REVIEW ARTICLE

Pathogenesis of Polycystic Ovary Syndrome: What Is the Role of Obesity?

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Both obesity and the polycystic ovary syndrome (PCOS) are commonly seen in women of reproductive age. Fifty percent of all patients with PCOS are obese, and the presence of obesity affects the clinical manifestations of PCOS. The underlying pathogenetic mechanisms appear to involve insulin resistance and hyperinsulinemia, the magnitude of which is greater in obese than in non-obese women with PCOS. Specific effects of obesity on the manifestations of PCOS, underlying mechanisms of the interactions between obesity and PCOS, and therapeutic implications of these interactions are discussed in this article.

POLYCYSTIC OVARY syndrome (PCOS) is the most common endocrinological disorder of reproductive age women. Although its etiology remains unknown and the pathogenesis is unclear, a variety of theories have been proposed to explain the development of PCOS. These theories classically included the "central hypothesis," which postulated a primary defect at the level of the hypothalamus/pituitary, and an "ovarian hypothesis," which proposed a primary defect of steroidogenesis in the ovary.

The most recent theories of the pathogenesis of PCOS have focused also on the role of insulin resistance and hyperinsulinemia. It has been proposed that, by a variety of mechanisms, hyperinsulinemia increases ovarian androgen production and contributes to the development of the hallmark abnormality of PCOS–anovulation. The insights into the role of hyperinsulinemia in the development of PCOS brought into focus the role of obesity, which is present in about 50% of patients with PCOS and which magnifies hyperinsulinemia observed in PCOS patients. In addition to enhanced hyperinsulinemia, other potential contributions of obesity to the development of PCOS involve a recently discovered hormone, leptin, produced in adipose tissue, and the enzymatic activity of adipose tissue as it relates to steroid hormone metabolism (eg, aromatase and 11β- hydroxysteroid dehydrogenase [11β-HSD]).

In the course of this article, we will first review current theories of the development of PCOS and then focus on the role of obesity in its pathogenesis. Specifically, we will review the contributions of insulin resistance/hyperinsulinemia, leptin, and adipose tissue steroid hormone metabolism to the development of PCOS. We will then examine differences between clinical presentations of PCOS in obese and non-obese women. We will conclude the article by discussing therapeutic approaches to PCOS, focusing, in particular, on therapeutic modalities which address pathogenetic contributions of obesity to the development of this common disorder.

DEFINITION, GENETICS, AND EPIDEMIOLOGY OF PCOS

In their original description of the syndrome, Stein and Leventhal[1] reported the condition characterized by enlarged ovaries, which contained multiple small subcapsular cysts and were associated with amenorrhea and hirsutism. PCOS, as it is understood today, is a disorder of unknown and probably heterogeneous etiology, which is characterized by chronic anovulation, menstrual irregularities, evidence of hyperandrogenism (either clinical, manifested as hirsutism, acne, male pattern balding, or biochemical, manifested by elevated serum adrenal and/or ovarian androgen concentration), and exclusion of other etiologic factors (nonclassical congenital adrenal hyperplasia, Cushing's syndrome, hyperprolactinemia, thyroid disease, and androgen-producing tumors).[2] These diagnostic criteria, adopted by the 1990 National Institutes of Health consensus conference on PCOS, do not require pelvic ultrasonography to evaluate ovarian morphology.

Demonstration of polycystic ovaries on ultrasound is not required for diagnosis of PCOS because, with the introduction of high-resolution ultrasonography, it became apparent that 20% of healthy women have morphologic features of polycystic ovaries, and only some of them have symptoms of hyperandrogenemia.[3] Furthermore, while 80% to 100% of women with PCOS have polycystic ovaries (which are defined sonographically by the presence of 8 or more small 4 to 8 mm follicles in each ovary in subcapsular location) so do many women with idiopathic hirsutism and other hyperandrogenic disorders.[4] Finally, ovarian vein catheterization studies proved that ovarian hyperandrogenism may be present with and without polycystic ovaries.[5]

Although the etiology of PCOS remains unclear, there is increasing evidence for a genetic component. Givens et al[6] were among the first to describe a familial presentation of this disease. The specific genetic defect, if any, causing familial PCOS is unknown. Autosomal dominant type of inheritance has been suggested by familial clustering of cases and high prevalence of PCOS (close to 50%) among siblings.[7,8]

PCOS is the most common endocrinopathy in women of reproductive age with a probable prevalence of 5% to 10%.

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Available data on the prevalence of PCOS are variable, in part due to ethnic differences and lack of consistent criteria for diagnosis. By the end of the 20th century, the prevalence of PCOS had ranged from 2% to 20% in general population studies.[9-11] In 1998, a prospective study that used the above criteria reported 3.4% prevalence of PCOS in African American and 4.7% in Caucasian women who presented for a routine pre-employment history and physical examination in the United States.[10] A survey in Greece reported 6.8% prevalence of PCOS, similar to that seen in the United States.[11] In some genetically homogeneous groups, such as, for example, the Pima Indians, who also have a very high prevalence of obesity and diabetes (the latter approaching 50%), the prevalence of PCOS has been reported as high as 21%.[12]

OBESITY, INSULIN RESISTANCE, AND HYPERINSULINEMIA

Women with PCOS are often insulin resistant and hyperinsulinemic.[13] Both insulin resistance and hyperinsulinemia are magnified in the presence of obesity, which is defined by body mass index (BMI = body weight in kilograms divided by height in meters^2) of 30 kg/m^2 or more. BMI of 25 to 29.9 kg/m^2 is defined as 'overweight', while BMI of less than 25 kg/m^2 is considered normal.[14]

Rabinowitz and Zierler[15] were among the first to demonstrate the presence of insulin resistance in obese individuals. Obesity in PCOS is usually of the central variety. Central obesity (also called visceral, android, abdominal, or male-type) can be diagnosed clinically by measuring the waist circumference (WC) or waist-to-hip circumference ratio (WHR).[14] WC larger than 102 cm for men and 88 cm for women or WHR greater than 0.95 in men and 0.85 in women confer high risk for metabolic complications in obese individuals with BMI between 25.0 and 34.9 kg/m^2.[16] Studies have shown that excess fat in the upper part of the body is associated with increased risk for diabetes, hyperlipidemia, hypertension, atherosclerosis, and insulin resistance[17] more often than peripheral fat excess (gynoid, female-type, or peripheral obesity).[18-21] Buffington and Kitabchi[22] studied insulin clearance in obese PCOS patients and concluded that the hyperinsulinemia may be due, in part, to defects in insulin clearance and peripheral tissue insulin degradation. Thus, in addition to its association with insulin resistance, fat localized in the upper body is correlated with significantly reduced overall clearance of insulin, which contributes to hyperinsulinemia.[23]

Although the mechanisms by which obesity causes insulin resistance are not fully understood, the 2 main pathogenetic hypotheses that have been proposed focus on the roles of free fatty acids (FFAs) and tumor necrosis factor-α (TNF-α).

FFAs, which are released from adipose tissue triglycerides via lipolysis, are the key mediators of impaired insulin sensitivity. Elevated circulating levels of FFA have been reported in PCOS patients,[24] and Ek et al[25] showed that visceral adipocytes in PCOS women exhibit significantly increased catecholamine-induced lipolysis in vitro.

Whether visceral adipose tissue differs from subcutaneous adipose tissue in the magnitude of FFA flux into liver is not quite clear. FFA released from the splanchnic bed account for only about 10% of the FFA reaching the liver.[26] Recent studies using catheterization of the hepatic vein to determine the maximal contribution of visceral and mesenteric fat depots to the increased fatty acid release in women with upper body obesity have concluded that the major source of FFA in circulation is not the visceral depot, but rather upper body nonsplanchnic subcutaneous adipose tissue.[27]

Increased FFA flux into the liver, irrespective of its source, decreases hepatic insulin extraction, increases gluconeogenesis, and produces hyperinsulinemia.[28,29] Additionally, high circulating FFA concentrations lead to peripheral insulin resistance by reducing glucose uptake by the skeletal muscle.[29,30]

TNF-α is produced by adipose tissue and leads to insulin resistance by stimulating the phosphorylation of serine residues of the insulin receptor substrate-1 (IRS-1). Consequently, tyrosine kinase activity of the insulin receptor β-subunit, the rate-limiting component of the insulin receptor signaling cascade, is inhibited.[31,32] Serum TNF-α concentrations have been reported to be high in hyperandrogenic women,[33] including women with PCOS.[33] The nature of the direct effects of TNF-α on ovarian cells, if any, is not understood. TNF-α was shown to enhance the proliferative actions of insulin and insulin-like growth factor-I (IGF-I) in rat ovarian theca cells,[34] but, on the other hand, it inhibited gonadotropin-stimulated steroidogenesis.[35]

In summary, regardless of its pathogenesis, insulin resistance is commonly present in obesity, particularly in its central or android form, which is the characteristic form of obesity in PCOS. As will be discussed later in this article (in the section comparing obese and non-obese women with PCOS), obesity magnifies insulin resistance and hyperinsulinemia in PCOS patients.

