The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited

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Polycystic ovary syndrome (PCOS) was hypothesized to result from functional ovarian hyperandrogenism (FOH) due to dysregulation of androgen secretion in 1989–1995. Subsequent studies have supported and amplified this hypothesis. When defined as otherwise unexplained hyperandrogenic oligoanovulation, two-thirds of PCOS cases have functionally typical FOH, characterized by 17-hydroxyprogesterone hyperresponsiveness to gonadotropin stimulation. Two-thirds of the remaining PCOS have FOH detectable by testosterone elevation after suppression of adrenal androgen production. About 3% of PCOS have a related isolated functional adrenal hyperandrogenism. The remaining PCOS cases are mild and lack evidence of steroid secretory abnormalities; most of these are obese, which we postulate to account for their atypical PCOS. Approximately half of normal women with polycystic ovarian morphology (PCOM) have subclinical FOH-related steroidogenic defects. Theca cells from polycystic ovaries of classic PCOS patients in long-term culture have an intrinsic steroidogenic dysregulation that can account for the steroidogenic abnormalities typical of FOH. These cells overexpress most steroidogenic enzymes, particularly cytochrome P450c17. Overexpression of a protein identified by genome-wide association screening, differentially expressed in normal and neoplastic development 1A.V2, in normal theca cells has reproduced this PCOS phenotype in vitro. A metabolic syndrome of obesity-related and/or intrinsic insulin resistance occurs in about half of PCOS patients, and the compensatory hyperinsulinism has tissue-selective effects, which include aggravation of hyperandrogenism. PCOS seems to arise as a complex trait that results from the interaction of diverse genetic and environmental factors. Heritable factors include PCOM, hyperandrogenemia, insulin resistance, and insulin secretory defects. Environmental factors include prenatal androgen exposure and poor fetal growth, whereas acquired obesity is a major postnatal factor. The variety of pathways involved and lack of a common thread attests to the multifactorial nature and heterogeneity of the syndrome. Further research into the fundamental basis of the disorder will be necessary to optimally correct androgen levels, ovulation, and metabolic homeostasis. **(*Endocrine Reviews* 37: 467–520, 2016)**

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Abbreviations: AE-PCOS, Androgen Excess-PCOS Society; AMH, anti-Müllerian hormone; BMI, body mass index; BMP, bone morphogenetic protein; CYP17, cytochrome P450c17; DAST, dexamethasone androgen-suppression test; DENND, differentially expressed in normal and neoplastic development; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; DM, diabetes mellitus; FAH, functional adrenal hyperandrogenism; FOH, functional ovarian hyperandrogenism; GDF, growth differentiation factor; GnRHag, GnRH agonist; GTT, glucose tolerance test; GWAS, genome-wide association studies; hCG, human chorionic gonadotropin; Hippo, hippopotamus; HOMA-IR, insulin resistance by homeostatic model assessment; 3βHSD2, Δ5-isomerase-3β-hydroxysteroid dehydrogenase type 2; IGT, impaired glucose tolerance; MNC, mononuclear cell; NGF, nerve growth factor; NIH criteria, National Institutes of Health 1990 conference diagnostic criteria; NOM, normal ovarian morphology; 17OHP, 17-hydroxyprogesterone; OSA, obstructive sleep apnea; PCOM, polycystic ovarian morphology; PCOS, polycystic ovary syndrome; PCOS-A, functionally atypical PCOS; PCOS-T, functionally typical PCOS; POR, cytochrome P450-oxidoreductase; RD, reductase; SDAST, short DAST; SHBG, sex hormone-binding globulin; SNS, sympathetic nervous system; SULT2A1, steroid sulfotransferase; VEGF, vascular endothelial growth factor; V-NOM, volunteer with NOM.

I. Historical Perspective

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in reproductive aged women, with a prevalence between 5% and 15%, depending on the diagnostic criteria applied (1, 2). PCOS was first described by Stein and Leventhal as a syndrome of oligo-amenorrhea and polycystic ovaries that was variably accompanied by hirsutism, acne, and obesity (3, 4). Demonstration of polycystic ovaries became required for PCOS diagnosis, which required gynecologic expertise, yet polycystic ovaries were found to be variably associated with the signs and symptoms that characterize the disorder (5).

Seminal contributions to our understanding of PCOS pathogenesis began with the 1958 report that urinary LH was elevated by bioassay in the 4 cases studied (6). The 1970 documentation by RIA that serum LH and the ratio of LH to FSH were typically high (7) led both to the adoption of altered gonadotropin secretion as an alternative diagnostic tool and to a focus of research on the putative neuroendocrine genesis of the syndrome. Shortly thereafter, plasma free testosterone was recognized as a marker for hyperandrogenism in hirsute amenorrheic women; subsequent studies suggested the hyperandrogenemia was of ovarian origin (8). During the 1980s, administration of testosterone to female-to-male transsexuals was found to cause polycystic ovaries (9), and ultrasonographic criteria for the identification of polycystic ovarian morphology (PCOM) were developed (10).

Meanwhile, significant insulin resistance was recognized to be related to hyperandrogenism and acanthosis nigricans (11) and to occur independently of obesity in the syndrome (12, 13). In vitro studies subsequently showed that insulin stimulates ovarian androgen production (14), particularly in synergy with LH (15, 16). These studies raised the possibility that hyperinsulinemia contributes to ovarian androgen excess.

In 1989, we published evidence that a GnRH agonist (GnRHag) test, which stimulates the coordinated function of the ovarian follicle in response to endogenous LH and FSH release, disclosed a previously unrecognized form of hyperandrogenism in women with classic PCOS: generalized ovarian steroidogenic hyperresponsiveness (17). 17-Hydroxyprogesterone (17OHP), and to a lesser extent androstenedione, responses were most consistently abnormal, and there was no evidence of a steroidogenic block. This suggested abnormal regulation (dysregulation) of 17-hydroxylase and 17,20-lyase activities, which are 2 activities of the single enzyme cytochrome P450c17 (CYP17) encoded by *CYP17A1* (17, 18).

We then found that most hyperandrogenic women (two-thirds of those with oligo-amenorrhea, 30% of eumenorrheic ones) had this type of androgenic ovarian dysfunction and that this was independent of serum LH elevation or PCOM in about half of cases (19, 20). This abnormality was termed functional ovarian hyperandrogenism (FOH), because the steroidogenic disorder is gonadotropin dependent (ie, any treatment that suppresses gonadotropin production suppresses androgen production), and there is not a requisite anatomic basis for the disorder.

This research, suggesting that PCOS was usually a form of FOH due to dysregulation of androgen secretion, gen-

erated a paradigm shift in 2 ways. First, the ovary was identified as the source of the disorder rather than the target of a neuroendocrine disturbance. Second, a steroidogenic disorder was attributed to enzyme dysregulation rather than deficient enzyme activity. This research also contributed to the subsequent redefinition of PCOS as otherwise unexplained hyperandrogenic anovulation, irrespective of the presence of polycystic ovaries or LH elevation, ie, National Institutes of Health 1990 conference diagnostic criteria (NIH criteria) (21).

These and subsequent studies by ourselves and others extended and confirmed these pathophysiologic findings and were summarized in a 1995 *Endocrine Reviews* paper (22). The evidence was consistent with the ovarian hyperandrogenism being a functional abnormality that requires normal adult LH levels but not LH elevation.

Furthermore, despite resistance to the effects of insulin on glucose metabolism in target tissues such as muscle, the ovary seemed to be responsive to the synergistic effect of hyperinsulinemia and LH on ovarian androgen secretion. The FOH of PCOS was ordinarily "primary," ie, not secondary to any known disorder, although unusual cases can be caused by severe insulin resistance, such as that arising from mutations of the insulin receptor, or by congenital virilizing disorders (22).

Subsequent studies have supported and amplified this hypothesis. Particularly noteworthy is the evidence that the disorder ordinarily is due to an intrinsic disorder of ovarian function (23) and the evidence for ovarian-sparing tissue-specific differences in insulin resistance in PCOS and obesity (24, 25). It is the purpose of the current review to update the evidence regarding the pathogenesis of PCOS and emphasize how new data are providing insights into diagnosis and treatment of the disorder. A review of the literature in English through April 2016 was conducted via PubMed, and data were summarized and integrated from the authors' perspectives.

II. Definition of PCOS

Two international consensus conferences have developed adult diagnostic criteria that widen the definition beyond NIH criteria (21) by incorporating the presence of PCOM, defined by consensus (26), as a diagnostic criterion for PCOS (Table 1). Rotterdam criteria are the broadest and encompass all combinations of otherwise unexplained clinical or biochemical evidence of hyperandrogenism, evidence of oligo-anovulation, and PCOM (27). Androgen Excess-PCOS Society (AE-PCOS) criteria (2006) encompass otherwise unexplained hyperandrogenism with either oligo-anovulation or PCOM (28); this allows a diagnosis of PCOS in women with hyperandrogenism who lack anovulatory symptoms ("ovulatory PCOS"), which comprises about 10% of cases.

Table 1. Diagnostic Criteria for PCOS

Adult Diagnostic Criteria (Rotterdam)

Otherwise unexplained alternative phenotypes:

1. Phenotype 1 (classic PCOS)[a]
   a. Clinical and/or biochemical evidence of hyperandrogenism
   b. Evidence of oligo-anovulation
   c. Ultrasonographic evidence of a polycystic ovary
2. Phenotype 2 (Essential NIH Criteria)[a]
   a. Clinical and/or biochemical evidence of hyperandrogenism
   b. Evidence of oligo-anovulation
3. Phenotype 3 (ovulatory PCOS)[a]
   a. Clinical and/or biochemical evidence of hyperandrogenism
   b. Ultrasonographic evidence of a polycystic ovary
4. Phenotype 4 (nonhyperandrogenic PCOS)
   a. Evidence of oligo-anovulation
   b. Ultrasonographic evidence of a polycystic ovary

Adolescent Diagnostic Criteria

Otherwise unexplained combination of:

1. Abnormal uterine bleeding pattern
   a. Abnormal for age or gynecologic age
   b. Persistent symptoms for 1–2 y
2. Evidence of hyperandrogenism
   a. Persistent testosterone elevation above adult norms in a reliable reference laboratory is the best evidence
   b. Moderate-severe hirsutism is clinical evidence of hyperandrogenism

Modified from Rosenfield, The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*. 2015;136:1154–1165 (50).

[a] AE-PCOS recognizes only hyperandrogenic phenotypes.

An independent panel reviewed the evidence through 2012 in an international workshop and recommended that Rotterdam criteria be adopted with specific identification of phenotype, of which there are 4 (29), listed next in order of decreasing clinical severity, which corresponds to decreasing specificity of the milder phenotypes (Table 1) (30–37). Phenotype 1 is the classic combination of all the reproductive endocrine features of the syndrome, namely evidence of hyperandrogenism, oligo-anovulation, and PCOM. Phenotype 2 is the combination of hyperandrogenism and oligo-anovulation (the essential NIH criteria). Phenotype 3 is the combination of hyperandrogenism and PCOM in the absence of oligo-anovulation (ovulatory PCOS), as endorsed by AE-PCOS. Phenotype 4 is the combination of oligo-anovulation and PCOM (nonhyperandrogenic PCOS). Although insulin resistance and obesity are common in PCOS, they are not recognized as diagnostic criteria. Likewise, alterations in gonadotropin secretion are not included in any of the definitions of the syndrome.

Hyperandrogenism severity decreases in these successive phenotypes, as does, in most populations, the severity

of insulin resistance, obesity, and LH elevation, with ethnic and environmental factors playing a role (30, 34–36). The hyperandrogenic phenotypes 1–3 have ovulatory dysfunction ranging successively from severe to nil, whereas phenotype 4 is anovulatory but lacks evidence of hyperandrogenism.

Phenotypes 3 and 4 are successively less specific and successively more contentious. Phenotype 3 permits a PCOS diagnosis in the presence of PCOM in mildly hirsute females with normal serum androgen levels (ie, those with hirsutism scores up to 2-fold above the normal upper limit), who would be considered to have idiopathic hirsutism according to The Endocrine Society Clinical Practice Guidelines (38), or in apparently normal asymptomatic women with subclinical hyperandrogenemia (39), as reviewed below (see section IV.B.2). The lack of hyperandrogenism in phenotype 4 makes it particularly debatable, and it is not considered here; many seem to have functional hypothalamic amenorrhea (40), and it is not recognized as constituting PCOS by AE-PCOS.

Weaknesses of these criteria have emerged. First, the documentation of hyperandrogenemia can be difficult: serum testosterone concentration undergoes episodic, diurnal, and cyclic variation (41) and attains mature levels approximately 1 year after menarche (42). Furthermore, methodologic problems in commercial testosterone assays have emerged (43, 44). Consequently many steroid assays are inaccurate, and the best steroid assays differ from one another modestly but significantly (45–48). For these reasons, hirsutism is often considered the clinical surrogate of hyperandrogenemia although half of mild hirsutism and a small proportion of moderate-severe hirsutism (hirsutism score >2-fold above normal upper limit) are not associated with hyperandrogenemia (38). Second, the consensus sonographic definition of PCOM (26) is prone to lead to overdiagnosis, particularly as it applies to antral follicle count criteria determined by the current generation of high-definition imaging techniques (49) and as it applies to adolescents and young women (30, 50). In adolescents, anovulatory criteria must be age- and pubertal stage-appropriate, and the paucity of normative data obviates the routine use of PCOM as a diagnostic criterion (Table 1) (30, 50, 51). Furthermore, all these criteria overlook the potential presence of the PCOS type of FOH in patients who present with hirsutism, obesity, or insulin-resistance signs such as acanthosis nigricans, but who lack clinical evidence of ovarian dysfunction (19, 52, 53).

Finally, the hyperandrogenism of PCOS improves during middle age, which is sometimes accompanied by normalization of menstrual regularity (54, 55). These changes seem related to the fall in follicle number during the premenopausal transition, which is accompanied by falling serum inhibin-B and rising FSH levels that maintain estradiol secretion (56). Although hyperandrogenism may remit during menopause (54), lifelong metabolic dysfunction persists and may increase postmenopausal cardiovascular disease risk (57). Criteria for the diagnosis of postmenopausal PCOS remain to be defined.

III. Normal Androgen Physiology

Understanding of the normal biochemical and molecular basis of steroidogenesis and of normal androgen physiology is necessary to understand the pathophysiology of PCOS.

Under normal circumstances, the ovaries and adrenal glands contribute about equally to testosterone production (58–60). Approximately half of testosterone originates from direct testosterone secretion by the ovaries and adrenal glands, whereas half is produced by peripheral conversion of circulating androstenedione, which itself arises from approximately equal ovarian and adrenal secretion.

Androgen production is not under direct negative feedback regulation by the neuroendocrine system in females, as is the case for estradiol and cortisol secretion (8, 61). Indeed, modest androgen excess interferes with female sex hormone negative feedback according to recent research (see section V.C).

Androgens are secreted by both the ovaries and adrenal glands in response to their respective tropic hormones, LH and ACTH. Intraglandular paracrine and autocrine mechanisms seem to play a major role in modulating androgen secretion in response to tropic hormone stimulation.

A. Biochemical and molecular overview of steroidogenesis

The rate-determining step for the formation of all steroid hormones in response to tropic hormones in both the gonads and adrenal glands is cholesterol side-chain cleavage, which is mediated by the enzyme cytochrome P450scc (encoded by *CYP11A*) (62). The ovarian steroidogenic response to LH is slow in the early follicular phase, and is accelerated in the luteinized preovulatory follicle as LH induces the steroidogenic acute regulatory protein, which delivers cholesterol into mitochondria (63).

Cytochrome P450c17 (*CYP17A1*) is the rate-limiting enzyme for the formation of androgens in the gonads and adrenal cortex (Figure 1) (22, 64). Its expression is absolutely dependent upon tropic hormone stimulation, LH in the ovary (65, 66) and ACTH in the adrenal cortex (67, 68), in a dose-dependent manner. This one enzyme possesses both 17-hydroxylase and 17,20-lyase activities. The

Figure 1.

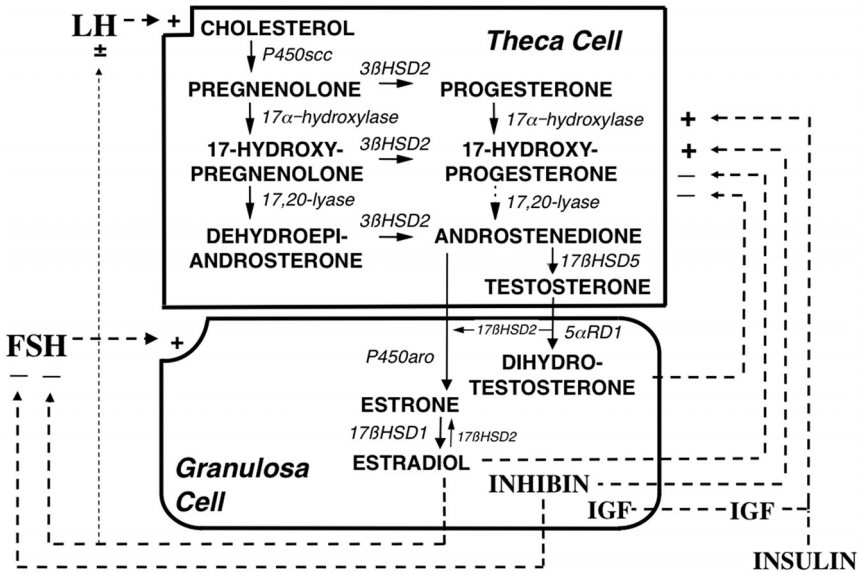

Figure 1. Depiction of the organization and regulation of the major steroid biosynthetic pathways in the small antral follicle of the ovary according to the 2-gonadotropin, 2-cell model of ovarian steroidogenesis. LH stimulates androgen formation within theca cells via the steroidogenic pathway common to the gonads and adrenal glands. FSH regulates estradiol biosynthesis from androgen by granulosa cells. Long-loop negative feedback of estradiol on gonadotropin secretion does not readily suppress LH at physiologic levels of estradiol and stimulates LH under certain circumstances. Androgen formation in response to LH appears to be modulated by intraovarian feedback at the levels of 17-hydroxylase and 17,20-lyase, both of which are activities of cytochrome P450c17 that is expressed only in theca cells. The relative quantity of androstenedione formation via 17OHP (dotted arrow) in the intact follicle is probably small, as is the amount of progesterone formed from granulosa cell P450scc activity in response to FSH (data not shown). 17βHSD2 activity is minor in the ovary, and estradiol is primarily formed from androstenedione. Androgens and estradiol inhibit (minus signs) and inhibin, insulin, and IGF-1 (IGF) stimulate (plus signs) 17-hydroxylase and 17,20-lyase activities. Pertinent enzyme activities are italicized: the 17-hydroxylase and 17,20-lyase activities of P450c17 are shown, otherwise enzyme abbreviations are as in the text. Modified with permission from Ehrmann et al, Polycystic ovary syndrome as a form of functional ovarian hyperandrogenism due to dysregulation of androgen secretion. *Endocr Rev*. 1995;16:322–353 (22).

first of these activities, 17-hydroxylase, is necessary for the formation of cortisol in the adrenal cortex and the potent sex steroids in the gonads. The second of these activities, 17,20-lyase, less efficiently acts sequentially on enzyme-bound 17-hydroxylated substrate to form 17-ketosteroids, eg, dehydroepiandrosterone (DHEA) or androstenedione. These 17-ketosteroids are, in turn, the precursors of all potent sex steroids in the gonads and adrenal zona reticularis. Specifically, P450c17 mediates conversion of pregnenolone by 17-hydroxylation to form 17-hydroxypregnenolone, which is transformed by 17,20-lyase activity to DHEA. DHEA is then converted by Δ5-isomerase-3β-hydroxysteroid dehydrogenase type 2 (3βHSD2) (*HSD3B2*) to androstenedione. Progesterone undergoes parallel P450c17 17-hydroxylation to 17OHP. Although 17OHP is a poor substrate for P450c17 17,20-lyase activity in theca cells or cell-free systems, formation of androstenedione has been documented in ovarian and adrenal homogenates and minces (69–71), compatible with the possibility that either paracrine interactions with granulosa cells may up-regulate 17,20-lyase activity for this substrate or that another enzyme accounts for this activity (22).

