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Insulin-Lowering Agents in the Management of Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is a medical condition that has brought multiple specialists together. Gynecologists, endocrinologists, cardiologists, pediatricians, and dermatologists are all concerned with PCOS patients and share research data and design clinical trials to learn more about the syndrome. Insulin resistance is a common feature of PCOS and is more marked in obese women, suggesting that PCOS and obesity have a synergistic effect on the magnitude of the insulin disorder. Hyperinsulinemia associated with insulin resistance has been causally linked to all features of the syndrome, such as hyperandrogenism, reproductive disorders, acne, hirsutism, and metabolic disturbances. Women with PCOS should be evaluated for cardiovascular risk factors, such as lipid profile and blood pressure. Modification of diet and lifestyle should be suggested to those who are obese. Several insulin-lowering agents have been tested in the management of PCOS. In particular, metformin is the only drug currently in widespread clinical use for treatment of PCOS. In a high percentage of patients, treatment with metformin is followed by regularization of menstrual cycle, reduction in hyperandrogenism and in cardiovascular risk factors, and improvement in response to therapies for induction of ovulation. (*Endocrine Reviews* 24: 633–667, 2003)

I. Introduction

POLYCYSTIC OVARY SYNDROME (PCOS) is the most common endocrinopathy in women and the most common cause of anovulatory infertility, affecting 5–10% of the population. It is characterized clinically by some evidence of androgen excess, such as hirsutism, seborrhea, acne, elevated plasma androgen levels, or a combination of these. Its association with menstrual abnormalities and infertility leads many affected women of reproductive age to attend gynecology and infertility clinics where the syndrome is diagnosed (Table 1). In recent years it has been widely recognized that most women with PCOS have some degree of insulin resistance (1). Abnormality of insulin secretion and action has been implicated in the pathophysiology of PCOS (2). As a consequence of insulin resistance, PCOS patients often have an atherogenic lipid profile and increased incidence of cardiovascular risk factors (3). Women with PCOS have the constellation of symptoms (insulin resistance, obesity, hypertension, and dyslipidemia) defining so-called syndrome X (4). There is currently much interest in the use of insulin-sensitizing drugs in women with PCOS. In a good percentage of cases, treatment with these drugs is followed by regularization of the menstrual cycle, reduction in hyperandrogenism, reduction in cardiovascular risk factors, and improved response to therapies for induction of ovulation. This review endeavors to define insulin resistance and its possible role in the pathogenesis of PCOS. The use of insulin-lowering drugs in this disorder is summarized.

Abbreviations: A, Androstenedione; BMI, body mass index; CVD, cardiovascular disease; DHEAS, dehydroepiandrosterone sulfate; FFA, free fatty acids; FSIVGTT, frequently sampled iv glucose tolerance test; GD, gestational diabetes; G:I ratio, glucose to insulin ratio; hCG, human chorionic gonadotropin; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; HSD, hydroxysteroid dehydrogenase; IGFBP, IGF-binding protein; IGT, impaired glucose tolerance; IRS, insulin receptor substrate; IVF, *in vitro* fertilization; LDL, low-density lipoprotein; NIDDM, non-insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test; 17OHP, 17-hydroxyprogesterone; PAI-1, plasminogen activator inhibitor-1; PCO, polycystic ovaries; PCOS, polycystic ovary syndrome; PI3K, phosphatidylinositol 3-kinase; PPARγ, peroxisome proliferator-activated receptor-γ; PRL, prolactin; QUICKI, quantitative insulin-sensitivity check index; T, testosterone.

II. Definition of PCOS

A. Endocrine profile

A lack of uniformity in the criteria used to diagnose PCOS adds to the confusion surrounding this syndrome. Diagnosis of PCOS is usually based on clinical symptoms and biochemical markers. The National Institutes of Health Consensus Conference (5) established definite and probable criteria for PCOS, including menstrual abnormalities and

TABLE 1. Endocrine profile, signs, and symptoms of PCOS

Hormonal profile	Hyperandrogenism	Reproductive abnormalities	Metabolic disturbances
LH/FSH[a]	Acne	Menstrual disturbances	Obesity
Androgen[a]	Hirsutism	Anovulation	Dysfibrinolysis
Estrogen[b]	Seborrhea	Infertility	Dyslipidemia
PRL[b]	Alopecia	Miscarriage	Diabetes
SHBG[c]	Acanthosis nigricans	GD	Hypertension
IGFBP-1[c]		Preeclampsia	CVD
Hyperinsulinemia			

[a] Increase in.
[b] No modification or increase in.
[c] Reduction in.

androgen excess and excluding adrenal hyperplasia and other causes of hyperandrogenism. Insulin resistance, elevated LH to FSH ratio, and ultrasonographic signs were defined as possible criteria.

The endocrine profile of women with PCOS is characterized by high plasma concentrations of ovarian and adrenal androgens, gonadotropin abnormalities, a relative increase in estrogen levels (especially estrone) derived from conversion of androgens, reduced levels of SHBG, and often high levels of prolactin (PRL) and insulin.

1. Gonadotropin secretion. Although the pathogenesis of PCOS is still controversial, an array of plausible pathophysiologies has emerged over the last several decades of study. Inappropriate gonadotropin secretion with elevated LH and relatively low FSH secretion is typical (6).

In women with PCOS, 55–75% have a high LH to FSH ratio (6–8) due more to increased levels of LH than low levels of FSH. Administration of GnRH evokes an exaggerated LH response (9). GnRH stimulation and gonadotropin pulsatility tests indicate hyperactivity of the hypothalamo-pituitary axis. It is still unclear whether the high levels of LH depend on a higher frequency of GnRH pulses, a greater amplitude of GnRH pulses evoking an increased pituitary response to GnRH, or a combinations of these (10).

During puberty, women with PCOS showed chronobiological abnormality of LH secretion, characterized by an approximately 8-h forward shift of the LH surge from the normal nocturnal sleep period to the afternoon (11). This shift could indicate primary hypothalamic-pituitary impairment in the pathogenesis of the syndrome. However, it has been shown that in women with PCOS, recovery of the LH pulsatile pattern after inhibition by GnRH agonist closely follows the pattern observed in normal controls (12), indicating that high LH levels could be the result of increased androgen levels. Indeed, other hyperandrogenic states, such as congenital adrenal hyperplasia, may exhibit increased LH levels (13). On the other hand, the evidence that testosterone (T) administration to eugonadal female-to-male transexuals lowered LH levels (14) and that antiandrogen treatment was not always followed by a reduction in LH levels in PCOS patients argues against the hypothesis that elevated LH secretion is the consequence of hyperandrogenemia.

Similarly, the relative reduction of FSH may be explained on the basis of a higher frequency of GnRH pulses (15, 16) or a different action of circulating and paracrine factors on pituitary function. It is not yet clear whether altered hypothalamo-pituitary function is an intrinsic factor in PCOS or secondary to steroid hormone anomalies. An altered hypothalamic neuroregulation in PCOS patients has been hypothesized according to the impaired opioid and dopaminergic tonus that has been shown in PCOS. However, the administration of naltrexone (an opioid antagonist), bromocriptine (a dopaminergic agonist), or metoclopramide (a dopaminergic antagonist) induces slight changes in LH pulsatility in women with PCOS (17, 18). Whatever the cause, an altered function of the GnRH-LH axis explains some symptoms of PCOS: 1) relatively low FSH levels may lead to incomplete or inefficient follicle maturation; and 2) high levels of LH may lead to theca cell hyperplasia, favoring increased androgen secretion.

2. Hyperandrogenemia. Hyperandrogenemia is a key feature of the syndrome; it is mainly of ovarian origin although an adrenal contribution cannot be excluded. Most, but not all, women with PCOS have high plasma levels of androgens. Androstenedione (A) and T are markers of ovarian androgen secretion, and dehydroepiandrosterone sulfate (DHEAS) is the best marker of adrenal secretion. Hyperandrogenemia is not always linked to hyperandrogenic symptoms such as acne or hirsutism; indeed, ethnic groups such as Asians show hyperandrogenemia without any skin manifestations (19, 20).

Ovarian catheterization studies (21, 22), human chorionic gonadotropin (hCG) stimulation studies (23), and suppression of gonadotropin secretion with combined estrogen and progestins (24) or GnRH analog all suggest that polycystic ovaries (PCO) overproduce androgens. The adrenal contribution, however, should not be ignored, because women with PCOS have higher plasma concentrations of T, free T, 17-hydroxyprogesterone (17OHP), A, and dehydroepiandrosterone than normal women (25). Acute administration of GnRH is followed by 3 times greater 17OHP production than in normal women (9, 25). This and other observations have led to the hypothesis that 17-hydroxylase and 17,20-lyase activities, constituents of the enzyme P450c17, are altered in PCOS. The consequence of the intrinsic dysregulation of this enzyme is a relative inhibition of 17,20-lyase with respect to 17-hydroxylase and thus an increase in the 17OHP to A ratio with respect to normal women. The increased response of 17OHP to stimulation with GnRH or hCG is one of the best known endocrine features of the syndrome (26–28). When LH is added to human thecal cell cultures, 17OHP production increases with respect to A production. Long-term cultures of replicating thecal cells from PCOS and

healthy women have shown that the former produce T, progesterone, and 17OHP more abundantly than the latter, even though the culture medium was free of LH (29). The clear message from these *in vitro* studies is that theca cells of PCOS patients have enhanced steroidogenic potential that is not limited to 17α-hydroxylase and 17,20-lyase activity. This *in vitro* biochemical phenotype may be the result of a stable metabolic imprint obtained *in vivo* or an intrinsic genetic variation.

Recent studies by McAllister and colleagues (30, 31) demonstrated that basal and forskolin-stimulated CYP17 gene transcription (the gene that encodes the cytochrome P450) is increased in PCOS theca cells. They established that increased androgen production is a stable phenotype of PCOS theca cells that not only results from preferentially increased CYP17 expression but involves the up-regulation of other steroidogenic enzymes, including CYP11A and 3β-hydroxysteroid dehydrogenase (3β-HSD). A comparison of 17β-HSD activity (which converts A to T) in normal and PCOS theca cells demonstrated that androgenic 17β-HSD activity per theca cell was not different in PCOS theca cells. It is likely that the increased production of T by PCOS theca cells is driven by increased androgen precursor production and not by altered 17β-HSD activity (30).

In women with PCOS, altered granulosa cell function, characterized by aromatase activity, may be present. Most studies have found that aromatase activity is higher in polycystic than in normal ovaries, providing a likely explanation of the hyperestrogenism often observed in this syndrome (9, 26, 30, 31). Women with PCOS are reported to have a significantly greater response of estradiol to a single dose of GnRHa than normal women (26).

Thecal cells of PCO synthesize more androgens than those of the normal ovary, and granulosa cells have high aromatase activity that converts this large quantity of substrate into estrogens. High aromatase activity could therefore be a reason for the normal quantities of androgens found in follicular fluid from PCOS patients.

It was recently proposed that hyperinsulinemia plays a role in steroidogenesis. The relationship between steroidogenesis and insulin resistance in PCOS is discussed below.

B. Reproductive abnormalities

In clinical practice, women with PCOS present with infertility (mean incidence, 74%), menstrual irregularity (dysfunctional bleeding, 29%; amenorrhea, 51%), hyperandrogenism (69%), and virilization (21%) (32).

Anovulation is usually chronic in PCOS and is associated with infertility and dysfunctional bleeding such as oligomenorrhea or amenorrhea. Periods of regular menses are also possible. Some women who report normal menses may be anovulatory. Carmina and Lobo (33) found that approximately 21% of hyperandrogenic women with normal menses were anovulatory. The menstrual irregularity of PCOS patients typically begins at menarche and although amenorrhea may occur, the usual presentation is oligomenorrhea. The proportion of PCOS patients with regular menses is thought to increase with age, reaching about 70% at 39–41 yr (34). No longitudinal data, however, are available on this matter.

Infertility is the presenting problem for about 40% of PCOS patients (35). If pregnancy is achieved, other reproductive problems, such as miscarriage, emerge (36, 37). The relationship between PCOS and miscarriage is a complex and unresolved area. Excellent papers have been published on this topic (36, 38–49); consequently the subject is only outlined here. PCOS is not predictive of miscarriage, but patients who miscarry have higher plasma levels of androgens than women with ongoing pregnancies (38). The miscarriage rate in PCOS is about 30% of all pregnancies, which is double the rate for early miscarriage in normal women. The exact mechanism is unknown. High levels of LH and androgens have been regarded as a cause for poor reproductive history (50, 51).

The unfavorable endocrine environment to which ovarian follicles are exposed could be at least partly responsible for a low percentage of pregnancies, because it affects oocyte quality and luteal phase efficiency. However, oocytes of women with PCOS are nearly always normal and, when removed from their unfavorable environment, have a similar fertilization percentage to oocytes of normal women (52).

Insulin resistance in PCOS may be considered a risk factor for gestational diabetes (GD) (53). Because patients with PCOS have insulin resistance, albeit with normal glucose tolerance, they may run a higher risk of diabetes when exposed to the diabetogenic effects of pregnancy. Indeed the prevalence of GD in PCOS patients has been reported to be 40–46%. The prevalence of PCOS in women with a history of GD has variously been reported to be as high as 20% (54), 39.4% (55), 41% (56), 44% (57), and 52% (58). Interestingly, these women showed higher fasting glucose and features reminiscent of syndrome X, such as higher body mass index (BMI), higher waist-hip ratio, higher fasting insulin, higher triglycerides, and lower insulin sensitivity than women with normal previous pregnancies (58). However, some studies have failed to find a significantly higher prevalence of PCOS in women with a history of GD (59, 60).

In a longitudinal study, Paradisi *et al.* (61) investigated carbohydrate metabolism in pregnant women with PCOS. They used oral glucose tolerance test (OGTT) and hyperinsulinemic-euglycemic clamp to show that women who developed GD had lower insulin sensitivity than women who did not, indicating that GD is associated with impairment of insulin metabolism as early as the first trimester.

A link between insulin resistance and hypertensive disorders in pregnancy has been widely reported. Insulin resistance, obesity, and increased risk of GD in PCOS patients suggest that hypertensive complications may be common in pregnancy. Preeclampsia is reported to be more frequent in PCOS patients than in normal women (62). In a case control study, the incidence of this disorder was found to be as high as 28.5% (63). In a recent retrospective study of 99 pregnancies in PCOS patients, it was found that the relative risk for preeclampsia was 2.2. However, logistic regression analysis indicated that nulliparity was the only significant risk factor for preeclampsia and that PCOS had no predictive value (54).

C. Long-term health consequences

Insulin resistance in young, otherwise healthy women raises the question of other cardiovascular risk factors, including impaired glucose tolerance (IGT), diabetes, hyperlipidemia, hypertension, and abdominal obesity as well as increased cardiovascular disease (CVD) itself (64). Because PCOS patients tend to be obese with abdominal deposition of body fat and insulin resistance, it has been proposed that they may have other metabolic features of so-called syndrome X. This syndrome is defined by a constellation of symptoms such as insulin resistance, obesity, hypertension, and hyperlipidemia. Indeed PCOS patients tend to have higher blood pressure, triglycerides, low-density lipoprotein (LDL)-cholesterol, and total cholesterol, with lower high-density lipoprotein (HDL)-cholesterol than age-matched controls (65).

The association between PCOS and endometrial disease has been reported for many years. Few studies have addressed the possibility of an association between PCOS and breast and ovarian cancer. Multiple factors other than insulin resistance, such as obesity and hormonal imbalance, may contribute to increase the long-term risks in PCOS. This makes it difficult to evaluate the independent role of each single risk factor.

Detailed reviews on long-term health consequences of PCOS are available (66–72), and thus only a brief overview will be presented here.

1. Hypertension. A link, independent of obesity, has been reported between hypertension and insulin resistance (73). A few studies have shown increased risk for hypertension in PCOS. Increased risk of arterial hypertension in older women with a history of PCOS has been repeatedly demonstrated (74, 75). In these retrospective cohort studies, the prevalence of treated hypertension was 3 times higher in women with a history of PCOS between the ages of 40 and 59 yr than in healthy controls (74). However, it should be acknowledged that the subjects included in that study had a history of PCOS only as documented by wedge resection and not by other clinical data. A higher prevalence of hypertension was found in a group of patients with a history of PCOS, aged 43–62 yr, matched for age and BMI with 56 controls (75). However, in a study comparing 28 patients with a history of PCOS aged 45–59 yr with 752 age- and BMI-matched controls, hypertension was diagnosed in 60% PCOS and 39% controls and the difference was not significant (76).

Normal ambulatory blood pressure was found to be similar in PCOS and normal body composition-matched women (77). However, careful comparison of blood pressure levels in young women with PCOS revealed an increase in mean and systolic blood pressure during 24-h blood pressure recording (78). A causal relation has been reported between insulin resistance and hypertension. Indeed, untreated hypertensive patients exhibited higher fasting and postprandial insulinemia than normotensive controls, and a direct correlation was found between plasma insulin levels and blood pressure (79, 80).

2. Dyslipidemia and dysfibrinolysis. Insulin resistance is associated with an unfavorable lipid profile with low LDL and high triglyceride levels (81). Hyperinsulinemia inhibits lipolysis with a consequent increase in levels of nonesterified fatty acids. High levels of nonesterified fatty acids led to increased triglyceride levels and reduced HDL levels.