ENDOCRINE ABNORMALITIES IN PCOS: THE ROLE OF OBESITY

Based on the putative primary abnormality, 3 major hypotheses of the pathogenesis of PCOS have been proposed. They are the central (luteinizing hormone [LH]) hypothesis, the peripheral (ovarian or adrenal) hypothesis, and the insulin hypothesis (Table 1).

Gonadotropins and the Central Hypothesis

In 1970 Yen et al[36] reported that increased serum luteinizing hormone (LH) concentration or increased LH to follicle-stimulating hormone (FSH) ratio were characteristic of the syndrome. The increased LH/FSH ratio became a commonly used diagnostic criterion in association with a biopsy confirming polycystic ovarian morphology.

While serum concentrations of FSH in anovulatory women with the PCOS are similar to those in the midfollicular phase of normal cycle, approximately 75% of women with PCOS exhibit elevated pooled LH serum concentrations compared with ovulatory control women.[37] The prevalence of this abnormality has ranged from 30% to 90%, depending on gonadotropin assays used and timing of sampling.[38] Gonadotropin-releasing hormone (GnRH) pulse amplitude and frequency determine the preferential gonadotropin secretion, slower pulses favoring FSH release, while more rapid pulses favoring increased LH

Table 1. Theories of the Pathogenesis of PCOS

Theory	Primary Defect	Origin of Abnormality	Mechanism
Central (LH) hypothesis	Increased LH pulse amplitude and frequency; elevated LH/FSH ratio.	?Primary abnormality in GnRH pulsatility[36] (rapid pulse generation). ?Sensitization of GnRH receptor to GnRH by endogenous opioids.[38,43] ?Reduced dopaminergic inhibition of LH release.[43]	-Increased LH secretion leading to hyperandrogenism.
Peripheral (ovarian or adrenal) hypotheses[54,55]	1. Intrinsic ovarian or adrenal defect leading to overproduction of androgens and anovulation.	Ovarian theca cell hypertrophy.[52] Steroidogenic and mitogenic abnormalities of granulosa cells.[40]	-Dysregulated function of the p450 C17-alpha in the ovaries and adrenal glands;[54-56,152] autocrine and paracrine local defects as well as blocked FSH activity at the ovarian level.[40]
	2. Inhibin B deficiency.	Product of granulosa cells, inhibin B locally enhances follicular development.[211]	-Deficiency of inhibin results in anovulation.[211]
Insulin/IGF hypothesis	Insulin resistance and hyperinsulinemia.	Postreceptor defect in insulin signaling.	-Hyperinsulinemia leads to inhibition of SHBG secretion and increase in free fraction of androgens.[40] -Direct stimulation of ovarian steroidogenesis by hyperinsulinemia.[152] -Synergism of insulin with LH leads to stimulation of theca cells, hyperandrogenism[59] and large cystic ovaries.[210]
	Increase in free IGF-I and IGF-II.[212]	Systemic and local excess of free IGFs.	-Potentiate LH-stimulated androgen synthesis in theca cells.[153] -Supresses IGFBP-1 synthesis.[159,160]

secretion. Abnormally rapid GnRH pulse generation is assumed to underlie abnormal LH secretion in PCOS.[36] Studies addressing the response of gonadotropins to GnRH have demonstrated an increased LH responsiveness,[39] with no differences in FSH response in PCOS patients when compared with normal women. This preferential LH responsiveness may be related to the elevated circulating concentrations of estrone, which, as will be discussed below, are commonly present in PCOS women. Conversely, selective suppressive effects on FSH response may be related to the effects of estradiol and inhibins, although the role of inhibins in decreased FSH responsiveness has not been clearly demonstrated in PCOS women.[38]

Whether the hypothalamic defect, manifested by rapid GnRH pulse generation, is the primary cause of PCOS or whether this abnormality is secondary to other hormonal phenomena, such as hyperinsulinemia, remains to be determined. It has been shown that insulin augments the effect of GnRH on gonadotropin secretion in vitro.[40] However, the ability of either insulin or IGFs to enhance gonadotrope sensitivity to GnRH-releasing hormone has not been consistently demonstrated in vivo.[40-42]

In addition to abnormal GnRH function, other central mechanisms involved in the pathogenesis of PCOS may include abnormalities of neuroendocrine modulators, such as endogenous opioids and dopamine.[38,43] There is evidence suggesting that endogenous opioid excess may play a role in sensitization of the gonadotrope to GnRH and that decreased dopaminergic inhibition, leading to increased prolactin and LH secretion, is present in women with this disorder.[43] Further, B-endorphin administration increases insulin secretion in humans, and opioid antagonists suppress glucose-stimulated insulin secretion, particularly in patients with central obesity.[44] Thus, in obese PCOS women, excessive activation of the opioid system may contribute to a higher degree of hyperinsulinemia than in non-obese patients.

Two studies by Arroyo et al[45] and Morales et al[46] addressed the role of obesity in the abnormalities of the gonadotropin axis in PCOS and reported consistent results: accelerated LH pulse frequency is seen in many patients with PCOS, regardless of body weight, but obese patients exhibit lower LH pulse amplitude and mean circulating LH values than lean patients. Factors proposed to account for these differences include variations of insulin and leptin levels, as well as differences in catecholamine and endorphine metabolism. The exact mechanism, however, remains unclear.

Today, an increased LH/FSH ratio is not required for diagnosis of PCOS, because this ratio can be elevated in women without identifiable ovarian pathology,[3] as well as in women

with other hyperandrogenic disorders, such as, for example, congenital adrenal hyperplasia.[47]

Regardless of the cause, LH hypersecretion, when present, is thought to contribute to stromal and thecal stimulation with a resultant increase in ovarian andogen secretion in PCOS.

Ovarian Androgens and the Ovarian Hypothesis

Evidence suggesting a primary ovarian role in androgen excess in PCOS women includes the following observations: increased synthesis of testosterone and androstenedione by the ovaries, documented by direct sampling of ovarian and adrenal venous blood[48,49]; suppression of serum testosterone and androstenedione concentrations in PCOS women treated with GnRH agonists, whereas dehydroepiandrosterone concentrations remains unaltered[50]; decreased serum androgen concentrations after ablation of ovarian interstitial tissue.[51]

An intrinsic ovarian defect has been postulated as a primary cause of PCOS. Thecal hypertrophy and overproduction of androgens are characteristic of the PCOS ovary.[52] Women with PCOS exhibit increased circulating levels of 17-OH progesterone, as well as increased ovarian 17-OH-progesterone response to stimulation with GnRH, GnRH-agonists, or human chorionic gonadotropin (hCG).[53] This exaggerated response suggests a dysregulated function of ovarian P450c-17-α-hydroxylase, an enzyme with 17-hydroxylase and 17,20-lyase functions. P450c-17-α-hydroxylase can be selectively induced to increase 17,20-lyase activity (leading to excessive androgen production) by serine phosphorylation.[54-56] Interestingly, almost 50% of women with PCOS have been reported to have an abnormally high level of serine phosphorylation of the insulin receptor β-subunit[13]; such insulin-receptor serine phosphorylation inhibits insulin receptor signaling. Thus, an abnormality of serine phosphorylation may account for both insulin resistance and excess androgen prodution in some women with PCOS.

Possibly because of increased 17α-hydroxylase activity and reduced aromatase activity (the latter abnormality will be discussed in more detail later in the article), serum concentrations of ovarian androgens (testosterone and androstenedione) are 50% to 150% higher in PCOS women than in control populations.[57-59] Further, serum concentration of sex hormone-binding globulin (SHBG) is decreased because of the inhibitory effect of hyperinsulinemia on SHBG production.[60] As a result, free testosterone concentration is increased out of proportion to total testosterone in women with PCOS.[40]

In a study performed in the United Kingdom, total testosterone and androstenedione concentrations in serum were similar in obese and non-obese women with PCOS, but serum SHBG concentrations were significantly lower and circulating free testosterone levels higher in obese compared with lean subjects. In addition, circulating concentrations of androsterone glucuronide, a marker of peripheral 5α-reductase activity, were higher in obese than in non-obese women.[60] These abnormalities may be due to hyperinsulinemia, which is more pronounced in the presence of obesity.

