in normal women since insulin levels in the 100 μU/ml (~600 pM) range are generally seen only after meals (1, 196). Furthermore, such transient meal-related increases in insulin do not result in increased androgen levels, whereas the more sustained increases produced by continuous insulin infusion can slightly increase androgen levels (196).

Studies in which insulin levels have been lowered for prolonged periods have been much more informative. This has been accomplished for 7 days to 3 months with agents that either decrease insulin secretion, diazoxide (199) or somatostatin (200), or that improve insulin sensitivity, metformin (201) or troglitazone (202). Circulating androgen levels have decreased significantly in women with PCOS in these studies. Sex hormone binding globulin (SHBG) levels have increased (199, 202), compatible with a major role for insulin in regulating hepatic production of this protein (203, 204). Abnormalities in apparent 17,20-lyase activity have improved in parallel with reduced circulating insulin levels consistent with insulin-mediated stimulation of this enzyme (205). However, estrogen levels also decreased significantly, suggesting that insulin has diffuse effects on steroidogenesis (202). Changes in estrogen levels were seen only when insulin levels were lowered with troglitazone and thus, alternatively, these changes might be the result of troglitazone-mediated increases in sex steroid metabolism, a recently reported action of this agent (Rezulin Package Insert, Parke-Davis, Morris Plains, NJ). It is also possible that troglitazone has direct effects on steroidogenesis. Indeed, the thiazolidinediones have been shown to have such effects on granulosa cell steroidogenesis (206).

Most of the reported actions of insulin on steroidogenesis are observed only in women with PCOS (197, 198) and are greatly enhanced by the addition of gonadotropins when measured in *in vitro* experiments (1, 20, 176, 190, 207). In the one study in normal women in which insulin levels were lowered by diazoxide administration, no significant changes in androgen levels were noted (208). These observations suggest that, if insulin is to produce ovarian hyperandrogenism in women, polycystic ovarian changes (*e.g.*, theca cell hyperplasia) must be present that predispose the ovaries to secrete excess androgens. In normal women insulin does not appear to have any acute effects on ovarian function under physiological circumstances (196, 197, 208).

Although insulin has been shown to stimulate gonadotropin release in isolated rat pituitary cells (209), human studies of insulin action on gonadotropin release have yielded conflicting results. Acute insulin infusion has not changed pulsatile LH or FSH release or gonadotrope sensitivity to GnRH in normal or in PCOS women, despite direct effects on gonadal steroidogenesis in PCOS women (197). Long-term suppression of insulin levels with diazoxide, which resulted in decreases in circulating testosterone levels, did not alter circulating LH levels (199). In contrast, decreases in LH levels were observed after 7 days of somatostatin-mediated insulin lowering (201), after metformin for 8 weeks (205), or after troglitazone for 3 months (202). It is possible that insulin-mediated changes in gonadotropin release contribute to the changes of steroidogenesis produced by insulin in humans (Fig. 18).

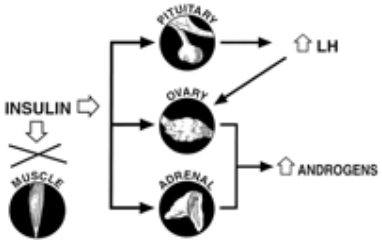

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Figure 18. Studies in which insulin levels have been lowered with the insulin-sensitizing agent, troglitazone, suggest that insulin is a general augmentor of steroidogenesis and LH release. [Figure is used with permission from A. **Dunaif**.]

Acute insulin infusions decrease DHEAS levels in men and women, suggesting that insulin is a negative modulator of adrenal androgen metabolism (176). When insulin levels are chronically lowered, however, circulating DHEA and DHEAS levels rise in men but not in women, suggesting that this regulation of adrenal androgen metabolism is sexually dimorphic (210). Lowering insulin levels with insulin-sensitizing agents, such as troglitazone, has resulted in decreases in DHEAS levels in PCOS women (202) (Fig. 18♠). The mechanism of this appears to be a direct action of insulin to increase adrenal sensitivity to ACTH in hyperandrogenic women (211). Insulin can directly decrease hepatic SHBG production (203), explaining the frequently observed inverse correlation between peripheral insulin and SHBG levels (204). Indeed, insulin rather than sex steroids appears to be the major regulator of SHBG production (204).