Several studies have examined the association between PCOS and dyslipidemia (65, 82–84), showing that PCOS patients have an atherogenic lipid profile with increased LDL and triglycerides and decreased HDL levels. Wild *et al.* (65) showed that, compared with controls, PCOS patients had serum levels of triglycerides twice as high and mean HDL levels 26% lower. Conway *et al.* (83) and Talbott *et al.* (85) showed that lean women with PCOS also had lower levels of HDL-2 subfraction than weight-matched controls. This was confirmed by Robinson *et al.* (86), who compared 11 lean PCOS patients with 22 BMI-matched controls and found a significant difference in HDL-2 levels, correlated with insulin resistance rather than BMI. However, not all studies found lipid abnormalities in women with PCOS, especially when they are matched to normal women for weight and body composition (87).

Insulin resistance is associated with alterations that accentuate thrombosis by increasing coagulation and inhibiting fibrinolysis (88). Plasminogen activator inhibitor-1 (PAI-1) is a potent inhibitor of fibrinolysis. Elevated PAI-1 levels have been reported in obese women (89) and lean PCOS patients (90), and a direct correlation with insulin resistance was shown. Elevated levels of fibrinogen, an independent risk factor for CVD, has been found in PCOS patients (89).

3. CVD. Women with PCOS display a higher prevalence of cardiovascular risk factors such as obesity, hyperinsulinemia, hypertension, dyslipidemia, and dysfibrinolysis and seem to be at high risk for developing CVD.

Wild *et al.* (91) studied 102 consecutive pre- and postmenopausal women undergoing cardiac catheterization to investigate chest pain. Coronary artery lesions were detected in 52 patients; the other 50 had normal coronaries. The women with artery lesions had a higher incidence of hirsutism and acne than the others, and a high waist-to-hip ratio was associated with both hirsutism and coronary artery disease.

Birdsall *et al.* (92) performed a similar study of 143 pre- and postmenopausal women undergoing catheterization. They also performed pelvic ultrasonography to detect PCO. PCO were detected in 42% of the women. Women with PCO had more coronary artery segments with stenosis exceeding 50%, indicating a trend toward greater severity of ischemic heart disease. They found that the extent of coronary artery disease was independently associated with PCO. However PCO as detected by ultrasonography should not be confused with PCOS. Indeed it has been demonstrated that only 33% of women with PCOS showed PCO morphology on ultrasound (93).

Support for these findings also comes from a long-term follow-up study of PCOS (94). A small group of women with PCOS (n = 33) were followed up after ovarian wedge resection performed between 1956 and 1965. It was calculated that PCOS patients had a 7.4-fold greater risk of myocardial infarction than age-matched controls and increased preva-

lence of central obesity as well as 7-fold higher prevalence of diabetes and 3-fold higher prevalence of hypertension.

This, however, contrasts with a recent much larger retrospective study by Pierpoint *et al.* (95), who reported a mortality rate in 1028 women diagnosed with PCOS between 1930 and 1979. There were 59 deaths: 15 resulted from circulatory disease and six from diabetes. The standard mortality rates, both overall and from CVD, were not higher in women with PCOS than in the general female population. However, there was a significant increase in mortality from diabetes.

The same research group (96) recently investigated cardiovascular mortality in women diagnosed with PCOS before 1979 and failed to find increased mortality from CVD in these women. However, PCOS patients had a high prevalence of nonfatal cerebrovascular disease.

To evaluate vascular disease in other sites, Guzick *et al.* (97) measured intima-to-media thickness of the common and internal carotid arteries in 16 women more than 40 yr of age with PCOS. They found a significant increase in carotid intima-media thickness compared with that in healthy women, but no significant differences in the number of atherosclerotic plaques. The same group (98) evaluated the presence of subclinical atherosclerosis in 125 women diagnosed with PCOS between 1970 and 1990. They found that 21.6% had ultrasonographic evidence of carotid plaques compared with 15.5% of controls; 72% of PCOS patients also had plaque thickness exceeding 50% of vessel diameter compared with 0.7% of controls, suggesting that lifelong exposure to this cardiovascular risk profile may lead to premature atherosclerosis in PCOS patients.

In summary, whether PCOS is an independent risk for CVD remains unclear. The only way to establish whether PCOS is associated with high cardiovascular morbidity and mortality would be to conduct a large prospective study following women with a definitive diagnosis of PCOS for several decades.

4. Diabetes. Insulin resistance is recognized as a major risk factor for type 2 diabetes (99). Another risk factor is pancreatic β-cell dysfunction (100), which is also found in PCOS (101), presumably making PCOS patients at increased risk for type 2 diabetes mellitus.

Multiple factors other than insulin resistance and β-cell dysfunction, such as obesity and family history of type 2 diabetes, may contribute to increase the diabetes risk in PCOS. This makes it difficult to evaluate the independent role of each single risk factor in the development of diabetes.

It has been reported that about 30% of obese women with PCOS have IGT. In a retrospective study Dahlgren *et al.* (74) observed that the prevalence of non-insulin-dependent diabetes mellitus (NIDDM) was 15% in PCOS patients compared with 2% in controls. Dunaif (102) suggested that up to 20% of PCOS patients have IGT or NIDDM by the third decade.

In a recent prospective controlled study performed in 254 women with PCOS (103), it was shown that 31% of patients had IGT and 7.5% NIDDM compared with 16% and 0%, respectively, in controls. Women aged 14–44 yr were studied from 1983–1998, and the prevalence of IGT and NIDDM was higher over 35 yr of age. By multiple regression, it was found that fasting glucose, PCOS status, waist-to-hip ratio, BMI, and age were predictors of glucose intolerance. However, in this prospective study 78% of patients had a BMI greater than 25 kg/m^2 and 73% were obese (BMI $>$ 27 kg/m^2). In a recent European follow-up study (104), the prevalence of diabetes was investigated in 346 PCOS patients aged 30–55 yr; 2.3% had diabetes compared with 1% of controls. When the women were divided into age groups, it was evident that more than 9% of PCOS patients aged 45–54 yr had diabetes compared with 2% of controls. The divergence with the U.S. results is probably due to the different BMI of patients. In the European study, only 44% of patients had a BMI greater than 25 kg/m^2 compared with 78% of patients in the U.S. study. However, both studies demonstrated that PCOS patients are at increased risk of IGT or overt diabetes during their third or fourth decade and that this risk is higher for obese than for lean patients.

Several studies have demonstrated that a family history of type 2 diabetes is frequently found in diabetic patients (105). A positive family history of diabetes is observed in a high percentage (>80%) of women with PCOS and diabetes compared with 30% among women with only PCOS (106), suggesting that a family history of diabetes may magnify the severity of insulin metabolism defects associated to PCOS. Due to the relevant pathogenetic role of insulin resistance in both PCOS and diabetes, some investigators hypothesized that among women with type 2 diabetes an higher percentage of PCOS should be found (107). Indeed the prevalence of PCOS among diabetic women seems to be 5-fold higher than normal (108). In conclusion the risk of type 2 diabetes is 5- to 10-fold higher in PCOS patients than in normal women. Obesity, insulin resistance, β-cell dysfunction, and positive family history may contribute to the increased diabetes risk in women with PCOS. All PCOS women should be screened for glucose intolerance. Physicians need to be aware that PCOS women are at high risk for IGT and type 2 diabetes and that these abnormalities are present in both lean and obese subjects.

5. Endometrial disease. The risk of endometrial disease is adversely influenced by several factors including obesity, unopposed estrogen, and infertility. All these factors are found in women with PCOS.

In 1970, Chamlian and Taylor (109) found that 25% of 97 cases of endometrial hyperplasia in women younger than 35 yr presenting with irregular uterine bleeding had sclerocystic ovaries (a criterion used at that time to support the diagnosis of PCOS).

Cancer incidence rates in a Mayo Clinic cohort of 1270 women with chronic anovulation, as defined by ovarian appearance consistent with PCOS and clinical evidence of chronic anovulation without hypoestrogenemia, were compared with population incidence rates (110). The relative risk for subsequent endometrial cancer associated with this syndrome was 3.1. Increased risk was also noted for premenopausal and postmenopausal cancer. In addition, 14 women reported concurrent diagnosis of chronic anovulation syndrome and endometrial cancer, consistent with a prevalence of endometrial cancer in this syndrome of approximately 1%.

A recent prospective study of 56 PCOS patients was conducted with the aim of predicting endometrial hyperplasia (111). The author found high prevalence of endometrial hyperplasia in these patients (35.7%). Of the 20 cases of endometrial hyperplasia, 12, three, and five were simple hyperplasia, complex hyperplasia, and hyperplasia with cytological atypia, respectively. Women affected were older (30–40 yr) and reported amenorrhea of 1–4 yr duration. Logistic regression analysis revealed that ultrasonographic endometrial thickness and intermenstrual interval were the only predictors of hyperplasia.

The true risk of endometrial disease in women with PCOS is difficult to ascertain. Studies have been limited to a relatively small number of cases of endometrial cancer identified specifically in PCOS. Furthermore the heterogeneous presentation of the syndrome makes it impossible to asceratin which factor (hyperinsulinemia, obesity, hormonal imbalance) has the most relevant role in the increased risk.

Clinically, it is generally accepted that in oligoamenorrheic or amenorrheic women with PCOS the induction of withdrawal bleeding to prevent hyperplasia is a prudent management.

6. Breast and ovarian cancer

Breast cancer is reported to be more common in PCOS patients (112); conversely, it has been argued that PCOS is protective against breast cancer (113).

In a large prospective study designed to examine the development of breast cancer in postmenopausal women (114), the prevalence of PCOS was found to be only 1.35%, suggesting that women presenting with the syndrome are not at increased risk for breast carcinoma. However, in a recent series of 786 women with a histological diagnosis of PCOS recorded between 1930 and 1979, breast cancer was the leading cause of death (95). Mortality was assessed from the mortality registry of deaths, and standardized mortality rates were calculated for women with PCOS and the normal population. The standardized mortality rate was 0.91 for all neoplasms and 1.48 for breast cancer [95% confidence interval (CI) 0.79–2.54] (95).

Few studies address the possibility of an association between PCOS and ovarian cancer (94, 110). Studies are limited to a small number of women with PCOS and results are conflicting.

In conclusion, the association between PCOS and breast and ovarian cancer has not been demonstrated. Although the links seem to be probable and logical, epidemiological evidence is still lacking.

D. Summary

PCOS is extremely prevalent and is considered the most frequently encountered and endocrinopathic condition. A lack of uniformity in the diagnostic criteria adds to the confusion surrounding the syndrome. The pathogenesis of PCOS is still controversial. It is likely to involve abnormalities in several systems. There has long been an association of abnormal gonadotropin secretion with this syndrome. Although the adrenal gland may contribute, hyperandrogenemia is principally ovarian in origin. During reproductive age, PCOS is associated with relevant reproductive morbidity including menstrual irregularity, anovulation, infertility, increased pregnancy loss, and complications of pregnancy. Insulin resistance is a common feature of PCOS. In the general population, insulin resistance and consequent hyperinsulinemia are associated with hypertension, dyslipidemia, dysfibrinolysis, CVD, and high risk of developing type 2 diabetes (metabolic syndrome X). It is mainly obese women with PCOS who are characterized by the presence of central obesity, insulin resistance, and dyslipidemia, which place them at a higher risk of developing diabetes as well as the possibility of CVD. However this may be true for a proportion of lean women.

Biochemical evidence regarding the potential for long-term risks of CVD is recognized. However, it remains unclear whether PCOS is associated with high CVD morbidity and mortality. Women with PCOS cluster risk factors for endometrial, breast, and ovarian cancer. However, the true incidence of endometrial, breast, and ovarian cancer in women with PCOS is not known.

III. Insulin Resistance in PCOS

A. Definition and prevalence

Insulin resistance has been defined as a state (of a cell, tissue, or organism) in which a greater than normal amount of insulin is required to elicit a quantitatively normal response (115). It leads to increased insulin secretion by β-cells and compensatory hyperinsulinemia. As long as hyperinsulinemia overcomes insulin resistance, glucose levels remain normal. If β-cell compensatory response declines, relative or absolute insulin insufficiency develops. Insulin secretion cannot keep pace with the underlying insulin resistance, which may lead to glucose intolerance and type 2 diabetes. Type 2 diabetes develops only in subjects with insulin resistance and concomitant β-cell dysfunction. A number of abnormalities are recognized as associated with insulin resistance. Reaven (100) defined this constellation of abnormalities as syndrome X. It is intended to refer to subjects with insulin resistance, hyperinsulinemia, and dyslipidemia. Patients often have elevated triglycerides and decreased HDL-cholesterol with high blood pressure. Other frequent abnormalities are high plasma levels of PAI-1, uric acid, and fibrinogen, together with endothelial dysfunction, which make for a high risk of CVD.

In 1921 Achard and Thiers (116) first reported a relationship between hyperandrogenism and insulin metabolism in their description of "diabetes des femmes à barbe." The subsequent description in 1976 (117) of virilization in young women with severe insulin resistance led to further investigations of insulin metabolism in PCOS patients.

Both obese and lean women with PCOS have a greater insulin response to oral glucose load than healthy women (118, 119). Dunaif *et al.* (1) used the euglycemic glucose clamp technique to demonstrate that PCOS-associated hyperinsulinemia was caused by insulin resistance. They recruited obese and nonobese PCOS patients and showed that insulin-stimulated glucose utilization, whether expressed per kilo-

gram total weight or per kilogram fat-free mass, was significantly lower than normal in both, indicating a type of insulin resistance that was independent of obesity and changes in body composition. With the development of easier techniques than the euglycemic clamp, such as the iv glucose tolerance test, more researchers have investigated insulin resistance in PCOS patients.

Approximately 60–70% of PCOS patients are obese, and it is well known that obesity is associated with insulin resistance. However, PCOS patients have evidence of insulin resistance beyond that of obese women in the general population. Most studies have shown that impaired insulin sensitivity is present without obesity (1, 119–122); however, any degree of obesity further impairs insulin action.

Although it is universally accepted that overweight PCOS patients are insulin resistant and their insulin sensitivity is lower than that of obese non-PCOS patients, contradictory results emerged for lean women with PCOS. On the whole, European studies have failed to find insulin resistance in nonobese women with PCOS (123–127), whereas North American studies have either found (1, 119, 122) or not found (128) impaired insulin sensitivity in lean women with PCOS. This discrepancy may be partly due to ethnic, genetic, nutritional, and lifestyle differences.

A high percentage of women with PCOS have abnormalities of carbohydrate metabolism, such as IGT or NIDDM. Indeed, in a large prospective study by Legro *et al.* (103), it was shown that 38.6% of PCOS patients had either IGT (31.1%) or diabetes (7.5%) compared with 14% and 0% of controls, respectively. Lean PCOS patients had IGT in 10% of cases and diabetes in 1.5%. Similar results were reported by Ehrmann *et al*. (129). They found IGT in 35% and NIDDM in 10% of cases.

In conclusion, although insulin resistance is associated with obesity, it is also found in normal-weight women with PCOS. Both obese and nonobese women with PCOS seem to be more insulin resistant and hyperinsulinemic than age- and weight-matched normal women. Because insulin resistance is implicated in the pathophysiology of diabetes, women with PCOS seem to be at high risk for abnormalities of carbohydrate metabolism such as IGT and NIDDM.

B. Diagnosis

Clinical assessment of insulin resistance relies on several tests, which include determination of insulin levels, either at baseline or after OGTT, assessment of sequential plasma glucose levels after iv administration of insulin (insulin tolerance test), estimation of an index of insulin sensitivity by applying the minimal model technique to data obtained from the so-called frequently sampled iv glucose tolerance test (FSIVGTT), and measurement of *in vivo* insulin-mediated glucose disposal by the euglycemic hyperinsulinemic clamp procedure (for review see Ref. 130). Recently, simple methods have been elaborated for assessing insulin sensitivity, such as homeostatic model assessment (HOMA) (131) and quantitative insulin-sensitivity check index (QUICKI) (132).

The most common assessment is done after oral glucose load, which represents a normal meal. The homeostatic response includes an increase in insulin secretion and insulin-dependent processes that lower glycemia. Although measurement of glycemia and insulinemia after OGTT is easy and readily available, OGTT results are difficult to reproduce and may be influenced by factors such as time of day, physical inactivity, previous carbohydrate or alcohol intake, and fasting interval before test (133). Furthermore, the results of this test must be interpreted in the context of plasma glucose levels. Indeed, any degree of hyperglycemia indicates impaired insulin secretion, further exacerbating insulin resistance and invalidating insulinemia as an index of insulin resistance. After oral glucose the increments in insulin do not depend entirely on glucose, but also on factors such as gut hormones and neural stimulation. Glycemia also changes in relation to gastric emptying and absorption.

The glucose clamp is regarded as the gold standard for assessing insulin action, and several studies have demonstrated insulin resistance in women with PCOS (Fig. 1). It is somewhat difficult to perform, requiring special equipment and trained personnel. Insulin is infused at a constant rate to achieve physiological suprabasal levels. Glycemia is monitored frequently and glucose is infused at variable rates to maintain a constant level of glycemia. When the glucose infusion rate has stabilized, this rate divided by the insulin level is defined as insulin sensitivity.

The FSIVGTT is another common technique used to assess insulin sensitivity in PCOS patients. It requires an iv injection of a fixed amount of glucose followed by frequent blood sampling for 180 min thereafter and subsequent modeling of the relevant plasma glucose and insulin data to derive indices of insulin sensitivity. FSIVGTT with minimal model analysis is a mathematical model with few parameters that has good fit with the insulin-mediated glucose disposal rate as determined by euglycemic hyperinsulinemic clamp (134).