Adrenal Androgens and Cortisol

Many studies have demonstrated adrenocortical hyperfunction in PCOS. Adrenal uptake of I-131-iodocholesterol and serum concentration of dehydroepiandrosterone sulfate (DHEAS) are increased in 20% of women with PCOS.[61-63] The most common adrenocortical disturbance in this syndrome, present in about 50% of women with PCOS, is excessive DHEAS response to adrenocorticotrophic hormone (ACTH) stimulation.[63] Adrenal glands may also contribute to the excess of testosterone and androstenedione in PCOS, because administration of metyrapone to women with PCOS causes an excessive increase in serum levels of these hormones.[64]

One of the theories of the pathogenesis of PCOS involves a hypothesis that increased cortisol metabolism leads to a compensatory increase in corticotropin secretion, in order to maintain normal circulating cortisol concentrations. Increased corticotropin secretion, in turn, leads to increased adrenal production of androgens.[65] In support of this hypothesis, increased mean serum concentrations and pulse frequency and amplitude of both ACTH and cortisol during daytime were noted in PCOS patients.[66]

Some investigators have proposed that cortisol metabolism may be affected in patients with PCOS because of abnormal activity of 11β-HSD. 11β-HSD exists in 2 isoforms: type 1 is expressed in liver, gonads, and adipose tissue and in vivo acts as a reductase generating active cortisol from inactive cortisone. The type 2 11β-HSD is predominantly present in mineralocorticoid target tissues, such as placenta and kidney, and converts active cortisol to the inactive compound cortisone. In a study by Rodin et al,[67] oxidized cortisol metabolites were increased in patients with PCOS when compared with control subjects, suggesting dysregulation and increased activity of type 2 11β-HSD, possibly leading to excess adrenal androgen production via the increase in corticotropin activity. When adjusted for BMI, however, no significant differences were found in the level of cortisol metabolites among obese compared with nonobese PCOS patients.

Plasma cortisol concentrations in obese subjects have often been found to be lower than in lean individuals.[68,69] However, investigators have not been able to find consistently any difference between obese and non-obese subjects in cortisol response either to ACTH stimulation[70] or to hypoglycemia.[71] The number and affinity of glucocorticoid receptors on mononuclear leukocytes in women with PCOS, obese or non-obese, does not differ from that in control women.[72]

In summary, although the circulating levels of adrenal androgen dehydroepiandrosterone sulfate (DHEAS) in patients with PCOS are often increased, the reason for this abnormality and the contribution of obesity, if any, to its development remain unclear. There is no clear-cut evidence that abnormal cortisol metabolism is present in either obese or non-obese PCOS women.

Estrogen and Progesterone

Progesterone deficiency and acyclical and continuous estrogen production are typical of PCOS.[73] Women with PCOS exhibit chronic anovulation, and plasma levels of progesterone are low. Progesterone acts as an inhibitor of GnRH pulse frequency, and low circulating levels of progesterone in PCOS women may contribute to rapid LH pulse frequency and elevated serum LH concentrations.[73] Reduction in LH pulse fre-

quency has been demonstrated in PCOS patients given a vaginal progesterone compound[74] or estrogen-progesterone containing contraceptives.[75] LH pulse frequency was decreased to a greater degree in normal women compared with PCOS women, suggesting reduced hypothalamic sensitivity to ovarian steroid hormones in PCOS.[76]

Estrogen production in the ovary requires both theca and granulosa cells. Testosterone and androstenedione produced by theca cells are converted to estradiol and estrone by P450arom activity in granulosa cells. Aromatase cytochrome P450 (P450arom) is a unique member of a superfamily of microsomal enzymes that catalyze the rate-limiting step in the conversion of C19 androgens (testosterone and androstenedione) into C18 estrogens (estradiol and estrone) in a wide variety of tissues, including the ovary, testis, placenta, brain, and adipose tissue.

Aromatase activity of granulosa cells is a function of follicular size.[77] PCOS ovaries are characterized by multiple small follicles, which enter developmental arrest before selection of a dominant follicle.[40] Because of the lower activity of aromatase in smaller follicles, follicular fluid from ovaries of women with PCOS is characterized by low concentration of estradiol.[78] In rats with experimentally-induced hyperinsulinemia, however, systemic aromatase activity appears to be increased.[79] Therefore, low aromatase activity in ovarian cells of PCOS women suggests the presence of a local aromatase inhibitor that counteracts the stimulatory effect of insulin. Insulin-like growth factor-binding proteins (IGFBPs), among other molecules, have been proposed as candidates for the role of ovarian aromatase inhibitors.[40]

It has been suggested that reduced aromatase activity may be responsible for hyperandrogenism in PCOS women.[80] Aromatase knockout (ArKO) mice (both female and male) develop intra-abdominal obesity due to increased adipocyte size and number. Moreover, these mice exhibit lower lean body mass, hyperinsulinemia, and fatty liver.[81,82] These features, however, have not been consistently observed in humans with aromatase gene mutation,[83,84] a rare disorder characterized in females by pubertal virilization and multicystic ovaries.[85]

There are conflicting observations concerning the etiologic role of reduced aromatase activity in hyperandrogenism. For example, both troglitazone and meformin inhibit aromatase activity, but also reduce systemic hyperandrogenism.[86,87] Further, aromatase inhibitors have been used to induce ovulation in anovulatory women, including women with PCOS.[88] Thus, the role of reduced aromatase activity in the development of hyperandrogenism in PCOS remains controversial.

It has been demonstrated in both in vivo and in vitro studies that peripheral aromatase activity is positively correlated with adiposity.[89,90] Adipocytes exhibit variable levels of P450arom activity, depending on their location. The highest level of activity is present in the buttocks, next highest in the thighs, and the lowest in the subcutaneous abdominal tissue.[91] Aromatase activity is higher in women with lower body obesity than in those with upper body obesity.[92]

An increased peripheral conversion of androgens to estrogens, combined with decreased SHBG levels, results in increased circulating bioactive estrogen levels in obesity. These factors contribute to a state of functional hyperestrogenism in obese compared with lean PCOS patients.[93] High estrogenic activity, mostly due to elevated levels of estrone, may contribute to positive feedback on LH secretion, leading to enhanced stimulation of androgen production in obese PCOS patients.[94] This issue is controversial, however, given the lower mean LH serum concentrations in obese compared with lean PCOS women reported in some studies.[45,46]

In summary, peripheral aromatase activity in obese PCOS women may be increased, contributing to the hyperestrogenic state. On the other hand, reduction in ovarian aromatase activity in PCOS may contribute to hyperandrogenism. Further studies are needed to clarify the role of aromatase in the pathogenesis of PCOS in both obese and non-obese women.

Leptin

Several decades ago, investigators at The Jackson Laboratory postulated the presence of recessive mutations in obese (ob/ob) and diabetes (db/db) mice, which exhibited hyperphagia, morbid obesity, insulin resistance, hypercortisolism, and infertility.[95] Subsequently, Coleman et al,[96] through cross-circulation studies, demonstrated that the ob gene encoded a factor that decreased appetite and increased metabolism, while the db gene encoded a receptor for this factor. These observations were confirmed with the discovery of the ob gene through positional cloning in 1994.[97] The ob gene encodes a 167-amino acid protein that acts as a circulating feedback signal to the brain to regulate adipose mass. Based on its ability to reduce the body weight, ob gene product was named “leptin” (from the Greek root “leptos,” meaning “thin”). The human ob gene is located on chromosome 7q32 and consists of 3 exons and 2 introns.[97]

Leptin is synthesized in adipose tissue. Its synthesis is regulated by many factors, including the state of nutrition and a variety of hormonal influences.[95,98] Leptin circulates in plasma in free and bound form. It can be bound to plasma proteins (5% to 20%) or to complexes with soluble leptin receptors (Ob-R).[95] Multiple variants of Ob-R mRNA, encoding at least 6 different leptin receptor isoforms, have been identified. All Ob-R isoforms share an identical extracellular ligand-binding domain at the N-terminus, but differ at the C terminus. Ob-Rb, the long receptor isoform, is the only receptor isoform that is able to activate janus kinase signal transduction and translation (JAK/STAT) pathway, the signaling system for the intracellular portion of the leptin receptor.[95]

Obesity in animals defective in leptin or leptin-receptor genes (ob,db, fa, and cp rodents) resembles hypothalamic obesity induced by ventromedial lesions,[99,100] suggesting that the anorexic actions of leptin are exerted mainly at the level of the hypothalamus.