The differential regulation of the 2 enzyme activities of P450c17 is incompletely understood. The preferential formation of androgen by P450c17 of the gonads and adrenal zona reticularis is largely dependent on 3 posttranslational factors that up-regulate its 17,20-lyase activity (64). First, this activity is especially sensitive to the molar abundance of the electron-transfer protein cytochrome P450-oxidoreductase (POR). Second, cytochrome *b5* (*CYB5A*) strongly promotes 17,20-lyase activity, principally by acting as an allosteric factor promoting the interaction of P450c17 with POR. Third, the serine/threonine phosphorylation of P450c17 itself by a specific MAPK (p38α) appears to selectively promote 17,20-lyase activity by also promoting the interaction of P450c17 with POR.

Androstenedione is the major precursor for both testosterone and estrogen synthesis in the ovaries (Figure 1) and adrenal cortex (Figure 2) (62). In the ovaries it is in part converted in theca cells by 17βHSD5 (*HSD17B5*; also termed α-ketoreductase type 1C3 [*AKR1C3*]) to form testosterone (72) and is in part aromatized in granulosa cells by cytochrome P450aro (*CYP19A1*) to form estrone. Androstenedione predominates over testosterone as the aromatase substrate, because it is available in 10-fold greater amounts (60, 62, 72–75). Estrone is then converted to estradiol by 17βHSD1 (*HSD17B1*).

Androgens are preferentially metabolized to dihydrotestosterone rather than estradiol in small ovarian follicles, before follicle selection, because of high steroid 5α-reductase (5αRD) (*SRD5A*) activity (76). This is carried out by both type 1 and 2 5αRD isozymes in theca, stroma, and granulosa cells, but the predominant reaction is type 1 activity in granulosa cells (77). 17βHSD2 reconversion

Figure 2.

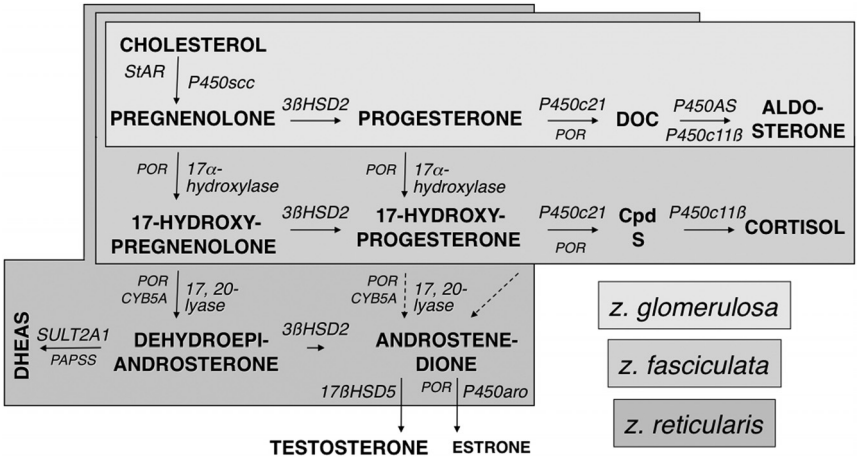

Figure 2. Depiction of the organization of the major steroid biosynthetic pathways in the adrenal cortex. The top row shows the pathway to aldosterone; the middle row shows the zona fasciculata pathway to cortisol; the lowest, darkly shaded row shows the zona reticularis steps to 17-ketosteroids that are not expressed in the other adrenal zones. Note similarities between the biosynthetic capacities of the zona reticularis and that of ovarian theca cells. Dotted pathways are minor. The zona reticularis is notable for its low 3βHSD2 activity (denoted by small arrow) and unique expression of cytochrome *b*5, a cofactor which enhances the 17,20-lyase activity of P450c17. Sulfotransferase 2A1 is uniquely expressed in the zona reticularis and rapidly converts DHEA to DHEAS. Compound S (Cpd S), 11-deoxycortisol. Corticosterone and 18-hydroxycorticosterone, the successive intermediates between deoxycorticosterone (DOC) and aldosterone, are not shown. The steroidogenic enzymes are italicized. The clinically relevant electron transfer enzymes also shown are *POR* and type 1 3′-phosphoadensosine-5′-phosphosulfate synthase (*PAPSS*). Formation of androstenedione from 17OHP and Cpd S does not seem attributable to CYP450c17. Modified with permission from Rosenfield, Identifying children at risk of polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2007;92:787–796 (431).

of testosterone to androstenedione and reconversion of estradiol to estrone are minor pathways.

B. Regulation of ovarian function

Androgens are not only obligate intermediates in the biosynthesis of estradiol. They also have complex effects on follicular growth (78), including up-regulation of aromatase activity (79). It is crucial for the function of the ovary that ovarian androgen secretion be coordinated with the formation of estrogen so that both are optimized for ovulation. Although these processes are critically dependent on LH and FSH concentrations, a variety of intrafollicular modulators are essential to the coordinated function of the ovarian follicle.

1. Regulation of gonadotropin secretion

Androgens are not under tight neuroendocrine negative feedback control by LH, nor is serum LH readily inhibited by modest increases in serum estrogen levels. FSH is reciprocally regulated by estradiol and inhibin in a sensitive, log-dose, negative-feedback loop (Figure 1) (22, 80–82).

It has long been apparent that the mature female neuroendocrine-gonadotropic axis is not very sensitive to negative feedback by androgens (8); only frankly virilizing testosterone levels are clearly gonadotropin suppressive (83). Congenital virilization (due to conditions such as 21-hydroxylase deficiency congenital adrenal hyperplasia), on the other hand, is a positive determinant of LH pulsatility but a negative determinant of the capacity to mount the LH surge necessary for ovulation, masculinizing the pattern of gonadotropin release (83). Congenital androgen effects appear to be mediated in part by permanent impairment of estradiol-induced progesterone receptor gene expression in the hypothalamus (84). Paradoxically, androgen receptor signaling enhances the capacity of females to mount an LH surge in response to estrogen positive feedback (85–87). Recently, it has been discovered that modest increases in serum androgen levels have a stimulatory effect on LH secretion, as discussed in the section on LH excess in PCOS (see section V.C).

2. Regulation of ovarian steroidogenesis

In small antral follicles steroidogenesis is organized as shown in Figure 1 (22). Theca cells produce androgens in response to LH, but granulosa cells do not do so because they do not express *CYP17A1* (62). Androgens then diffuse from theca cells to granulosa cells, where they are substrates for estrogen formation in response to FSH because granulosa cells differentially express *CYP19A1*, which encodes aromatase (62).

a. Homologous desensitization to LH. The expression of thecal steroidogenic enzymes is absolutely dependent upon LH in a dose-response relationship (22). The normal secretory dose-response curve is asymptotic, however, because as LH rises, desensitization of ovarian responses to LH commences (88–90). Animal models indicate desensitization is in part mediated by down-regulation of LH receptor-binding sites by ligand binding (22, 91), an endocytic process that involves recycling of receptors via membrane raft microdomains or degradation (92–94), and in part by down-regulation of the 17,20-lyase activity of P450c17 (22). Thus, as LH stimulation approaches maximal, 17OHP secretion rises, yet normally androgen production increases very little. Judging from serum

17OHP and steroidogenic responses to the LH analog human chorionic gonadotropin (hCG) in normal women, desensitization normally has commenced by half-maximal stimulation of the LH receptor (Figure 3) (90, 95), whereas maximal stimulation causes about a 5-fold increase in 17OHP and small (2-fold) increases in sex steroids (96). Serum 17OHP responses assessed at 4-hour intervals in response to interim dose changes of pulsatile LH suggest that elevating the serum LH 2-fold affects steroid output in normal women comparably with half-maximal LH receptor stimulation (97); however, it is dubious whether 4 hours are sufficient to ascertain the effect full effect of LH on steroidogenesis.

b. Modulation of LH action. Modulation of ovarian androgenic responsiveness to LH involves a number of hormones and growth factors acting in paracrine, autocrine, and endocrine fashions, as modeled in Figure 1 (22). Substantial evidence indicates that estrogen inhibits P450c17 activity by a short-loop (paracrine) negative feedback mechanism (98–100). Intraovarian androgens inhibit thecal P450c17 activity, but the testosterone effect is not reversed by antiandrogen (101), so it is possible that this androgen effect is estrogen mediated. These inhibiting modulators are counterbalanced by growth factors of granulosa cell origin that are under FSH control and by hormones and cytokines extrinsic to the ovary that amplify P450c17 activities (22, 102–105). Insulin, IGFs, and inhibin are the best recognized of these modulators (Figure 1). Insulin and IGFs up-regulate P450c17 activities (102) and in rat studies have been shown to up-regulate LH receptor sites (16, 91, 106). This counters the normal process of homologous desensitization to LH and thereby potentiates LH-induced androgen synthesis (22, 88). Insulin is approximately equipotent with IGF-1 in this regard, which makes it unlikely that insulin acts through the IGF-1 receptor, because its affinity is about 500-fold less than that for its cognate receptor. More recent studies have indicated that insulin acts through the insulin receptor itself: the effect on human theca cells is specifically neutralized by an antibody to the insulin receptor (105), and selective knockout of theca cell insulin receptors attenuates the androgenic response to hCG in mice (25). Furthermore, the hyperandrogenic anovulation induced by an obesogenic diet in association with 10- to 20-fold elevation of serum insulin in wild-type mice does not occur in transgenic littermates that lack the theca cell insulin receptor (25). Insulin also directly up-regulates 17βHSD5 gene expression and activity, stimulating testosterone formation from androstenedione (107).

Studies in women with PCOS have demonstrated that a previously unrecognized protein variant, differentially expressed in normal and neoplastic development (DENND)1A.V2, is a facilitator of steroidogenesis in androgen-producing cells, as discussed below in the section

Figure 3.

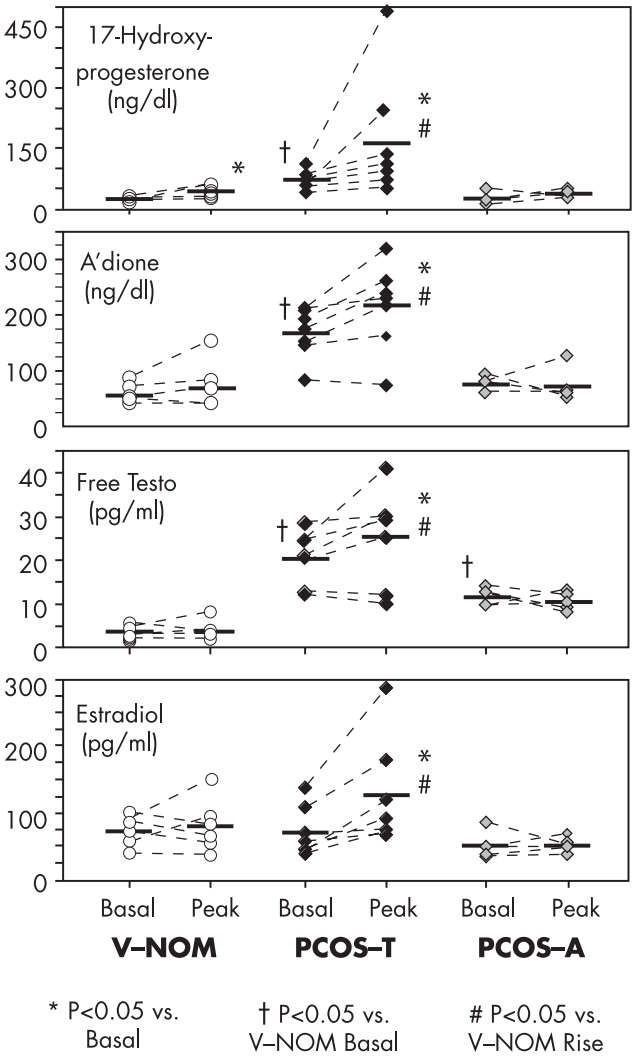

Figure 3. Response to half-maximal hCG stimulation during overnight dexamethasone suppression of normal and PCOS subjects. After bedtime dexamethasone 0.25 mg/m^2, 500-IU hCG was administered im at 8 AM; a basal blood sample was drawn before hCG, and the peak response to hCG was sampled after a repeat dexamethasone dose 24 hours later. Subjects were healthy volunteers with normal ovarian morphology (V-NOMs), PCOS patients with functionally typical FOH (ie, 17OHP hyperresponsiveness to GnRHag; PCOS-T), and PCOS patients with functionally atypical FOH (ie, normal 17OHP responsiveness to GnRHag; PCOS-A). V-NOM had a small but significant rise in serum 17OHP but not in other steroids. PCOS-T had hyperresponsiveness of all steroids. PCOS-A, a heterogenous group, had elevated basal serum free testosterone, but normal hCG-responses of all steroids. To convert to SI units, multiply 17OHP by 0.0303 (nM), androstenedione (A'dione) by 0.0340 (nM), free testosterone by 3.47 (pM), and estradiol by 3.67 (pM). Regraphed from data of Hirshfeld-Cytron et al, Characterization of functionally typical and atypical types of polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2009;94:1587–1594 (95).

on etiology of PCOS/genetic traits (see section VI) (108). At this time, its mechanism of action is unclear.

Inhibin-B, a peptide secreted by granulosa cells of small antral follicles in response to FSH, seems essential for androgenic responses to LH and may be modulated by androgens (95, 109). It seems necessary but not sufficient for up-regulation of CYPA1 gene expression (110). Patients with inactivating mutations of FSH lack hyperandrogenism, despite very high levels of LH. Such patients have low inhibin-B levels, and when exogenous FSH is given, inhibin-B rises dramatically, and the response of theca cell steroids to hCG/LH increases markedly (103, 111). Inhibin-B seems to play a permissive role, rather than a stimulatory role, in determining theca cell LH responsiveness because our data indicate that FSH administration normally suppresses testosterone levels, seemingly through the paracrine action of other granulosa cell factors under its control (95).

Numerous other small molecules of granulosa cell and oocyte origin both positively and negatively modulate LH action in theca cells so as to optimize the follicular environment for oocyte maturation (22). These factors include TGF-β superfamily members such as bone morphogenetic proteins (BMPs), other growth and differentiation factors, cytokines such as TNFα, and microRNAs (90, 112–119).

Catecholaminergic overactivity also amplifies the ovarian steroidogenic response to hCG in rodents, with signaling via β2-adrenoreceptors on thecal cells (120–123). In a unique rodent model of PCOM induced by a single injection of estradiol valerate, central sympathetic nervous system (SNS) activity is increased resulting in increased adrenomedullary noradrenergic activity, increased ovarian sympathetic nerve stimulation, and increased intraovarian synthesis of nerve growth factor (NGF), a sympathetic neurotrophin secreted by thecal cells. This is followed by the development of PCOM, anovulation, and increased androgen responses to hCG. These changes are reversed by transection of the superior ovarian nerve. Transgenic mouse NGF overexpression causes increased androgen production in response to pregnant mare serum, a higher prevalence of follicular cysts after sustained increases in LH, enhanced sympathetic outflow, increased body fat with disproportionate hyperinsulinemia, and glucose intolerance (121, 123).

In summary, androgen blood levels are not tightly controlled by direct negative feedback by the pituitary trophic hormones, as is the case for estradiol and cortisol. Rather, intraglandular paracrine and autocrine mechanisms seem to play a major role in the regulation of ovarian androgen secretion. Due to the process of homologous desensitization to LH, once serum LH levels approximate high-normal, intraovarian modulation of LH action seems to be the major factor determining ovarian androgen formation. Insulin counters the desensitization process and sensitizes the ovary to LH.

3. Folliculogenesis and its regulation

The transition from the resting primordial to the growing primary follicle stage ("initial recruitment/activation") is independent of serum gonadotropins (114, 124, 125). Primordial follicle formation in animal models is directed by the oocyte-specific chemokine S100A8 (126) and BMP2 (127) signaling. Primordial follicles are maintained in a dormant state by mesenchymal-epithelial cell interactions, intraovarian paracrine signals, and oocyte-secreted factors. Among these, oocyte liver kinase b1 (128) and somatic cell forkhead transcription factors inhibit activation (129, 130) and BMP4 promotes apoptosis (131, 132), whereas oocyte kit ligand and phosphatidylinositol-3-kinase are important stimulatory signaling pathways (114). Anti-Müllerian hormone (AMH), of granulosa cell origin, is the major hormonal paracrine inhibitor of primordial follicle progression: *Amh*-null mice undergo accelerated depletion of the primordial follicle pool, although at a slower rate than do than do *Foxo*-null mice (129, 133). Estrogen receptor expression is critical for development of the granulosa cell layer (134). Development of primary follicles depends on germ cell/oocyte factors. Growth differentiation factor (GDF)9 successively stimulates granulosa cell differentiation and then initiates theca cell differentiation, in conjunction with kit ligand, BMP6, and BMP15 (114). These primary theca cells express LH receptors and produce androgen. Insulin (114, 135) and androgen (136) promote the primordial-primary follicle transition, although the androgen effect does not occur at normal androgen levels judging from the results of the androgen receptor deletion studies discussed below.