Most data on insulin resistance in PCOS patients have been obtained by OGTT, euglycemic clamp, or FSIVGTT. Other

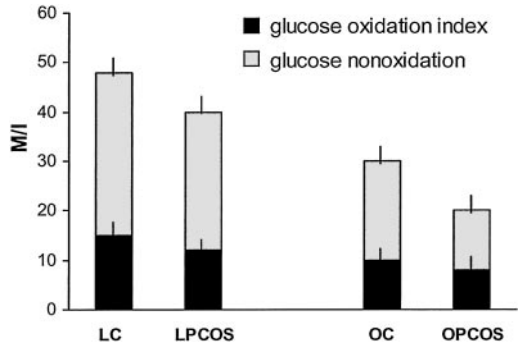

FIG. 1. Mean insulin sensitivity index (M/I, μmol/kg·min/mU·liter), glucose oxidation (*black section*), and nonoxidation (*open section*) indices expressed as μmol/kg·min/mIU·liter during the euglycemic hyperinsulinemic clamp in PCOS and controls. Indirect calorimetry was performed with a computerized flow-through canopy gas analyzer system in connection with the euglycemic clamp to reveal insulin-stimulated glucose metabolism, *e.g.*, the rates of glucose oxidation and nonoxidation. During the euglycemic clamp, the M/I tended to be lower in lean PCOS (LPCOS) and obese PCOS (OPCOS) subjects compared with the lean controls (LC) and obese controls (OC), respectively. [Adapted with permission from L. C. Morin-Papunen *et al.*: *Hum Reprod*: 15:1266–1274, 2000 (127). © European Society of Human Reproduction and Embryology. Reproduced by permission of Oxford University Press/Human Reproduction.]

available techniques have not been used because of high incidence of adverse events (insulin tolerance test and insulin suppression test). Except for OGTT, techniques such as euglycemic clamp and FSIVGTT are time-, labor- and cost-intensive measures and are not feasible for large-scale screening of the population.

To avoid complex procedures or widely changing glucose levels, the HOMA focuses on basal fasting insulin and glucose levels (R_{HOMA}: glucose × insulin/22.5). It has been demonstrated that the correlation between HOMA and clamp-derived insulin sensitivity is surprisingly good considering the simplicity of the formula (131). A recent formula defined as QUICKI has been elaborated (132). Similar to HOMA, QUICKI is a formula based on fasting insulin and glucose values [QUICKI = 1/(log insulin + log glucose)]. It has been shown to strongly correlate with insulin sensitivity as measured by HOMA (132).

The characteristics of the most common tests for assessment of insulin sensitivity are summarized in Table 2.

To facilitate the determination of the presence of insulin resistance in PCOS patients, simple fasting markers of impaired insulin sensitivity have been correlated with dynamic insulin tests. The fasting glucose to insulin (G:I) ratio may be useful as a screening test for insulin resistance in obese white PCOS patients (135). The G:I ratio was significantly correlated with insulin sensitivity calculated by FSIVGTT (r = 0.73). A fasting G:I ratio below 4.5 predicted insulin resistance with a sensitivity of 95%, a specificity of 84%, a positive predictive value of 87%, and a negative predictive value of 94%. The main limit of this parameter is that it was derived from the data of a group of obese women and, therefore, is unlikely to be a good measure of insulin resistance in nonobese PCOS patients. Baseline fasting G:I ratio was previously shown to have good correlation with NIDDM (136). However, more studies on large groups are needed to validate its utility for predicting insulin resistance in PCOS patients. Simple prediction models for insulin sensitivity in women with PCOS were recently developed. Mathematical models predicting insulin sensitivity as measured by euglycemic clamp have been constructed (137) (Fig. 2). The three models were based on waist-circumference and fasting insulin, serum triglycerides, or subscapular skin fold. Of the three models, the one based on waist circumference and fasting insulin best predict insulin resistance. The mathematical models were derived from an unselected population of PCOS patients with BMIs of 17.6–37.4 kg/m^2, making this model applicable to both lean and obese patients.

As a significant correlation has been found between insulin resistance and abnormalities in ovarian function, it has been proposed that the higher the insulin resistance in PCOS

TABLE 2. Characteristics of the most common tests for assessment of insulin sensitivity

	Glucose clamp	G:I ratio	OGTT	Insulin tolerance test	Insulin suppression test	FSIVGTT	HOMA	QUICKI
Quantitative	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Invasive	Yes	No	No	Yes	Yes	Yes	No	No
Simple		+++	+++	++	++	++	+++	+++
Economical	No	Yes	Yes	Yes	Yes	No	Yes	Yes
Reproducible	+++	+	+	++	+	++	+	+
Ability to measure glucose tolerance	+	+	++	++	+	++	+	+
Correlation with the clamp		Not good	Not good	Good	Good	Good	Good	Good

+++, High; ++, moderate; +, low.

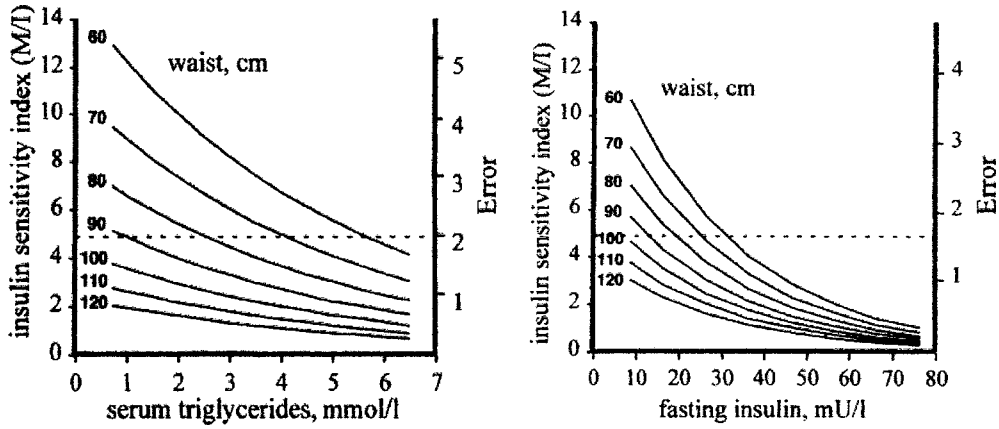

FIG. 2. Nomograms indicating the relationship between waist girth and the insulin sensitivity index (M/I), obtained during a euglycemic hyperinsulinemic clamp, for different values of fasting insulin or serum triglycerides in women with PCOS. Models were constructed on 72 women with PCOS; insulin sensitivity was measured by the euglycemic hyperinsulinemic clamp. The normal ranges of insulin sensitivity were calculated from 81 nonhirsute, normally menstruating women with normal ovaries and similar BMIs and ages as the women with PCOS. *Left axis*, Predicted values of M/I; *right axis*, prediction error at that level of M/I. The nomograms also report the fifth percentile (*dashed lines*) of the distribution of the insulin sensitivity index for the reference group of normal women (4.9 U). Taking into account the prediction errors in a model based on fasting insulin predicted values of M/I less than 3.8 would indicate insulin resistance, whereas the M/I threshold for a model based on triglycerides would be 3.5. [Reproduced with permission from G. Gennarelli *et al.*: *Hum Reprod* 15:2098–2102, 2000 (137). © European Society of Human Reproduction and Embryology. Reproduced by permission of Oxford University Press/Human Reproduction.]

TABLE 3. Clinical and biochemical findings suggesting insulin resistance

Finding	Ref.
Obesity	103, 126, 127, 139
Waist-to-hip ratio > 0.85	137, 139
Subscapularis skin fold > 50 mm	137
Acanthosis nigricans	140–143
Fasting insulin > 30 mU/liter	126, 137
G:I < 4.5	135
Serum triglycerides > 5.5 mmol/liter	137
Amenorrhea	138

patients, the lower the probability of spontaneous ovulation (138). This implies that moderate or severe insulin resistance is associated with severe oligomenorrhea or amenorrhea.

Clinical and biochemical findings usually associated with insulin resistance are reported in Table 3. These findings may help clinicians to identify women at high risk for impaired insulin sensitivity.

In conclusion, the choice of a test to assess insulin sensitivity depends upon several considerations; however, a correlation can be drawn to relate the complexity of a test to the quality and quantity of information generated. For research purposes, the most informative techniques, such as the euglycemic clamp or the iv glucose tolerance test, should be preferred. In daily practice, the clinician could use simpler indices such as fasting insulin levels, OGTT, G:I HOMA, and QUICKI indices.

C. Pathogenesis of insulin resistance

As mentioned above, insulin resistance is defined as a pathological condition in which target cells fail to respond to ordinary levels of circulating insulin. At the molecular level, impaired insulin signaling results from mutations or posttranslational modifications of the insulin receptor or any of its downstream effector molecules. Insulin resistance could be accounted for by a defect in insulin binding to its receptor or to a shortage of insulin receptors; however, there is recent evidence to suggest that insulin resistance is most often due to a postbinding defect in insulin action.

1. Insulin receptor. Insulin binds the extracellular part of its receptor, which is heterotetrameric, consisting of two α- and two β-subunits. Binding activates intracellular tyrosine kinase in the transmembrane β-subunits. Receptor autophosphorylation triggers receptor kinase activity toward intracellular protein substrates (110), defined as insulin receptor substrates (IRSs).

The IRS family is composed of four related proteins (IRS-1 to -4) (144). These activated intermediates bind and activate other molecules, amplifying and diversifying the signal generated by insulin binding to its receptor. By activating different intermediates, insulin stimulates glucose and amino acid uptake, glycogen synthesis, lipogenesis, and mitogenesis. Any change in one of these processes (in binding of insulin to its receptor or in the postbinding signal cascade) could theoretically lead to a reduced cellular response to insulin, causing insulin resistance.

Insulin receptors are expressed in all ovarian compartments (for review see Ref. 145). Insulin receptor has been demonstrated in granulosa and thecal cells and stromal tissues (146, 147). Insulin itself, IGFs, sex steroids, and other circulating factors are involved in insulin receptor expression and regulation in the ovary (145).

2. Insulin binding. Although the pathogenesis of insulin resistance in PCOS is unclear, there is evidence that it springs from many defects, not always coexisting. A reduction in cell-surface insulin receptors has been reported in studies performed with blood cells of PCOS patients (148, 149). Although blood cells are not a classic insulin target, this finding was recently confirmed in adipocytes from lean and obese PCOS patients (150). Indeed, a marked reduction in adipocyte insulin receptor binding was found in both groups, and this appeared to be due to a reduction in insulin receptor number as opposed to a reduction in receptor affinity. However, when the study was repeated with cultured skin fibroblasts from PCOS patients, this observation was not confirmed (151). Although a significant reduction in adipocyte GLUT-4 (an insulin-regulated glucose transporter) content has been observed in PCOS, independent of obesity (152), this defect could be secondary to abnormal insulin receptor signaling.

Discrepancies in studies investigating insulin receptor binding may be due to methodological difficulties. Apart from adipocytes, insulin binding has been studied in blood cells and fibroblasts, which are not insulin target tissues. Insulin binding may depend partly on the hormonal and metabolic environment of the subjects. Hence, differences in BMI, glucose levels, insulin levels, and insulin sensitivity between studies may lead to different results. Insulin binding defects are not generally thought to play a major role in the pathogenesis of PCOS-associated insulin resistance.

3. Insulin signaling defects. Greater attention has been paid to postbinding defects in signal transduction. In an attempt to characterize postbinding defects of insulin signaling, Dunaif (153) found that increased insulin receptor serine phosphorylation decreased its protein kinase activity. Studies of insulin receptors purified from PCOS skin fibroblasts have shown reduced insulin-stimulated receptor autophosphorylation, which appears to be the consequence of serine phosphorylation. Purification studies suggest that a factor extrinsic to the receptor (perhaps serine kinase) was responsible for serine phosphorylation (154).

Interestingly, serine phosphorylation modulates the activity of the key enzyme, P450c17, that regulates sex steroid synthesis. This suggests that a single genetic defect involving serine protein kinase could be a common cause of insulin resistance and androgen hyperproduction (155) but this hypothesis has not been confirmed. Furthermore, the defect in postbinding signaling in PCOS seems to be selective, because fibroblast cell lines from women with PCOS have significantly decreased insulin-stimulated glucose incorporation into glycogen but similar insulin-stimulated thymidine incorporation, suggesting that the defect in insulin action in PCOS is limited to the metabolic, and does not involve the mitogenic, action of insulin. In the original report, only 50% of PCOS patients exhibited a marked increase in insulin receptor β-subunit phosphoserine (154), but PCOS patients

with "normal" insulin receptors may be highly insulin resistant. This indicates that there may be other defects in the postbinding signaling that produce insulin resistance.

Indeed, a significant decrease in skeletal muscle insulin-mediated activation of IRS-1-associated phosphatidylinositol 3-kinase (PI3K) was recently demonstrated (156). Current evidence suggests that IRS-mediated activation of PI3K controls insulin-stimulated glucose transport and carbohydrate metabolism (157). Interestingly, the authors found greater expression of IRS-2 in skeletal muscle of PCOS patients than controls, suggesting a compensatory change. However insulin-mediated glucose uptake was lower in PCOS, suggesting that IRS-2-PI3K activity did not completely compensate for defective IRS-1-PI3K activity.

Lower insulin-stimulated insulin receptor tyrosine autophosphorylation was recently observed in PCOS ovaries with respect to normal ovaries (158). This could be significant, because ovaries are the main organ implicated in PCOS. Different patterns of IRS-1 and-2 expression in normal and polycystic ovaries has subsequently been demonstrated (159). IRS-1 expression is reduced in granulosa cells of ovaries from women with PCOS, and IRS-2 expression is increased in thecal cells.

Studies with adipocytes isolated from PCOS patients demonstrated reduced insulin sensitivity for induction of glucose transport, although the number of receptors and insulin affinity were unchanged (160). Treatment of adipocytes with an adenosine agonist normalized insulin sensitivity. Adenosine is thought to act as an autocrine/paracrine factor binding to G protein-linked receptors. Hence, adenosine pathways may modulate a serine protein kinase that phosphorylates insulin receptors or substrates (160, 161).

In conclusion, there may be multiple changes in the cellular pathways that mediate insulin action and signaling that underlie insulin resistance in PCOS (Fig. 3). It is therefore possible that different genetic mutations and consequently different molecular abnormalities result in the same phenotype.

4. Insulin resistance as key to the syndrome. Whatever the pathogenesis of insulin resistance, the resulting hyperinsulinemia is seen as a cause of the main features of PCOS, namely hyperandrogenism and anovulation. The first problem was to explain why the ovaries of an insulin-resistant subject remained sensitive to hyperinsulinemia. A first possibility was that insulin acted via IGF-I receptors. This theory was soon jettisoned because it was shown that insulin only binds these receptors at very high concentrations that never occur in PCOS patients. It has now been demonstrated that defects in insulin signaling may exist in some, but not all, tissues of an individual, so such hypotheses could be no longer necessary. Indeed, it was recently shown that the action of insulin on the ovaries is mediated by inositolglycan mediators and is therefore distinct from the insulin-activated tyrosine phosphate cascade that enhances glucose utilization (162). This indicates that the pathways of induction of insulin signaling are also separate in the ovaries and that the action of insulin on steroidogenesis is maintained even in cases of insulin resistance.

Hyperinsulinemia may increase androgen production in PCOS by stimulating the ovaries directly, or indirectly through stimulation of LH secretion and inhibition of IGF binding protein (IGFBP) and SHBG synthesis and secretion. Finally, insulin may also stimulate adrenal androgen secretion (Fig. 4).

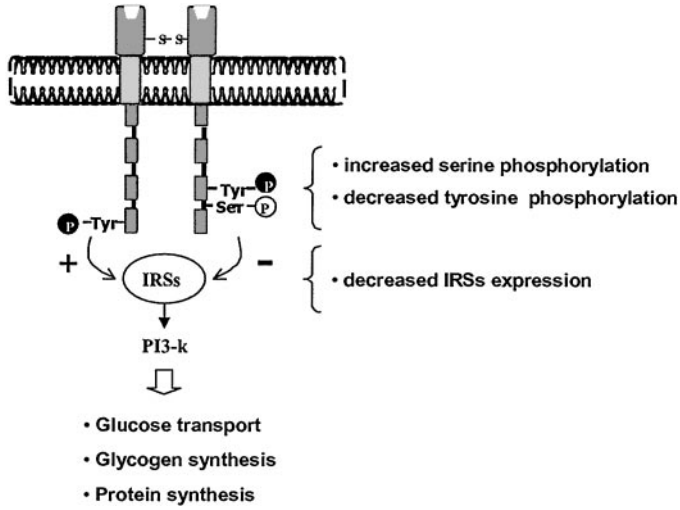

FIG. 3. Multiple defects in the cellular pathways that mediate insulin action and signaling may underlie insulin resistance in PCOS. Increase in serine phosphorylation and decrease in tyrosine phosphorylation and in IRS expression and activation may be involved in the pathogenesis of PCOS-associated insulin resistance.

a. Insulin and the ovary. Human ovaries have specific receptors for insulin (146, 163), which suggests that the hormone has a role in regulating ovarian function. A series of *in vitro* studies (145) demonstrate that insulin stimulates steroidogenesis by granulosa and thecal cells. The direct action of insulin on granulosa cells, and hence on aromatase, seems to be minor (164), whereas its effect on FSH-induced estradiol production by granulosa cells is more pronounced (164).

Studies on thecal cells are more interesting and show that insulin acts as a cogonadotropin in steroidogenesis (165). Insulin has been shown to stimulate proliferation of thecal cells (166), to increase LH-stimulated androgen secretion (167–169), to increase P450c17 mRNA levels (170), to up-regulate LH receptors (171), and to up-regulate ovarian IGF-I receptors (145).