In common forms of human obesity (unlike in leptin-deficient ob/ob mouse), leptin mRNA and circulating protein levels are high, indicating leptin resistance in obese subjects.[101-103] Leptin resistance may be secondary to defects in leptin transport or abnormalities in leptin signaling.[104] Excessive amounts of insulin or high-fat diets may cause leptin resistance.[105,106] An acute increase in caloric intake causes an increase of circulating leptin up to 40% over baseline levels within 12 hours, without any change in BMI.[107] Starvation-induced reduction of

circulating leptin levels has been reported as well.[108] Insulin, glucocorticoids, and TNF-α stimulate leptin secretion.[95] The stimulatory effect of insulin on leptin synthesis, however, is seen only long-term.[109] It appears that under steady-state energy balance conditions circulating leptin concentrations reflect the amount of triglyceride stored in adipocytes, while in non-steady-state conditions, leptin acts as a sensor of energy balance.[95]

Almost a century ago, Marshall and Peel[110] observed that "over-fat" animals were sterile. The sterility was reversible with starvation.[110] In 1922, Evans and Bishop[111] reported that inadequate nutrition also affected reproduction. Frisch[112] observed that menarche occurs only when a certain "critical" mass of body weight is reached. The mechanism of the relationship between reproduction and body weight was a mystery until the discovery of leptin. Leptin treatment reverses the hypogonadism in ob/ob mouse independently of the effect on body weight.[113] Similarly, leptin ameliorates starvation-induced hypogonadism.[114] Moreover, leptin accelerates puberty in normal mice[115] and its circulating levels increase before puberty.[116-118] Thus, it has been proposed that leptin acts as a signal from adipose tissue to the brain indicating that the critical amount of fat mass necessary for initiation of puberty and maintenance of reproductive function has been reached. Although Ob-Rs have been found in the ovaries and the testes, it remains unknown whether the effect of leptin on reproduction is mediated by its central action (on hypothalamus), peripheral action (on gonads), or both.

Most studies have shown that serum leptin concentrations in women with PCOS do not differ from those in normal women with similar adiposity.[119-122] Besides, serum leptin concentrations did not correlate with circulating androgen levels in most studies.[119,120] Studies of the relationship between leptin and circulating insulin concentrations produced variable results, from no correlation[120,123] to positive correlation.[119,121] The same lack of consistency was seen in studies that examined correlation between leptin and circulating LH levels.[119-121,123]

Insulin sensitizers seem to have no effect on circulating leptin levels in women with PCOS if the weight of the subject remains stable. For example, troglitazone treatment did not affect the level of leptin in PCOS patients.[120] Metformin therapy reduced the level of leptin in both control women and in PCOS patients in some studies, but the values of circulating leptin levels in these studies were not corrected for weight reduction.[124,125]

In summary, the circulating concentrations of leptin in women with PCOS do not appear to differ from those in weight-matched, normal women. Thus, the role of leptin in PCOS and, particularly, in the development of hyperandrogenism in PCOS remains under debate.

Insulin and the IGF System

Endogenous insulin has a circulatory half-life of 3 to 5 minutes. The normal circulating concentration of insulin ranges from approximately 10 μU/mL in the fasting state to approximately 50 μU/mL postprandially. In insulin-resistant conditions, such as PCOS or type 2 diabetes, circulating insulin concentrations can be as high as 35 μU/mL in a fasting state and 180 μU/mL postprandially. In syndromes of extreme insulin resistance caused, for example, by a mutation in the insulin receptor gene, circulating insulin levels can be increased many-fold.[40]

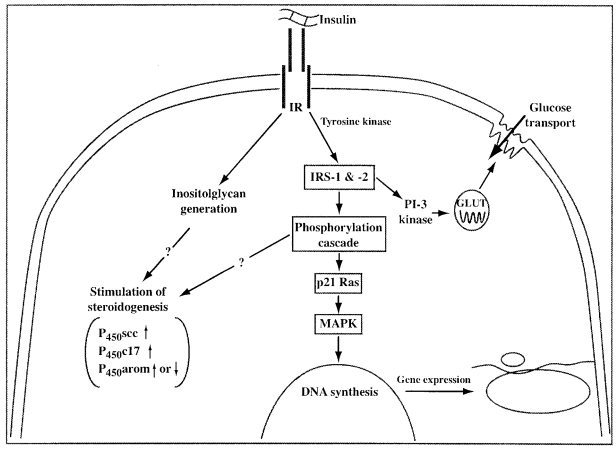

Fig 1. Insulin receptor and its signaling pathways. See text for details. (Adapted and reprinted from Poretsky et al[40] with permission. © The Endocrine Society.)

Insulin action is initiated by insulin binding to its receptor on the surface membrane of target cells. Insulin receptors are heterotetrameric glycoproteins composed of 4 subunits. Two larger α-subunits are extracellular and bind the insulin molecule, while two smaller β-subunits are mainly cytoplasmic and contain a tyrosine kinase, the main signaling component of the insulin receptor. Tyrosine kinase becomes activated following insulin binding to the α-subunits. A cascade of intracellular protein phosphorylation, beginning with two members of the insulin receptor substrate (IRS) protein family (IRS-1 and -2), is then initiated. Subsequent activation of phosphatidylinositol-3-kinase (PI-3 kinase) leads to increased glucose transport by glucose transporter-4 (GLUT-4) proteins.

Some actions of insulin may involve inositol-glycan second messengers, which are produced by the insulin-sensitive hydrolysis of a glycolipid in the plasma membrane. The inositolglycan pathway may participate in the mediation of insulin effects on ovarian steroidogenesis[40,126] (Fig 1).

As mentioned above, many women with PCOS are insulin resistant and therefore exhibit compensatory hyperinsulinemia.[127] The etiology of insulin resistance in PCOS is not completely understood. The defect of insulin receptor action appears to be at postbinding level and to involve a unique abnormality of postreceptor insulin signaling and glucose transport, distinct from that seen in patients with type 2 diabetes.[128]

Several molecular mechanisms have been suggested to explain the origin of insulin resistance in PCOS: lower content of GLUT4 glucose transporter,[129] excessive phosphorylation of insulin receptor on serine residues (which reduces signal transduction), or depletion of cellular adenosine.[130] Dunaif et al[130] demonstrated that many obese women with PCOS have defect in tyrosine autophosphorylation of the insulin receptor. It appears that the basal unstimulated phosphorylation is occurring

on serine, rather than tyrosine residues, and that the tyrosine phosphorylation is decreased.[128]

Archard and Thiers[131] original description of "diabetes of bearded women" in the 1920s suggested a link between insulin and ovarian function. In one of the first studies addressing the relationship between insulin resistance and hyperandrogenism, Burghen et al[132] observed that circulating insulin levels correlated with circulating testosterone levels in obese women with PCOS. Kitabchi et al[133] later found that the direct correlation between hyperinsulinemia and gonadal hyperandrogenemia might be a race-related phenomenon, possibly limited to African American women with PCOS.

The hypothesis suggesting that hyperandrogenism causes significant insulin resistance has been proposed. Indeed, chronic excessive use of androgen in powerlifters[134] or in female-to-male transsexuals[135] has been shown to decrease insulin sensitivity. A study involving use of oxymetholone, a testosterone-like compound, has demonstrated a positive correlation between its use and the presence of insulin resistance.[136] However, oophorectomy,[137,138] GnRH agonists,[139,140] or antiandrogenic agents[141] do not produce a significant change in insulin sensitivity in most studies despite eliminating hyperandrogenism in PCOS patients. Further, the degree of insulin resistance produced by androgens appears to be much less severe than that seen, for example, in women with hyperandrogenemia associated with syndromes of insulin resistance and acanthosis nigricans.[142,143] Finally, some PCOS women do not exhibit insulin resistance despite hyperandrogenism.[144]

Evidence of possible salutary effects of hyperandrogenism of adrenal origin, as opposed to ovarian origin, on insulin sensitivity was developed by Schriock et al.[145] Their study evaluated the insulin response to standard oral glucose tolerance test (OGTT) in 26 women, finding divergent correlations of circulating DHEAS and testosterone with insulin levels and insulin receptor binding to erythrocytes. The investigators concluded that, unlike testosterone, DHEAS might enhance insulin binding and action.

Thus, it appears that hyperandrogenism of ovarian origin may produce mild reduction of insulin sensitivity and may contribute to the development of insulin resistance in PCOS. However, because significant hyperandrogenism develops in women whose insulin resistance is clearly a primary phenomenon, for example, in women with mutations of insulin receptor or with insulin-receptor antibodies,[143,146] the hypothesis proposing that hyperinsulinemia may affect ovarian function has been developed.[142] Insulin receptors and IGF receptors are expressed in all types of ovarian cells, and multiple theories of effects of insulin and IGFs in the ovary have been proposed.[147,148]

In early studies, Channing et al[149] examined the effects of insulin on porcine granulosa cells. They found increased responsiveness of granulosa cells to gonadotropin stimulation in the presence of insulin. Other investigators have reported insulin-induced stimulation of androgen, estrogen, and progesterone production by both granulosa and theca cells.[150,151] In vitro, insulin directly stimulates ovarian androgen secretion via its effects on 17-α-hydroxylase, 17,20-lyase, and p450 enzymes.[152] Insulin also directly stimulates LH secretion from cultured pituitary cells and sensitizes gonadotrophs to GnRH stimulation.[59,153]