A host of local factors then regulate further follicle growth and development; for example, BMP15 synergizes with GDF9 to stimulate granulosa cell proliferation (137). Only upon reaching the early antral follicle stage does follicle development become strictly dependent on FSH action (103, 125, 138). FSH actions on both growth and steroidogenesis are facilitated by androgen (139), insulin and IGF signaling (89, 138). Inhibin-B, produced by the granulosa cells of small antral follicles at a stage before aromatase becomes highly inducible by FSH (140, 141), seems to be the prime ovarian regulator of FSH secretion via negative feedback on gonadotropes (82).

AMH is an important intrafollicular modulator of follicle growth and FSH action (142). It is a TGF-β superfamily member that is produced by the granulosa cells of small growing follicles. As follicles grow, intrafollicular AMH levels rise sufficiently to inhibit both recruitment of primordial follicles to the primary follicle stage and FSH

stimulation of aromatase activity. Because estradiol inhibits AMH production, there exists an intrafollicular short negative feedback loop confining AMH expression to follicles up to about 8 mm in diameter. Thus, AMH appears to act as a follicular gatekeeper, ensuring that each small antral follicle produces little estradiol before selection of the dominant follicle, which allows a direct ovarian-pituitary dialogue regulating the development of the follicle selected to undergo ovulation.

The serum AMH level is an indicator of the number of growing follicles. AMH levels reflect intrafollicular androgenic status (109), probably because androgens stimulates the early phases of follicular growth (136, 143). The AMH level also independently indicates the size of the follicular ("ovarian reserve") pool (39, 144).

Androgens have complex effects on follicular development that indicate paracrine interactions between theca and granulosa cells (79, 145). As expected from human data indicating that virilization causes polycystic ovaries (9), systemic induction of hypertestosteronemia (4–30 ng/mL for 3–10 d) in nonhuman primates (primates) activates the earliest stages of follicle growth so as to stimulate the growth of small, but not large, antral follicles: granulosa cell androgen receptor and FSH receptor content increase, as do granulosa and theca cell proliferation and cortical thickening (136, 146, 147). Notably, androgen receptor expression precedes FSH receptor expression in human granulosa cells (148). Androgen receptor deletion studies indicate that androgens, in conjunction with gonadotropins (61), normally are important for follicle development from the preantral through the early antral follicle stage and for up-regulating aromatase activity via granulosa cell actions (149, 150). Androgens also synergize with FSH to luteinize follicles by inducing LH receptors (138, 139, 151, 152). In vitro study of primate follicle maturation from the secondary to small antral follicle stage over a 40-day period in serum-containing medium indicates that 10-ng/mL testosterone promotes preantral follicle growth, but 50-ng/mL testosterone or dihydrotestosterone inhibits it (153). In excess, androgens impair selection of the dominant follicle of women (154); this appears likely to result from premature luteinization of follicles (155).

Luteinization of granulosa cells, as indexed by the development of LH receptors, commences as selected follicles reach 5 mm in the midfollicular phase of the menstrual cycle, and in the preovulatory (dominant) follicle LH receptors rise 10-fold more (138, 152). FSH is the primary inducer of these LH receptors. Androgens and estrogens synergize in FSH induction of LH receptors and the subsequent augmentation of progesterone and estradiol formation in luteinized granulosa cells (138, 139, 147, 152). Insulin amplifies these granulosa cell steroidogenic responses to FSH and LH (89, 156). As the dominant follicle emerges, LH signaling comes to predominate, and FSH and androgen receptor expression wane (138, 152, 157). Only the preovulatory follicle continues to grow, seemingly because of its high LH receptor content and an androgen to estrogen ratio that favors estradiol. Reciprocally with dominant follicle emergence, the companion cohort of follicles is growth-inhibited, atresia commences and the androgen to estrogen ratio favors androgen (138). This atresia has been attributed to the combination of relatively low gonadotropin and androgen receptor expression (149, 150, 157).

The biochemical rigidity of the stroma influences follicular development and steroidogenesis (125, 158, 159). The hippopotamus (Hippo) signaling pathway, a serine/threonine kinase signaling cascade that is regulated by the biomechanics of the microenvironment, is growth restrictive and has been postulated to play a role in regulating follicle growth as developing follicles move from the relatively dense cortex, where primordial follicles reside, to softer medullary regions (125). Vascularization of the stroma is a determinant of stromal rigidity and is regulated by the vascular endothelial growth factor (VEGF) family of cytokines (145). They and their receptors are produced by granulosa cells, theca cells, and stroma and are up-regulated by androgens (114, 145, 160). VEGFs not only stimulate endothelial proliferation and permeability, which affect delivery of circulating hormones and cytokines to follicles, they directly restrain primordial follicle growth (160).

In summary, paracrine-acting growth factors originating in germ cells, oocytes, granulosa cells, theca cells, and stroma are the predominant regulators of early follicular development, with FSH becoming essential for follicle growth from the early antral stage. Normal thecal androgen production supports antral follicle growth and development. Androgens do so in part by up-regulating granulosa cell expression of FSH receptors and then augment FSH induction of granulosa cell LH receptors, thus luteinizing granulosa cells and sensitizing them to both gonadotropins. Insulin amplifies the luteinization process. Androgen excess stimulates the excess proliferation of small antral follicles while causing follicle maturation arrest and PCOM. Antral follicle growth is accompanied by increased production of AMH, which normally acts as a gate-keeper that inhibits primordial follicles from entering the growth phase.

C. Regulation of adrenal androgen production

The zona reticularis of the adrenal gland (Figure 2) resembles the theca cell compartment of the ovary in its

expression of the core enzymatic pattern for androgen production. Zona reticularis cells become discernable in the central adrenal cortex at 3 years of age. As it begins to develop into a continuous zone at about 5–6 years of age, it becomes discernable as "adrenarche," the maturational increase in adrenal androgen production that is indexed by increased DHEA sulfate (DHEAS) production (161).

Adrenarche represents a change in the pattern of the adrenocortical secretory response to ACTH. It is characterized over time by disproportionately increasing responsiveness of Δ^5-steroid intermediates (17-hydroxypregnenolone and DHEA) compared with Δ^4-steroids (eg, 17OHP and androstenedione) in the presence of stable responses of cortisol (162, 163).

A unique zona reticularis enzyme expression profile underlies this adrenarcheal pattern of adrenal secretion. Zona reticularis cells express low 3βHSD2, but high CYB5, and steroid sulfotransferase (SULT2A1) activities (Figure 2) (64, 164, 165). The combination of low 3βHSD2 and high CYB5 activity diverts pregnenolone formation from cortisol to DHEA, and the unique expression of SULT2A1 rapidly sulfates DHEA, forming DHEAS, which accounts for the high secretion of DHEAS by the adrenal cortex (166). SULT2A1 thus acts as a "sump" to direct steroidogenesis to this relatively inert terminal product and prevents adrenal DHEA from being converted into biologically active androgens (165). Seladin-1 (24-dehydrocholesterol RD), expressed in both zona fasciculata and reticularis cells, enhances DHEA, but not cortisol, secretion in vitro, which suggests that it enhances 17,20-lyase activity (167). Enhanced expression of HSD17B5 by this zone accounts for the small but significant adrenal contribution to testosterone secretion (168).

Zona reticularis development requires ACTH, but its determinants are otherwise poorly understood. The stimulus has long been thought to be of pituitary origin (169, 170). Adrenarche is not directly related to the pubertal maturation of the neuroendocrine-gonadotropin-gonadal axis. Adiposity is strongly related to adrenarche, and insulin, IGF-1, and leptin have been suggested as determinants of this relationship (171–177). Adrenarche is severely attenuated in somatotropin- and thyroxine-replaced patients with deficiency of the pituitary-specific transcription factor Pit-1/POU1F: because this gene defect causes a congenital deficiency of somatotropin, thyrotropin, and prolactin, and the former hormones were replaced, a role for prolactin is suggested (178). IL-6 is another candidate because it is strongly expressed in the zona reticularis of the adrenal cortex and stimulates DHEA secretion (179). On the other hand, a BMP4 signaling system has been identified in the zona reticularis that is inhibitory to androgen formation (180). Notably, the theca cell steroidogenesis facilitator protein DENND1A.V2 has been localized to the zona reticularis (see section V.A.1.). Other pathways that potentially modulate adrenal androgen formation have been identified in a human adrenocortical carcinoma cell line (181). We favor the hypothesis that whatever the factor(s) responsible for adrenarche, it affects the growth or differentiation of zona reticularis precursor cells, so that they acquire the enzymatic properties that permit them to respond to ACTH by secreting androgens.

D. Regulation of peripheral androgen production

Peripheral formation of testosterone from androstenedione primarily occurs in liver, skin and fat (107 , 182–184). Skin and fat tissue express 3βHSDB1 and 17βHSD5 activities as well as P450aro activity. Although early direct studies of steroid metabolism did not reveal it (185), recent evidence suggests that adipose tissue excess is an important contributor to androgen as well as estrogen excess (47).

No clear picture has emerged of tissue-specific regulation of androgen production in nonendocrine sites. Adipose tissue has become recognized in recent years as an endocrine organ that is an important site of generation of sex steroids and inflammatory cytokines in obesity (186, 187). The ratio of androgenic 17βHSD to aromatase activity in visceral fat is positively correlated with central adiposity, and experimental aromatase deficiency in animals and humans is associated with visceral adiposity (187), implicating increased local androgen production in central adiposity (reviewed in section V.B.1). Notably, insulin up-regulates adipocyte 17βHSD5 gene expression and activity in sc fat (107). Furthermore, 17βHSD5 expression in sc fat correlates with body mass index (BMI) (184). Insulin, glucocorticoids, and inflammatory cytokines also stimulate aromatase activity in adipocytes or preadipocytes (188, 189).

Hepatic 17βHSD5 gene expression, hence testosterone formation from androstenedione, appears to be down-regulated by insulin in liver (107). On the other hand, 5αRD activity, which promotes androgen action, is up-regulated in hyperandrogenic states by mechanisms that involve both insulin resistance (190) and possibly androgen excess itself (191).

Sex hormone-binding globulin (SHBG) is of hepatic origin; it is an important factor in androgen action and metabolism. The SHBG concentration determines the fraction of serum testosterone and other 17β-hydroxysteroid ligands (eg, estradiol, dihydrotestosterone) that are free or bound to albumin. It is thus a major determinant of ligand egress from serum to androgen target tissues and to liver for clearance from the circulation (192). SHBG levels

are raised by estrogen and suppressed by androgen, insulin resistance in obesity, and hypothyroidism (192, 193). Although the low SHBG in obese individuals has long been attributed to hyperinsulinemia (194), recent evidence suggests that monosaccharide excess itself, signaling via inflammatory cytokines, mediates the SHBG response to obesity (193, 195). Rarely, mutations cause very low levels of SHBG (196).

E. Summary of normal androgen physiology

Androgen and androgen precursors normally are produced by the ovaries and adrenal cortices in about equal amounts in response to LH and ACTH, respectively. About half of testosterone arises from peripheral metabolism of secreted precursors in liver, skin, and fat, where the factors regulating these conversions are less clear, although insulin stimulates testosterone formation in fat. Androgens are not under tight neuroendocrine negative feedback control. Rather, the ovarian androgenic response to LH appears to be normally modulated by intraovarian mechanisms so as to optimize androgen and estrogen formation so as to promote follicular maturation, because although androgens are essential substrates for estradiol formation, in excess, they hinder ovulation. In part, this modulation seems to be accomplished by homologous desensitization of theca cells to LH, which minimizes the androgenic response to high LH levels commencing with desensitization at the level of the LH receptor. In part, modulation seems to be accomplished by counterbalanced paracrine down-regulatory and up-regulatory mechanisms that primarily act on the rate-limiting step in sex steroid formation, P450c17 activity. Excess insulin is an extraovarian modulator that has the potential to override normal intraovarian down-regulatory mechanisms that control ovarian androgen production.

IV. Source of Androgen Excess in PCOS

A. Testing to determine the source of androgen in PCOS

To attempt to understand PCOS pathophysiology, we have functionally categorized PCOS patients according to whether the source of androgen excess is primarily the ovaries, the adrenal glands, both, or neither (Table 2 and figure 5 below) (47). Test procedures to determine the source of androgen are outlined in Table 3. Our studies reviewed here are based on RIA methods for testosterone, androstenedione, and 17OHP that have a precision of approximately 12% and have been validated against liquid chromatography-tandem mass spectrometry (47).

The ovarian hyperandrogenism of PCOS is demonstrated directly by the GnRHag test or the hCG test and indirectly by the dexamethasone androgen-suppression test (DAST) (Table 3). The GnRHag test determines the coordinated function of the ovarian follicle. Leuprolide acetate 10 μg/kg sc (or a comparable dose of any other short-acting GnRHag) stimulates endogenous LH and FSH release that peaks at 3–4 hours and persists for 24 hours; this in turn stimulates increased secretion of sex steroids and their precursors, with serum levels peaking at 18–24 hours (17, 22, 197). In the absence of evidence of a steroidogenic block, an elevated 17OHP response is typical of PCOS. Ovarian steroidogenic enzyme deficiency, which is rare, can be detected by an abnormal pattern of steroid intermediates in response to the test (17, 197, 198). hCG is an LH analog: 5000 IU im stimulates steroidogenic responses comparable with those of a GnRHag test at 24 hours (95, 96, 199). We perform the GnRHag test after the DAST so as to blunt coincidental adrenal secretion, which may otherwise occasionally confound the ability to interpret the results.

DAST indirectly tests ovarian androgenic function by suppressing ACTH-dependent adrenal androgen production. In the presence of normal adrenocortical suppres-

Table 2. Functional Classification of PCOS According to Source of Androgen Excess

PCOS Functional Type	Source of Androgen	GnRHag Test 17OHP Response	DAST Testosterone Response	ACTH test DHEA Response	Prevalence Among PCOS
PCOS-T	Primary FOH (typical FOH)	High[a]	High in 92.5%	High in 28% (associated FAH)	67%[b]
PCOS-A	Primary FOH (atypical FOH)	Normal[a]	High	High in 30% (associated FAH)	20%
	Primary FAH (isolated FAH)	Normal	Normal	High	5%
	PCOS without FOH or FAH (PCOS-A of obesity or idiopathic PCOS-A)	Normal	Normal	Normal	8%

Based on data of Rosenfield et al, Determination of the source of androgen excess in functionally atypical polycystic ovary syndrome by a short dexamethasone androgen-suppression test and a low-dose ACTH test. *Hum Reprod.* 2011;26:3138–3146 (47).

[a] High vs normal denotes defining characteristics; percentages indicate experimentally determined prevalence of abnormality.

[b] Prevalence determined from an age-matched subgroup (n = 60) of an original cohort (n = 99), in which 69% had PCOS-T. Modified with permission from Rosenfield, Polycystic ovary syndrome in adolescents. In: Rose BD, ed. *www.uptodate.com*. UpToDate. Waltham, MA; 2014.

Table 3. Test Procedures to Determine Source of Female Androgen Excess

Test	Rationale	Method	Outcome Measures	Interpretation[a]
GnRHag	Endogenous LH and FSH release stimulates coordinated function of ovarian follicles	Leuprolide acetate 10 μg/kg sc (for maximum stimulation)	Ovarian steroid secretion peaks at 20–24 h	17OHP >152 ng/dL without steroidogenic block indicates typical FOH (PCOS-T)
hCG	Exogenous administration of LH analog stimulates theca-interstitial cells	hCG 3000 IU/m^2 (for maximum stimulation)	Ovarian steroid secretion peaks at 24 h	17OHP >152 ng/dL without steroidogenic block indicates typical FOH (PCOS-T)
LDAST	Long DAST: dexamethasone profoundly suppresses adrenal androgens over several days	Dexamethasone 0.5 mg QID per os × 4–5 d	Free testosterone, DHEAS, cortisol: sample early morning d 5	Free testosterone ≥8 pg/mL with DHEAS <70 and cortisol <1 μg/dL characteristic of FOH
SDAST	Short DAST: dexamethasone rapidly suppresses adrenal testosterone and cortisol	Dexamethasone 0.25 mg/m^2 per os at 12 noon	Total testosterone, cortisol: sample 4 PM (4 h)	Total testosterone >26 ng/mL, cortisol <5 μg/dL suggests FOH
ACTH	Exogenous ACTH stimulates adrenal steroidogenesis	Cosyntropin ≥10 μg/m^2 (for maximum stimulation)	DHEA,17OHP, steroid intermediates, cortisol peak at 30–60 min	DHEA 1500–3000 μg/dL without steroidogenic block indicates FAH

Testing in early follicular phase of menstrual cycle. Conversion multipliers to SI units: 17OHP 0.0303 (nmol/L), cortisol 0.0276 (μmol/L), free testosterone 3.47 (pmol/L), total testosterone 0.0347 (nmol/L), DHEA 0.0347 (nmol/L), and DHEAS 0.0271 (μmol/L). Data from Rosenfield et al, Determination of the source of androgen excess in functionally atypical polycystic ovary syndrome by a short dexamethasone androgen-suppression test and a low-dose ACTH test. *Hum Reprod*. 2011;26:3138–3146 (47), Levrant et al, A pilot study of the human chorionic gonadotrophin test for ovarian hyperandrogenism. *Hum Reprod*. 1997;12:1416–1420 (96), and Rosenfield, The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*. 2015;136:1154–1165 (50).

[a] Norms are those in our laboratory; results may differ elsewhere.

sion, an inappropriately elevated serum testosterone post-DAST indicates an ACTH-independent source of androgen, which is ordinarily of ovarian origin. In circumstances where an adrenal virilizing disorder is suspected, a "long" (4–5 d) DAST is indicated (200–203), but a "short" (4 h) DAST suffices for most suspected PCOS (Table 3), because the long and short tests yield similar results (47). Notably, the differences in testosterone responses between these 2 tests were greater than expected from assay precision in about 25% of cases, suggesting that biologic variability in ovarian function affects test results.

Adrenal hyperandrogenism is demonstrated by a rapid ACTH test: cosyntropin is administered iv and peak steroid responses occur at 15–60 minutes. Although the test is ordinarily performed using cosyntropin 250 μg, this is a supramaximal dose, and 10 μg/m^2 elicits a similar peak response. The low-dose ACTH test (1.0-μg cosyntropin) is more physiologic; it usually elicits nearly as great a peak response that promptly wanes, and in PCOS, does not elicit such a wide spectrum of elevated steroid intermediates as do larger doses (47). DHEAS is a simple correlate of this adrenal androgenic dysfunction ($r = 0.708$).

B. FOH in PCOS

PCOS is a diagnosis of exclusion by standard criteria (Table 1). Therefore, it is necessary to consider other causes of hyperandrogenism in the differential diagnosis, although they account for only 10%–20% of adults presenting with hyperandrogenic symptoms (Table 4) (50, 204–206).