In summary, studies in which insulin levels have been lowered by a variety of modalities indicate that hyperinsulinemia augments androgen production in PCOS (Fig. 18♠). Moreover, this action appears to be directly mediated by insulin acting through its cognate receptor rather than by spillover occupancy of the IGF-I receptor. Intrinsic abnormalities in steroidogenesis appear to be necessary for this insulin action to be manifested since lowering insulin levels does not affect circulating androgen levels in normal women. Further, in many PCOS women, lowering insulin levels ameliorates but does not abolish hyperandrogenism.

B. Possible genetic association of PCOS and insulin resistance

1. Family studies of PCOS. Familial aggregation of PCOS suggesting a genetic etiology has been clearly established (1, 212, 213, 214). Cooper *et al.* (212) reported that a history of oligomenorrhea was more common in the mothers and sisters of PCOS women than in controls. Probands reported that male relatives had increased "pilosity" (212). The proposed mechanism of inheritance was autosomal dominant with decreased penetrance. Givens and colleagues have reported multiple kindreds showing affected women in several generations and have examined some males in considerable detail (1, 215). Diagnostic criteria for PCOS were hirsutism and enlarged ovaries. There was a high frequency of metabolic disorders, such as NIDDM and hyperlipidemia, in both male and female pedigree members. In one kindred there were several males with

oligospermia and one with Klinefelter's syndrome (47, XXY). Elevated LH/FSH ratios were present in some males and 89% of their daughters had PCOS. This would suggest inheritance in either an autosomal or X-linked dominant manner.

Ferriman and Purdie (216) studied 700 women; affected status was assigned on the basis of hirsutism and enlarged ovaries (assessed by gynecography). The frequency of various abnormalities in relatives was determined by history provided by the proband; no relatives were examined. Oligomenorrhea and infertility were most common in women who had both hirsutism and enlarged ovaries. Forty-six percent of female relatives were reported to be similarly affected. There was an increased incidence of baldness reported in male relatives. Similar results were found in a study of 132 Norwegian PCOS probands identified by ovarian wedge resection (217). Information on pedigree members was obtained by questionnaire. Female first-degree relatives had a significantly increased frequency of PCOS symptoms (*i.e.*, hirsutism, oligomenorrhea, infertility), and male first-degree relatives had a significantly increased frequency of early baldness or "excessive hairiness" compared with controls. Human leukocyte antigen typing in PCOS has had conflicting results; an initial report showed no human leukocyte antigen association, whereas a follow-up study reported an association with DQA1[1]0501 (218, 219). There have been case reports of polyploidies and X-chromosome aneuploidies in PCOS (220, 221). Larger studies, however, have found normal karyotypes (222).

Studies from the United Kingdom have phenotyped women on the basis of polycystic ovarian morphology detected by ultrasound (223). Familial polycystic ovary morphology was observed in 51 of 62 pedigrees (92%). The proportion of females affected in all sibships was 80.5% (107 of 133), which would exceed the expected ratios for either an autosomal dominant or an X-linked dominant mode of inheritance. However, not all women in each kindred were examined and, thus, an accurate ratio of affected to unaffected women could not be established for segregation analysis. Further, the male phenotype was not sought.