Despite the in vitro evidence that insulin can stimulate ovarian steroidogenesis, in vivo studies which examined the effects of acute administration of insulin on androgen production in normal women and in PCOS patients have rendered conflicting results (ranging from increased circulating androgen levels to no change to a decline in circulating androgens).[40] On the other hand, studies in which reduction of circulating insulin levels was achieved (such as those involving weight loss, metformin, thiazolidinediones, or diazoxide) have consistently reported a reduction in circulating androgen levels.[40]

The fact that supraphysiologic concentrations of insulin, often many-fold higher than found in PCOS individuals, are required in some in vitro studies to stimulate ovarian steroidogenesis, suggested that insulin may cross-react with type 1 IGF receptor to activate steroidogenesis in ovarian cells. Studies using anti-insulin receptor and anti-IGF-1 receptor antibodies, however, have demonstrated that insulin action in ovarian cells from both normal and PCOS women is usually mediated by the insulin receptor,[40,126] although nonclassical insulin receptor signaling mechanisms can be involved in the ovarian effects of insulin.[154] For example, PI-3 kinase, an enzyme whose activation is crucial for glucose transport, may not be needed for insulin-induced stimulation of progesterone synthesis or for inhibition of IGFBP-1 production in human ovarian cells.[154] Activation of mitogen-activated protein kinase (MAPK), another key enzyme which is phosphorylated following insulin receptor activation and which is responsible for the growth-promoting effects of insulin,[155] may not be necessary for some of the ovarian effects of insulin. In support of this hypothesis, a recent study addressing insulin and IGF-I signaling pathways involving MAPK, demonstrated that while MAPK participated in IGF-I-induced progesterone synthesis and inhibition of IGFBP-1 production in human granulosa cells, insulin-induced stimulation of progesterone or inhibition of IGFBP-1 did not require MAPK activation.[156] Involvement of alternate pathways of insulin action, as well as insulin activation of type I IGF-I receptor in cases of extreme hyperinsulinemia, explains why the ovary may remain sensitive to insulin, while classical organs for insulin action (liver, fat, and muscle) are insulin resistant.[157,158]

The IGF-system is closely related to insulin and participates in the regulation of ovarian function. In vitro studies have shown that IGF-I, a 70-amino acid polypeptide homologous with proinsulin, affects ovarian function in animal and human cells.[79] IGF-II, a 67-amino acid polypeptide with 70% homology with IGF-I and 50% homology with proinsulin, appears to be the principal IGF peptide in the human ovary, with effects similar to those of IGF-I. It appears that the effects of both IGF peptides (IGF-I and IGF-II) in the ovary are mediated by the type 1 IGF receptor.[40] Hyperinsulinemia may enhance IGF action in the ovary by upregulating ovarian type 1 IGF receptors.[40,79]

The activity of IGFs is modulated by low-molecular weight-binding proteins, called IGFBPs, which bind IGFs with high affinity. Synthesis of one of these proteins, IGFBP-1, is inhibited by insulin.[159] Hyperinsulinemia may contribute to hyperandrogenism through inhibition of ovarian IGFBP-1 synthe-

Table 2. Insulin Effects Related to Ovarian Function

Insulin Effect	Organ
Directly stimulates steroidogenesis	Ovary
Acts synergistically with LH and FSH to stimulate steroidogenesis	Ovary
Stimulates 17-α-hydroxylase	Ovary
Stimulates or inhibits aromatase	Ovary, adipose tissue
Upregulates LH receptors	Ovary
Promotes ovarian growth and cyst formation (synergistically with LH and hCG)	Ovary
Downregulates insulin receptors	Ovary
Upregulates type 1 IGF receptors or hybrid insulin/type 1 IGF receptors	Ovary
Inhibits IGFBP-1 production	Ovary, liver
Inhibits SHBG production	Liver
Potentiates the effect of GnRH on LH and FSH	Hypothalanmus, pituitary

Adapted and reprinted from Poretsky et al.[40] © The Endocrine Society.

sis,[160] because low intrafollicular levels of IGFBP-1 may lead to increased intraovarian concentration of unbound IGFs. Because circulating IGFBP-1 is derived from the liver, where its production is also under inhibitory control of insulin, serum IGFBP-1 levels are negatively correlated with insulin in PCOS patients.[161]. Thus, in the presence of hyperinsulinemia, both circulating and intraovarian concentrations of bioavailable IGFs are increased, possibly contributing to stimulation of steroidogenesis.[40]

Body weight affects the growth hormone (GH)/IGF-I system in PCOS patients. In non-obese PCOS patients (whose insulin resistance, although present, is not as significant as in obese PCOS women) IGF bioavailability seems to be increased, not only as a result of insulin-induced IGFBP-1 suppression, but also because of excessive GH-induced stimulation of hepatic IGF-I production.[40,162] Indeed, increased GH pulse amplitude has been found in lean PCOS women.[46] On the other hand, in obese patients, reduced GH pulse amplitude and 24-hour mean circulating GH levels have been reported.[94] This decrease in circulating GH levels is probably caused by decreased pituitary responsiveness to growth hormone-releasing hormone (GHRH) in obese PCOS patients.[46] The reduced circulating GH concentrations in obese PCOS women are probably related to obesity itself, as demonstrated by studies in obese patients without PCOS.[163] Further, increased GH clearance in obese subjects contributes to the decreased GH/IGF system activity.[164]

These findings have led to a hypothesis proposing that, although both hyperinsulinemia and excessively activated IGF system participate in the development of PCOS in non-obese and obese women, the IGF system is more important in the pathogenesis of PCOS in non-obese women, while hyperinsulinemia is more important for the development of PCOS in the presence of obesity[94] (Table 2).

OBESE AND NON-OBESE PCOS PATIENTS: DIFFERENCES IN CLINICAL MANIFESTATIONS

Onset of clinical symptoms in PCOS usually occurs perimenarcheally, often in association with obesity. Women with PCOS have a wide range of phenotypic manifestations, most likely because of underlying genetic differences in etiology.

A hallmark feature of PCOS is irregular, infrequent, and unpredictable uterine bleeding. Some patients experience bleeding less than 6 times per year, while in others, it may occur monthly, but with lack of ovulation.

The most common symptom in young women is excessive facial hair growth (hirsutism), followed by acne. These symptoms are present in about 80% of patients. The Ferriman-Gallwey score can be used to assess the degree of hirsutism (Fig 2).[165]

Many patients present with infertility due to anovulation.[1] Recent evidence suggests that there is increased prevalence of miscarriage among patients with PCOS.[166,167] In addition, the rate of pregnancy is lower than the rate of ovulation induction, which may be a reflection of inefficient fertilization in PCOS.[168]

Overall, approximately 50% of women with PCOS are obese.[169,170] As discussed above, obese women with PCOS typically exhibit an increased WHR characteristic of an android or central pattern of obesity.[171]

The prevalence of obesity in PCOS varies between studies and between ethnic groups. A prospective study from Spain, for example, reported that the prevalence of PCOS in Spain was similar to that in the US population, but the prevalence of obesity in Spanish PCOS women was considerably lower than in most studies from North America, with a mean BMI in Spanish patients of 23 kg/m^2.[172] Asian PCOS patients exhibit less obesity and hirsutism than their US counterparts, even though the prevalence of biochemical hyperandrogenism is similar in these 2 groups.[173]

Distinct clinical presentations observed in different ethnic groups of PCOS patients are sometimes attributed to the differences in the prevalence of obesity among populations. For example, a high prevalence of obesity in Pima Indians may produce features of PCOS in this population that are different from those of a lean Japanese population. A recent study concluded that both the prevalence and the degree of insulin resistance were higher in Mexican American women with PCOS than in Caucasian PCOS patients.[174]

The differences in biochemical and clinical features between obese and non-obese PCOS patients allow to determine, to some degree, the contributions of obesity to the clinical manifestations of PCOS (Table 3).

Differences in menstrual function have been reported, with

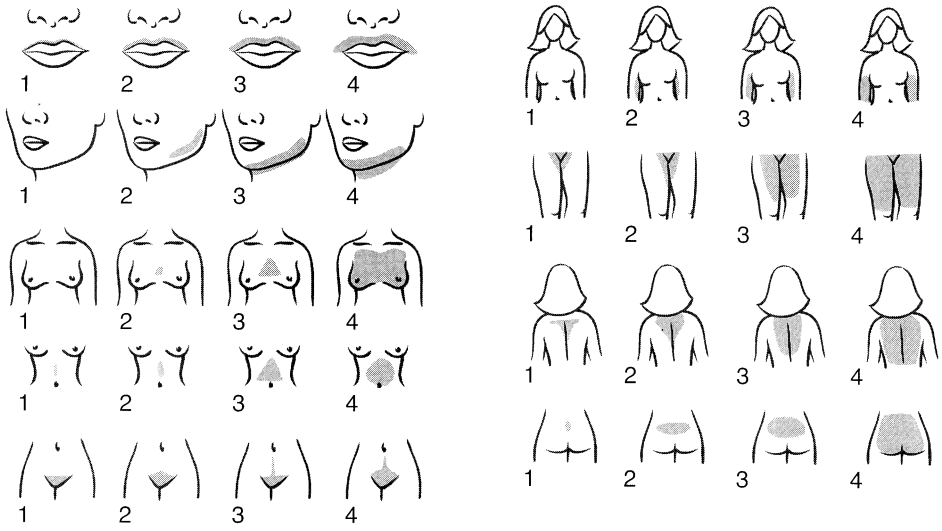

Fig 2. Grading the severity of hirsutism. Grade 1, minimal; grade 4, virilization. (Adapted and reprinted with permission.[58])

obese patients exhibiting a greater prevalence of oligoamenorrhea and anovulation than non-obese women.[94]

In a large study of 1,741 patients by Balen et al,[175] the presence of obesity was associated with an increased prevalence of infertility. This finding is consistent with the results of a recent study by Gill et al,[176] who reported higher rates of successful ovulation in response to pulsatile GnRH administration among lean PCOS patients than among obese PCOS subjects.