The unique ovarian dysfunction of PCOS (primary FOH) was first demonstrated by GnRHag testing (22).

Table 4. Differential Diagnosis of Hyperandrogenemia

A. Physiologic adolescent anovulation
B. Functional gonadal hyperandrogenism
   1. PCOS:Primary FOH (common form of PCOS)
   2. Secondary FOH
      a. Virilizing congenital adrenal hyperplasia
      b. Adrenal rests of the ovary
      c. Ovarian steroidogenic blocks
      d. Insulin resistance syndromes
      e. Acromegaly
      f. Epilepsy ± valproic acid therapy
   3. Disorders of sex development
   4. Pregnancy-related hyperandrogenism
C. FAH
   1. PCOS:primary FAH (uncommon form of PCOS)
   2. Virilizing congenital adrenal hyperplasia
   3. Other glucocorticoid-suppressible FAH
      a. Hyperprolactinemia
      b. Cortisone RD deficiency (and apparent RD deficiency)
      c. Apparent DHEA sulfotransferase deficiency
   4. Glucocorticoid-nonsuppressible FAH
      a. Cushing's syndrome
      b. Glucocorticoid resistance
D. Peripheral androgen metabolic disorders
   1. Obesity
   2. Idiopathic hyperandrogenism
   3. Portohepatic shunting
E. Virilizing tumors
F. Androgenic drugs

Modified with permission from Rosenfield, The diagnosis of polycystic ovary syndrome in adolescents. *Pediatrics*. 2015;136:1154–1165 (50).

The pattern of steroidogenesis indicated generalized overactivity of the entire ovarian steroidogenic cascade involved in sex steroid secretion. An elevated 17OHP response was the most consistent abnormality in classic PCOS, which suggested a prominent abnormality at the level of P450c17 activities. Subsequent clinical studies have shown that ovarian steroidogenesis in PCOS is typically similarly hyperresponsive to both the endogenous LH and FSH surge elicited by administration of GnRHag challenge or by hCG challenge (90, 96, 199, 207).

FOH is the common denominator in the vast majority of PCOS. However, as reviewed next, FOH is neither always typical nor always demonstrable in PCOS (Table 2).

1. Spectrum of ovarian androgenic function in PCOS: Typical and atypical FOH

Initially, we reported that the first 7 women with classic PCOS who underwent GnRHag testing had 17OHP hyperresponsiveness in comparison with 16 nonhirsute eumenorrheic women in the midfollicular phase of their menstrual cycle (17). Then, we demonstrated that most (58%) hyperandrogenic women (n = 40) had this PCOS type of 17OHP hyperresponsiveness to GnRHag testing conducted post-DAST in comparison with 13 controls (19). Eighty-seven percent of those with abnormal responses to GnRHag were oligomenorrheic. This abnormality in hyperandrogenic women correlated well (r = 0.75; r^2 = 0.56) with elevated plasma free testosterone in response to the 5-day DAST, with concordance in 85% of women, in contrast to the findings that polycystic ovaries or elevated LH levels occurred in only about half.

Although subsequent reports confirmed the presence of a significant difference in 17OHP responses to GnRHag between PCOS and control women, they differed widely in their estimates of the prevalence of this abnormality, with some reporting its presence in only a distinct minority (208, 209). We believe this low prevalence is due to failure to exclude women with PCOM from the reference control group, for reasons discussed in the following section.

From 2000 to 2007, we sought to determine the prevalence of FOH in hyperandrogenic women with anovulatory symptoms (PCOS), controlling for the presence of PCOM (197). We performed a GnRHag test and a DAST in 99 consecutively consenting PCOS patients who presented to our clinics and who met NIH criteria (elevated serum free testosterone and ovulatory dysfunction) and compared them with nonhirsute eumenorrheic volunteers, the reference group being volunteers with ultrasonographically normal ovarian morphology (V-NOM) (n = 21). Volunteers were studied in the midfollicular phase of their menstrual cycles (d 4–10) so as to match them for follicular status as well as possible with PCOS. The study protocol included assessment for PCOM, AMH levels, and glucose tolerance. We found that 69% of PCOS, defined by NIH criteria, had typical 17OHP hyperresponsiveness to GnRHag (197).

To better understand the differences between those PCOS patients with 17OHP hyperresponses (functionally typical PCOS [PCOS-T]) and those who lacked 17OHP hyperresponsiveness (functionally atypical PCOS [PCOS-A]), we then analyzed age-matched subsets of PCOS-T (n = 40), PCOS-A (n = 20), and nonhirsute eumenorrheic volunteers, the reference group being V-NOM. We determined the sources of androgen in these 2 functional types of PCOS and related the findings to glucose intolerance in 1 report (47), related ovarian androgenic function to markers of folliculogenesis (PCOM and serum AMH) in another (39), and then integrated these findings (30). Approximately half of the study subjects were adolescents (>1.0 y postmenarcheal and 11.0–17.9 y of age); adults were 18.0–39.9 years old. Within the volunteer and PCOS groups, adolescents and adults had similar baseline androgen, 17OHP, and LH levels (30). The data are displayed in Figure 4 (30).

Among PCOS-T, those with 17OHP hyperresponsiveness (Table 2 and Figure 5), hyperandrogenism was more severe than in PCOS-A, and the great majority (92.5%) also had an abnormal short DAST (SDAST) and PCOM (Figure 4Ac). Serum AMH was increased in 81%, and the increase was significantly greater than that of any other group. Coincidental FAH was present in 28%. Impaired glucose tolerance (IGT) and frank diabetes were present significantly more often than in PCOS-A or controls; in this series, the only diabetic cases were in PCOS-T. In addition, estradiol secretion was hypersensitive to submaximal hCG stimulation (Figure 3) and FSH administration did not result in the normal inhibition of baseline serum testosterone in PCOS-T (95).

PCOS-A is a functionally heterogeneous group (Table 2 and Figure 5). Sixty percent had an abnormal DAST (Figure 4Ab), which defines an atypical form of FOH (Table 2, atypical FOH). FAH coexisted with FOH in 30% of this subgroup. PCOM (65%) and AMH elevation (39%) were found significantly less frequently than in PCOS-T. The prevalence of glucose intolerance was significantly less than in PCOS-T and did not differ from that in the control subjects.

The other 40% of PCOS-A had a normal DAST (Figure 4Aa), ie, no evidence of an ovarian source of androgen. They had significantly milder hyperandrogenemia. This "nonovarian PCOS" itself seems functionally heterogeneous (Table 2). ACTH testing showed "isolated FAH" (Table 2) in 15% of PCOS-A; two-thirds of these had an elevated baseline DHEAS level. However, 85% of the

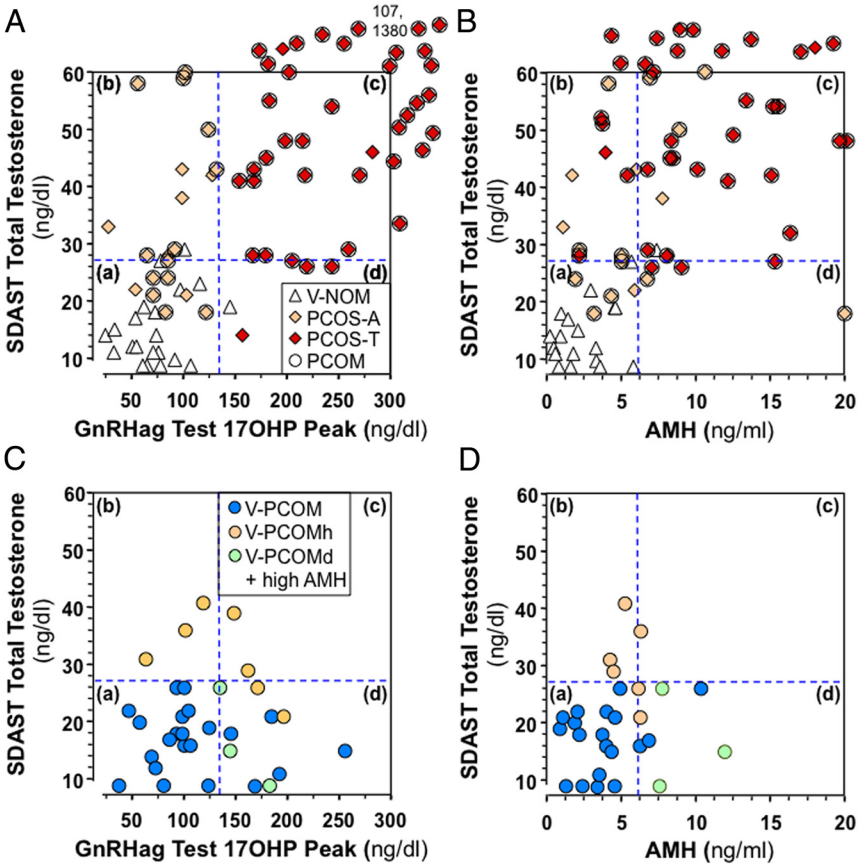

Figure 4. Figure 4. Scatterplots demonstrating relationships among tests for ovarian hyperandrogenism, PCOM, and serum AMH concentrations. Subjects are patients with PCOS identified by NIH criteria (n = 20 PCOS-A, n = 40 PCOS-T) and age-matched healthy eumenorrheic nonhirsute volunteers with normal or PCOM (V-NOM n = 21, V-PCOM n = 32, respectively). Serum for AMH was available in 92% of PCOS and 82% of these volunteers (39). PCOM was defined according to modified Rotterdam criteria: in adults, it was defined as an ovary more than 10.5 cc (in adolescents, >10.8 cc) using the formula for a prolate ellipsoid and/or more than or equal to 10 follicles 2–9 mm in diameter in the maximum plane (27, 39). Dotted lines show normal ranges for the V-NOM reference group; thus (a) quadrant panels show normal ranges. A, PCOS-T is defined by an elevated 17OHP response to the GnRHag test (c and d). SDAST results correlate with GnRHag results ($r = 0.671$, $P < .0001$). SDAST is abnormal in 92.5% of PCOS-T. PCOS-A is defined by lack of 17OHP hyperresponse to GnRHag. The SDAST divides PCOS-A into those with (b) and without (a) ovarian androgenic dysfunction. SDAST indicates that 60% of these PCOS-A cases have atypical FOH (b) and 40% of PCOS-A cases have normal ovarian androgenic function (a). B, SDAST relationship to baseline AMH levels. C, 22% (n = 7) of asymptomatic V-PCOM have baseline hyperandrogenemia (hyperandrogenic PCOM [V-PCOMh]). These all proved to have FOH, as indicated by an abnormal SDAST or GnRHag test (b–d). Adolescent V-PCOM tended to have this asymptomatic FOH less often (1/9) than adult (6/23) V-PCOM. Dysregulated PCOM (V-PCOMd), defined by an abnormal 17OHP response to GnRHag in the absence of baseline hyperandrogenemia (d), was found in 25% (n = 8) V-PCOM. D, Mild AMH elevation was found in V-PCOM independently of hyperandrogenemia. So as to best illustrate differences among groups, very high values (post-SDAST testosterone up to 107 ng/dL and post-GnRHag l7OHP up to 1380 ng/dL) are plotted off-scale. To convert to SI units, multiply total testosterone by 0.0347 (nM), 17OHP by 0.0303 (nM), and AMH by 7.125 (pM). Reproduced with permission from Rosenfield, The polycystic ovary morphology-polycystic ovary syndrome spectrum. *J Pediatr Adolesc Gynecol*. 2015;28:412–419 (30). Since publication of these data, accumulated evidence suggests that in adolescents mean ovarian volume more than 12 cc is a more appropriate criterion for PCOM than more than 10.8 cc (50, 51). Doing so alters the constitution of the V-NOM and V-PCOM groups. With this adjustment and the addition of data on 4 contemporaneously studied but previously overlooked healthy volunteers, our current upper limits for V-NOM (n = 31) are: baseline-free testosterone 9.3 pg/mL and AMH 6.3 ng/mL; SDAST total testosterone 26 ng/dL; and postdexamethasone GnRHag 17OHP 152 ng/dL (50).

nonovarian subgroup (25% of PCOS-A) lacked FAH, so evidence for a glandular source of androgen was lacking. All in this small subgroup were obese, and we have attributed their androgen excess to excessive peripheral testosterone formation by excessive adipose tissue ("PCOS-A of obesity" in Table 2) (47).

We conclude that there is a spectrum of pathophysiologic dysfunction in PCOS that generally corresponds to clinical severity (30): the PCOS-T group, which has an ovarian secretory pattern suggestive of dysregulation of steroidogenesis prominent at the level of the 17-hydroxylase/17,20-lyase activities of P450c17 (Figure 1), constitutes two-thirds of PCOS and is significantly more clinically severe than PCOS-A, although considerable clinical overlap exists. Furthermore, these data suggest heterogeneity in the pathophysiologic basis of FOH. It remains to be proven whether these pathophysiologic categorizations have clinical utility beyond possibly identifying a subpop-

Figure 5.

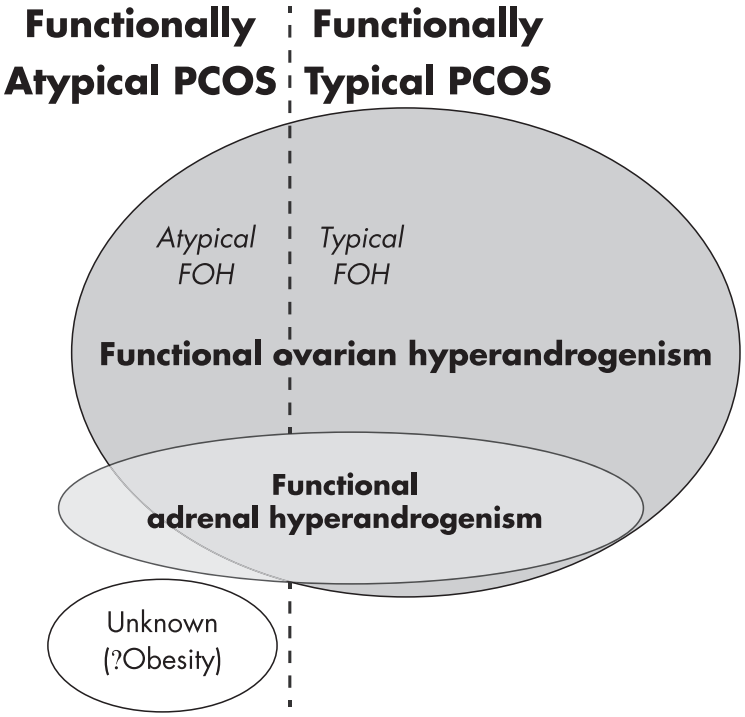

Figure 5. Relationships among sources of androgen in PCOS. About two-thirds of cases have functionally typical PCOS (PCOS-T) that is due to typical FOH, in which there is hypersensitivity to LH, characterized by hyperresponsiveness of 17OHP to a GnRHag or hCG test. The remaining one-third of PCOS is functionally atypical, lacking 17OHP hyperresponsiveness. This is a heterogeneous group, most of which have atypical FOH, in which ovarian androgen excess is indicated only by a DAST. A small number are due to isolated FAH. About one-quarter of FOH also have FAH. In a minority of cases, the source of androgen cannot be identified as ovarian or adrenal; most of these are associated with obesity. Modified and reproduced with permission from Rosenfield, Polycystic ovary syndrome in adolescents. In: Rose BD, ed. *www.uptodate.com*. Waltham, MA: UpToDate; 2014.

ulation of PCOS patients whose androgen excess arises from simple obesity and so would be expected to be reversible by weight loss (210, 211) or distinguishing adolescents with PCOS from those with physiologic anovulation (53). Biochemical categorization is expected to prove useful in developing phenotype-genotype correlations, as discussed later: only when we understand the etiology of PCOS will it be possible to truly assess the sensitivity and specificity of these tests.

2. Spectrum of ovarian androgenic function in asymptomatic women with PCOM

PCOM is a common finding among healthy women. Many of these women have mild PCOS features, ie, irregular menstrual cycles and/or hirsutism (212, 213). When care has been taken to exclude those with such symptoms, groups of apparently normal women with PCOM, including some with documented ovulatory cycles, have been shown to have subclinical androgenic ovarian dysfunction that is intermediate between that of women with normal ovaries and those with PCOS (31, 207, 214).

AMH serum concentrations in normal subjects with PCOM are also intermediate between those of women with NOM and those with PCOS (215–217). Data vary as to whether insulin resistance is associated with PCOM in healthy adult volunteers (31, 214, 218).

We have studied the ovarian androgenic function of normal females with PCOM in some detail. We compared nonhirsute eumenorrheic volunteers with PCOM (V-PCOM) to both the otherwise entirely similar reference group of females with NOM (V-NOM) and to PCOS, all groups age-matched, using the above protocol (39, 197), as shown in Figure 4.

Healthy adolescent and adult volunteers had similar ovarian function, except that V-NOM adolescents by definition had slightly larger ovaries (volume $\leq$ 10.8 cc) than V-NOM adults and slightly lower FSH and higher AMH levels (30, 197). V-PCOM are heterogeneous, with a spectrum of ovarian function (Figure 4, C and D). At one end of the V-PCOM spectrum was a subgroup of 40% with an extreme variation of normal size and morphology; they had ovarian androgenic function test results and serum AMH like that of V-NOM (Figure 4, Ca–Da). At the other end of the V-PCOM spectrum, was a subgroup of 22% with "hyperandrogenic PCOM" (V-PCOMh in Figure 4C, b–d); although asymptomatic, they had baseline hyperandrogenemia, and so seemed to meet the definition of ovulatory PCOS (Table 1, phenotype 3) since eumenorrheic; all also had a positive SDAST and/or GnRHag test, which indicates subclinical FOH. They also had mildly increased adrenal androgenic function: DHEAS was 159 $\pm$ 58, SD, μg/dL, significantly higher than that of V-NOM (80 $\pm$ 41 μg/dL), unlike any other V-PCOM subgroup ($P < .01$); they also tended to have a higher DHEA response to ACTH ($P = .15$). DHEAS and DHEA peaks were, respectively, elevated (>180 and > 1100 ng/dL) in 42% and 14% of this subgroup.

Between these extremes lay 2 different kinds of ovarian functional variants (39). On the normal side of the spec-

Figure 6.