A more recent study prospectively examined the families of probands consecutively identified on the basis of polycystic ovarian morphology on ultrasound (224). The first-degree relatives in 10 families were evaluated by history, measurement of physical indices, and hirsutism as well as serum levels of androgens, 17-hydroxyprogesterone, gonadotropins, and PRL. Transabdominal ultrasound was performed in female first-degree relatives. Only 54% of women with polycystic ovaries had an elevated total testosterone or LH level consistent with the endocrine *syndrome*. Glucose and insulin levels were assessed in obese but not lean probands. Twenty-two males were screened; eight had premature (before age 30 yr) fronto-parietal hair loss, 10 did not, and four were too young to assess. Female affected status was assigned on the basis of ultrasound evidence of polycystic ovaries. If male affected status was considered to be premature balding, and a history of menstrual irregularity was used to assign postmenopausal affected status, the segregation ratio for affected families, excluding the proband, was 51.4%, consistent with an autosomal dominant mode of inheritance with complete penetrance (224). Studies in monozygotic twins, however, have not found complete concordance of polycystic ovary morphology, arguing against this mode of inheritance (133). The study contained only 19 pairs of monozygotic twins as well as a sample of 15 dizygotic twins. The prevalence of polycystic ovary morphology was ~50% in these twins, who were recruited from a twin registry, which was twice the prevalence reported in other randomly selected groups of women (3). This raises concern about the accuracy of the detection of polycystic ovaries.

Family studies have been constrained by small sample sizes and/or failure to examine all available family members. In several studies, PCOS affected status has been assigned on the basis of ovarian morphology rather than hormonal abnormalities. Only one study has proposed a male phenotype on the basis of examination of male relatives, and this study was constrained by a small sample size (224). Nevertheless, these studies strongly suggest that PCOS has a genetic component, most likely with an autosomal dominant mode of transmission (220, 224). If this is true, are there other phenotypes in affected kindreds? The studies cited above have suggested that premature male balding may be a male phenotype (216, 224). This finding could be an artifact, since it is also possible that bald men choose to marry hirsute women. Recent studies in these families, however, suggesting linkage of this phenotype with a candidate gene in the steroidogenic enzyme pathway (Ref. 225; see below), if verified, would confirm genetically that this is a male phenotype.

Our studies have suggested that insulin resistance may be an additional male phenotype as well as a prepubertal and postmenopausal female phenotype (132, 220) (Table 4♣). This has been also reported recently in a series of Australian PCOS kindreds. In the small number of families that we have studied (132, 220), when women of reproductive age are insulin resistant, they usually have possessed the other endocrine features of PCOS. The one insulin-resistant prepubertal girl was also hyperandrogenic and developed chronic anovulation after menarche consistent with the diagnosis of PCOS (132). That insulin resistance and hyperandrogenism may be a prepubertal phenotype is supported by recent studies suggesting that PCOS develops in insulin-resistant girls with premature adrenarche (58, 226, 227) (Table 4♣). Our studies suggest that hyperandrogenism without insulin resistance is another phenotype in female PCOS kindred members of reproductive age (228) (Table 4♣). Finally, we have found postmenopausal hyperandrogenic female family members with normal insulin sensitivity, which may represent an additional postmenopausal phenotype (132) (Table 4♣). This possibility is supported by the study of Dahlgren and colleagues (53), which found that postmenopausal women with a history of PCOS had higher androgen levels than age-matched control women. We have found that hyperandrogenism and insulin resistance can segregate independently in PCOS kindreds (132). It is not yet possible to determine whether this reflects separate genetic traits or variable penetrance of a single defect. These studies also indicate that there is considerable phenotypic variation, even within kindreds.

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Table 4. Additional familial phenotypes

2. Candidate genes for PCOS. The biochemical reproductive phenotype in PCOS is characterized by increased LH secretion and acyclic FSH release (2, 12). The ovaries (in response to LH) and, often, the adrenals secrete excessive androgens, and there is decreased ovarian aromatization of androgens to estrogens (12). The circulating androgens feed back on the hypothalamic-pituitary axis (directly or via their

extragonadal aromatization to estrogen) to increase LH relative to FSH release, producing a self-sustaining syndrome (34, 35, 36, 37, 42). The defect that initiates these reproductive disturbances in PCOS is unknown, but it can be shown experimentally that factors that increase either androgen secretion or LH release can reproduce these disturbances (1, 2, 12, 38, 39). Thus any factor regulating gonadotropin secretion or action, adrenal or ovarian steroidogenesis, and/or extragonadal aromatization could be a plausible candidate gene for the reproductive phenotype of PCOS. Indeed, polycystic o