It is known that obesity has a direct relationship with the degree of hirsutism in PCOS patients. In a study performed in the United Kingdom in 1990, for example, obese women with PCOS had a greater prevalence of hirsutism than non-obese patients (73% compared with 56%).[60]

Stuart et al[177] found significant correlations between hyperandrogenism, insulin resistance, and the presence of acanthosis nigricans. On physical examination, acanthosis nigricans is found with greater frequency in obese than in non-obese patients with PCOS,[169,178] probably reflecting a higher prevalence and magnitude of insulin resistance among obese PCOS patients.

As discussed above, women with the PCOS have a higher prevalence and degree of both hyperinsulinemia and insulin resistance than weight-matched controls. Both lean and obese women with the syndrome have evidence of reduced insulin sensitivity, but insulin resistance is most marked when obesity is present.[171] Impaired glucose tolerance and type 2 diabetes mellitus are more prevalent in PCOS patients compared with weight-matched controls, with the highest risk in obese PCOS patients.[169] In a prospective study that included 254 PCOS patients, 31% of obese subjects had impaired glucose tolerance, and 7.5% had overt diabetes. By comparison, 10% of non-obese PCOS patients had impaired glucose tolerance, and 1.5% had diabetes, prevalence rates still 3 times higher than in an age-matched control population.[179]

Along with the increased risk for diabetes mellitus, the prevalence of dyslipidemia is increased in women with the syndrome. Elevated circulating levels of triglycerides, very–low-density lipoprotein (VLDL), and total cholesterol, accompanied by low levels of high-density lipoprotein (HDL), have been reported in PCOS. Dyslipidemia is present in both obese and non-obese patients, but higher BMI correlates with higher triglyceride levels.[180]

The association of PCOS with hypertension has not been firmly established. Initial studies noted higher blood pressure among PCOS patients, but the difference was not statistically significant after adjustment for BMI.[169] A cross-sectional study involving non-obese PCOS patients undergoing ambulatory blood pressure monitoring demonstrated no increase in the prevalence of hypertension.[181] Thus, the available evidence suggests that hypertension in patients with PCOS is related to obesity rather than to PCOS itself.

Overall, given the prevalence of risk factors for atherosclerosis in women with PCOS, a higher prevalence of cardiovascular events in these patients can be expected. Some studies suggest that this might be true,[182] but definitive, prospective

Table 3. Clinical and Biochemical Features of Obese Versus Non-Obese Patients With PCOS

Features	Obese PCOS	Lean PCOS
Hirsutism[60]	++	+
Total testosterone/androstenedione ratio[60]	↑	↑
Sex hormone-binding globulin[60]	↓	Normal
Free testosterone[60]	↑↑	↑
Androsterone glucuronide[60]	↑↑	↑
Oligomenorrhea/anovulation[94]	++	+
Infertility[175]	++	+
Acanthosis nigricans[169,178]	++	+
Insulin resistance[171]	++	+
IGT/diabetes mellitus[169,179]	++	+
Hyperlipidemia[180]	+	–
Hypertension[169,181]	+ (?)	–
Cardiovascular risk[182]	++ (?)	+ (?)
Endometrial carcinoma risk[9]	++	+

Abbreviation: IGT, impaired glucose tolerance.

Table 4. Selected Studies of Therapeutic Modalities and Their Effects in Obese Women With PCOS

Therapeutic Modality/ References	Year	Insulin Sensitivity	T	Free T	SHBG	Other
Weight loss						
Kiddy et al[190]	1992	↑	↔	↓	↑	
Guzick et al[191]	1994	↑	↓	↓	↑	
Huber-Buchholz et al[189]	1999	↑	↔	↔	↔	LH ↓
Metformin						
Velazquez et al[192]	1994	↑	↓	↓	↑	LH ↓
Acbay et al[195]	1996	↔	↔	↔	↔	
Nestler et al[152]	1996	↑	↓	↓	↑	LH ↓
Moghetti et al[194]	2000	↑	↓	↓	NS	
Pasquali et al[124]	2000	↑	↓	↓	↔	Leptin ↓
Arslanian et al[193]	2002	↑	↓	↓	↔	
Troglitazone						
Dunaif et al[196]	1996	↑	↓	↓	↑	LH ↓
Ehrmann et al[197]	1997	↑	↓	↓	↔	
Antiandrogens						
Diamanti-Kandarakis[204]	1995	↔	↔	↔	NS	DHEAS ↓
De Leo et al[203]	1998	↔	↓	↓	↑	LH ↓
Oral contraceptive agents						
Pasquali et al[206]	1999	↑	↓	NS	↑	
Cibula et al[207]	2001	↔	↓	NS	↑	

Abbreviations: NS, not studied; ↑, increase; ↓, decline; ↔, no change.

studies are lacking. Recent studies have demonstrated higher plasma homocysteine and tissue plasminogen activator (t-PA) concentrations[44,183] in PCOS subjects, suggesting that early development of atherosclerosis in this syndrome may not depend exclusively on abnormal lipid levels.

With obesity being a risk factor for endometrial carcinoma, obese PCOS patients have higher prevalence of this malignancy than non-obese PCOS women.[9] Anovulation, unopposed estrogen stimulation, and hyperinsulinemia may play a role in the increased risk of this gynecologic carcinoma in PCOS patients. The data for other malignancies in PCOS are less conclusive.

Recently, an association of PCOS with obstructive sleep apnea has been reported.[184] Even in lean PCOS subjects, the prevalence of obstructive sleep apnea was higher than in weight-matched controls. Obese PCOS patients had a higher prevalence of sleep apnea than non-obese patients (19% *v* 8%). The reason for the association between sleep apnea and PCOS remains unknown.[184]

The clinical course of pregnancy in PCOS patients is affected by the presence of obesity. Gjonnaess et al[185] and Urman et al[186] studied PCOS patients who conceived after ovarian electrocautery; an 8% prevalence of gestational diabetes was found, higher than the 2% to 5% seen in control populations. This increased prevalence of gestational diabetes was found only in overweight PCOS women, but not among non-obese PCOS patients.[185,186]

Obesity itself is a risk factor for preeclampsia and gestational non-proteinuric hypertension.[187] In the study by Gjonnaess mentioned above, an increased risk of preeclampsia was found among obese PCOS patients, but not among lean women with PCOS. On the contrary, in a retrospective analysis of 81 pregnant PCOS patients in the Netherlands, De Vries et al[188] found that an increased risk of preeclampsia was present even after adjustment for BMI. Thus, the respective roles of BMI and PCOS in the development of hypertension during pregnancy remain unclear.

In summary, the presence of obesity in PCOS has implications for some of the clinical features and biochemical abnormalities of the disease. Specifically, obesity appears to play a role in the prevalence of hirsutism, hyperandrogenism, menstrual irregularities and infertility, glucose intolerance and overt diabetes mellitus, endometrial carcinoma, the level of bioactive estrogen activity, and hypertension. In other areas, such as hyperlipidemia and pregnancy, the role of obesity is less clear. Prospective studies are needed to clarify whether the presence of PCOS, with or without obesity, represents a cardiovascular risk factor.

THERAPY OF PCOS: IMPACT OF OBESITY (Table 4)

Weight Loss

There have been numerous studies of the effects of weight loss in obese women with PCOS. Many of these studies, however, were uncontrolled, had a small sample size, and were conducted over a short period of time. Even with these limitations, the studies showed fairly consistently that weight loss resulted in the improvement of hormonal and menstrual parameters in obese PCOS patients. These benefits appear to be related to the improvement in insulin sensitivity that occurs with weight loss.