Although *in vitro* studies have generally shown major effects of insulin on ovarian steroidogenesis, *in vivo* studies tend to be divergent. The results of studies involving infusion of insulin *in vivo* followed by measurement of plasma levels of androgens have been both negative (172–175) and positive (176–179). The reason is probably related to the brief duration of the studies; a few hours is not long enough to detect insulin-induced steroidogenesis. Studies in which circulating levels of insulin were reduced by administration of diazoxide (180), somatostatin (181), acarbose (182), metformin (183, 184), and troglitazone (185) are more convincing. As illustrated in *Section IV*, reduction of hyperinsulinemia may be followed by a decrease in basal plasma levels of androgens in response to administration of GnRH (183, 186, 187) or hCG (28).

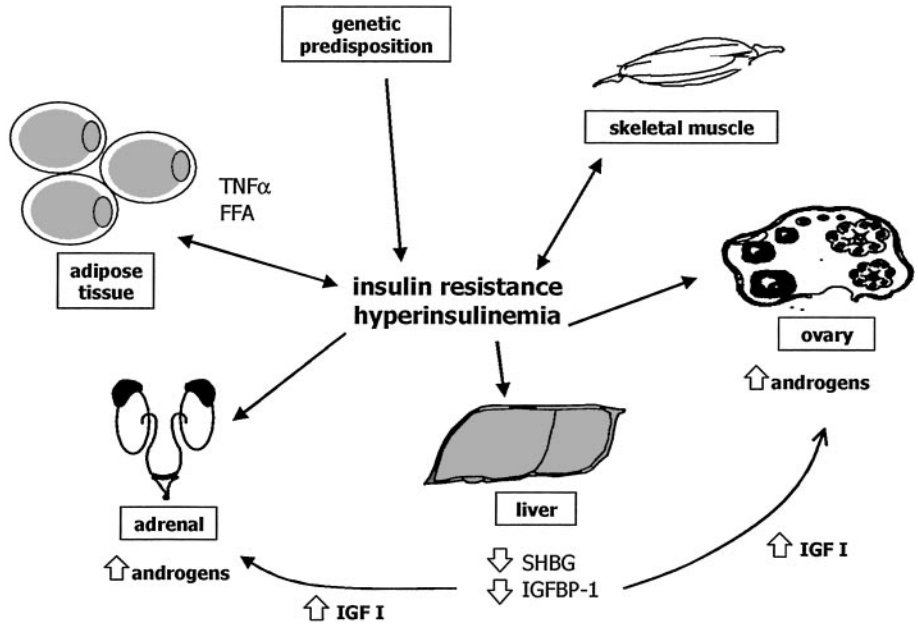

FIG. 4. Pathogenesis of insulin resistance and role of hyperinsulinemia in the pathophysiology of PCOS. Genetic predisposition, obesity, and body fat location may have independent effects on insulin sensitivity. Hyperinsulinemic insulin resistance could have a central role in the pathogenesis of PCOS. Hyperinsulinemia may increase androgen levels by stimulating ovarian steroidogenesis and inhibiting IGFBP and SHBG synthesis and secretion. A role for insulin on adrenal steroidogenesis has been hypothesized.

b. Insulin and gonadotropin. There is evidence to suggest that insulin of pancreatic origin penetrates the brain (188), and insulin receptors have been identified in various brain regions, particularly the hypothalamus (189). In mice with neuron-specific disruption of the insulin receptor gene, a reduction in circulating LH and enhancement of LH response to GnRH have been observed (190). This suggests that insulin signaling in the brain could be essential for normal regulation of the hypothalamic-pituitary-ovarian axis. Insulin receptors have also been identified in the pituitary (189), and insulin has been demonstrated to modulate pituitary activity *in vitro* (191). In the well-known study of Adashi *et al.* (192), insulin was shown to stimulate both basal and GnRH-stimulated release of LH and FSH in rat pituitary cells.

In vivo studies, however, have not completely clarified the role of insulin in gonadotropin secretion. Those based on insulin infusion have failed to demonstrate changes in gonadotropin responses to GnRH or pulsatile gonadotropin release (178). Correlation studies suggest that LH levels and pulse amplitudes are inversely related to insulin levels and to the degree of insulin resistance (122, 193). Insulin could therefore inhibit, rather than stimulate, gonadotropin secretion. Indeed, obese hyperinsulinemic women with PCOS have decreased LH levels compared with lean women (193, 194).

Studies in which insulin levels were reduced by means of drugs are more univocal. As illustrated below, insulin-lowering drugs, according to some but not all authors, bring about a reduction in basal levels of LH (195–198) and of the LH response to GnRH (183, 186, 187). However, it should not be forgotten that spontaneous or induced ovulations rapidly lower LH levels and may be a confounding factor.

c. Insulin and IGF- and sex hormone-binding proteins. Insulin regulates androgen metabolism, not only affecting synthesis and secretion, but also indirectly regulating circulating levels of SHBG, which has high affinity for sex hormones. As plasma concentrations of SHBG become lower, the free or bioavailable androgen fraction becomes greater.

In vitro studies indicate that insulin suppresses SHBG production by cultured hepatoma cells (199), and *in vivo* studies indicate an inverse correlation between insulinemia and SHBG plasma levels in hyperandrogenic women and also in the general population (200–203). A reduction in hyperinsulinemia in PCOS patients leads to a significant increase in circulating levels of SHBG, which is certainly a main reason for improvement of hyperandrogenism in response to therapy (185, 202, 204).

Insulin also inhibits liver production of IGFBP-1, which in turn leads to an increase in the free fraction of IGF-I. Insulin suppresses IGFBP-1 gene transcription. An inverse relationship between fasting insulin and IGFBP-1 has been demonstrated in PCOS patients and the general population (205–207). Insulin reduces not only hepatic synthesis of IGFBP-1 but also the intraovarian pool (166).

Several studies have found a significant increase in the IGF-I to IGFBP-1 ratio in women with PCOS (208–210). As a consequence, increased bioavailability of IGF-I to thecal tissue may subserve a cogonadotropin role, inducing hyperandrogenism by autocrine and paracrine mechanisms; indeed, IGF-I has been shown to stimulate estrogen production by granulosa cells (211) and to act synergistically with FSH and LH in controlling granulosa cell aromatase levels (212, 213). IGF-I synergizes with LH to stimulate androgen production (165, 167), probably via its receptors on thecal cells (165).

Furthermore IGF-I, like insulin, could indirectly control ovarian steroidogenesis by affecting hypothalamic-pituitary function. Indeed, IGF-I has been shown to positively regulate GnRH gene expression (214, 215) and to increase basal and GnRH-stimulated pituitary gonadotropin release (216). Insulin-lowering treatment increases IGFBP-1 plasma levels, thereby leading to a reduction in the IGF-I to IGFBP-1 ratio

and reducing the free fraction of IGF-I available in peripheral tissues (217, 218).

d. Insulin and the adrenal. As mentioned above, adrenal hyperandrogenism has been considered a characteristic feature of PCOS. Because insulin resistance is a main component of the endocrine pattern of PCOS, many studies have evaluated the hypothesis that adrenal hyperandrogenism could be secondary to hyperinsulinemia. The key enzyme of androgen synthesis, P450c17, is expressed both in the gonads and adrenals and is encoded by the same gene (219); therefore, the same factor (*i.e.*, insulin) probably regulates enzyme activity at both sites.

Physiological concentrations of insulin and IGF-I were found to increase P450c17 mRNA levels irrespective of ACTH, in cultured adrenocortical cells (170, 220, 221). Insulin and IGF-I also increased type II 3β-HSD mRNA levels and had less effect on 21-hydroxylase mRNA levels (170).

Alternatively, some *in vitro* studies have shown a stimulatory effect of insulin on adrenal steroid production (222), and others have shown inhibitory effects (223). Nor do the findings of *in vivo* studies agree. Indeed, an inverse correlation has been shown between plasma levels of insulin and DHEAS in human males (224), and certain epidemiological studies have shown low levels of DHEAS in states characterized by insulin resistance and hyperinsulinemia, such as obesity (225). Moreover, acute insulin infusion in healthy women is reported to cause a drop in DHEAS levels (175). Short-term insulin infusion resulted in an increased response of some steroid intermediates to ACTH stimulation and suggested that insulin may drive an increase in 17-hydroxylase and relative impairment of 17,20-lyase activity (226).

Hyperinsulinemia in women with PCOS affects adrenal androgen production; the response of A and 17OHP to ACTH was greater in hyperinsulinemic women than in normoinsulinemic women with PCOS (227). Studies in which circulating levels of insulin are reduced by administration of metformin (187, 228) confirmed that insulin has a role in regulating adrenal steroidogenesis. Metformin administration was followed by a significant reduction in the response of 17OHP, T, and A to ACTH (228), indicating reduced adrenal steroidogenesis in response to lower circulating insulin levels. Pharmacological reduction of insulin levels was associated with a significant decrease in 3β-HSD activity in C21 steroids (which converts 17OHP into 17OH pregnenolone), in 17β-HSD activity (which converts T to A), and an increase in 17,20-lyase activity in the D4 pathway (which converts A to 17OHP) (187). No changes in 3β-HSD activity in C19 steroids and 17,20-lyase activity in the D5 pathway were observed. These observations may indicate a stimulating effect of insulin on 3β-HSD and 17β-HSD activity and an inhibiting effect on 17,20-lyase activity.

As in the case of the ovaries, the major abnormalities in adrenal steroid secretion seem to be dysregulation of 17-hydroxylase and 17,20-lyase activities. As in the ovaries, insulin may largely affect the regulation of adrenal steroidogenesis.

5. Role of obesity. Acquired factors seem to play an important role in the pathogenesis of PCOS-associated insulin resistance. Indeed, obese PCOS patients are more insulin resistant than lean ones. Although the association of obesity and insulin resistance is universally accepted, the mechanisms by which increased adipose tissue causes insulin resistance remain unknown. It is significant that the sites of adiposity are not equal in this regard. Intraabdominal fat deposits (central obesity) are much more strongly associated with impaired insulin sensitivity. Central obesity is thought to induce insulin resistance by expressing and secreting several peptide hormones and cytokines. Increased production of free fatty acids (FFA) may inhibit insulin clearance and induce defects in cell uptake of glucose and glycogen synthesis (229, 230).

a. Emerging role of TNF-α. Adipocytes are well known for their relevant role as energy storage depots. New data have established an additional role for adipocytes, that of secretory cell. Adipocytes secrete peptide hormones and cytokines, including TNF-α, which helps maintain hemostasis.

Expression of the cytokine TNF-α is greater in adipose tissue and muscle of obese animals. The degree of TNF-α expression is correlated positively with the degree of obesity (231). Circulating levels of this factor are elevated in obese subjects and decrease with weight reduction (232).

TNF-α has many effects on adipocyte function including inhibition of lipogenesis and stimulation of lipolysis. TNF-α exerts a direct effect on insulin signaling by stimulating serine phosphorylation of IRSs with consequent impairment of cellular response to insulin (233). Interestingly, high circulating TNF-α has been reported in normal-weight PCOS patients and even higher levels in obese PCOS patients (234, 235). Thus, increased circulating TNF-α may be involved in the pathogenesis of insulin resistance associated with PCOS.

D. Summary

Hyperinsulinemic insulin resistance is characteristic of many, if not all, women with PCOS. Women with this syndrome also display a high prevalence of glucose intolerance and type II diabetes. Generally, the euglycemic hyperinsulinemic clamp is considered the gold standard for the measurement of insulin sensitivity *in vivo*. Because of its cost, time, and technical demands, other simpler methods, such as HOMA and QUICKI, may be used in clinical practice. Some clinical and biochemical findings may be useful to detect the presence of insulin resistance.

Pathogenesis of insulin resistance in PCOS is still a matter of debate. Defects in insulin binding to its receptor or, most probably, defects in downstream effectors of the insulin receptor may be the molecular sites of insulin resistance in PCOS. Obesity and body fat location have important independent effects on insulin sensitivity. Adipocyte-derived factors (FFA and TNF-α) seem to directly affect the cellular action of insulin.

Hyperinsulinemic insulin resistance is considered to have a central role in the pathogenesis of PCOS. Hyperinsulinemia may lead to inappropriate gonadotropin secretion and to adrenal hyperandrogenism. However, experimental evidence suggests that hyperinsulinemia could produce hyperandrogenism by directly increasing ovarian androgen synthesis and reducing SHBG plasma levels.

IV. Insulin-Lowering Strategies

On the basis of the theory that insulin resistance and hyperinsulinemia may be a relevant contributor to the pathophysiology of PCOS, it has been hypothesized that insulin-lowering agents, by reducing hyperinsulinemia, might improve endocrine and reproductive abnormalities with PCOS.

A. Metformin

There is now a large body of data documenting the clinical efficacy of metformin in the treatment of PCOS-associated insulin resistance. Metformin is an "old" drug (Fig. 5), mainly used to lower blood sugar in NIDDM. Its mechanism of action is still not entirely understood. Metformin and phenformin were introduced in 1957. Phenformin was withdrawn from clinical use in many countries in the late 1970s, when an association with lactic acidosis was recognized. Metformin became available for use in the United States in 1995. It is administered orally and improves insulin sensitivity that is impaired in NIDDM. Its efficacy is considered similar to that of sulfanilylurea.

1. Mechanism of action

a. Effect on glucose production. Metformin has been shown to significantly reduce basal hepatic glucose production in subjects with NIDDM (236, 237). It inhibits hepatic glucose production by 9% to 30%. In isolated hepatocytes, therapeutic concentrations of metformin enhance the suppression of gluconeogenesis by insulin and reduce glucagon-stimulated gluconeogenesis (238, 239). Even if the glucose-lowering effect of metformin is attributed to decreased hepatic glucose production and increased peripheral glucose utilization, other factors could contribute. Indeed, metformin therapy has been associated with a reduction in FFA levels due to decreased adipose tissue release (240, 241). FFA have been implicated in the pathogenesis of insulin resistance because of their effect in increasing hepatic gluconeogenesis and inhibiting glucose uptake and oxidation in skeletal muscle (242). Some studies found a small but significant decrease in body weight, which seems due to reduced calorie intake (243, 244). Metformin could reduce hepatic glucose production and fatty acid levels through genetic mechanism. Indeed, when hepatocytes were cultured in the presence of metformin, expression of genes for regulatory proteins of fatty acid oxidation and gluconeogenesis decreased, whereas expression of genes encoding proteins involved in glycolysis increased (245).

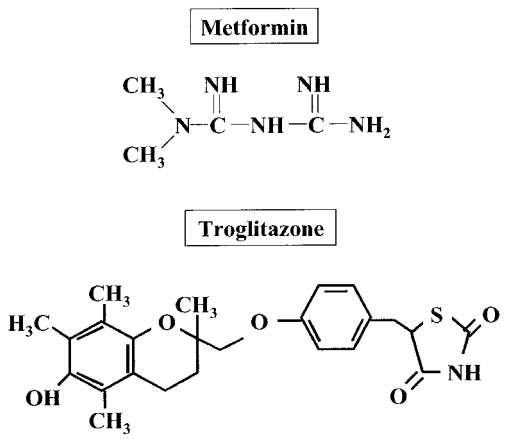

FIG. 5. Chemical structures of metformin and troglitazone.

b. Effect on peripheral glucose utilization. Metformin increases insulin-stimulated glucose utilization (estimated by means of the hyperinsulinemic clamp) by up to 50% in subjects with NIDDM (246) or normoglycemic insulin resistance (247). This effect has been predominantly attributed to an increase in nonoxidative glucose metabolism (240) with glucose oxidation being less affected. Nonoxidative glucose metabolism includes storage as glycogen, conversion to lactate, and incorporation into triglycerides (Table 4).

Metformin seems to facilitate the translocation of glucose transporters (GLUT) from intracellular sites to the plasma membrane, enhancing insulin-stimulated glucose transport into cells (248, 249). In cultures of skeletal muscle cells from insulin-resistant subjects, metformin increases insulin-stimulated glucose transport (250).

c. Effect on insulin levels and insulin receptor. A significant reduction in insulin and proinsulin levels in lean and overweight patients with NIDDM has been reported (251, 252). This effect may be secondary to the glucose-lowering effect of metformin. A report of the Biguanide and the Prevention Risk of Obesity has indicated that metformin significantly reduces fasting insulin levels in nondiabetic subjects. After 12 months, 164 patients taking metformin showed a reduction in plasma insulin of about 36 pmol/liter (253). Insulin binding to its receptor is reduced in NIDDM. Attempts have been made to determine the effect of metformin on the extent of insulin binding. Some studies have reported improved binding of insulin on erythrocytes and monocytes in healthy subjects and in obese and lean patients with NIDDM (254).

Other studies, however, found no change in either the number of insulin receptors on erythrocytes and monocytes or the affinity of insulin for its receptor (246, 255). Moreover, the extent of insulin receptor binding did not appear to be

TABLE 4. Effects of metformin on glucose metabolism and side effects

Glucose utilization	
Peripheral glucose utilization	[a]
Oxidative glucose metabolism	[a]
Nonoxidative glucose metabolism	[a]
Glucose transporter expression	[a]
Intracellular glucose transport	[a]
Glucose production	
Hepatic glucose production	[b]
Insulinemia and insulin receptor	
Insulinemia	[b]
Insulin receptor binding	[a]
Insulin receptor tyrosine kinase activity	[a]
Side effects and toxicity	
Diarrhea	
Nausea	
Abdominal discomfort	
Lactic acidosis	

[a] Increase in.
[b] Reduction in.

correlated with the clinical and metabolic response to metformin, indicating that these effects are probably mediated at the intracellular level (256). Indeed, it has recently been shown that metformin enters the cell and directly stimulates the tyrosine kinase activity of the intracellular portion of the β-subunit of the insulin receptor (257).

d. Side effects and toxicity. Reversible gastrointestinal side effects occur in about 30% of patients taking metformin. These effects include diarrhea, nausea, abdominal discomfort, anorexia, and a metallic taste in the mouth. These effects are not severe and can be avoided by taking metformin with food or by commencing therapy with a low dose. The newly available sustained-release formulations seem to be associated with a reduction in gastrointestinal side effects (258).