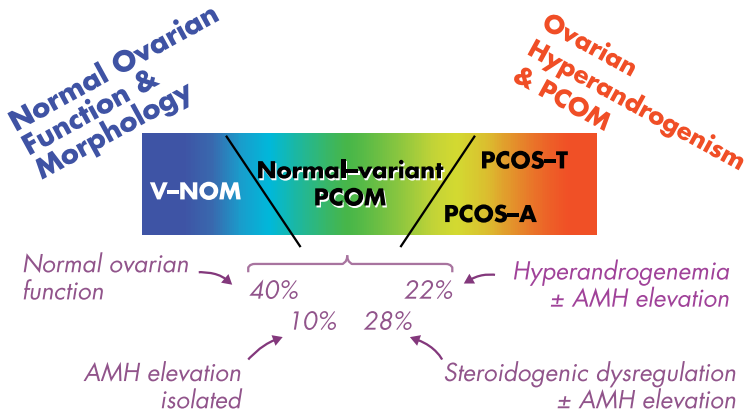

Figure 6. Schematic representation of the spectrum of ovarian function found in eumenorrheic nonhirsute V-PCOM (normal-variant PCOM) in relation to that of normal and PCOS women. Approximately 40% of V-PCOM are functionally variations of normal: this group has ovarian function like that of similar V-NOMs. Another 10% of V-PCOM has elevated AMH in the absence of any evidence of ovarian steroidogenic dysfunction, which suggests an isolated increase in folliculogenesis unrelated to ovarian androgenic dysfunction. The remaining half of V-PCOM have some degree of PCOS-related steroidogenic dysregulation, often with AMH elevation. Of these, nearly half (22% of the V-PCOM group) have biochemically hyperandrogenic PCOM, ie, subclinical FOH that suggests ovulatory PCOS. The remainder have isolated dysregulation of ovarian steroidogenesis (ie, isolated in the sense that 17OHP hyperresponsiveness to GnRHag testing occurs in the absence of hyperandrogenemia). Based on data in Figure 4; percentages are averages derived from different denominators for GnRHag test (n = 32) and AMH (n = 28) determinations in V-PCOM.

trum was a V-PCOM subgroup (10% of V-PCOM) with an isolated AMH elevation (V-PCOM in Figure 4Dd) that indicates increased folliculogenesis. On the PCOS side of the spectrum was a V-PCOM subgroup with "dysregulated PCOM," ie, steroidogenic hyperresponsiveness to GnRHag (17OHP elevation without hyperandrogenemia) (Figure 4Cd); these constituted 28% of V-PCOM, one-third of whom had mildly elevated serum AMH. It seems probable that this dysregulation indicates a very mild and subclinical degree of intraovarian androgen excess. In a multivariate model, AMH correlated independently across all groups (healthy volunteers with or without PCOM and PCOS) with SDAST testosterone ($P = .001$), but not peak 17OHP response to GnRHag ($P = .5$). Summary multiple regression analysis across all groups (Figure 4) shows that serum AMH is independently ($R^2 = 0.3$) related to the presence of ovarian hyperandrogenism ($P < .001$) and to that of PCOM ($P = .014$).

The picture that emerges from this biochemical spectrum of differences in ovarian function among V-PCOM subjects is that apparently normal women with PCOM occupy a middle position on a spectrum of ovarian function between women with clearly normal ovarian function and those with PCOS (Figure 6). About half have no evidence of steroidogenic dysfunction and, thus, no relation to PCOS. On the other hand, about half of V-PCOM have subclinical evidence of a PCOS-related dysregulation of ovarian steroidogenesis. However, most V-PCOM appear to be ovulatory and at low risk of developing symptomatic PCOS (31, 197, 207, 214, 219). How is this to be interpreted? The subgroup of eumenorrheic young women who have subclinical hyperandrogenemia, which suggests ovulatory PCOS, may well represent a carrier state, or occasionally a risk factor, for PCOS (197), although this remains to be established. The spectrum of ovarian androgenic ovulatory dysfunction may well be wider than ascertained through the presence of PCOM or hyperandrogenemia. Among apparently normal eumenorrheic women, a prospective study indicated that about 8% had sporadic anovulatory cycles and that serum free testosterone and AMH levels were significantly increased in these cycles, although within the normal range (220).

On the other hand, a substantial minority of our asymptomatic volunteers have isolated (normoandrogenemic) PCOS-like dysregulated steroidogenic function. Many of this subgroup have elevated AMH levels, which is compatible with a very mild degree of intraovarian androgen excess promoting folliculogenesis (79, 143) without interfering with ovulatory function.

Those V-PCOM with AMH elevation, some of whom have absolutely normal steroidogenic function and some of whom have subclinical evidence of steroidogenic dysregulation (Figure 6), are expected to have an increased population of growing follicles (39, 144). Thus, they can be predicted to have a slightly prolonged reproductive lifespan (221).

In conclusion, about half of asymptomatic V-PCOM have evidence of an ovarian steroidogenic dysfunction related to PCOS, including some who seem to have ovulatory PCOS. They are postulated to be carriers for, or at risk for, PCOS.

C. Functional adrenal hyperandrogenism (FAH) in PCOS

Less than 10% of FAH can be accounted for by well-established pathophysiologic entities, the most common of which is nonclassical virilizing congenital adrenal hyperplasia with a prevalence approximating 5% (Table 4). Most FAH is idiopathic (primary), ie, it cannot be incon-

trovertibly assigned to any of these well-established disorders.

Primary FAH is defined as 17-ketosteroid hyperresponsiveness to ACTH that is otherwise unexplained, which practically speaking involves ruling out steroidogenic blocks (17). The steroidogenic pattern of response to ACTH resembles an exaggerated adrenarche (22). DHEA is the sole hyperresponsive 17-ketosteroid when testing is performed with low-dose ACTH; it was abnormal in 27% of our PCOS series, with similar prevalence in both PCOS-T and PCOS-A (47). DHEA hyperresponses are accompanied by 17-hydroxypregnenolone hyperresponses ($r = 0.773$), which suggests a relationship between these two entities. Higher-dose ACTH testing yielded a higher prevalence estimate for FAH in PCOS (46%), and androstenedione hyperresponses accounted for most of the difference (22). The pattern of adrenal secretion is compatible with dysregulation of zona reticularis steroidogenesis prominent at the level of the 17-hydroxylase/17,20-lyase activities of P450c17 (Figure 2). The FAH of PCOS is accompanied by an average 50% increase in adrenal volume that correlates with hyperandrogenemia severity (222).

About one-quarter of FOH have the common (primary) type of FAH (Figure 5). This agrees with most estimates of FAH prevalence in PCOS from serum DHEAS measurements (28). However, the magnitude of the DHEAS correlation with DHEA responsiveness to ACTH ($r = 0.7$) is such that there is considerable nonspecificity in this estimate, which seems due to the high (~70%) heritability of DHEAS serum levels (223–225).

D. Other sources of androgen in PCOS

A glandular source of androgen cannot be identified in approximately 8% of hyperandrogenic patients despite thorough testing. Those who present with hirsutism and normal menses, but lack a polycystic ovary, are traditionally given a diagnosis of idiopathic hyperandrogenism.

In our series of PCOS patients who met NIH diagnostic criteria, the subset with no demonstrable ovarian or adrenal dysfunction was obese (Figure 4a and Table 2) (47). We postulated that their excess adipose tissue was both the cause of the testosterone excess (because adipocytes are a site of conversion of circulating androstenedione to testosterone) and the cause of the ovulatory dysfunction (because obesity suppresses LH levels) (83, 226–229), as discussed below (see section V.B.2). These patients with the PCOS-A of obesity were characterized by mild hyperandrogenemia (normal total testosterone, mildly elevated free testosterone, normal DHEAS, low SHBG), and most had normal-size ovaries, normal LH levels, and normal AMH levels; all had insulin resistance that was similar to that of the other PCOS-A subgroups.

The possibility is unexplored that some cases in whom there is no glandular source of androgen are caused by hereditary defects in the peripheral metabolism of steroids, such that testosterone formation from precursors is excessive.

E. Summary

The common denominator of the great majority (87%) of PCOS patients is FOH. Two-thirds of PCOS cases have 17OHP hyperresponsiveness to GnRHag or hCG stimulation (functionally typical FOH). Two-thirds of the remainder have FOH detectable by DAST, in which testosterone remains elevated after suppression of adrenal androgen production. About 3% of PCOS have isolated FAH. The remaining PCOS cases are mild and lack evidence of steroid secretory abnormalities; most of these are obese, and we postulate that their excess adipose tissue accounts for their PCOS. These relationships among the sources of androgen in PCOS are summarized in Table 2 and Figure 5.

V. Pathophysiology of PCOS: Abnormal Regulation of Steroidogenesis and Ovarian Function

The above in vivo data suggest that the fundamental defect in most PCOS is FOH due to an otherwise unexplained (primary) unique type of steroidogenic hyperactivity that seems to disturb the intraovarian processes that normally coordinate ovarian androgen and estrogen secretion (22).

The PCOS ovary is typically hypersensitive to LH stimulation. The initial studies of the responses to GnRHag suggested abnormal steroidogenic dose-response relationships in response to LH that were consistent with partial escape from desensitization (198). Subsequent studies directly demonstrated hypersensitivity to submaximal hCG test doses associated with a similar pattern of increased androgen responsiveness (Figure 3) (90, 95). Initially, the possible causes of this dysregulation of androgen secretion were postulated to include insulin excess, which is known to sensitize the ovary to LH by interfering with the normal process of homologous desensitization to LH as discussed above, or an intrinsic imbalance among intraovarian regulatory systems (198).

Support for an intrinsic theca cell defect has come from both in vivo and in vitro studies. In vivo, ovarian steroidogenic hyperfunction in response to submaximal acute hCG challenge persists after the ovarian quiescence achieved by 1–3 months of gonadotropin suppression (95, 207). In vitro studies have shown the presence of an intrinsic theca cell abnormality that is independent of LH

receptor status by demonstrating that an overactive steroidogenic phenotype is constitutively present in isolated theca cells and persists through long-term passage in cell culture, which suggests an inherent defect(s) (23, 72).

These findings support the concept that FOH is usually the essence of PCOS. The intraovarian level of androgens in FOH would seem to be higher than those in FAH and such extraovarian disorders as nonclassical virilizing congenital adrenal hyperplasia in the presence of similar circulating levels of androgen. Only frankly virilizing extraovarian disorders would be expected to boost intraovarian androgen levels to those of FOH. Androgen excess, as noted above, has been demonstrated to enhance the initial recruitment of primordial follicles into the growth pool and thus play a role in initiating the growth of small antral follicles (79, 136, 143). Androgen excess also initiates premature luteinization (138, 139, 147), which hinders ovulation by impairing selection of the dominant follicle (155, 157). Androgen excess has also been shown to cause the classical PCOS histopathologic and gross anatomic changes that constitute PCOM (9, 230).

However, PCOS is a multisystem disorder. A metabolic syndrome, underpinned by insulin resistance and obesity, is common in PCOS, and the insulin resistance is excessive for the degree of adiposity (13, 231–233). PCOS is a state in which tissue-selective resistance to the glucose-metabolic effects of insulin seems to be paradoxically associated with ovarian sensitivity to insulin, such that the compensatory hyperinsulinemia of insulin resistance contributes to ovarian androgen excess (24, 234). Insulin excess appears to do so primarily by leading to a partial "escape" from desensitization of ovarian responsiveness to LH, with the consequence that ovarian steroids are hyperresponsive to LH (see sections III.B.2.b and V.B.1.a) (22, 88, 89).

LH excess is common in the disorder. LH is necessary for the expression of gonadal steroidogenic enzymes and sex hormone secretion. Consequently, PCOS is LH dependent (hence, "functional"), and any treatment or disorder that suppresses LH levels suppresses ovarian steroidogenesis. However, the moderate increase of LH that characterizes PCOS seems unlikely to ordinarily be the primary cause of the ovarian androgen excess, due to the normal process of LH-induced desensitization of theca cells.

A. Dysregulation of ovarian function in PCOS

1. Dysregulation of steroidogenesis in PCOS

a. Intrinsic theca cell dysfunction. In vitro studies have provided convincing evidence for a thecal cell defect that can account for excess androgen production and the steroidogenic secretory pattern observed in response to gonadotropin stimulation. They show that isolated thecal cells overexpress most steroidogenic enzymes, particularly cytochrome P450c17, and LH receptors (23, 235).

Critical evidence in support of inherent ovarian steroidogenic dysfunction in PCOS came from the demonstration by the McAllister group that "augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries" of PCOS patients (23). Theca cells were obtained at hysterectomy from 3- to 5-mm follicles of polycystic ovaries of women meeting NIH criteria for PCOS and asymptomatic fertile women. They were cultured on fibronectin-coated plates in a highly enriched medium including 20% serum; at confluence they were frozen at −70°C. Subsequently, cells were thawed and passaged 3–4 times (22–38 population doublings); then experiments were performed in serum-free medium from age- and follicle-sized matched normal and PCOS subjects. These studies showed that progesterone, 17OHP, and testosterone production per cell were markedly increased in PCOS theca cell cultures. Moreover, basal and cAMP-stimulated pregnenolone, progesterone, and DHEA metabolism were increased dramatically in PCOS theca cells. PCOS theca cells were capable of substantial metabolism of precursors into testosterone, reflecting expression of androgenic 17βHSD activity. cAMP-stimulated *CYP11A* and *CYP17A1* expressions were augmented in PCOS theca cells compared with normal cells, whereas no differences were found in steroidogenic acute regulatory protein mRNA expression. Collectively, these observations establish that increased *CYP11A* and *CYP17A1* mRNA expression, as well as increased P450c17, 3βHSD, and 17βHSD enzyme activity per theca cell, and consequently increased production of progesterone, 17OHP, and testosterone, are stable properties of PCOS theca cells (23). Subsequent study indicated that increased synthesis of testosterone precursors, particularly via increased P450c17 and 3βHSD activity, is the primary factor driving enhanced T secretion in PCOS (72). Stimulated *HSD17B5* mRNA was not consistently overexpressed in PCOS, but the statistical power of the study was too low to reach a firm conclusion about the contribution of *HSD17B5* gene expression to the increased 17βHSD activity in PCOS.

Recently, drawing upon genome-wide association screening data discussed later, McAllister's group reproduced the PCOS theca phenotype in vitro by forced overexpression of a DENND isoform (A1) that is increased at the mRNA and protein level in PCOS theca cells (108). DENND1A encodes a protein (connecdenn 1) associated with clathrin-coated pits where cell-surface receptors reside that is located in the cytoplasm and nuclei of theca

cells. Alternative splicing of DENND1A generates 2 transcripts, and it is the shorter transcript, DENND1A.V2, that is differentially expressed in PCOS and normal theca cells. "Forced overexpression of DENND1A.V2 in normal theca cells resulted in a PCOS phenotype of augmented *CYP17A1* and CYP11A1 gene transcription, mRNA abundance, and androgen biosynthesis. Knockdown of DENND1A.V2 in PCOS theca cells reduced androgen biosynthesis and *CYP17A1* and CYP11A1 gene expression" (108).

A polyclonal antibody specific to the unique C-terminal sequence of DENND1A.V2 also reduced androgen biosynthesis and *CYP17A1* and *CYP11A1* mRNA in cultured PCOS theca cells. Additionally, urinary excretion of exosomal DENND1A.V2 RNA in PCOS women was significantly increased in comparison with normal. Thus, DENND1A.V2 potentially plays a key role in the hyperandrogenemia associated with PCOS. It seems likely that excess DENND1A.V2 expression will prove to underlie typical FOH.

The mechanism by which DENND1A.V2 stimulates steroidogenesis is currently unknown. DENND1A is a member of the connecdenn family of proteins, which are clathrin associated adjacent to the inner cytoplasmic membrane and which are involved in protein trafficking, endocytotic processes, and receptor recycling (236). Thus, it is tempting to speculate that it affects LH action by upregulating LH receptor signaling, much as insulin does in causing escape from LH-induced receptor desensitization.

b. Adrenocortical androgenic dysfunction in PCOS. FAH often coexists with FOH but may occur in its absence (Table 2). The FAH of PCOS is a unique type of adrenal dysfunction that is characterized by DHEA hyperresponsiveness and hypersensitivity to ACTH of DHEA without evidence of a block in steroidogenesis (22, 47). 17-Hydroxypregnenolone responses to ACTH are highly concordant with DHEA responses in adults and are more often abnormal in adolescents, who have not completed adrenarche (237). Submaximal ACTH stimulation elicits selective DHEA and 17-hydroxypregnenolone hyperresponses in 28% of PCOS patients (47). High-dose ACTH testing demonstrates FAH more often (46%) and elicits additional androstenedione, 17OHP, and/or 11-deoxycortisol hyperresponses; 18% of FAH detected by high-dose ACTH testing is comprised of androstenedione, 17OHP, and/or 11-deoxycortisol hyperresponsiveness in the absence of DHEA hyperresponsiveness (22). The broader spectrum of steroidogenic abnormality in response to high-dose ACTH testing is possibly because PCOS patients have an increased capacity for adrenal androgen secretion (238). FAH is reflected in a high DHEAS level in 20%–30% of patients (239). Adrenal gland volume is reportedly increased about 50% and to correlate positively with age, DHEAS, 17OHP, and testosterone levels and negatively with LH levels (222).

Originally, FAH was attributed to exaggerated adrenarche (22). Subsequently, FAH came to be mistaken for deficiency of *3βHSD2*, which is now known to be a rare disorder of steroidogenesis (240, 241). The DHEA responses of FAH are not of the magnitude found in *HSD3B2* deficiency (*HSD3B2* mutations are not found unless DHEA responses are >5 SD above the mean norm). A functional deficiency of this enzyme activity has been suggested (242).

However, dysregulation of steroidogenesis seems to be the most parsimonious explanation for FAH. The steroidogenic pattern in response to ACTH usually is consistent with 17,20-lyase hyperactivity (22, 240, 243) and overactivity of the early enzymatic steps common to adrenal zona reticularis and ovarian steroidogenesis (eg, P450scc and P450c17 activities). The resultant adrenal secretory pattern differs from the ovarian secretory pattern because of the characteristically different patterns of steroidogenic enzyme expression of these glands: the consequence is that adrenal androgen biosynthesis flows predominantly into DHEA and DHEAS due to the lower 3β-HSD2 and higher sulfotransferase activity of the adrenal zona reticularis than of ovarian theca cells (Figure 1 vs Figure 2). Although no direct evidence exists in support of this hypothesis of adrenal P450c17 activity dysregulation, it is notable that DENND1A is reportedly expressed in the adrenal zona reticularis as well as in ovarian theca cells (236). Furthermore, insulin administered in vitro alters adrenal steroidogenesis much as it does ovarian steroidogenesis (176, 177), and insulin infusion results are consistent with potentiation of 17α-hydroxylase and 17,20-lyase activities in response to ACTH (244). 17-Hydroxypregnenolone responses to ACTH correlate with fasting insulin levels and are lowered by metformin therapy (245). Free fatty acid overload has also been implicated in adrenal hyperandrogenism (246, 247).