Huber-Buchholz et al[189] found a 71% improvement in insulin sensitivity index and a 33% reduction in fasting circulating insulin concentration in anovulatory obese PCOS women who responded to weight loss with ovulation. Kiddy et al[190] found a reduction in fasting circulating insulin concentrations in patients who achieved greater than 5% weight loss. Guzick et al[191] randomized 12 obese PCOS women to 12 weeks of

treatment with a weight-reduction diet or to 12 weeks with weight-maintaining diet. Fasting insulin levels declined markedly in the weight-loss group; there was no change of circulatory insulin concentrations in the control group.[191] Thus, the above studies demonstrated that calorie restriction leading to weight loss produces a reduction in circulating insulin levels in obese women with PCOS, suggesting an improvement in insulin sensitivity.

Improved insulin sensitivity is associated with reduction of hyperandrogenism. Kiddy et al[190] evaluated the effects of long-term calorie restriction in 24 obese PCOS women. Thirteen women who had lost more than 5% of weight had an increase in circulating concentrations of SHBG and a decrease in circulating free testosterone compared with 11 women who lost less than 5% of weight; the latter group exhibited no significant changes in these values.[190]

Confirming that weight loss is necessary to achieve an improvement in hyperandrogenism, Huber-Buchholz et al[190] evaluated obese women with PCOS who were treated with diet and exercise, but achieved no significant weight loss. The investigators found no statistically significant changes in the circulating concentrations of SHBG and free testosterone in these women.[189]

Nevertheless, hypocaloric diet, with or without weight loss, appears to be associated with an improvement of menstrual function in women with PCOS. In a study by Huber-Buchholz et al,[189] after a 6-month diet and exercise program that promoted a healthy lifestyle, but produced no rapid weight loss, 9 of 18 obese PCOS patients ovulated; of these, 2 became pregnant. In a study by Kiddy et al,[190] 9 of 11 patients who had lost at least 5% of weight had improvement in menstrual function, manifested by regular menstrual periods and conception. Guzick et al[191] evaluated serum progesterone values to assess ovulation in 6 obese PCOS women treated with weight reduction. Four of these women acheived ovulatory circulating progesterone levels compared with only 1 of 6 obese PCOS patients with no weight loss.[191]

Several groups of investigators have studied the effects of weight loss on serum LH concentrations and found inconsistent responses. Some studies showed no changes in either basal LH levels or in LH pulse frequency or amplitude,[190,191] even with improvement in reproductive function.[190] Alternatively, a 39% decline in circulating LH levels in obese PCOS women who responded to weight loss with ovulation was reported; women who did not resume ovulation did not exhibit significant changes in circulating LH concentration.[189]

In summary, most studies show that weight loss in obese women with PCOS leads to hormonal, menstrual, and metabolic improvement. Weight loss, especially more than 5% of the baseline weight, is associated with increased serum concentrations of SHBG and reduced serum concentrations of free testosterone in obese women with PCOS. The mechanism by which weight loss leads to a reduction of hyperandrogenism appears to involve improved insulin sensitivity with a resultant decline in circulating insulin levels. Exercise, even in the absence of weight loss, also has some beneficial effects, probably because of associated reduction in insulin resistance. Exercise may lead to changes in body fat distribution, with a decrease in abdominal fat and an increase in lean muscle mass, but with no change in weight.

Insulin Sensitizers

Metformin. Metformin, which belongs to the biguanide class of oral antihyperglycemic agents, has been shown to improve the metabolic, hormonal, and menstrual parameters in obese women with PCOS.

In the first major study of metformin in obese PCOS women, Velazquez et al[192] followed for 8 weeks 26 women with PCOS who had mean BMI of 29 kg/m^2. The women received 1,500 mg metformin daily. The effect of metformin on insulin secretion and glucose metabolism was assessed by an OGTT. After 8 weeks of treatment, there was no significant change in the area under the glucose curve, but there was a 35% decrease in the area under the insulin curve. The insulin area to glucose area ratio decreased by 31%, demonstrating an improvement in insulin sensitivity with metformin.[192] In a study by Arslanian et al,[193] 15 obese adolescents with PCOS and impaired glucose tolerance, diagnosed using an OGTT, were treated with 850 mg metformin twice a day for 3 months. After this course of treatment, OGTT parameters improved in 8 patients. The glucose area under the curve and the insulin area under the curve declined, but there were no differences in steady-state circulating glucose or insulin concentrations before and after treatment.[193] In a study of obese PCOS women treated with metformin, Nestler and Jacubowicz[152] demonstrated a reduction of the mean area under the insulin curve after oral glucose challenge.

Not all studies have shown consistent improvement of insulin sensitivity with metformin therapy. In a double-blind study by Moghetti et al,[194] 23 women were randomly assigned to treatment with metformin (1,500 mg/d) or placebo for 6 months. Eighteen of these patients and 14 additional patients were subsequently given metformin in an open trial for 11 months. The mean BMI was 30 kg/m^2. At baseline, both groups had similar fasting glucose and insulin plasma concentrations. After 6 months of metformin therapy, fasting plasma insulin concentration decreased in the metformin-treated group; there were no changes in the placebo group. Insulin sensitivity, assessed by a euglycemic-hyperinsulinemic glucose clamp, improved. There was, however, no statistically significant difference between circulating glucose or insulin levels before and after treatment when the patients underwent 2-hour OGTT.[194] Acbay and Gundogdu[195] followed 16 women with PCOS who were treated with placebo for 8 weeks followed by metformin for 10 weeks. Insulin sensitivity was assessed by an intravenous insulin tolerance test. There was no difference in BMI or in the areas under the curve for insulin or glucose in metformin versus placebo groups.[196]

As with weight loss, the reduction in insulin resistance achieved with metformin in most studies was associated with improved hormonal and menstrual parameters,[193,194] In the study by Velazquez et al,[192] at baseline, all patients had abnormal menstrual cycles, 90% exhibited hirsutism, and 85% had elevated circulating concentration of free testosterone. After treatment with metformin, mean circulating levels of free testosterone declined by 52%, total testosterone declined by 44%,

androstenedione declined by 29%, DHEAS declined by 21%, free androgen index was reduced by 49%, and LH declined by 35%. Concurrently, circulating SHBG concentration increased by 33% and FSH increased by 95%. Three patients became pregnant.[192]

Adrenal steroidogenesis can also be affected by metformin, suggesting that hyperinsulinemia may contribute to the increased adrenal androgen production in PCOS. In a study by Nestler et al,[152] 12 women with PCOS were randomly assigned to receive metformin, while 13 received placebo. In the metformin group, mean basal serum concentration of 17-α-hydroxyprogesterone decreased by 51% and peak 17-α-hydroxyprogesterone serum concentration after leuprolide injection decreased by 38%; no changes were observed in the placebo group.[152] After 3 months of metformin treatment, Arslanian et al[193] reported significantly lower plasma concentrations of androstenedione, 17-hydroxyprogesterone, and 17-hydroxypregnenelone in response to ACTH stimulation in women with PCOS.

Studies of the combined effects of metformin and hypocaloric diets leading to weight loss have been performed. Pasquali et al[124] evaluated the effects of combined hypocaloric diet and metformin in 40 obese PCOS patients. All patients followed a low-calorie diet for 1 month prior to being randomized to metformin or placebo in a double-blind design for 6 months. The metformin-treated group demonstrated improvement in menstrual cycles and hirsutism compared with the placebo group. Fasting plasma insulin concentrations declined significantly in both treatment arms, but glucose-stimulated insulin secretion decreased only in patients treated with metformin. The area under the curve for insulin decreased in PCOS patients treated with metformin, but not in the placebo arm. There were no changes in LH, FSH, DHEAS, or progesterone, but a decline in circulating total testosterone concentration in the PCOS patients treated with metformin was observed. Circulating leptin levels also decreased in patients treated with metformin in contrast to the placebo group.[124]

Kowalska et al[125] examined the combined effects of a hypocaloric diet and metformin in 23 obese PCOS patients, 19 obese patients without PCOS, and 11 normal-weight healthy women. The obese patients were treated with a hypocaloric diet and metformin for 4 to 5 months. Fasting insulin, LH, and testosterone serum concentrations decreased significantly only in obese PCOS patients treated with diet and metformin. Circulating leptin levels declined in both groups of PCOS patients, but not in normal-weight healthy women. Six of the 11 obese PCOS patients developed more regular menstrual cycles, and 2 patients conceived.[125] Both of these studies demonstrated that metformin therapy, combined with weight loss, could improve insulin sensitivity, hyperandrogenism, and menstrual irregularity in patients with PCOS.

In summary, treatment with metformin in most studies was associated with an improvement in insulin sensitivity. Improved insulin sensitivity, in turn, was associated with a reduction in circulating androgen levels and improvement in menstrual and ovulatory symptoms. However, not all studies have shown consistent results, with a few showing no improvement in insulin sensitivity with metformin treatment. Possible explanations for this discrepancy may be the variability in the doses of metformin and the effect of metformin on BMI. Metformin, used in conjuction with weight loss, appears to produce a more consistent effect on insulin sensitivity and androgen profile than metformin without weight loss.