Reduced gastrointestinal folic acid and vitamin B12 has been reported, although without clinical symptoms (259). The worst toxic effect of metformin is probably lactic acidosis, which is fortunately not common. The US Food and Drug Administration recently reported a rate of five cases/100,000 treated patients (260). Lactic acidosis due to metformin therapy is associated with a mortality rate of about 50%. Mortality increases with the degree of renal impairment. Thus, strict observation of the exclusion criteria for metformin treatment should reduce this risk. Exclusion criteria include liver disease, heart or respiratory failure, alcohol abuse, and kidney disease. Metformin should not be administered if serum creatinine is above 1.5 mg/dl for men and 1.4 mg/dl for women. A case of metformin-induced hepatitis was recently described, probably a rare idiosyncratic reaction, with high serum transaminase and intrahepatic cholestasis (261).

Metformin does not seem to have teratogenic activity. In a recent study, its embryotoxicity was investigated in mice (262). Toxicity manifested at concentrations of at least 100 μg/ml, which is never achieved *in vivo*. Indeed, during controlled clinical trials, plasma levels of metformin do not exceed 5 μg/ml, even at maximum doses.

2. Effects on insulin resistance. Improvement in insulin resistance is generally accompanied by an improved metabolic profile, a significant reduction in hyperandrogenemia, and an improvement in ovarian function and menstrual cycles. In most studies, insulin resistance has been evaluated by OGTT (Fig. 6) (184, 186, 187, 195, 198, 218, 263–269) or methods based on tolerance to insulin or glucose infusion (264, 270, 271). Neither method, however, is regarded as reproducible, so it is preferable to consider only studies that evaluated insulin resistance by euglycemic clamp (263, 272, 273). In the study by Diamanti-Kandarakis *et al*. (272), 13 women with PCOS (mean BMI, 33.6 ± 6 kg/m^2) took 850 mg metformin twice a day for 6 months. The euglycemic clamp showed a significant increase in glucose utilization rate, irrespective of changes in body weight. The same results were obtained by the Finnish group (263), which found a significant reduction in fasting glucose and fasting insulin and an increase in fasting glucose oxidation and rates of glucose utilization during the clamp. The authors suggest that the reduction in hyperinsulinemia observed may be due to improvements in hepatic extraction and insulin sensitivity during therapy with metformin. They also found that metformin reduced basal levels of FFA, which, as mentioned above, have a role

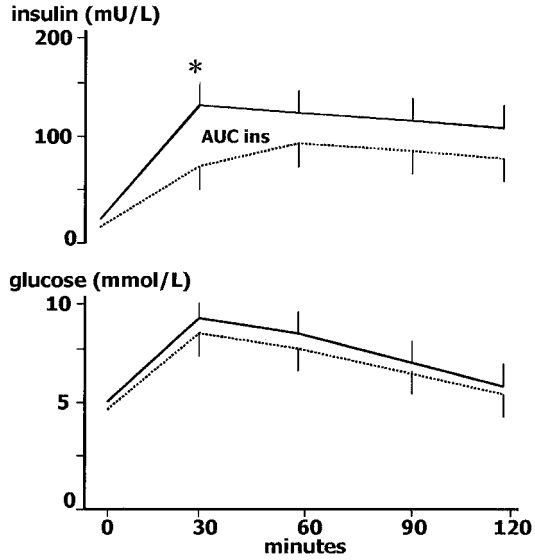

FIG. 6. Integrated insulin response to OGTT was significantly lower after 6 months of metformin therapy in women with PCOS *(single line*, PCOS women before therapy; *dotted line*, PCOS women after therapy). *, $P < 0.05$. AUC ins, Area under the curve insulin. [Adapted with permission from L. C. Morin-Papunen *et al*.: *J Clin Endocrinol Metab* 85:3161–3168, 2000 (263). © The Endocrine Society.]

in reducing glucose disposal in skeletal muscle, leading to impaired insulin sensitivity. Because the waist-to-hip ratio of the patients decreased after therapy, they concluded that the reduction in insulin resistance induced by metformin was secondary to its effects on adipose tissue.

Twenty-three young women (mean BMI, 30 ± 1.1 kg/m^2) were randomly assigned to treatment with metformin (500 mg 3 times a day) or placebo for 6 months. Euglycemic clamp carried out before and after therapy showed a significant improvement in insulin sensitivity. Insulin-stimulated glucose uptake increased from 0.14 to 0.2 μmol/kg·min (corrected for ambient plasma insulin). This increase was independent of changes in body weight (273).

Two original papers investigating the effect of metformin on insulin resistance in women with PCOS report negative results (264, 270). The reasons for partly conflicting results are not clear. A possible explanation (as discussed in *Section IV.A.5.a*) is that only about 50% of women with PCOS respond to metformin and this could be why some studies did not report benefits from metformin therapy. In the study of Ehrmann *et al.* (270), the effects of metformin therapy were evaluated for 3 months in 14 women with PCOS (65% Caucasian and 35% Afro-American). Insulin sensitivity was measured by IVGTT. A slight but significant reduction in the response of total T to leuprolide was found, but metformin therapy did not improve insulin resistance. A possible explanation may be the obesity of this particular population (mean BMI, 39 ± 7.7 kg/m^2). Substantial reductions in BMI are reported to be necessary to improve insulin sensitivity in subjects with BMI exceeding 30 kg/m^2 (274). The balance of opinion seems to favor beneficial effect of metformin over and above those due to weight loss only.

3. Effects on hyperandrogenism. Most studies on this subject suggest that insulin-lowering agents may affect the entire

spectrum of endocrine, metabolic, and reproductive abnormalities in PCOS patients. However, not all studies that have assessed the effects of metformin in hyperandrogenic women have confirmed these findings (264, 270). Interestingly, where insulin levels were reduced by treatment, serum androgens were lowered as well. The two studies that did not find changes in insulin levels likewise did not report changes in circulating levels of androgens (264, 270).

In an uncontrolled trial that assessed 26 obese women with PCOS before and after treatment with 1500 mg metformin/d for 8 wk, a reduction in insulin concentrations and in serum-free T was reported (275). Serum SHBG increased by 23%. After this report, a number of mostly uncontrolled short-term studies assessed the effects of this drug in insulin-resistant hyperandrogenic women. Nestler and Jakubowicz (183) reported a controlled study in 25 obese women with PCOS, many with acanthosis nigricans, a marker of severe insulin resistance. After 4–8 wk of metformin therapy, significant reductions in serum insulin, free T, and LH and an increase in SHBG were observed. The women also showed a reduction in 17OHP response to GnRH stimulation, a hallmark of ovarian hyperandrogenism. These effects were independent of changes in body weight. The observation that the effects of metformin were independent of body weight was subsequently confirmed (184).

Control studies available to date indicate that treatment with 1500–1700 mg metformin/d for at least 2–3 months is associated with a significant reduction in plasma levels of various androgens: total T (from −25 to −60%) (184, 198, 218, 268, 269, 276), free T (from −20 to −73%) (183, 184, 218, 265, 271, 273), A (from −25 to −50%) (184, 218), and 17OHP (−50%) (183). Only two studies of the 10 available report a significant reduction in basal levels of DHEAS (from −28% to −40%) (184, 218).

The favorable effect of metformin on hyperandrogenism in PCOS may be due to 1) reduced pituitary secretion of LH; 2) reduced ovarian secretion of androgens; 3) reduced adrenal secretion; or 4) increased levels of SHBG.

Metformin therapy was followed by a significant reduction in basal levels of LH (from 8.5 to 2.8 mIU/ml). Administration of GnRH analog was followed by a reduced LH response at the end of treatment (183). Hence, hyperinsulinemia associated with PCOS enhances both endogenous (basal) and exogenous (leuprolide-stimulated) release of LH. Increased ovarian steroidogenesis may be due to an insulin-induced abnormality in the dynamics of gonadotropin secretion rather than to direct stimulation of ovarian steroidogenesis by insulin. The same results were obtained in lean women with PCOS (184). A significant reduction in basal levels of LH has also been reported in other studies (195–198, 266), and the reduced response to GnRH has been confirmed as well (186, 187). However, there have been numerous studies that did not find any change in basal (28, 271, 277, 278) or GnRH-stimulated levels of LH (271, 273).

The reduction of plasma levels of LH is not a primary event in the reduction of hyperandrogenism induced by metformin. Indeed, many studies have reported a reduction in plasma androgens but not a concomitant reduction in LH, indicating that in these cases the reduction of steroid synthesis cannot be secondary to reduced stimulation of LH. On the other hand, it is possible that spontaneous or induced ovulation or a reduction in androgens may lead to a secondary reduction in LH. Indeed, it has been demonstrated that androgens returned to pretreatment levels when metformin was suspended and that this rise preceded the rise in LH, sustaining the hypothesis that a primary disorder of androgen hypersecretion is the cause of LH hypersecretion (196).

Various studies have been conducted with the aim of testing the hypothesis that the reduction in hyperinsulinemia induced by metformin is followed by a reduced ovarian response in terms of steroid secretion. The stimulations most used in these studies were GnRH and hCG. Women with PCOS generally have increased P450c17 activity as shown by increased 17α-hydroxylase and, to an lesser extent, increased 17,20-lyase activity, resulting in excessive ovarian androgen production. A hallmark of increased ovarian P450c17 activity is an exaggerated 17OHP response to stimulation by GnRH (9, 25). In a randomized controlled trial, Nestler and Jakubowicz (183) hypothesized and demonstrated that hyperinsulinemia stimulates ovarian cytochrome P450c17. One month of metformin therapy was followed by a reduced response of 17OHP to GnRH stimulation. However, metformin administration led to a reduction in LH response to GnRH. The decline in androgen production was attributed to the direct effect of insulin on ovarian enzymes but it may also have been due to a reduction in LH response to GnRH. The link between hyperinsulinemia and supranormal activity of cytochrome P450c17 was recently demonstrated by using hCG challenge, which is a more direct ovarian stimulus than GnRH for detecting modifications in ovarian steroidogenesis (28, 279). Metformin therapy was associated with reduced 17OHP response to hCG, confirming results obtained previously (183) (Fig. 7). The observation of a reduced ovarian steroidogenic response to GnRH has subsequently been confirmed (184, 186, 187, 273).

The reduced secretion of ovarian androgens after metformin therapy has been ascribed, therefore, to reduced circulating levels of insulin, although other mechanisms may also be involved. Indeed, the reduction in insulin levels after metformin therapy in PCOS patients is closely correlated with an increase in IGFBP-1 and a decrease in IGF-I to IGFBP-1 ratio, indicating a reduction of IGF available to peripheral tissue (217) (Fig. 8). A significant increase in IGFBP-1 after metformin therapy was recently confirmed (218).

Furthermore, a direct effect of metformin on androgen production by thecal cells cannot be excluded. It was demonstrated recently that metformin has a direct inhibitory effect on A production by human ovarian theca-like tumor cells (280). Treated with metformin, these cells showed reduced expression of steroidogenic acute regulatory protein, which is considered the rate-limiting step in steroid hormone production in the gonads. As metformin is known to directly increase insulin receptor tyrosine activity (281), the authors speculated that the decrease in androgen production could be due to an increase in tyrosine activity in metformin-treated cells and subsequently to a decrease in steroidogenic enzyme activity.

As mentioned previously, adrenal steroidogenic pathways are regulated much like those in the ovary. Insulin and IGF-I

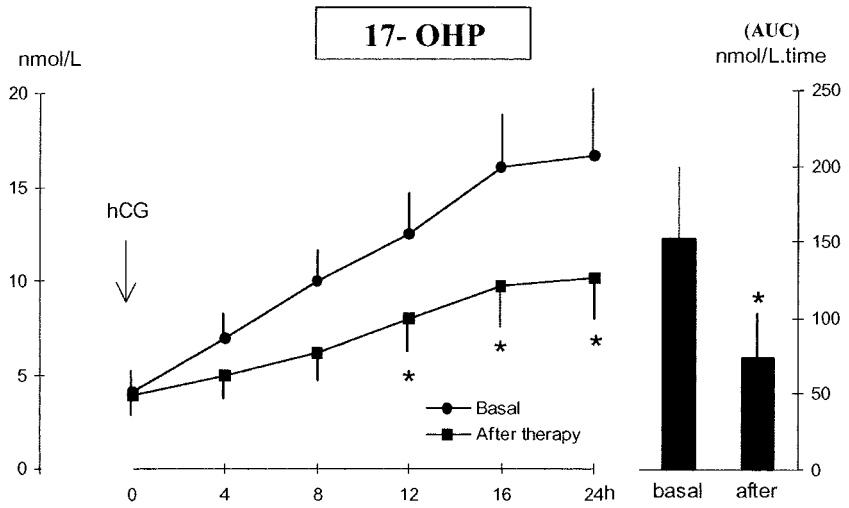

FIG. 7. 17-OHP response to hCG administration was significantly lower (*, $P < 0.05$) after metformin administration than before. Metformin was given at a dose of 500 mg three times a day for 30–32 d. AUC, Area under the curve. [Reproduced with permission from A. la Marca *et al.*: *Hum Reprod* 15:21–23, 2000 (28). © European Society of Human Reproduction and Embryology. Reproduced by permission of Oxford University Press/Human Reproduction.]

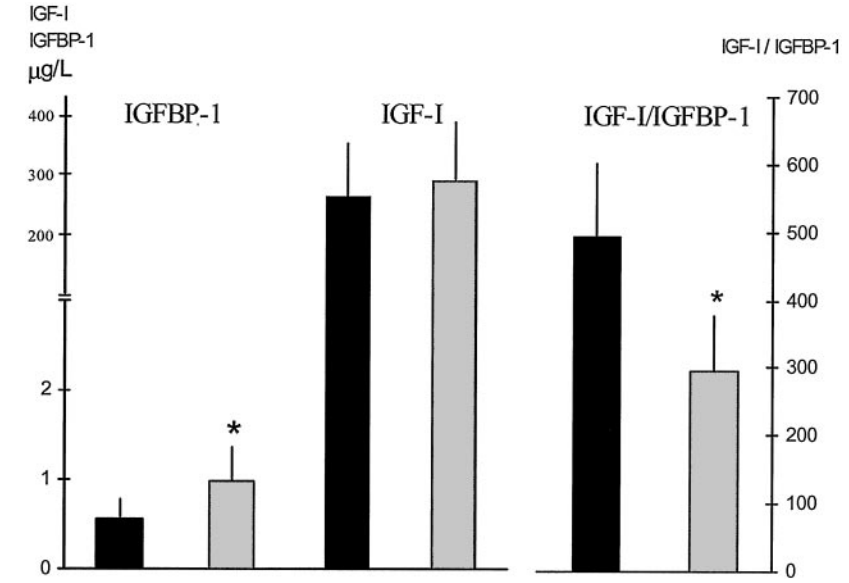

FIG. 8. Plasma IGF-I and IGFBP-1 levels before (*black*) and after (*gray*) metformin treatment. (*, $P < 0.05$). IGF-I to IGFBP-1 ratio before (*black*) and after (*gray*) metformin treatment (*, $P < 0.05$). Metformin was given at a dose of 500 mg three times a day for 1 month. [Reproduced with permission from V. De Leo *et al.*: *J Clin Endocrinol Metab* 85:1598–1600, 2000 (217). © The Endocrine Society.]

seem to regulate adrenal synthesis and secretion in a positive manner. The effect of metformin therapy on adrenal secretion in response to ACTH was recently investigated (228) in nonovariectomized PCOS women, and a significantly reduced response of 17OHP and A was found, which is compatible with a reduction in adrenal cytochrome P450 activity. Utilizing the ratios of substrates to products to indicate enzyme activity before and after metformin administration, the reduction in hyperinsulinemia was associated with a decrease in 17α-hydroxylase and 17,20-lyase activities.

Insulin influences androgen status not only by affecting ovarian and adrenal production, but also by regulating liver production of SHBG. This protein has a high affinity for binding T, the free fraction of which is responsible for steroid action in peripheral tissues. By reducing SHBG levels, hyperinsulinemia increases delivery of T to the tissues. Metformin therapy is generally associated with an increase in SHBG levels. A month of metformin therapy was followed by an increase in SHBG from 29 nmol/liter to 80 nmol/liter (183). An increase in SHBG levels after metformin therapy has also been reported in many other (184, 186, 197, 198, 218, 228, 265, 271, 272, 276, 277) but not all studies (196, 267, 263, 270, 273). The reasons for this discrepancy are not simple to identify. This may indicate, therefore, that the principal mechanism by which hyperandrogenism is reduced in PCOS is via reduced glandular synthesis and is reduced only secondarily by increased SHBG.

4. Acne and hirsutism. Despite the reduction in circulating levels of androgens, short-term treatment with metformin seems to have little effect on the skin manifestations of hyperandrogenemia in PCOS patients. A significant improvement in acanthosis nigricans, as diagnosed by the presence of papillomatous hyperpigmentation at the nape of the neck and/or in the axillae, was found in patients treated with metformin for 6 months. However, no objective criteria were available to assess the improvement (272).