Other causes of FAH have been suspected. The unopposed hyperestrogenism of PCOS has been suspected of playing a role in enhancing DHEA secretion by modulating steroidogenic enzyme activity (248); one group reported that steroid ratios indexing 17,20-lyase activity post-CRH stimulation correlated with plasma estradiol in PCOS subjects whose estrogen levels were manipulated. Recent data suggest that intraadrenal end-product inhibition of 3βHSD activity by cortisol might play a role in increasing adrenocortical DHEA secretion (249). Increased cortisol turnover due to elevated peripheral 5αRD activity or decreased peripheral 11β-HSD1 (cortisone

RD) activity have been suggested to elicit compensatory ACTH hypersecretion that maintains cortisol levels at the expense of adrenal hyperandrogenism (239, 250). The latter might be related to a common functional polymorphism in the *HSD11B1* gene that has been related to FAH (251). On the other hand, obesity may contribute to both pathways (239), and androgens may inhibit 11β-HSD1 (252). The possible role of 5αRD activity in PCOS is discussed in more detail below (see section V.D).

In summary, dysregulation of zona reticularis steroidogenesis akin to that of theca cells seems to be the most parsimonious explanation for the FAH of PCOS, but there is a paucity of direct evidence.

2. Granulosa cell dysfunction and disordered folliculogenesis

a. Granulosa cell dysfunction contributes to thecal androgen excess. A large body of clinical observations and animal studies suggests that theca cell function is modulated by paracrine factors produced by granulosa cells in response to FSH (Figure 1). Inhibin-B, a peptide that is reciprocally regulated by FSH in a negative feedback loop, is essential and permissive for thecal androgen production, as noted earlier (95). The fall in inhibin-B as follicle number diminishes during menopause may well account for the waning hyperandrogenism of PCOS patients during middle age, as noted above (54, 56). In typical FOH, serum inhibin-B levels are hyperresponsive to FSH, even after prolonged gonadotropin suppression (95). Ovarian inhibin-B betaglycan receptor expression is also increased in PCOS (253). Whether inhibin-B excess plays a role in the hyperandrogenemia of PCOS is unclear because normally FSH predominantly seems to reduce testosterone levels (95). This normal FSH effect is not found in typical FOH. Although this lack of testosterone suppression by FSH might be ascribed to the inhibin-B hyperresponsiveness to FSH, in vitro observations suggest that normal granulosa cells exert a restraining effect on thecal androgen secretion in vivo that is lacking in PCOS; isolated thecal cells from clinically normoandrogenic ovulatory subjects with polycystic ovaries have been reported to secrete as much 17OHP and androgen in culture as did those from hyperandrogenic anovulatory PCOS patients (254). Other granulosa cell peptides are known to induce expression of LH receptors and steroidogenic enzymes during early theca cell development (255, 256) and may mediate the granulosa cell effects on theca cells in PCOS.

b. Disordered folliculogenesis and PCOM. The ovaries of PCOS patients classically show an excessive number of small antral follicles and various degrees of theca cell hyperplasia and hypertrophy ("hyperthecosis" and "luteinization" in histologic terms), stromal hyperplasia and hypertrophy, and cortical thickening (257). The increased number of small (2–9 mm) antral follicles results from an increased proportion of follicles leaving the resting (primordial follicle) phase to become growing (primary) follicles and eventually small antral follicles that have a prolonged lifespan when the follicle maturation arrest occurs that hinders dominant follicle development (258, 259). Androgen excess can account for all of these morphologic changes (9, 136) and insulin excess likely contributes, but it is unclear whether intrinsic disturbances exist in the ovarian follicle itself.

Enhanced follicle recruitment in PCOS has been attributed to androgen excess promoting the number of small follicles (143). In the rhesus monkey, testosterone implants that raised serum levels 5- to 10-fold rapidly increased the number of primary follicles as well as healthy preantral and small antral follicles by 3- to 5-fold (136). Cystic follicle development may be promoted by androgenic inhibition of follicular 11β-HSD1 increasing cortisol locally (252). Due to their roles in folliculogenesis, GDF9 and BMP15 have been examined in PCOS, but aside from reduced expression of GDF9 in the early stages of follicle development, no convincing evidence has emerged to support their involvement in the abnormal folliculogenesis (137, 260).

Increased folliculogenesis is responsible for the increased AMH production by polycystic ovaries; the increase in AMH is due in part to the increase in follicle count (142), and in part due to increased AMH production per granulosa cell (261, 262). The androgen-induced increase in folliculogenesis has been postulated to cause the AMH elevation of PCOS (143).

Premature luteinization of granulosa cells is indicated biochemically by their acquisition of LH responsiveness at an inappropriately early stage (156). Premature luteinization seems attributable to both androgen excess (9, 136) and insulin excess (156). Both granulosa and theca cells overexpress LH receptors (235), which increases LH responsiveness. Androgen increases LH responsiveness by an indirect mechanism; LH-dependent excessive thecal androgen production causes increased granulosa cell FSH receptor expression (147), which in turn increases thecal LH receptor expression (138, 139, 152). Insulin augmentation of gonadotropin action (89) can account for follicular LH responsiveness to develop at a premature stage (156). The large pool of prematurely luteinized follicles is particularly hyperresponsive to LH. Consequently, unlike midfollicular phase normal women, PCOS women inappropriately secrete estradiol as well as excessive androgen in response to LH/hCG (Figure 3) (95).

Prematurely luteinized granulosa cells are also hypersensitive to FSH. Estradiol is overproduced in response to

FSH (95, 263, 264) and seems important for further stimulation of granulosa cell and follicular growth (265, 266). Inhibin-B is hyperresponsive to FSH in typical FOH (95), despite low follicular fluid levels and normal baseline serum levels (140), and combines with the inappropriate estradiol secretion to inhibit FSH secretion and lower FSH levels, and thereby prevent frank hyperestrogenism. However, if challenged with exogenous FSH, as during fertility treatment, PCOS women are at risk of developing ovarian hyperstimulation syndrome (267).

The mechanism of follicular maturation arrest is a subject of debate. Granulosa cell IGF receptor and IGF-binding protein gene expression, which are, respectively, enhanced and inhibited by FSH, are consistent with follicular maturation arrest (268). Premature luteinization may also play a role in follicle maturation arrest by inhibiting further follicle proliferation and so hinder dominant follicle emergence (156). Follicle maturation arrest may be an indirect consequence of the increase in early folliculogenesis via the AMH inhibitory effect on the response of antral follicles to FSH (142).

VEGF serum levels and ovarian gene expression are increased in PCOS, as is ovarian vascularity (114, 145). It has been postulated that this hypervascularity contributes to the relatively dense cortex of the PCOS ovary (159) and inappropriately delivers inflammatory cytokines, and that these factors aggravate the disorder and contribute to ovarian hyperstimulation risk (114, 145). The sclerotic cortex has also been postulated to inhibit the growth-restraining Hippo signaling pathway and contribute to proliferation of granulosa, theca, and stromal cells (125). Local factors, such as VEGF and Hippo, have been postulated to be important mediators of the ovulation induced by ovarian surgery (125, 269).

Differential expression profiling of granulosa cell cDNA is being used as a means to detect the nature of signaling pathway abnormalities in PCOS (270–272). These data suggest that there are multiple and complex disturbances of biological functions: regulation of fatty acid metabolism, insulin and kinase signaling, cell-cell signal transduction, immune, oxidative metabolism, oxidative stress, and inflammatory responses.

Oocyte gene expression is also dysregulated in PCOS and the possibility has been raised that this might be related to the increased risk for pregnancy loss of some PCOS patients (273). A comparison of high quality oocytes from 9 PCOS and 10 unmatched controls undergoing in vitro fertilization showed the PCOS oocytes to have abnormal gene expression profiles that are associated with defective meiosis or early embryonic development. A high proportion of the differentially expressed genes contain androgen and peroxisome proliferator-activated receptors, which suggests that excessive androgen and epigenetic metabolic signals contribute to the reduced developmental competency of PCOS oocytes. However, in vitro oocyte maturation rate, fertilization rate, and grade 1 embryo rate were reported to be significantly higher for PCOS than in controls, and the pregnancy, miscarriage, and live birth rate outcomes were reportedly normal for the number and quality of transferred embryos (274, 275). Factors other than oocyte quality, such as obesity and placental compromise, seem to determine pregnancy outcomes of PCOS women.

3. Summary

Theca cells from the polycystic ovaries of PCOS patients ("classic PCOS") have provided convincing in vitro evidence for an intrinsic defect that can account for excess androgen production and the steroidogenic secretory pattern observed in vivo. This defect is independent of normal endocrine and paracrine regulatory processes. After long-term passage, these cells in culture overexpress most steroidogenic enzymes, particularly cytochrome P450c17. Overexpression of DENND1A.V2, a protein identified via genome-wide association screening data discussed later, has reproduced this PCOS phenotype in normal theca cells in vitro. FAH is likely to have a similar basis, but no direct evidence exists.

Granulosa cell dysfunction appears to contribute to theca cell overproduction of androgens. The data suggest paracrine defects in FSH inhibition of theca cell function that involve inhibin-B and likely other as-yet-unidentified factors. Granulosa cells prematurely luteinize primarily as a result of androgen and insulin excess. Folliculogenesis is excessive as indicated by PCOM and AMH elevation; although this may be secondary to androgen excess, the possibility cannot be excluded that these abnormalities may be a manifestation of an intrinsic defect in the intraovarian regulation of folliculogenesis (155).

B. Relationship of metabolic syndrome to PCOS

Metabolic syndrome is defined by a cluster of hyperglycemia, central obesity, hypertension, and dyslipidemia. Expression of these individual components can vary between individuals. It ordinarily results from the interactions of insulin resistance and obesity with age (276, 277). Metabolic syndrome occurs in up to one-third of PCOS adolescents and nearly half of PCOS adults (278–282). Among PCOS patients, an abnormal degree of insulin resistance is reported in about one- to two-thirds (although insulin resistance per se is not a defining feature) (13, 233, 283–285); obesity prevalence is similar, with considerable variability among populations (1). A number of observations suggest that among obese women with PCOS, met-

abolic abnormalities related to insulin resistance and obesity are in many instances more important in the mechanism of anovulation in PCOS than androgen excess (286–288).

Type 2 diabetes mellitus (DM) itself is related to PCOS (289, 290), and deterioration of glucose tolerance can be accelerated in PCOS (291). Glucose intolerance and diabetes arise when β-cell failure compromises the ability of insulin secretion to compensate for insulin resistance. There is a strong heritable component to β-cell dysfunction in women with PCOS (292), and glucose intolerance, ie, β-cell failure, seems to be specifically associated with PCOS-T rather than PCOS-A (47).

1. Role of insulin-resistant hyperinsulinism in PCOS pathogenesis

Insulin resistance is not only common in PCOS, it is often excessive for the degree of adiposity and is found in nonobese PCOS women (13, 24, 231–234). Insulin resistance in PCOS is characterized by reduced sensitivity and responsiveness to insulin-mediated glucose utilization primarily in skeletal muscle and adipose tissue, although the nature of these defects differs (24, 293). The insulin resistance of PCOS typically has a prominent intrinsic element, although in some cases, it may simply be acquired because of exogenous obesity. The mechanism characteristically involves constitutive, tissue specific, postbinding defects in receptor signaling that selectively affect metabolic pathways but not mitogenic or steroidogenic, actions (22, 232, 234, 294). Intracellular serine kinases account for phosphorylation of the insulin receptor and insulin receptor substrate-1 that decreases insulin activation of the phosphatidylinositol-3-kinase signaling pathway that activates glucose transport; serine kinase phosphorylation also activates mitogenic pathways mediated by ERK/MAPK (232). PCOS-related commonalities in insulin signaling in the ovary and other tissues have been noted. Constitutive activation of serine kinases that contribute to resistance to insulin's metabolic actions in skeletal muscle has been proposed to contribute to the increased 17,20-lyase activity of P450c17 in steroidogenic tissue, but commonalities in kinase signaling pathways involving both effects has been elusive (64). Postreceptor insulin signaling through the transcriptional coregulator protein Kruppel-like factor 15 in steroidogenic tissues and adipose tissue up-regulates testosterone formation via increased *HSD17B5* gene expression and also stimulates adipogenesis in fat depots (107); this signaling appears to be intact in the face of insulin resistance (25).

A paradox is created by the tissue-selective resistance to the metabolic effects of insulin: the hyperinsulinemia compensatory for resistance to the glucose-metabolic effect of insulin elicits excess insulin action in some tissues in the presence of resistance to insulin action in others. This insulin-resistant hyperinsulinism (hyperinsulinism) is a major extraovarian factor in the steroidogenic dysregulation and DM-related comorbidities of PCOS (231, 232, 295). Mitogenic signaling and protein metabolism also remain sensitive to insulin, according to studies in cultured skin fibroblasts from PCOS patients (232, 296).

a. Insulin resistance and ovarian dysfunction. Insulin has been shown to stimulate PCOS theca cell (297) and normal granulosa cell (105, 298) steroidogenesis through the insulin receptor rather than through the IGF-1 receptor. The initial report of antibody blockade of the insulin receptor showing an IGF-1 receptor mediated effect of insulin on PCOS thecal steroidogenesis (297) was of dubious significance because supraphysiologic doses of insulin were used in PCOS theca cells. However, selective knockout of theca cell insulin receptors in transgenic mice has been shown to ameliorate the hyperandrogenic anovulation of insulin resistance induced by an obesogenic diet; this indicates that hyperandrogenic anovulation was caused by insulin signaling through the theca cell insulin receptor (25). Although this mouse model differs from human PCOS in some regards (eg, LH levels are high in obese mice, normal in obese PCOS), this constitutes proof of principle that insulin signaling in the ovary is preserved in a state of resistance to the metabolic effects of insulin. Insulin signaling in the GnRH neuron similarly seems to be preserved (299).

Hyperinsulinemia augments LH stimulation of ovarian androgen production by up-regulating LH-binding sites and enhancing androgen production in response to LH at the level of cytochrome P450c17, as discussed earlier (Figure 1). Insulin and IGF-1 also stimulate expression of adrenal P450c17 and 3βHSD2 activities (176, 177), with lesser effects on other steroidogenic steps (300). Insulin potentially augments LH-stimulated androgen production through several other mechanisms. Insulin may also act via the IGF-1 receptor, atypical IGF-1 receptors, or a hybrid receptor that contains a combination of α- and β-subunits of both receptors (22). It has also been postulated that, by lowering levels of IGF-binding protein 1, insulin may raise the fraction of IGF-1 that is bioavailable (22).

All known forms of insulin resistance are associated with PCOS (22). PCOS is evident in patients with the extreme insulin resistance of hereditary insulin receptor mutations or lipodystrophy (22, 301). The PCOS of such extreme generalized insulin resistance may well be the result of extreme hyperinsulinemia promiscuously signaling through the IGF-1 receptor in the ovarian thecal cell. The

IGF-1 excess state of acromegaly also is associated with PCOS (302).

The severe insulin resistance of pseudoacromegaly is characterized by a selective defect in postreceptor insulin signaling that only affects the glucose metabolic pathway mediated by phosphoinositide 3-kinase, whereas mitogenic signaling via the MAPK system remains intact (52, 303). In this syndrome, as in ordinary PCOS where the mechanisms are less clear (232), hyperinsulinemia appears to cause clinical symptoms (in this case, acromegaloid overgrowth) and to augment ovarian androgen production by signaling through one arm of the insulin signaling cascade via the insulin receptor itself.

More modest forms of insulin resistance also are associated with PCOS, including both major forms of DM. As an example, premenopausal women with type 2 DM have a PCOS prevalence of 30%–40% (304, 305). Type 1 DM also is associated with PCOS; this association is thought to be due to the supraphysiologic systemic doses of insulin required to control glycemia in the absence of the efficient hepatic glucose utilization that normally results from insulin secretion into the portal system (306). In both types of diabetes, insulin levels are not sufficiently high to act via the IGF-1 receptor

All treatments that cause a reduction in serum insulin levels, whether by weight loss, bariatric surgery, or by administration of somatostatin, metformin, or insulin-sensitizing thiazolidinediones, significantly improve ovulation and hyperandrogenemia in PCOS (1, 22, 210, 287, 288, 307, 308). The extent to which hyperinsulinemia is fundamental to PCOS pathophysiology has been a subject of debate. About half of PCOS patients experience improvement in the PCOS symptoms when they lose weight, and patients with the most severe ovarian dysfunction are those least likely to benefit symptomatically from weight loss (309). This is consistent with the concept that it is the patients with the atypical FOH of obesity, discussed earlier, that can expect the greatest symptomatic benefits from weight loss (47). Metformin, an antidiabetic drug, is well established to transiently reduce BMI, improve menstrual frequency, and lower testosterone levels by about 20%, which has a limited, if any, efficacy for hirsutism (41, 310, 311). Well-controlled studies indicate that metformin therapy offers no advantage over lifestyle modification in regards to weight, insulin levels, menstrual frequency, or ovulation (57, 211, 312, 313). The efficacy of thiazolidinedione therapy appears to be similar (313), but these agents are seldom used because they promote adipogenesis. Dietary supplementation with the insulin sensitizers myo- or D-*chiro*-inositol has shown anecdotal (314) or inconsistent effects on PCOS ovarian function (315).

Ibáñez et al have issued a series of positive reports of a prolonged randomized nonblinded trial comparing combined metformin-thiazolidenedione-antiandrogen therapy with estrogen-progestin therapy in hyperinsulinemic PCOS adolescents (316). They found these treatments to similarly lower serum total testosterone levels (but probably not free testosterone because marked differences in SHBG responses exist) with 94% normalization of menstrual irregularity while conferring markedly superior metabolic outcomes. Their experience has led them to equate PCOS with the hyperinsulinemia of insulin resistance ("Hyperinsulinaemic androgen excess is the most common cause of hirsutism, acne and menstrual irregularity in adolescent girls"; see Ref. 317). However, their Catalan PCOS population is both nonobese and hyperinsulinemic, which does not seem representative of the type of PCOS described in the United States and Europe. To illustrate this, we have applied their hyperinsulinemia criteria post hoc to a comparable subset of our study population that was studied by similar methodology (197). We compared the 14 nonobese PCOS adolescents (15.9 ± 1.4, SD, years old, BMI 24.7 ± 3.0), which represents 26% of our cohort of 53 adolescent PCOS, with our 19 nonobese control adolescents (15.0 ± 1.6 y, BMI 22.9 ± 2.4). Four of this PCOS subset, 1 of whom had asymptomatic diabetes by glucose tolerance test (GTT) criteria, had greater insulin resistance by homeostatic model assessment (HOMA-IR) (318) than the controls ($P < .05$). However, this PCOS subset was not significantly hyperinsulinemic, neither compared with our controls nor by the criteria of Ibáñez et al, which were met by 36%–37% of both our groups (316, 319).