Thiazolidinediones. The thiazoledinediones are a group of insulin-sensitizing medications commonly used in the treatment of type 2 diabetes mellitus. The effects of one thiazolidinedione, troglitazone, have been studied in PCOS patients. A study by Dunaif et al,[196] in which 25 women with PCOS were randomized to receive either 200 mg or 400 mg troglitazone and were compared with 12 normal (control) women, reported an improvement in insulin sensitivity and reduction in circulating DHEAS and estrone levels in the groups that received 200 mg or 400 mg troglitazone daily. The group that received 400 mg troglitazone daily also exhibited a decline in circulating free testosterone, androstenedione, and LH concentrations and an increase in circulating SHBG levels. In 2 patients, ovulatory menses resumed.[196] In a study of 13 obese women with PCOS who were treated with 400 mg troglitazone for 12 weeks, Ehrmann et al[197] reported a significant decrease in fasting and 2-hour plasma glucose concentration during OGTT, improvement in insulin sensitivity, and a decrease in total and free serum testosterone concentrations. Leuprolide-stimulated serum levels of 17-OH progesterone, androstenedione, and total testosterone were also significantly reduced with treatment.[198]

In a randomized, double-blind, controlled study of rosiglitazone and metformin in lean patients with PCOS, preliminary results showed a greater increase in ovulation with metformin alone versus rosiglitazone alone, and no greater benefit with combination therapy. Serum free testosterone decreased in the metformin group and also in the rosiglitazone group.[198] Mercado-Asis et al[199] reported that patients previously treated with troglitazone maintained normal menses when their therapy was changed to rosiglitazone, and newly diagnosed patients treated with rosiglitazone also had restoration of menses within 6 weeks of initiating treatment. In a preliminary report of a randomized, double-blind, placebo-controlled trial of 40 women with PCOS, administration of 30 mg pioglitazone for 3 months resulted in a reduction of fasting serum insulin levels, increased insulin sensitivity, reduction in free androgen index, increase in SHBG, and resumption of ovulatory function.[200]

It appears, therefore, that troglitazone has beneficial effects in obese PCOS women. Since troglitazone has been removed from the market because of liver toxicity, definitive studies with other thiazolidinediones need to be completed.

D-chiro-inositol. Inositol-phosphoglycans are involved in mediating insulin action.[202] It has been proposed that, at least in some patients, insulin resistance in PCOS may be due to a deficiency of D-chiro-inositol-phosphoglycan mediators.[201,202] Nestler et al[202] hypothesized that repletion of D-chiro-inositol stores would lead to improved insulin sensitivity. Forty-four obese women with PCOS were given either 1,200 mg D-chiro-inositol daily or placebo for 6 to 8 weeks. The treatment group demonstrated a significant reduction in the mean area under the insulin curve after oral glucose load, a 55% decrease in free testosterone, a 47% reduction in DHEAS, and reduction in circulating triglyceride levels; an increase in circulating SHBG concentrations was also observed. The placebo group demon-

strated none of these changes. There were no changes seen in basal LH concentrations in either group. Nineteen of the 22 women treated with D-chiro-inositol ovulated versus only 6 of the 22 patients in the placebo group.[202] Thus, D-chiro-inositol holds promise for the treatment of insulin resistance and hyperandrogenism in patients with PCOS.

Antiandrogens

Antiandrogens, such as flutamide or spironlactone, are beneficial in the treatment of the hyperandrogenism associated with PCOS and may improve insulin sensitivity. In a study of 8 teenagers given flutamide for 6 months, De Leo et al[203] reported significant reductions in circulating levels of LH, androstenedione, and free and total testosterone, an increase in serum SHBG, and resumption of regular menses. In a study by Diamanti-Kandarakis et al,[204] 10 obese and 8 lean PCOS women and 13 lean control women were treated with flutamide, 250 mg/d, for 3 months. Patients in the flutamide-treated group exhibited significant improvement in hirsutism and reduction in circulating androstenedione and DHEAS levels, in contrast to the control group. There were no changes in circulating free testosterone levels and no improvement in insulin sensitivity in either group.[204] In a study of hyperandrogenic patients treated with anyone of 3 agents (spironolactone, flutamide, or buserelin) Moghetti et al[205] found an improvement in insulin sensitivity, with lean patients responding better than obese patients.

Oral Contraceptive Agents

Oral contraceptive agents have been used extensively in the treatment of PCOS. Pasquali et al[206] studied 37 obese patients with PCOS approximately 10 years after their initial evaluation. Sixteen women took estrogen-progesterone combination and 21 women did not. In the treatment group, circulating basal insulin levels and fasting C-peptide levels decreased, but no change was found in the insulin area under the curve during OGTT in comparison to baseline. The control group exhibited no changes in basal insulin levels, but demonstrated an increase in the insulin area under the curve and in both fasting and stimulated C-peptide levels compared with baseline. Serum SHBG concentrations increased only in the estrogen-progesterone group, but there were no changes in total testosterone in either group. Thus, this study demonstrated that estrogen-progesterone combination can enhance insulin sensitivity and that, if left untreated, insulin resistance in obese PCOS patients tends to progress.[206] In a comparison of combined oral contraceptives in lean versus obese PCOS women, Cibula et al[207] found significant improvements in hyperandrogenism in lean PCOS patients with only a moderate improvement in the obese PCOS patients.

Estrogen has been combined with the antiandrogen cyproterone acetate. Dahlgren et al[208] compared the effects of ethinylestradiol plus cyproterone versus the antiandrogen goserelin in 32 women with PCOS over a 6-month period. In the ethinylestradiol-cyproterone-treated group, serum androgen concentrations decreased and hirsutism improved, but there was a reduction in insulin sensitivity. Patients treated with goserelin had an improvement in both circulating androgen levels and insulin sensitivity. The investigators speculated that the estrogen component of the combined therapeutic regimen was responsible for the reduction in insulin sensitivity.[208]

In a study comparing metformin versus ethinylestradiol plus cyproterone in obese PCOS women, Morin-Papunen et al[209] reported a reduction in circulating insulin concentrations, improvement in glucose utilization, and improvement in insulin sensitivity in the metformin-treated group, in contrast to an increase in the glucose area under the curve and no improvement in insulin sensitivity in the ethinylestradiol-cyproterone-treated group.

Thus, oral contraceptives may improve the androgen profile of PCOS patients, with a greater benefit in lean than obese PCOS patients; their effects on insulin sensitivity are controversial.

Summary

In summary, many studies assessing the role of diet, exercise, and multiple therapeutic agents in the treatment of PCOS have been published (Table 4). Although many of these studies are not double-blind, randomized, or long-term, they show benefits of treating obese women with PCOS with a variety of approaches. Because insulin resistance and hyperinsulinemia may contribute to the pathogenesis of PCOS, weight loss as first-line therapy will benefit obese patients with PCOS by reducing circulating insulin concentration and, consequently, androgen levels, and producing a resumption of regular ovulatory periods. Metformin can be suggested as a second-line treatment for most obese women with PCOS. Further studies of D-chiro-inositol in PCOS women will demonstrate whether this agent is safe and effective for the treatment of insulin resistance and hyperandrogenism in PCOS patients. Antiandrogens and oral contraceptives are effective in reducing androgen levels, but their effects on insulin sensitivity are inconsistent. The effects of rosiglitazone, pioglitazone, and other thiazolidinediones in PCOS patients need to be studied in detail.

CONCLUSIONS

PCOS is a common disorder, which, in all likelihood, is etiologically diverse. Obesity is present in approximately 50% of PCOS patients. The differences in clinical manifestations between obese and non-obese PCOS patients are mostly quantitative in nature. Although obese PCOS patients do not exhibit manifestations that are not seen in non-obese women with PCOS, obesity contributes to the manifestations of PCOS by increasing the magnitude of hyperandrogenism and by increasing the rates of anovulatory cycles and infertility.

The pathophysiologic mechanisms by which obesity makes these contributions to the clinical picture of PCOS appear to be related to hyperinsulinemia which, in turn, is induced by insulin resistance. Although insulin resistance is present in both obese and non-obese PCOS patients, the magnitude of both insulin resistance and hyperinsulinemia is greater in obese than in non-obese women with PCOS. Hyperinsulinemia impacts ovarian function and morphology not only by stimulating androgen production directly and in synergism with gonadotropins, but also by activating the

ovarian IGF-system (specifically by inducing expression of ovarian-type 1 IGF receptors and by inhibiting IGFBP-1 production in both liver and ovary), by inhibiting SHBG production in the liver, and by contributing to ovarian growth and cyst formation.[40,210] Therapeutic modalities directed at the reduction of hyperinsulinemia (weight loss or insulin-sensitizing agents) appear to ameliorate symptoms of PCOS and restore normal ovarian function in obese women with PCOS.

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