Administration of metformin for 3 months was found to reduce the acne score by 14%, indicating that insulin-sensitizing therapy has little effect on acne, which is one of the main reasons young women with PCOS seek medical intervention (277).

Six months of metformin therapy in adolescent girls with PCOS was reported to be associated with a significant reduction in Ferriman-Gallwey score (from 16.16 to 10.7) (186, 197). However, only 3 months after the end of therapy, the score returned to its previous values. Long-term use of metformin does not bring about a reduction in hirsutism, despite the striking improvement in androgenemia and menstrual regularity (273).

When metformin is compared with the classical combination of ethinyl estradiol and cyproterone acetate, its relative inefficacy on skin manifestations of PCOS is apparent (263).

5. Reproductive function

a. Menstrual cycle and ovulation. The effect of metformin therapy on menstrual cyclicity has been extensively investigated (Table 5). Menstrual irregularity is a main symptom of PCOS, and its severity reflects the degree of insulin resistance of these patients (138).

A significant improvement in the frequency of menstrual cycles has been reported. In 1997 Velazquez *et al.* (195) evaluated the effect of 6 months of metformin therapy in 22 women with PCOS and irregular cycles. At the end of therapy, 21 of 22 women observed an improvement in menstrual frequency with 86% of ovulatory cycles, as assessed by progesterone measurement. Similar improvement has also been reported in other small uncontrolled studies (186, 196, 267, 272, 276, 285).

Metformin administration for 5 wk led to ovulatory cycles in 34% of women compared with 4% in the placebo group (265). In a recent trial (273), 23 PCOS patients were randomly assigned in double-blind fashion to treatment with metformin or placebo for 6 months. After treatment, the mean frequency of menstruation increased due to a striking improvement in about 50% of subjects. Menstrual frequency increased from 0.22 to 0.59 cycles per month (Fig. 9). Assay of progesterone in luteal phase showed that 79% of cycles were ovulatory. Patients were divided into "responders" and "nonresponders" according to whether or not regular cycles were achieved. Multiple logistic regression analysis of the data revealed that responders to metformin had high insulin levels, less severe menstrual abnormalities, and lower serum A levels before therapy. The reason why about half the women did not respond is probably due to the heterogeneity of the pathogenesis of PCOS. It is hypothesized that only patients in whom hyperinsulinemia plays a major pathogenetic role in anovulation can benefit from therapy with insulin-lowering drugs.

b. Ovulation induction. The first pharmacological approach to induction of ovulation in women with PCOS is clomiphene citrate. About 75% of patients ovulate in response to this drug, and most do so at a dose of 50 or 100 mg; the maximum dose is generally regarded as 250 mg/d. The number of nonresponders is therefore high. These women are defined as "clomiphene resistant" and are generally obese and more insulin resistant than responders (290). Because increasing obesity is associated with increasing hyperinsulinemia, the

TABLE 5. Summary of studies on the effect of metformin on reproductive function in women with PCOS

Authors (Ref.)	Dose and duration	Design and dimension	Regular cycles	Ovulation rate	Ovulation induction	Pregnancy rate	Pregnancy outcome
Velazquez *et al.* (275)	1.5 g/d × 8 wk	O, n = 26	↑	↑		↑	
Velazquez *et al.* (195)	1.5 g/d × 8 wk	O, n = 40	↑	↑		↑	
Ehrmann *et al.* (270)	2.55 g/d × 12 wk	O, n = 20	=	=			
Diamanti-Kandarakis *et al.* (272)	1.7 g/d × 6 months	O, n = 16	↑			↑	
Morin-Papunen *et al.* (267)	1.5 g/d × 4–6 months	O, n = 31	↑				
Nestler *et al.* (265)	1.5 g/d × 5 wk	RP, n = 61		↑	↑		
Glueck *et al.* (276)	1.5–2.25 g/d × 1.5–24 months	O, n = 43	↑				
De Leo *et al.* (282)	1.5 g/d × 4–8 wk	CO, n = 20			↑		
Unluhizarci *et al.* (271)	1 g/d × 12 wk	O, n = 17	↑				
Pirwany *et al.* (196)	1.7 g/d × 8 wk	O, n = 20	↑	↑			
Moghetti *et al.* (273)	1.5 g/d × 6 months	RPDB, n = 23	↑	↑			
Kolodziejczyk *et al.* (277)	1.5 g/d × 12 wk	O, n = 39	↑				
Pasquali *et al.* (268)	1.7 g/d × 6 months	RPDB, n = 20	↑				
Morin-Papunen *et al.* (263)	1 g/d 1st–3rd; 2 g/d 4th–6th months	R, n = 32	↑				
Ibanez *et al.* (186)	1.275 g/d × 6 months	O, n = 10	↑				
Glueck *et al.* (283)	1.5–2.55 g/d throughout pregnancy	O, n = 22					↑[a]
Vandermolen *et al.* (278)	1.5 g/d × 7 wk	RPDB, n = 28			↑		
Stadtmauer *et al.* (284)	1–1.5 g/d × 3–4 wk before IVF	Retro, n = 46			↑	↑	
Kowalska *et al.* (198)	1.5 g/d × 4–5 wk	O, n = 23	↑				
Vrbikova *et al.* (187)	1 g/d × 5–6 months	O, n = 24	↑				
Ibanez *et al.* (197)	1.275 g/d × 6 months	O, n = 18	↑	↑			
Glueck *et al.* (285)	1.5–2.55 g/d × 5–26 months	O, n = 11	↑	↑			
Kocak *et al.* (286)	1.7 g/d × 3–4 wk	RPDB, n = 56			↑	↑	
Jakubowicz *et al.* (287)	1.5 g/d throughout pregnancy	Retro, n = 65					↑[a]
Fleming *et al.* (288)	1.7 g/d × 14 wk	RPDB, n = 94	↑	↑			
Glueck *et al.* (289)	2.55 g/d throughout pregnancy	O, n = 33					↑[b]

O, Observational; R, randomized; RP, randomized, placebo; CO, cross-over; RPDB, randomized, placebo, double-blind; Retro, retrospective. ↑, Increase in; ↓, reduction in; =, no modification in.

[a] Reduced first trimester abortions.

[b] Reduced incidence of GD.

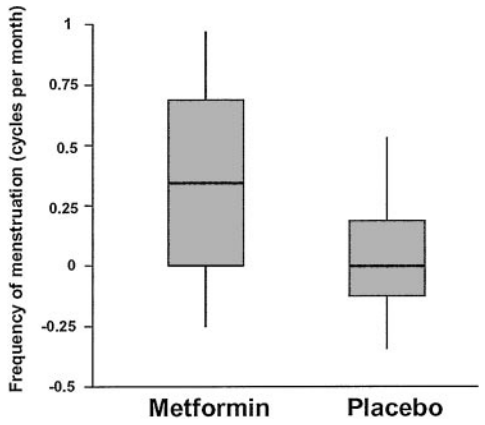

FIG. 9. Boxplot of the differences in frequency of menstruation (cycles per month) before and after therapy in the metformin (1.5 g/d for 6 months) and the placebo groups ($P = 0.002$ between groups). The *heavy bars* represent the median values; the *lower and upper limits of the boxes* represent the interquartile range (25th and 75th percentiles, respectively); the *I bars* indicate the extreme values. [Adapted from P. Moghetti *et al.*: *J Clin Endocrinol Metab* 85:139–146, 2000 (273). © The Endocrine Society.]

high degree of hyperinsulinemia in obese women with PCOS may account for their low responsiveness to clomiphene.

Presuming that the pathogenetic basis of clomiphene resistance was hyperinsulinemia, Nestler *et al.* (265) investigated the effect of metformin therapy on the response of PCOS patients to induction of ovulation with clomiphene. Sixty-one obese PCOS patients were pretreated for 5 wk with metformin or placebo and then given clomiphene citrate (50 mg for 5 d) to induce ovulation. Compared with placebo, metformin therapy resulted in a more than 10-fold increase in clomiphene-induced ovulation (90% of women ovulated).

Significant improvements in ovulation and pregnancy rates as a result of clomiphene treatment after metformin in women with clomiphene-resistant PCOS were recently reported in a randomized, double-blind, placebo-controlled trial (278). The women recruited for the study were unresponsive to 5-d courses of 150 mg clomiphene/d and were given increasing doses of clomiphene from 50 to 150 mg/d if they were still anovulatory after taking metformin. In the metformin and placebo groups, nine of 12 women (75%) and four of 15 women (27%) ovulated and six of 11 (55%) and one of 14 (7%) conceived, respectively. After these good results, the authors suggested that metformin treatment should precede ovulation induction with gonadotropins in women with clomiphene-resistant PCOS, because the first treatment is less expensive and has a lower risk of multiple births.

It has been shown that the risk of ovarian hyperstimulation syndrome in PCOS increases with increasing insulin resistance. This again emphasizes the important role of insulin as follicle growth factor (291).

Based on this concept, a reduction in insulin resistance by means of metformin therapy was associated with a more "physiological" ovarian response to exogenous gonadotropins (282). Indeed, combined metformin-FSH therapy was followed by recruitment of fewer follicles and by lower estradiol levels at the moment of hCG administration. The percentage of cycles in which hCG was withheld because of excessive follicular development was therefore significantly lower in cycles treated with metformin (282).

In PCOS patients undergoing *in vitro* fertilization (IVF)-embryo transfer or intracytoplasmic sperm injection, combined metformin-gonadotropin treatment has been shown to be better than gonadotropin alone (284) with recruitment of fewer follicles and lower plasma concentrations of estradiol. The authors also showed that metformin treatment was associated with an increased number of mature oocytes and four-cell embryos on d 3 of transfer. Fertilization rates and clinical pregnancy rates were better in the metformin group (increases from 43% to 64% and 30% to 70%, respectively). If these results are confirmed in future trials, routine use of metformin with gonadotropins will have to be seriously considered for PCOS patients undergoing IVF-embryo transfer.

In conclusion, metformin administration increases both spontaneous and clomiphene-induced ovulation by greater than 8- to 10-fold. In women undergoing gonadotropin ovulation induction, pretreatment with metformin may allow a reduced rate of hyperstimulation and may reduce the risk of multiple gestations. Evidence for improved fertilization rates and clinical pregnancy rates is accumulating.

c. Pregnancy. Because metformin therapy improves spontaneous and clomiphene- or gonadotropin-induced ovulation and conception rates in women with PCOS, the safety of metformin administration during pregnancy has to be proven. It is classified as a category B drug, which means that *in vitro* studies did not demonstrate teratogenicity.

A high miscarriage rate has been reported in PCOS patients (36, 37, 50, 51). It has been shown that metformin administration during pregnancy was associated with an early pregnancy loss rate of 8.8%, as compared with 41.9% in the nontreated PCOS women (287).

An independent risk factor for miscarriage in PCOS women is hypofibrinolysis associated with high levels of PAI activity (42). Metformin therapy has been found to normalize PAI activity levels in women with familiar thrombophilia and thrombofibrinolysis, reducing the incidence of miscarriage in the first trimester (292). The same authors administered metformin before conception and continued it into pregnancy and found a much lower incidence of miscarriage in the first trimester (from 73% to 10%).

Metformin therapy is associated with a significant increase in circulating levels of glycodelin (218), a protein whose circulating concentrations may reflect endometrial function (293). Glycodelin is thought to inhibit endometrial immune response to the embryo (294). In patients treated with metformin, follicular and luteal phase serum glycodelin exhibited 20-fold and 3-fold increases, respectively, compared with placebo. The resistance index of uterine spiral arteries also decreased significantly after metformin treatment. This reduction is thought to favor embryo implant and maintenance of pregnancy (295).

In aggregate, the above studies suggest that metformin therapy before conception and during pregnancy might not only reduce miscarriage rate, but also other frequent complications, such as GD.

In a recent prospective study (289), it was observed that metformin administration during pregnancy was associated

with a 10-fold reduction in GD. Thirty-three nondiabetic obese PCOS women conceived while taking metformin. Of these patients, 28 took metformin throughout pregnancy. GD developed in one of 33 (3%) women with metformin therapy and in 22 of 72 (31%) women without metformin ($P = 0.0009$). The odds ratio for GD in women taking metformin *vs.* those who did not was 0.093 (95% confidence interval, 0.011–0.795). Authors observed a significant reduction in body weight in women taking metformin before conception (from 102 to 88 kg; BMI from 33.9 to 30.3 kg/m^2). No modifications in BMI were observed during pregnancy. Furthermore, all women were instructed in a 1500-cal diet (with 44% of calories as carbohydrate). Body weight loss before pregnancy and caloric restriction during pregnancy may have contributed largely to reduced development of GD, confounding the interpretation that metformin, rather than weight loss alone, was responsible for the positive results.

6. Metabolic disturbances. PCOS is associated with a series of metabolic disorders that together confer increased risk of CVD. Treatment with insulin-lowering drugs should therefore lead to an improved metabolic profile in these women. A reduction in mean systolic and diastolic blood pressure in PCOS patients after metformin treatment has been reported (275, 276). However, 4–6 months of therapy were not followed by significant changes in blood pressure (267). Apart from the studies of Ibanez and co-workers (186, 197), which indicate a reduction in total and LDL-cholesterol in quite young women with PCOS, metformin generally seems to have little effect on lipid profile.

B. Thiazolidinediones

1. Troglitazone: mechanism of action. Troglitazone is the first of a new group of oral antidiabetic drugs (Fig. 5) that act by enhancing insulin effects at peripheral target sites. In March 2000, troglitazone was withdrawn from the market due to liver toxicity. As troglitazone is not available, the primary goal of this section is to discuss the pathophysiology of insulin in PCOS using such a drug as probe.

a. Effect on peripheral glucose utilization. Its main indication was the treatment of type 2 diabetes mellitus. Available clinical trials with troglitazone consistently show improvement in glycemic control, as well as reduction in insulinemia. The mechanism of action of troglitazone has not been fully elucidated (Table 6). It may improve insulin sensitivity by a direct effect on peripheral tissue sites (such as skeletal muscle and adipose tissue) or by indirect effects on other metabolic parameters. At the cellular level, troglitazone binds and activates the peroxisome-proliferator-activated receptor-γ (PPARγ), a hormone receptor located in the cell nucleus (296). PPARγ is predominantly expressed in adipose tissue (297), muscle, and liver (298). It regulates the transcription of several insulin-responsive genes involved in the control of glucose and lipid metabolism. In rats, by activating PPARγ, troglitazone is thought to promote adipocyte differentiation, increasing the number of small adipocytes that exhibit higher insulin sensitivity than large adipocytes (299). The resulting increased glucose uptake by small adipocytes may contribute to the improvement of glucose disposal observed in diabetic rats treated with troglitazone (299).

TABLE 6. Effects of troglitazone on glucose metabolism and side effects

Glucose utilization	
Peripheral glucose utilization	[a]
Glucose transporter expression	[a]
Intracellular glucose transport	[a]
Adipose PPARγ activation	
Glucose production	
Hepatic glucose production	[b]
Insulinemia and insulin receptor	
Insulinemia	[b]
Pancreatic β-cell dyfunction	[a]
Side effects and toxicity	
Weight gain	
Edema	
Diarrhea	
Nausea	
Headache	
Liver toxicity	

[a] Increase in.
[b] Reduction in.

However, activation of PPARγ does not explain all the effects of troglitazone on glucose metabolism. Indeed, various preclinical studies have demonstrated an acute effect of troglitazone on glucose metabolism (PPARγ independent). Troglitazone acutely decreased plasma glucose in diabetic animals (300) and increased glucose disposal in hyperinsulinemic clamp studies (301). Hepatic gluconeogenesis is acutely reduced by troglitazone in isolated hepatocytes (302).

Troglitazone exerts additional effects at the cellular level that may prove to be clinically important. Indeed, it decreases the release of FFA and TNF-α (299, 303). FFA suppress glucose uptake in skeletal muscle and increase glucose production by the liver. TNF-α interferes with insulin action by reducing insulin-stimulated tyrosine phosphorylation of insulin receptors (304). Therefore the reduction of FFA and TNF-α release from adipose tissue may be a way by which troglitazone improves glucose disposal in skeletal muscle and decreases hepatic glucose production.

b. Side effects and toxicity. Troglitazone is generally well tolerated. The most frequently reported side effects include diarrhea, nausea, headache, increase in body weight, edema, and weakness, which rapidly reverse when the drug is discontinued (305). Patients receiving the drug as monotherapy are not at risk for hypoglycemia. Troglitazone produces small reductions in neutrophil count, hemoglobin, and hematocrit, which are thought to be the result of a dilution effect (306).

A major side effect of troglitazone is liver toxicity. Up to November 1997, 135 cases of severe hepatotoxicity and six deaths were reported. The UK Medicine Control Agency withdrew the drug on the grounds that the risks overweighed the benefits. By March 2000, 60 patients had died as a result of liver dysfunction and 10 had received liver transplants. The incidence of troglitazone-induced acute liver failure has been estimated at 1 in 8,000–20,000 patients treated (307).

The U.S. Food and Drug Administration recommended monthly monitoring of liver function in the first 6 months of

therapy and every 2 months for the rest of the first year of therapy; however, in March 2000 troglitazone was withdrawn from the market. Troglitazone is a category B drug, which means that no teratogenicity has been found *in vitro*.

2. Effects on insulin resistance. Available data on the effect of troglitazone on insulin resistance in PCOS come from a small number of studies all conducted in the United States (185, 204, 308). In a randomized study, Dunaif *et al.* (185) examined the effect of two doses of the drug (200 and 400 mg/d) administered for 3 months in very obese patients (mean BMI, 42.9 kg/m^2). The author found a significant reduction in fasting and 2 h postglucose load insulin levels and that integrated insulin response to glucose decreased. A frequently sampled IV glucose tolerance test (FSIVGTT) was performed before and at the end of troglitazone administration. Insulin sensitivity increased significantly in both groups (200 and 400 mg/d), but improvement of insulin sensitivity was much more marked at the higher dose.