Another group has reported that metformin and rosiglitazone, alone and in combination, similarly improve ovulation rates and testosterone levels in nonobese women with PCOS and normal indices of insulin sensitivity (320). These data are compatible with insulin-promoting hyperandrogenism independently of hyperinsulinemic insulin resistance and are consistent with the concept that levels of insulin in the normal range modulate the androgenic response to LH (88). This possibility is consistent with experimental data in which selective knockout of theca cell insulin receptors attenuated the androgenic response to hCG in both lean and obese transgenic mice (25).

On the other hand, caution must be exercised in attributing metformin actions to specific targeting of insulin resistance. Metformin's "insulin-sensitizing effect" is more accurately an insulin-lowering effect that appears to be a consequence of the reduction of hepatic glucose output and inhibition of gastrointestinal glucose absorption. Increasing glucose effectiveness occurs via effects on mitochondrial complex I adenosine triphosphate generation

and activation of cAMP-activated protein kinase (321–326). These biochemical processes have the potential to exert a broad spectrum of effects, among which are known to be direct inhibition of steroidogenesis (327–329) and rarely lactic acidosis (330).

Liraglutide, a glucagon-like peptide receptor 1 agonist, in a 12-week trial was recently reported to be as efficacious as metformin in treating PCOS (331). Liraglutide improves glucose homeostasis by enhancing the early insulin response to meal ingestion and inhibiting glucagon secretion; it also inhibits appetite. Weight loss, HOMA-IR, glucose tolerance, and serum free testosterone were similar after liraglutide and metformin. Indeed, monotherapy outcomes in the most obese and insulin-resistant subset of their study group were significantly better than with metformin. Furthermore, the liraglutide effect was additive with that of metformin (332). The possibility that these effects were due in part to direct effects on the hypothalamic-pituitary-ovarian axis (333) cannot be excluded.

b. Insulin resistance and adipose tissue biology. Insulin signaling is of major importance to the size and function of the adipose tissue depot: it stimulates adipogenesis (development of preadipocytes into adipocytes) and lipogenesis while inhibiting lipolysis (334, 335). The critical periods for establishment of the adipocyte population are fetal life and adolescence, after which lipid accumulation occurs primarily by cell hypertrophy (335). The overexpansion of PCOS fat depots suggests that insulin signaling in fat is intact in PCOS.

Studies on adipocytes from women with PCOS have revealed impaired insulin sensitivity (293, 336–338) and reduced glucose transport (24, 293, 336). In some studies this has been shown to be related to lower expression of the insulin-sensitive glucose transporter-4 (GLUT-4) (339, 340). Defects in tyrosine phosphorylation in the insulin signaling pathway have been identified, although not consistently (24, 340). Epigenetic changes such as microRNA overexpression have also been reported in association with reduced glucose transport in abdominal adipocytes of women with PCOS (340).

However, in contrast to skeletal muscle, in PCOS, the abdominal adipocyte cell lineage has no intrinsic defect in insulin-stimulated glucose transport, the first step of lipogenesis, and glycogen formation. Testosterone causes an acquired defect in lipogenesis by impairing sensitivity of glucose transport to insulin in both abdominal and visceral adipocytes (24, 341–344). Androgen excess may also contribute to adipocyte insulin resistance by suppressing production of the insulin-sensitizing adipokine adiponectin (345–347).

Androgens have been shown to inhibit differentiation of human and murine preadipocytes at a point before peroxisome-proliferator activated receptor expression (345, 348), although adipocyte stem cell commitment to abdominal preadipocytes is increased according to the prenatally androgenized monkey model of PCOS (349). These data suggest the potential for a local negative feedback regulatory loop for adipogenesis whereby androgen generated by sc adipocytes, as they differentiate in response to insulin (107), in turn impairs insulin-stimulated adipocyte lipogenesis (342, 348, 349). This loop remains to be documented, as does the extent to which these effects occur in visceral fat.

Although the relationship between hyperandrogenism and insulin resistance appears to primarily result from the effect of the compensatory insulin secretion on steroidogenesis, excess androgen may modestly reduce insulin sensitivity in vivo. Long-term testosterone-induced virilization of female-to-male transsexuals and female nonhuman primates fed a high-fat diet reduced insulin sensitivity of glucose uptake (343, 350, 351). On the other hand, androgen action via 5αRD type 1 promotes insulin sensitivity in males (352, 353). In PCOS, reducing androgen levels by antiandrogen or GnRHag treatment has improved insulin sensitivity in only a minority of studies (343).

The central fat accumulation in PCOS and the occasional presentation of pseudo-Cushing's syndrome as a manifestation of severely hyperinsulinemic PCOS (52) are consistent with insulin adipocyte actions being intact in PCOS and suggest a relation of hyperinsulinemia to glucocorticoid action (354, 355). Glucocorticoids augment insulin stimulation of adipogenesis and lipogenesis and redistribute fat to visceral stores (335, 354) while causing insulin resistance and attenuating secretion of insulin by the pancreatic β-cell (356). Local cortisol generation by adipocyte HSD11B1 is up-regulated in obesity and PCOS; a role for insulin in this process is unclear (357). On the other hand, in contrast to fat-specific insulin receptor knockout, which reduces fat stores by half (334), fat-specific glucocorticoid receptor deletion (358) does not affect baseline fat stores or fat distribution on either standard or high-fat diets, although it protects against cortisol-induced obesity. Thus, the mechanisms of glucocorticoid-insulin interactions in the regulation of adipogenesis and adipocyte function are unclear.

In summary, about half of PCOS women have an abnormal degree of insulin resistance, and this usually has a constitutive basis in which insulin resistance selectively affects tissue-specific metabolic, but not mitogenic or steroidogenic, insulin actions. The compensatory insulin-resistant hyperinsulinemia sensitizes ovarian theca cells to secrete androgen in response to LH and seems to have a similar effect on the adrenal androgenic response to ACTH. Parsimony suggests that hyperinsulinemia is un-

likely to play a primary pathogenetic role in the pathogenesis of most PCOS, because it is an inconsistent and usually mild feature of the syndrome, particularly in the developmental (adolescent) phase of the syndrome. Furthermore, it does not account for the intrinsic theca cell dysfunction characteristic of classic PCOS. However, it may play a primary pathogenetic role in those functionally PCOS-A patients in whom it is a prominent clinical feature (52, 53). Regardless, it is an important aggravating factor in PCOS pathogenesis in about half of cases., and any treatment that lowers insulin levels improves hyperandrogenism. The available data are consistent with the concept that levels of insulin in the normal range modulate androgen production, most notably those in response to LH. Stimulation of adipogenesis and lipogenesis and inhibition of lipolysis by insulin excess also appear to contribute to the obesity of PCOS.

2. Role of obesity in PCOS pathogenesis

PCOS is the most common obesity-related endocrine syndrome in females. The prevalence of obesity in PCOS case series is influenced by ethnicity (359) and by referral patterns (360). Most evidence indicates that body fat content is excessive for BMI (118, 346, 361–364). One-third or more of normal-weight women with PCOS have abdominal obesity, whereas obese PCOS women accumulate fat globally. Obesity plays roles in PCOS via insulin resistance and generating testosterone from circulating androstenedione while suppressing gonadotropin production.

a. Obesity and insulin resistance. As noted in the preceding section, insulin-resistant hyperinsulinemism likely is a major factor in the excessive adipogenesis and lipogenesis of PCOS, and obesity in turn seems to aggravate the hyperandrogenism of PCOS by exaggerating insulin resistance.

Regional differences in adipose tissue lipolysis have been reported in PCOS that contribute to the enlarged adipocyte size of the sc depot and promote later development of obesity in PCOS (365). Visceral fat contributes more to the insulin resistance of PCOS than does abdominal fat because of its enhanced lipolytic response to catecholamines, the major lipolytic stimulus in man (335, 366–369). The enhanced visceral fat lipolysis of PCOS appears to cause hepatic insulin resistance by the lipotoxicity of the excess free fatty acids released into the portal circulation (368, 369). The nature of the independent relationship of visceral fat to muscle insulin resistance is unclear, but a potential hepato-muscular endocrine role for fibroblast growth factor 19 (FGF19) has been suggested (370).

The relationship of androgens to the excess visceral fat lipolysis of PCOS has been unclear. This excessive lipolysis is not simply explicable by androgens. In human sc fat, but not visceral fat, androgens directly inhibit lipolysis in vitro (365, 368). In nonhuman primates, androgen administered in vivo appears to indirectly inhibit visceral fat lipolysis via suppression of luteal phase progesterone levels (351). A preliminary report in this primate model indicates that the coadministration of a high-fat/calorie diet with testosterone stimulated visceral fat lipolysis and increased insulin resistance while suppressing luteal phase progesterone (371). Thus, although the mechanism remains unclear, testosterone in conjunction with a high-calorie diet seems to promote visceral fat accumulation and insulin resistance in females by a combination of inhibiting lipolysis and promoting lipogenesis.

Proinflammatory cytokines arising from the mononuclear cells (MNCs) of adipose tissue are another mediator of the insulin resistance of PCOS. Abdominal adipocyte hypertrophy triggers an inflammatory response (335), which is aggravated in PCOS by hyperandrogenism priming MNCs to secrete proinflammatory cytokines in response to glucose and saturated fat ingestion (118, 372–374). Glucose ingestion by PCOS women stimulates a MNC prooxidant inflammatory response that promotes insulin resistance and atherogenesis and may be deleterious to pancreatic β-cell function.

Although inflammation from excess abdominal adipose tissue is an important element in the pathogenesis of insulin resistance, it seems insufficient to promote insulin resistance in the absence of increased body weight, as indicated by the following observations (118, 372). Normal-weight women with PCOS who lack excessive abdominal adiposity are insulin resistant in the presence of normal baseline serum levels of proinflammatory cytokines such as TNF-α, although their MNCs have an abnormal proinflammatory cytokine response to glucose ingestion. However, the third of normal-weight PCOS women with excess abdominal adiposity have elevated fasting TNF-α, and TNF-α levels of PCOS women rise as their BMI increases, in parallel with insulin resistance.

Obstructive sleep apnea (OSA) is characterized by sleep fragmentation and hypoxemia, and both obesity and male sex are risk factors for it. Women with PCOS have at least a 5-fold higher risk for OSA than similarly obese women without PCOS (375, 376). In some studies, insulin resistance has been found to be a stronger predictor of OSA than age, BMI, or circulating testosterone concentrations. Its effects seem to be additive with androgen; in one study, women with PCOS taking oral contraceptives may be less likely to have sleep disordered breathing, analogous to the lower likelihood of sleep disordered breathing among postmenopausal women on hormone replacement therapy (377). Conversely, the severity of OSA is a highly

significant predictor of the fasting concentrations of glucose and insulin as well as the 2-hour glucose concentration during an oral GTT and HOMA-IR. Several well-controlled experimental studies in healthy human subjects involving sleep restriction as a model of OSA and assessments of glucose metabolism by iv GTT or euglycemic-hyperinsulinemic clamp have shown that sleep restriction cause a reduction of insulin sensitivity ranging from 18% to 24% without simultaneous increases in insulin levels, thus resulting in reduced glucose tolerance and an increased risk of diabetes (378). Thus, there appears to be a bidirectional cause-and-effect relationship between insulin resistance and OSA.

An important link between OSA and insulin resistance may be its relationship to overactivity of the SNS (379), which is related to PCOS independently of its relationship to adiposity (373). PCOS women with OSA have elevated norepinephrine levels over a 24-hour sleep-wake cycle compared with PCOS women without OSA (376). The higher muscle sympathetic nerve activity of PCOS compared with weight-matched controls likewise seems related to OSA (380–382). Improvement of OSA by treatment with continuous positive airway pressure has been shown to significantly reduce insulin resistance and norepinephrine levels (376).

SNS overactivity promotes inflammatory cytokine production and is associated with cardiovascular instability (122, 373). It has been proposed that the SNS may also contribute to the PCOM/PCOS phenotype via the sympathetic neurotropin NGF. Ovarian catecholaminergic nerve fibers and NGF production are excessive in women with PCOS and PCOM (121).

b. Obesity and gonadotropins. Evidence is accumulating that obesity is associated with suppression of serum gonadotropin levels independently of insulin resistance, at least in part. Inverse associations between BMI and LH production have been reported in about half of studies of healthy eumenorrheic women with BMIs up to 40 kg/m^2 (83, 383–385). A series of studies have shown consistently blunted follicular phase LH levels, suppressed LH pulse amplitude, and subtle FSH suppression in morbidly obese eumenorrheic women who are clinically normoandrogenic (226, 229, 386). These lowered gonadotropins had a subtle but important effect on follicular function: estrogen production, normal during the follicular phase of the menstrual cycle, was significantly depressed at the time of the midcycle ovulatory surge, after which corpus luteum insufficiency ensued. These studies further suggested that obesity-related inflammatory cytokine excess mediates suppression of pituitary gonadotropin release and that both are improved by transdermal estradiol administration to slightly boost estradiol levels (229).

In PCOS, BMI is likewise inversely related to baseline mean LH levels (83). The fall in LH levels with obesity is attributable to a fall in pulse amplitude (384, 385, 387). Although the early LH response to GnRHag is significantly elevated in PCOS, it is significantly lower in the obese than in nonobese subset (83, 385, 387). Baseline and stimulated LH are normal in most PCOS patients with BMI more than 40.

The blunting of LH pulsations in obese PCOS is at least in part due to accelerated metabolism of LH (388). Clearance of gonadotropins from the circulation is related to the sulfonation and sialylation patterns of the component isoforms: sulfonated isoforms are cleared more rapidly than sialylated ones (389). Although sialylation of LH molecules is increased in PCOS, the percent of sulfonated LH isoforms is proportional to BMI in PCOS (390). Thus, the increase in sulfonated LH isoforms in obese PCOS seems likely to account for their accelerated LH turnover. Because LH turnover seems to be the major determinant of LH bioavailability, this change would be expected to decrease LH in vivo bioactivity (391).

The mechanism by which obesity suppresses LH pulse amplitude is unclear. Because obesity causes insulin resistance and because PCOS patients are more insulin-resistant than BMI-matched controls (13), insulin resistance is a major candidate mediator. However, studies of the effects of insulin on gonadotropin production by diverse methods have not yielded a consistent picture (392–394). The possibility also exists that estrogen production in excess adipose tissue plays a role in suppressing women's LH pulse amplitude, as reported in men (395), and LH bioactivity (396), perhaps by affecting LH sialylation.

Obese women with hyperandrogenic anovulation without FOH or FAH constituted 8% of women in our PCOS series. We have proposed that simple obesity explains their mild hyperandrogenic anovulation (the PCOS-A of obesity) (47). As noted in the preceding paragraph, obesity itself is associated with suppressed ovulation and LH levels (83, 227, 228). Furthermore, obesity itself can account for excess peripheral formation of testosterone independently of PCOS (184, 397, 398). Adipocytes convert circulating androstenedione to testosterone via type 5 17βHSD, which is up-regulated by insulin (107, 184). The expression of this enzyme in sc fat correlates with BMI and falls with weight loss in simple obesity. The extent to which the hyperandrogenic anovulation of obese women is due to simple obesity remains to be determined in a prospective study. PCOS symptoms may improve with 5%–10% weight loss (399). However, 25%–50% weight loss may be required in the very obese,

and correction of anovulation may require correction of both hyperandrogenism and metabolic syndrome (288). The hyperandrogenism of most morbidly obese PCOS patients can be corrected upon the substantial weight loss achieved by bariatric surgery (210, 288, 400). We postulate that it is those who lack an ovarian source of androgen excess that achieve resolution of hyperandrogenism with weight loss.

On the other hand, in most PCOS cases, whose hyperandrogenism is significantly greater, adipose tissue excess seems to contribute negligibly to testosterone excess, judging from androstenedione to testosterone ratios as an index of peripheral conversion of secreted androstenedione (401).

In summary, the major role of obesity in PCOS pathogenesis seems to be related to an increase in insulin resistance, thereby aggravating FOH. However, simple obesity may sometimes cause a mild PCOS picture that is atypical in that anovulation results from LH suppression rather than FOH.

C. Relationship of LH excess to PCOS

Increased LH relative to FSH was the first laboratory abnormality identified in classic PCOS. Mean LH levels correlate positively with LH pulse frequency (384, 385). Patients with PCOS have an increased LH pulse frequency, which is particularly striking in overnight studies, because these women typically lack the normal nocturnal slowing of pulse frequency that is the residual effect of ovulation in the previous cycle on the subsequent early follicular phase (384, 402). PCOS LH pulse amplitude is also increased, which is reflected in the size of the pulse induced promptly by GnRH or GnRHag administration (83). Elevated LH levels occur in about half of PCOS patients (197, 384).

Elevated LH has been thought to play a role in the pathogenesis of PCOS by increasing androgen production and secretion by ovarian theca cells (Figure 1). LH is necessary for the expression of gonadal steroidogenic enzymes and sex hormone secretion (Figure 1). Consequently, PCOS is LH dependent (hence, functional), and any treatment or disorder that suppresses LH levels suppresses ovarian steroidogenesis.

However, there are reasons to doubt that serum LH elevation is often the primary cause of FOH. For one, about half of PCOS subjects, particularly obese cases, with a documented ovarian source of hyperandrogenism were demonstrated to have normal baseline and GnRH-stimulated LH levels, as discussed in the previous section (83). For another, normal homologous desensitization of theca cells begins limiting the androgenic response to LH/hCG at submaximal stimulation, as discussed above. Nevertheless, LH is capable of overstimulating steroidogenesis in the presence of the escape from desensitization that occurs with hyperinsulinism. The hyperinsulinism common in PCOS would thus seem to contribute to the frequent ovarian steroidogenic hypersensitivity to LH stimulation (Figure 3). LH excess also seems to play an important role in the PCOS that follows congenital virilization (see section VI).

Recent research also supports the concept that the increase in LH is the result of abnormal sex steroid feedback rather than the cause of androgen excess (83). The elevated mean LH and, particularly, LH pulse frequency, of PCOS are less sensitive to negative feedback by combined estrogen-progestin administration than are those of controls (80, 403). In particular, higher concentrations of progesterone are required to suppress LH pulse frequency in the presence of luteal phase estradiol levels in PCOS (403). Furthermore, sensitivity to estrogen-progestin negative feedback is restored by antiandrogen treatment of PCOS (404). These data indicate that androgen excess interferes with the hypothalamic inhibitory feedback of female hormones, particularly progesterone. This conclusion is supported by a recent reexamination of the effects of testosterone infusion using deconvolution analysis to quantify the pulsatile properties of LH secretion in a 12-hour overnight study, in which 7 normal postmenarcheal and 7 PCOS adolescent girls were tested (405). In normals, when testosterone levels were increased 3-fold (to 120 ng/dL = 4.1nM), LH pulsatile secretion increased 50% ($P < .05$), whereas basal LH secretion over the 12-hour period did not change. When testosterone levels were raised 6-fold (to 245 ng/dL = 8.5nM), mean serum LH fell 22% ($P < .05$) due to a fall in basal LH secretion and a return of pulsatile secretion to normal. PCOS adolescents' LH levels were resistant to testosterone: only when testosterone was raised 4-fold to 300 ng/dL (10.4nM) were effects seen: testosterone significantly further increased LH pulsatile secretion and reduced basal LH secretion. Thus, induction of modest hyperandrogenemia appears to stimulate increased LH pulsatility in both normal and PCOS females. It is unclear why the dose-response relationship is shifted in PCOS; it may be due to the different antecedent progesterone or testosterone milieu, or other factors may be involved, for example, in the prenatally androgenized mouse model of PCOS AMH has been shown to directly activate GnRH neurons and increase LH secretion (406).