Subsequently, Ehrmann *et al.* (204) did a similar study in which 13 very obese PCOS patients (mean BMI, 39.9 kg/m^2) took 400 mg troglitazone/d for 3 months. BMI was unchanged at the end of therapy, but there was a significant reduction in fasting glucose, glucose response to OGTT (Fig. 10), fasting insulin, and integrated insulin response to glucose load. Insulin sensitivity, as determined by FSIVGTT, showed a marked improvement (Fig. 11). Three different doses of troglitazone (150, 300, and 600 mg/d) were compared (308). The highest dose of troglitazone, 600 mg/d, reduced fasting insulin by 53%, fasting glucose by 5.7%, glucose response to OGTT by 12.2%, and insulin response to

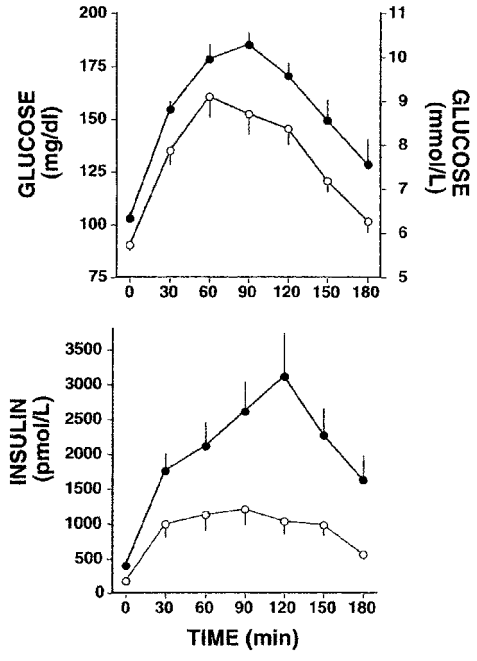

FIG. 10. Troglitazone administration (400 mg daily for 12 wk) was followed by a significant reduction ($P < 0.05$) in both glucose and insulin response to OGTT (*closed circles*, PCOS women before therapy; *open circles*, PCOS women after therapy). [Reproduced with permission from D. A. Ehrmann *et al.*: *J Clin Endocrinol Metab* 82:2108–2116, 1997 (204). © The Endocrine Society.]

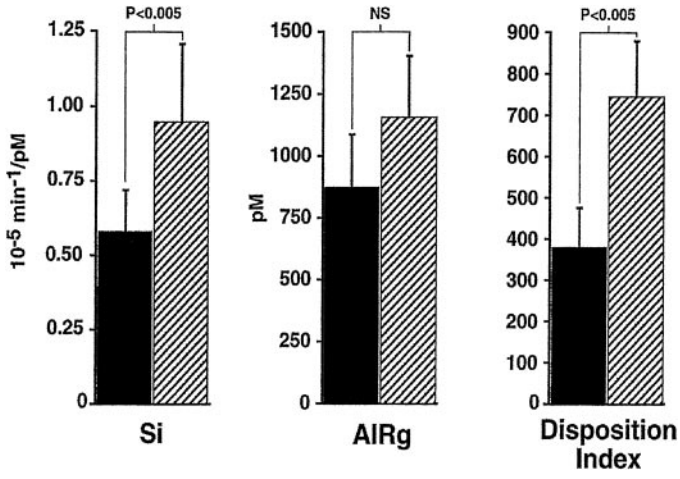

FIG. 11. Measures derived from the FSIVGTT before (*solid*) and after (*hatched*) treatment with troglitazone (400 mg daily for 12 wk). The insulin sensitivity (Si) and the disposition index (a measure of β-cell secretory function adjusted for insulin sensitivity) significantly increased after troglitazone treatment. AIRg, First-phase insulin secretion. [Reproduced with permission from D. A. Ehrmann *et al.*: *J Clin Endocrinol Metab* 82:2108–2116, 1997 (204). © The Endocrine Society.]

OGTT by 44.9%. At the lowest dose, the beneficial effects on insulin resistance were much smaller.

3. Effects on hyperandrogenism. All studies that examined the effects of troglitazone on PCOS-associated hyperandrogenism have reported positive results. After 3 months of therapy with 400 mg troglitazone/d, free T dropped 25–35% and SHBG increased by 25–66% (185, 204). A significant reduction in A, estradiol, estrone, and DHEAS with no change in plasma levels of LH has been reported (185). The same positive effects have also been found in populations of different ethnic groups, such as Japanese women (309). The women recruited in the latter study had a BMI of 28.7 kg/m^2 and were therefore much less obese than the women in the North American studies. Doses of 300 and 600 mg/d were associated with 26.6% and 37.8% reductions in free T and 45% and 71.5% increases in SHBG, respectively. No modifications were observed for LH, FSH, and total T (308).

Like metformin, troglitazone may affect hyperandrogenism by 1) reducing pituitary gonadotropin secretion; 2) reducing ovarian androgen secretion; 3) reducing adrenal androgen secretion; and 4) increasing plasma levels of SHBG. Ehrmann *et al.* (204) examined pituitary reserve by the GnRH analog test before and after troglitazone treatment. LH and FSH response to leuprolide was not affected by insulin-lowering treatment. The absence of changes in LH levels and the observation of significant correlations between reduction in insulin resistance and reduction in androgen response to GnRHa (204) indicate that the reduction in ovarian steroidogenesis plays a major role in improving hyperandrogenism after troglitazone therapy. The therapeutic effect of the drug is probably mediated by the reduction in insulin resistance and hyperinsulinemia. However, the drug could also have a direct effect on the ovaries. Indeed, troglitazone inhibits cholesterol biosynthesis in cultured hamster ovary cells (310) and inhibits P450c17 enzyme activity (311). PPARγ

receptors were recently demonstrated in pig ovaries. Addition of troglitazone to cultures of porcine theca cells resulted in a 53–69% decrease in LH- and/or insulin-stimulated A and T production (312).

Adrenal changes in response to troglitazone therapy have not yet been studied; however, it seems that treatment for 12 wk with this drug is associated with a reduction in DHEAS (185), a hormone of primarily adrenal origin. In addition to reduced ovarian, and possibly adrenal, secretion of androgens, the increase in plasma concentrations of SHBG certainly plays a role in reducing plasma levels of androgens during troglitazone therapy. Once again, the only available data are from the multicenter trial (308), which, due to the high number of patients, is the only study having great significance.

A dose-related decrease in Ferriman-Gallwey score during troglitazone treatment has been reported (308). The mean percentage reduction was 7% for women treated with 300 mg/d and 17% for women treated with 600 mg/d.

4. Reproductive abnormalities

a. Menstrual cycle and ovulation. Summary of studies on the effects of troglitazone administration on reproductive function in PCOS is reported in Table 7. The best data on this aspect come from the PCOS/troglitazone study group (308). In this multicenter study, three doses of troglitazone (150, 300, and 600 mg/d) were given for 44 wk. The mean rate of ovulation increased in a dose-related fashion and was 0.62 and 0.47 for the high and intermediate doses, respectively. In all, 57% of PCOS patients treated with the highest dose of troglitazone ovulated more than 50% of the time, compared with 12% in the placebo group. In the 600 mg/d group, about 60% of cycles were ovulatory. The mean number of days to first ovulation was 53 d and 107 d for women treated with troglitazone and placebo, respectively (Fig. 12). An improvement in the menstrual cycle was expected after the improvement in ovulation, and indeed, the improvement in menstrual cycle regularity mirrored that of ovulation.

An interesting analysis done by the authors was to determine factors that may be predictive of ovulation after troglitazone treatment. Patients with a higher ovulatory rate were older and less obese and had more episodes of vaginal bleeding before entering the study, lower fasting insulin levels, lower free T, and higher SHBG levels than patients with low ovulatory rates. This indicates that the women who are more affected are also less responsive to treatment. This is further evidence that the relationship between insulin levels and response remains unclear. About 5% of patients achieved pregnancy after treatment with 600 mg troglitazone/d, in comparison with 1.9% of patients receiving placebo (314).

b. Ovulation induction. Two reports are available on the effects of troglitazone on the response of PCOS patients to clomiphene (309, 313). Both are nonrandomized, uncontrolled studies. In the study by Hasegawa *et al.* (309), 13 PCOS patients (mean BMI, 28.7 kg/m^2) took troglitazone for 12 wk. If ovulation did not occur, the patients took clomiphene. During troglitazone administration, 37 cycles (26 on troglitazone alone and 11 on combined troglitazone and clomifene) were obtained. On the combined treatment, ovulation was confirmed in about 73% of the cycles, whereas in the troglitazone-only cycles, the ovulation rate was about 43%. In the study by Mitwally *et al.* (313), 18 clomiphene-resistant PCOS patients were recruited. They were followed for a total of 68 treated cycles. Almost half the treatment cycles (n = 33) involved troglitazone alone. In these cycles ovulatory rate was 61%. In combined clomiphene-troglitazone cycles, ovulation rate was 68.5%. Troglitazone used alone or concomitantly with clomiphene induced ovulation in a total of 83% of patients. Seven patients (39%) achieved pregnancy. Two patients miscarried during the first trimester; the others delivered healthy full-term babies.

5. Metabolic disturbances. Unlike metformin, troglitazone therapy is not associated with significant changes in blood pressure in PCOS patients (185, 204). The effects of troglitazone on lipids in clinical trials of patients with NIDDM have generally been variable and minor, with the notable exception of substantial reductions (13–26%) in triglyceride levels (315, 316). Troglitazone increased HDL- and LDL-cholesterol levels, and the LDL to HDL ratio did not change significantly. No significant change in total cholesterol, LDL, HDL, or triglycerides was observed after short-term therapy with troglitazone in PCOS (204, 309). A limited reduction in FFA levels was observed (309). However, troglitazone therapy

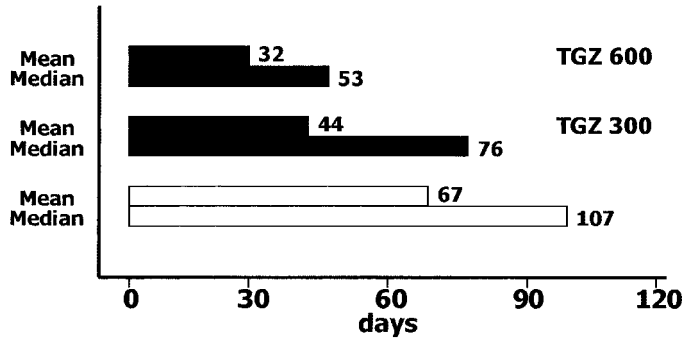

FIG. 12. Median time to first ovulation in women with PCOS treated with troglitazone (TGZ) 600 and TGZ 300 was significantly less ($P < 0.05$) than women treated with placebo (*white*) [Adapted with permission from R. Azziz *et al.*: *J Clin Endocrinol Metab* 86:1626–1632, 2001 (308). © The Endocrine Society.]

TABLE 7. Summary of studies on the effect of troglitazone on reproductive function in women with PCOS

Author (Ref.)	Dose and duration	Design and dimension	Regular cycles	Ovulation rate	Ovulation induction	Pregnancy rate	Pregnancy outcome
Dunaif *et al.* (185)	200–400 mg/d × 3 months	RDB, n = 25	↑	↑			
Mitwally *et al.* (313)	400 mg/d × 12 wk	Retro, n = 66		↑	↑		
Hasegawa *et al.* (309)	400 mg/d × 12 wk	O, n = 13		↑	↑		
Azziz *et al.* (308)	150–600 mg/d × 44 wk	RPDB, n = 410	↑	↑			

O, Observational; RDB, randomized, double-blind; RPDB, randomized, placebo, double-blind; Retro, retrospective. ↑, Increase in.

has been associated with significant reductions in PAI-1 and tissue plasminogen activator (tPA) plasma levels (204), which correlated with the reduction in insulin levels.

6. Rosiglitazone and pioglitazone. Rosiglitazone and pioglitazone are new drugs of the thiazolidine class released in the United States for the treatment of type 2 diabetes. They significantly reduce plasma levels of glucose in diabetics, improving insulin sensitivity (317–320). Like other thiazolidines, rosiglitazone and pioglitazone activate nuclear PPARγ receptors, which are mainly expressed in fatty tissue; these receptors cause reduced release of FFA and TNF-α, which are both involved in the pathogenesis of insulin resistance (321, 322).

7. Clinical efficacy. Few studies investigating the efficacy of rosiglitazone and pioglitazone have been conducted. Both drugs lower fasting plasma glucose, HbA1c, fasting insulin, and C peptide levels when administered in patients with type 2 diabetes. Both drugs increase levels of HDL and LDL and lower FFA levels, but only pioglitazone significantly lowers triglyceride levels (323–327).

Only one case report (328) concerning the use of rosiglitazone in PCOS is available. A 25-yr-old woman with oligomenorrhea, hirsutism, and a BMI of 25.0 kg/m^2 took 4 mg rosiglitazone/d for 5 months. Before treatment, total T levels were 47 ng/dl, and free T was 9.5 pg/ml; after treatment, they dropped to 41 ng/dl and 7.6 pg/ml, respectively. Insulin sensitivity was tested before and after treatment by the somatostatin suppression test, which revealed a drop in steady-state glucose infusion rate from 244 mg/dl to 178 mg/dl. This indicated a marked improvement in insulin sensitivity. The menstrual cycle became regular and ovulatory. Treatment was suspended because the patient achieved pregnancy.

These results indicate that rosiglitazone improved insulin sensitivity in this PCOS patient, reducing free T levels by 20% and restoring ovulation. Obviously randomized control trials are needed to confirm these effects.

Dose-dependent weight gain of 0.5–3.7 kg has been noted in the trials. Weight gain is likely to be multifactorial and could be the result of increased adipogenesis, increased appetite, and edema (323, 324, 329). Some studies report that the weight gain is associated with a reduction in waist-to-hip ratio, supporting the hypothesis that there is a shift of fat distribution from visceral to sc adipose depots, which may confer less cardiovascular risk (320, 324). Rosiglitazone and pioglitazone administration is associated with an increase in plasma volume of 6–7% and is contraindicated in patients with heart failure. To date, hepatotoxicity does not appear to be a significant problem with these newer agents; however, the product information for both drugs states that rosiglitazone and pioglitazone are contraindicated in patients with alanine aminotransferase levels more than 2.5 times the normal value.

C. Other drugs

Several insulin-sensitizing and insulin-lowering drugs have been tested as medical therapy of PCOS (Table 8). Some of these drugs have not had a role in clinical practice; diazoxide and somatostatin could be used only in a research setting as physiological probes.

1. Diazoxide. Dioxazide lowers circulating insulin levels (330) and has been used in studies investigating the link between hyperinsulinemia and PCOS (180, 331).

a. Mechanism of action. Dioxazide is a benzothiadiazine derivative, like thiazide diuretics, but does not have diuretic properties as it lacks a sulfonamide group. Dioxazide causes hyperpolarization of artery smooth muscle cells, activating ATP-sensitive potassium channels, followed by relaxation of vascular smooth musculature (332). *In vivo*, the drug acts exclusively on arterioles, with reflex activation of the sympathetic nervous system with water and salt retention. Heart output may double if heart rate and myocardial contractility are stimulated. Renin secretion increases, and the combination of increased output, water, and salt retention and high concentrations of angiotensin II counteracts the antihypertensive effects of diazoxide. Diazoxide directly suppresses plasma insulin. Its mechanism of action is via α-adrenergic stimulation of pancreatic β-islet cells (333).

b. Clinical efficacy. Diazoxide was found to significantly reduce plasma levels of insulin in five PCOS patients (180). After 100 mg diazoxide/d for 10 d, serum insulin levels decreased by about 80% with respect to pretreatment values, fasting glucose increased by 25%, and insulin levels dropped by 30%. These results were confirmed in a more recent study by Krassas *et al.* (334), who examined eight obese PCOS patients, evaluating insulin, glucose, leptin, androgen, and SHBG levels before and after treatment with diazoxide for 10 d. Insulin decreased by about 30% and glucose increased by 30%. Leptin showed a significant increase, androgens decreased, and SHBG increased.

A significant reduction in insulin and total and free T levels has been found in the five obese women with PCOS treated with diazoxide (180). Levels of A, DHEAS, estradiol, estrone, and progesterone did not change, and the A to estrone ratio dropped significantly. These results indicate that diazoxide

TABLE 8. Mechanism of action and side effects of other insulin-sensitizing and insulin-lowering drugs tested in PCOS

Drug	Mechanism of action	Side effects
Diazoxide	Insulin secretion[a]	Hyperglycemia, salt retention, hyperuricemia, hot flushes, palpitations
Acarbose	Carbohydrate digestion[a]	Abdominal discomfort
Somatostatin analog	Insulin secretion[a]	Abdominal discomfort
D-Chiro-inositol	Insulin post-binding signaling[b]	Not reported
Rosiglitazone and pioglitazone	PPARγ activation	Weight gain, edema

[a] Reduction in.
[b] Increase in.

does not have a direct effect on ovarian synthesis of androgens, but has an indirect effect through suppression of insulin secretion.

The main side effects of diazoxide are water and salt retention and hyperglycemia. Diazoxide administration in PCOS women led to an increase in BMI of 1.5 kg/m^2 due to water retention (180). BMI returned to initial values on suspension of treatment. Water and salt retention seems to be the result of stimulation of the renal sympathetic nerves and hemodynamic changes in the kidneys. Diazoxide also increases plasma concentrations of FFA by stimulating lipolysis through circulating catecholamines. It causes hyperuricemia by inhibiting excretion of uric acid by the renal tubules (335). Patients complained of hot flushes, palpitations, and dyspnea (331). Thus, diazoxide is not clinically useful due to its side effect profile.

2. Acarbose

a. Mechanism of action. Acarbose is a synthetic disaccharide that reversibly inhibits α-glucosidase activity of intestinal villi. It therefore slows digestion of complex carbohydrates and the more readily absorbed mono- and disaccharides. Inhibition of this enzyme causes a reduction in glucose absorption and therefore a decrease in postprandial hyperglycemia (336, 337).

b. Clinical efficacy. Only eight randomized placebo-controlled studies have examined the effect of acarbose on insulin sensitivity in patients with glucose intolerance or type 2 diabetes (338–345). The drug (100 mg, three times a day for 4 months) reduced steady-state plasma glucose by about 20% in an insulin suppression test with somatostatin, glucose, and insulin infusions. Laube *et al.* (344) reported that acarbose treatment (100 mg, three times a day) for 12 wk increased steady-state glucose infusion rate by 45% as assessed by hyperglycemic clamp. Acarbose therefore seems to improve insulin resistance in subjects with glucose intolerance and hyperinsulinemia, probably by reducing postprandial hyperglycemia. Studies in diabetes type 2 patients, however, did not show any improvement in insulin resistance, despite a reduction in postprandial hyperglycemia (343, 345).

There are little data in the literature on the effects of acarbose on hyperandrogenism. In hyperinsulinemic hyperandrogenic premenopausal women treated with acarbose, a significant reduction in postprandial hyperglycemia, hyperinsulinemia, and androgen levels was observed (346). The effects of acarbose therapy (300 mg/d for 3 months) on hirsutism, androgen concentrations, and insulin levels in hyperinsulinemic PCOS patients was investigated (182). All showed a reduction in acne and seborrhea, and menstrual regularity was restored in eight of 30 women. This clinical improvement was associated with a significant reduction in insulin response to glucose load, a significant reduction in LH, total T, and A, and a significant increase in SHBG. Serum concentrations of FSH, DHEAS, PRL, and 17OHP did not show significant changes. The reduction in androgen concentrations was probably caused by a reduction in ovarian cytochrome P450 activity. The reduction in LH may be related to the reduction in insulin levels.

The abnormal state of nutrient digestion induced by acarbose therapy also accounts for its distinctive profile of adverse events, which hinders compliance and continuance of therapy in some patients. The main side effects reported for acarbose are gastrointestinal; these include malabsorption, flatulence, and abdominal bloating (341, 344). Most withdrawals are due to gastrointestinal symptoms. Few studies investigating the effect of acarbose in women with PCOS have been conducted; hence there is no reason to recommend acarbose therapy in women with PCOS rather than other insulin-lowering drugs.

3. Somatostatin

a. Mechanism of action. Somatostatin is a 14-amino acid peptide found in tissues of the central and peripheral nervous system, pancreatic D cells, and glandular epithelium. In the pituitary, it inhibits secretion of GH and TSH and, under certain conditions, also PRL and ACTH. It inhibits all endocrine and exocrine secretions of the pancreas, intestine, and gallbladder. Like most peptide hormones, it acts by binding specific membrane receptors, of which there are various subtypes, some specific for certain tissues (347). Receptor binding leads to activation of G proteins, inhibiting cAMP production and reducing intracellular concentrations of free calcium.

Because of its wide range of effects, use of somatostatin has been proposed for the therapy of various diseases; however, its brief half-life (2–3 min) and ubiquitous action greatly reduce the possibility of pharmacological use. In 1982, Bauer *et al.* (348) synthesized an octapeptide, indicated as SMS 201–995 or octreotide, that proved to be 45 times more active than native somatostatin at inhibiting GH secretion in rats and 11 times more active in inhibiting glucagon. Ocreotide has a long half-life (90–120 min after sc administration) related to its rigid conformational structure.

b. Clinical efficacy. In 1990, Prelevic *et al.* (181) treated 10 PCOS patients, seven obese (BMI $>$ 25 kg/m^2), with octreotide (100 μg sc, two times a day for 7 d) and observed a significant reduction in insulin response to OGTT. Basal insulin and C peptide were substantially unchanged. Similar results were reported in 1992 (349), after short-term treatment of 12 PCOS patients, eight of whom were obese (BMI $>$ 25 kg/m^2). These results demonstrate the capacity of octreotide to reduce insulin levels in PCOS patients. A significant reduction in insulin response to OGTT after short-term treatment with octreotide was recently confirmed (350).

Short-term treatment of PCOS patients with octreotide induced a significant reduction in the amplitude of LH pulsatility, without affecting frequency of release (181, 349). LH secretion induced by buserelin was significantly reduced after octreotide treatment. Estradiol, T, and A showed significant reductions after treatment and reduced secretion after buserelin stimulation. Octreotide therefore reduces hyperandrogenism, inhibiting pituitary secretion of LH, and also lowers insulin levels. A reduction in LH release after administration of GnRH was observed in hyperinsulinemic PCOS patients; normoinsulinemic PCOS patients did not show such a reduction. This finding means that octreotide does not directly inhibit pituitary secretion of LH.

Administration of octreotide also caused a significant re-

duction in IGF-I and an increase in IGFBP-3 (351). Recent evidence shows that long-term treatment with octreotide significantly reduces adrenal response to ACTH and CRH. In a small observational study, combined human menopausal gonadotropin-somatostatin treatment was found to be associated with regular and balanced follicle growth (349) and with reduced risk of ovarian hyperstimulation. This means that if octreotide is added to gonadotropins in a combined treatment, it improves ovarian performance in PCOS.

The most frequent side effects of short-term octreotide treatment are reported to be diarrhea and intestinal cramps (352, 353).

4. D-Chiro-inositol

a. Mechanism of action. D-Chiro-inositol is a new insulin-sensitizing drug. The sponsor has recently discontinued studies of this drug for PCOS. This sugar is incorporated in a cell-membrane phospholipid, inositolphosphoglycan, involved in intracellular insulin signal transduction. Interaction of insulin with its receptor may activate this alternative transduction pathway, causing hydrolysis of the phospholipid and formation of intracellular messengers involved in oxidative rather than nonoxidative metabolism of glucose (354). Various studies (355, 356) have confirmed the involvement of this system in insulin-dependent glucose metabolism, showing reduced urinary excretion of D-chiro-inositol in subjects with reduced glucose tolerance or type 2 diabetes. Other authors (357, 358) have observed less of this sugar in muscle of subjects with type 2 diabetes than in controls. In an experimental study (355), administration of D-chiro-inositol reduced hyperglycemia in diabetic rats and improved glucose tolerance in normal rats. These results suggested that the mechanism of insulin resistance may be partly due to a deficit of the phospholipid containing D-chiro-inositol. Insulin acts via the phosphatidylinositol system even in ovarian thecal cells, where it stimulates T synthesis (162).

b. Clinical efficacy. Administration of D-chiro-inositol (1200 mg/d) to 22 obese PCOS patients for 6–8 wk was reported to reduce the insulin curve after OGTT from 13,417 ± 11,572 to 5,158 ± 6,714 U/ml·min (359). Diastolic and systolic blood pressure dropped by 4 mm Hg and plasma triglycerides decreased from 184 ± 19 to 110 ± 13 mg/dl. The women showed a significant reduction in plasma T and 19 of 22 ovulated during therapy. No side effects have yet been reported.

D. Weight loss

Because a high percentage of PCOS patients are obese, the role of weight loss in the management of this syndrome may be significant. The literature is encouraging on this point, although most studies have not included a control group (360, 361); statistical samples have also been small (362–364) and heterogeneous (360). Furthermore, studies have also been of short duration and have not provided follow-up information.

Pasquali *et al*. (360) analyzed the effects of a low-calorie diet (1000–1500 kcal/d) for 8 months in 20 obese women with PCOS (BMI, 32 kg/m^2). All lost about 10 kg, and insulin values after OGTT were found to be significantly lower than before weight loss and similar to those of women of normal weight. Similar results have been reported by other authors (361, 363, 364), who all agree that weight loss has a positive effect on hyperinsulinemia in women with PCOS. The effect did not seem to require great weight loss, but became evident with losses of 2–5% (365).

In addition to a reduction in insulin resistance, weight loss also involves a parallel improvement in endocrine status of PCOS patients. Significant improvements in hirsutism and ovulation, with restoration of regular cycles and an increased incidence of spontaneous pregnancies in 30% of patients, have also been reported (360–364, 366). A significant reduction in total T and A, with increased SHBG and reduced free T, are also reported. Some authors found a reduction in basal levels of LH (360), which, together with the increase in SHBG and reduced levels of insulin, could explain the improvement in hyperandrogenism in obese women with PCOS.

Similar improvements have been reported with modest weight reductions (2–5%). Nine of 18 patients in one study (365) experienced regular cycles and two of 18 became pregnant.

Weight loss certainly improves lipid profile and cardiovascular status in obese patients without PCOS (367), but this effect has not yet been specifically evaluated in a sufficient number of obese PCOS patients. Andersen *et al*. (368) analyzed total cholesterol, HDL, and triglycerides before and after a low-calorie diet in nine obese PCOS patients. After 24 wk, total cholesterol had fallen by 15% and HDL by 10%. The differences, however, were not significant because only six women completed the study.

E. Summary and clinical recommendations

In the past, the management of PCOS focused particularly on improvement of hirsutism and restoration of ovulation. However, the finding that hyperinsulinemia and insulin resistance are implicated in the pathogenesis of the syndrome, and that these alterations have major implications for health in the long term, led researchers to evaluate therapeutic strategies to control the insulin-glucose disorder in PCOS.

Short-term dietary intervention studies have consistently shown that weight loss may normalize reproductive function and hyperandrogenism and improve metabolic variables in overweight women with PCOS. Among the diabetic population, long-term studies of lifestyle modification have shown sustained benefit. Although there are no studies examining the long-term effects of weight loss in PCOS, changes in lifestyle should always be suggested. Lifestyle modification is a safe and inexpensive initial management for overweight women with PCOS seeking to improve reproductive function and metabolic profile. Moderate exercise, reduction in smoking, dietary modification, and reduction in alcohol and caffeine consumption would probably be of great benefit. However, not all obese women will achieve weight reduction, and not all women with PCOS are overweight or obese.

Because improvement in hyperinsulinemia and insulin resistance has been proposed as a primary goal, several insulin-lowering drugs have been introduced in the manage-

ment of PCOS. Of the drugs examined in the present review, only metformin and the new thiazolidinediones (rosiglitazone and pioglitazone) should be recommended in clinical practice. Others (somatostatin analog, diazoxide, chiro-inositol, and acarbose) have a role in a research setting only as physiological probes.

Metformin is the most thoroughly investigated insulin-lowering agent used to treat PCOS. One of the main reasons PCOS patients seek medical advice is menstrual irregularity. Oligomenorrhea and amenorrhea are usually linked to absence of ovulation. Available studies indicate that 6 months of metformin therapy induces an improvement in menstrual regularity in at least 50% of patients. It is currently not regarded as advisable to extend therapy if the response is negative. Although confirmation is needed, it appears that responders have high insulin levels, low androgen levels, and less severe menstrual abnormalities before therapy. This suggests that the best candidates for metformin therapy have oligomenorrhea rather than amenorrhea and are overweight rather than obese. In our opinion, insulin-lowering therapy is not the first priority in patients having hirsutism or acne as their principal problem. There have been very few studies into the effect of metformin on hirsutism and acne and the results have been discordant. Randomized prospective trials with appropriate long-term follow-up are needed to establish the effects of metformin on these two disorders, both of which cause major loss of quality of life and self-image, making more effective therapies, such as the contraceptive pill or antiandrogens, worthwhile. Contraceptive pills should be used with caution in patients with insulin resistance. Debate about whether oral contraceptives increase insulin resistance continues. Reports have shown that unbalanced estrogen may reduce insulin sensitivity (369). The use of progestin-only contraceptives may well increase the risk of diabetes, whereas the use of combined sex steroid preparations does not appear to significantly attenuate peripheral insulin action (370). These observations led to doubts about the long-term safety of contraceptive pills in women with PCOS. Close analysis of the effects of different oral contraceptives on insulin sensitivity is required. Research is underway into the efficacy of combining metformin and antiandrogens for dermatological problems linked to PCOS.

It has long been known that the first pharmacological approach to induction of ovulation in infertile women with PCOS is clomiphene citrate. This drug induces ovulation in at least 75% of patients. However, a combination of clomiphene and metformin seems more effective than either alone. Patients not responding to combined treatment are obviously candidates for gonadotropin therapy. Again, available studies suggest that metformin should be continued during controlled ovarian hyperstimulation. Indeed, metformin appears to reduce the risk of ovarian hyperstimulation and hence multiple pregnancies. When controlled ovarian hyperstimulation is followed by IVF, administration of metformin seems to be associated with improved oocyte quality, fertilization rates, and number of embryos. Recent data (requiring confirmation) indicate that continued administration of metformin during pregnancy reduces the rates of early pregnancy loss and the incidence of GD. If this finding is confirmed, metformin becomes established as the only available treatment for the high miscarriage rate in PCOS.

Adolescents with PCOS are a special problem. Several observational studies on adolescent girls with PCOS showed that 6 months of metformin therapy was followed by a high percentage of ovulation (76%) (197). In sexually active adolescents, metformin-induced restoration of ovulation could lead to unwanted pregnancy. These subjects therefore need to be counseled on contraception. In many cases it could be more useful to give the contraceptive pill. Recent studies show that adding metformin to oral contraceptive treatment may improve insulin sensitivity and may further suppress the hyperandrogenemia in women with PCOS (371). If these results are confirmed, combined therapy with metformin-oral contraceptive could be useful in cases requiring contraception, such as adolescent girls. Alcohol, the most common drug used during adolescence, is contraindicated in patients receiving metformin. Hence, if metformin is prescribed for adolescent girls, it should be accompanied by information to the patient and family regarding risks, benefits, and alternatives. It was recently shown that long-term metformin administration in patients at risk for diabetes type II is associated with a 31% reduction in the incidence of this type of diabetes (372). In diabetics, metformin also reduces the risk of cardiovascular complications (373). No research has yet been concerned with the effect of metformin on cardiovascular complications and the incidence of diabetes in women with PCOS. If positive results are obtained, metformin therapy could be used not only for women of reproductive age with PCOS but also in postmenopausal women with a history of PCOS.

Metformin has tedious gastrointestinal side effects such as nausea, vomiting, and diarrhea. To minimize these side effects, the authors start metformin at 500 mg/d with dinner for 1 wk, then increase to 500 mg twice daily with lunch and dinner for 1 wk, and finally 850 mg twice daily with lunch and dinner. The dose may be further increased if necessary but should not exceed 2.55 g/d. Metformin is unlikely to cause serious hypoglycemia even if a meal is missed. Before prescribing the drug, doctors should check for contraindications (see *Section IV.A.1.d*). Renal function should always be checked and drinking of alcohol should be discouraged.

The new thiazolidinediones (rosiglitazone and pioglitazone) appear promising for the treatment of insulin resistance associated with PCOS. However, data are available only for troglitazone, a drug with high liver toxicity that was withdrawn from commerce in 2000. Rosiglitazone and pioglitazone are safer and have proved effective in improving insulin sensitivity. There is little difference between the two agents although pioglitazone may have a more favorable effect on lipid profile than rosiglitazone. Randomized controlled trials on the effects of these drugs in PCOS are necessary before their use can be recommended. Rosiglitazone and pioglitazone are category C drugs that have been demonstrated to retard fetal development in animal studies. Because their use could restore ovulation and fertility, their presumed embryotoxicity would limit their use.

V. Conclusions

Women with PCOS exhibit a unique and intrinsic form of insulin resistance. Insulin resistance is associated with compensatory hyperinsulinemia. Many women with PCOS are also overweight or obese. Excess adiposity directly impairs peripheral insulin sensitivity. Insulin resistance seems to place women with this syndrome at high risk for diabetes, hypertension, dyslipidemia, and CVD.

Due to the role of hyperinsulinemia in the pathophysiology of PCOS, several insulin-sensitizing and insulin-lowering drugs have been tested. Some will soon be the basis of medical therapy of PCOS (biguanides, thiazolidinediones); others (diazoxide, somatostatin analogs) have a role only in research. Insulin-lowering agents examined in this review act at different levels of insulin-glucose metabolism. All drugs seem to have similar clinical efficacy on PCOS-associated symptoms. This suggests that reproductive abnormalities of women with PCOS may be directly related to hyperinsulinemia and not to any specific mechanism of insulin resistance.

Insulin-lowering agents have proven to be an effective new therapeutic tool in women with PCOS. The balance of opinion seems to favor beneficial effects of insulin-lowering agents on insulin sensitivity, hyperandrogenemia, menstrual irregularity, and metabolic disorders in a large subset of affected women.

PCOS is a heterogeneous group of disorders. Future studies need to characterize the endocrine and metabolic profile of the various subgroups, as insulin-lowering agents seem to be an effective therapeutic tool for a subset of patients, but not for all women with this syndrome.

Note Added in Proof

During the publication of this article, both Pioglitazone and Rosiglitazone administration in women with PCOS have been shown to be followed by reduction in insulin reistance and hyperandrogenism (374, 375) and regularization of spontaneous and clomiphene citrate-induced ovulation (376, 377).

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