The androgen effect can be accounted for by increased gonadotrope responsiveness to GnRH: the testosterone elevation of PCOS is associated with enhanced GnRH-stimulated early LH secretion (17, 95, 197, 407). In PCOS, as in normal men, GnRHag administration elicits exaggerated early LH release (Figure 7), reflecting a large gonadotrope pool of readily releasable LH, consistent with

Figure 7.

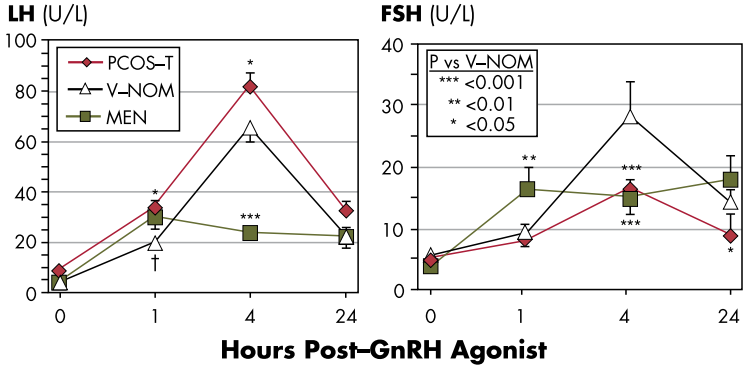

Figure 7. Effects of PCOS and sex on response to GnRHag. The early LH responses (1 h after 10-μg/kg GnRHag) of PCOS-T subjects resemble those of normal men ($P < .05$ vs V-NOM), whereas their surge responses (4 h after GnRHag) resemble those of normal women ($P < .001$ vs men). The FSH responses of PCOS-T subjects are significantly less than those of V-NOM from 4–24 hours and below those of normal men at 1 and 24 hours after GnRHag ($P < .05$). PCOS-T and V-NOM are previously reported age-matched groups (47), except NOM in postmenarcheal adolescents has been redefined as mean ovarian volume up to 12.0 cc, consistent with current consensus (30, 51). Adult male data shown for comparison were previously reported using a slightly different GnRHag sampling protocol (407); †, all head-to-head comparisons of normal male and female responses have shown a significant sex difference in the early releasable pool of LH (17, 407).

this being an effect of the ambient testosterone level (95, 407). However, unlike normal men, and like normal women, GnRHag elicits a subsequent LH surge (Figure 7), which seems to reflect the capacity of female gonadotropes to form the large pool of newly synthesized LH that men are incapable of forming. Notably, GnRHag elicits a lower FSH surge (seemingly secondary to negative feedback by the excessive estrogen and inhibin production), which may contribute to ovulatory inefficiency.

In summary, the data suggests that the response of LH to androgen is biphasic: mild testosterone excess is stimulatory, severe excess is inhibitory. LH is necessary for the expression of steroidogenic enzymes, and any treatment that suppresses LH levels improves ovarian hyperandrogenism. On the other hand, the phenomenon of homologous desensitization normally limits the role of LH in causing ovarian androgen excess. Thus, the data are compatible with LH excess in PCOS being the result rather than the cause of FOH. However, in the presence of escape from desensitization, such as is induced by hyperinsulinism, ovarian theca cells become more sensitive to LH, which aggravates ovarian hyperandrogenism. For these reasons, and because LH excess is an inconstant feature of PCOS, it is difficult to attribute a primary role to LH excess in the pathogenesis of most PCOS. However, LH excess seems to play a prominent role in the PCOS that follows congenital virilization.

D. Modulation of androgen action in PCOS

Although testosterone appears to be the main circulating androgen (38), the possibility has been raised that atypical adrenal androgens such as 11-ketotestosterone, which has 25% of testosterone's potency, might contribute to androgen action (408). Although serum bioactivity can essentially be accounted for by testosterone in normal women (409), the extent to which such atypical androgens contribute in hyperandrogenic remains to be systematically determined.

The formation of DHT from testosterone by 5αRD is a major determinant of androgen action. Evidence of increased global peripheral 5αRD activity in PCOS has been consistently obtained from evaluation of urinary androgen and cortisol metabolites (250). Indexes of increased 5αRD activity were increased in both nonobese and obese PCOS and significantly greater in the obese than nonobese subjects. The cause is unknown. The possibility of differential expression of "backdoor pathway" enzymes for the formation of DHT, such as 5αRD1/2, α-ketoreductase type 1C2, and 17β-HSD6 (62), in PCOS ovarian theca cells and adrenal zona reticularis cells has been raised by a preliminary report (410). It is possible that the increased 5αRD activity is a consequence of hyperandrogenism because 5αRD activity is up-regulated by androgen action (191, 411). Insulin appears to up-regulate 5αRD activity (250); this effect may be exerted on the type 1 isozyme (412), which in turn up-regulates insulin sensitivity (352, 353). Genetics may play a role, because 5αRD gene variants are associated with PCOS prevalence (413). The increased 5αRD activity has been postulated to play a role in adrenal hyperandrogenism (250). It could also potentiate androgen actions in other organs or tissues, such as the pilosebaceous unit and granulosa cells (77, 412, 413). Serum allopregnanolone, a 5α-reduced progesterone metabolite that is a γ-amino butyric acid receptor agonist, has been reported to be significantly higher in PCOS than in BMI-matched controls (25 vs 17 ng/dL; 0.8 vs 0.5nM) and proposed to play a role in appetite dysregulation (414).

Androgen receptor alternate splice variant heterozygosity has recently been reported in the luteinized granulosa cells of 62% of Han Chinese with PCOS undergoing

in vitro fertilization; it is absent in controls (415). Either an insertion or a deletion isoform were coexpressed with wild-type receptor and accounted for the androgen receptor overexpression in these PCOS cells. They reduced nuclear translocation of wild-type receptor, which has an overall negative effect on androgen action. In isolation, neither transduced the normal up-regulation of aromatase by dihydrotestosterone, whereas the insertion variant transduced up-regulation of *CYP17A1* by dihydrotestosterone. The expression pattern was tissue specific, not being found in peripheral lymphocytes, where wild-type receptor was found to be up-regulated. The variants were associated with more severe hyperandrogenemia, which was attributed to deficiency of aromatase activity secondary to deficient up-regulation by androgen receptor signaling. They were also associated with lowered expression of genes related to folliculogenesis and ovulation. The mechanism by which these gene variants arise appears to be locally regulated and in part may be epigenetically determined. It has been postulated that these changes are an adaptation of the PCOS ovary to the abnormally hyperandrogenic environment rather than a contributor to the hyperandrogenism (416).

The length of androgen receptor CAG trinucleotide repeats is known to be inversely related to the efficacy of androgen receptor activity. Many studies have examined the associations of polymorphic CAG repeats in the androgen receptor gene, or of X-inactivation favoring short repeats, with PCOS risk, but with inconsistent results (417). A 2013 metaanalysis demonstrated no evident association between the CAG length variations in the AR gene and PCOS risk, whereas the CAG length appeared to be positively associated with testosterone levels in PCOS patients (418).

In summary, although such clinical observations as hirsutism in the apparent absence of hyperandrogenemia and anovulation in association with isolated mild FAH suggest that androgen action may be excessive at the target tissue level in some individuals, information is scarce about the mechanism of such putative effects. Improved knowledge about the role of atypical androgens and postreceptor mechanisms of androgen action will be necessary to understand the role of tissue responsiveness to androgen in PCOS pathogenesis.

E. Summary: Unified model of PCOS pathophysiology

Our working model of PCOS pathogenesis is shown in Figure 8. Approximately 90% of PCOS have an abnormal SDAST or GnRHag test (the most specific of current diagnostic tools for testing ovarian androgenic function), whereas only about half of these have abnormally elevated

Figure 8.

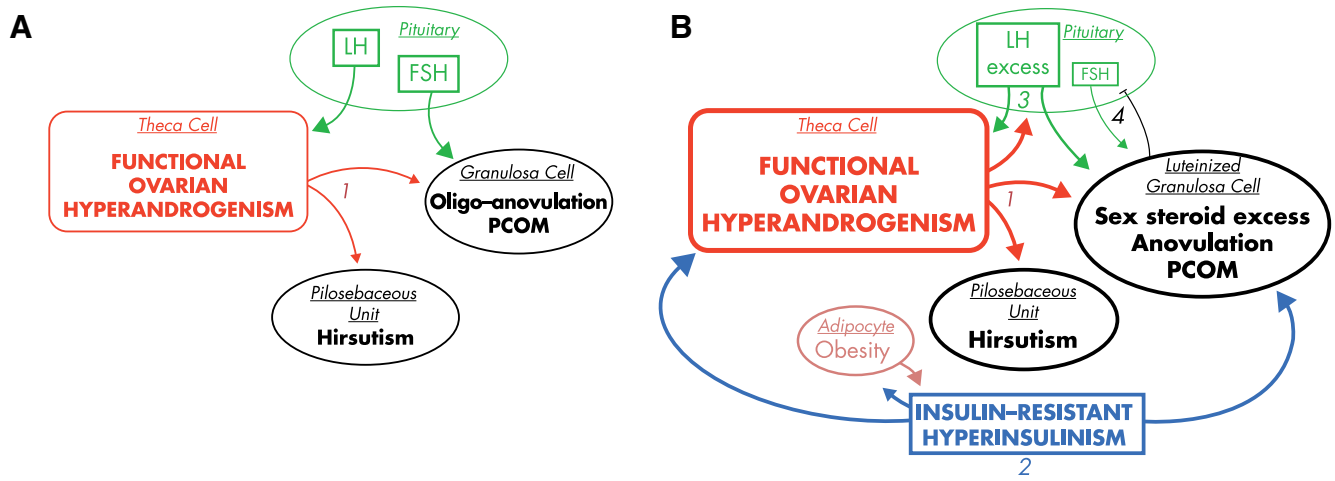

Figure 8. Unified minimal model of PCOS pathophysiology. A, Ovarian hyperandrogenism is nearly universal in PCOS and can account for all the cardinal clinical features of the syndrome: hyperandrogenemia, oligo-anovulation, and polycystic ovaries (1). Pituitary LH secretion is necessary to sustain the ovarian androgen excess but is not sufficient to cause it. B, About half of patients with FOH have insulin-resistant hyperinsulinism (2). Insulin-resistant hyperinsulinism acts on theca cells to aggravate hyperandrogenism, synergizes with androgen to prematurely luteinize granulosa cells, and stimulates adipogenesis. The increased hyperandrogenemia provokes LH excess (3), which then acts on both theca and luteinized granulosa cells to worsen hyperandrogenism. LH also stimulates luteinized granulosa cells to secrete estradiol (4), which suppresses FSH secretion. These hyperinsulinism-initiated changes in granulosa cell function further exacerbate PCOM and further hinder ovulation. Obesity increases insulin resistance, and the resultant increased hyperinsulinism further aggravates hyperandrogenism. Heaviness of lines and fonts represents severity. Both FOH and insulin resistance typically have an intrinsic basis. This model does not exclude the possibility that the unknown intrinsic ovarian defects that underpin the ovarian steroidogenic dysfunction also involve granulosa cell folliculogenesis as well. The figure also does not depict other associated defects, such as the FAH that often accompanies the ovarian hyperandrogenism and the contribution of excess adiposity to peripheral androgen production and gonadotropin suppression.

insulin and/or LH. Thus, the common denominator in PCOS appears to be FOH. FOH typically has a steroidogenic abnormality suggestive of the constitutive biochemical dysfunction that is characteristic of classic PCOS theca cells. (A similar adrenal dysfunction seems to account for the commonly associated FAH.) FOH can account for all the clinical features that characterize PCOS: hirsutism, anovulation, and polycystic ovaries or, in severe cases, hyperthecosis. In about half of cases, tissue-specific resistance to the metabolic effects of insulin causes compensatory hyperinsulinemia. This aggravates anovulation and the development of PCOM by up-regulating thecal androgen production in response to LH and synergizing with androgen to cause premature luteinization of ovarian follicles; it also stimulates adipogenesis. Then, 2 vicious cycles of feed-forward effects occur. The modest hyperandrogenemia causes secondary LH elevation by interfering with female hormone negative feedback; in the presence of hyperinsulinemia, this LH excess aggravates the ovarian dysfunction. Hyperinsulinemia also promotes adiposity, which in turn aggravates the insulin-resistant state.

In most PCOS, the cause of ovarian hyperandrogenism seems to be intrinsic, and there is evidence for a constitutive basis for much of the insulin resistance as well. In the absence of intrinsic ovarian dysfunction, modest hyperandrogenemia of extraovarian origin (adrenal or peripheral sources) or severe insulin resistance are unusual causes of hyperandrogenic anovulation and polycystic ovaries.

VI. Etiology of PCOS: A Complex Trait

A number of hereditary and environmental factors contribute to ovarian hyperandrogenism and/or insulin resistance. Polycystic ovaries, androgen levels, and insulin resistance have hereditary components. Environmental factors may be congenital or acquired and include intrauterine factors such as androgen exposure and prenatal nutrition, whereas acquired obesity is a major postnatal factor influencing the phenotype. The complex interactions generally mimic an autosomal dominant trait with variable penetrance: the disorder is correlated in identical twins ($r = 0.7$) (419); about half of sisters are hyperandrogenic, and half of these also have oligo-amenorrhea and thus PCOS (420, 421); and polycystic ovaries appear to be inherited as an autosomal dominant trait (421, 422). Although estimates vary widely, 3%–35% of mothers of women with PCOS also have PCOS, as do about 25% of sisters (423, 424), and metabolic syndrome prevalence is high in parents and siblings (280, 425, 426). The syndrome's phenotypic diversity is affected by ethnic diversity (427–429).

A. Heritable traits and genetic linkages

Familial cases of PCOS that appeared to be inherited as an autosomal dominant trait with variable penetrance were recognized long ago (430). Twin studies suggest that there is a strong contribution of familial factors to PCOS pathogenesis (419, 431). In a Dutch twin-family study, the correlation between monozygotic twins for PCOS was 0.71, and for dizygotic twin or nontwin sister pairs, the correlation was 0.38. Heritable traits that have been identified as PCOS risk factors are maternal PCOS, PCOM, hyperandrogenemia, and metabolic syndrome.

1. Maternal PCOS

Maternal PCOS is a risk factor for PCOS in daughters. One set of studies compared the singleton daughters, about two-thirds of whom were pubertal, of 99 Chilean women with PCOS (defined by NIH criteria) with those of 88 control women (432, 433). A study in subsets of these groups showed that in infancy and early childhood the daughters of women with PCOS had higher serum AMH (434); however, these findings were not replicated in a Nordic PCOS population in which an unknown proportion had nonhyperandrogenic or ovulatory PCOS phenotypes (435). From midchildhood onwards, the daughters of the Chilean women with PCOS had larger ovaries and higher insulin responses to an oral GTT than the daughters of women without PCOS (ethnic or environmental influences may play a role because evidence of insulin resistance did not emerge until late puberty in an American study of PCOS daughters) (436). Peripubertally, higher DHEAS levels emerged. In late puberty, higher basal testosterone and higher 17OHP responses to GnRHag testing emerged during the late stages of puberty. Half of the daughters of women with PCOS who were postmenarcheal had higher testosterone levels than any control daughters, which is consistent with the familial distribution of hyperandrogenemia found by Legro et al (420).

2. Polycystic ovarian morphology

Two studies suggest that the PCOM of PCOS is inherited in an autosomal dominant fashion (422, 437). Most PCOS adolescents with PCOM have either a mother with a polycystic ovary, which is usually asymptomatic, or a father with metabolic syndrome (280).

A presumed second factor, in concert with the intrinsic genetic susceptibility defect in the ovary, would appear necessary to result in the clinical phenotype of PCOS (hyperandrogenemic anovulation) in the affected daughter. This was suggested by the results of a study of 214 sisters of 125 PCOS probands, defined by NIH criteria (80%) or by broader Rotterdam criteria (20%), which showed that 70.5% had a polycystic ovary (421). Of the sisters with a

PCOM, 25% had clinically hyperandrogenic anovulation, 42% had hirsutism or oligomenorrhea, and 33% were asymptomatic. The group of sisters with a polycystic ovary resembled their proband sisters in both androgenic and glucose-metabolic traits, indicating significant heritability and cosegregation of these traits.

Genetic studies have not taken into account the heterogeneity of steroidogenic function of the polycystic ovary (30). On one hand, about half of asymptomatic young women with PCOM have no biochemical evidence of ovarian androgenic dysfunction and thus represent a variation of normal. On the other hand, half of asymptomatic women with PCOM have biochemical evidence of ovarian androgenic dysfunction (Figure 6), and nearly half of these have biochemical hyperandrogenemia and thus seem to have ovulatory PCOS although eumenorrheic and normal to all appearances, as discussed above. We have postulated that such women are PCOS carriers and may be at risk of PCOS with excessive weight gain, although the latter outcome seems to be uncommon (219). However, oligomenorrheic women with PCOM and functional hypothalamic amenorrhea and a very elevated AMH level are at high risk for hyperandrogenic PCOS when hypothalamic function normalizes with weight gain (438, 439).

3. Hyperandrogenemia

Approximately one-half of sisters of PCOS probands have an elevated serum testosterone level (420). However, only one-half of these sisters with excess androgen have menstrual irregularity, and the other half are asymptomatic. This suggests that these asymptomatic patients with excess androgen only become symptomatic in the presence of another precipitating factor(s).

4. Metabolic syndrome and DM

Metabolic syndrome and its core pathogenetic constituents, insulin resistance and obesity, have heritable components (421, 440, 441). Perhaps less appreciated is the evidence that defective insulin secretion is also highly heritable in PCOS (289, 290, 292) and is closely associated with PCOS-T (47).

Parental factors related to metabolic syndrome (ie, insulin resistance and/or obesity) are strongly associated with the pathogenesis of PCOS. There is a high prevalence of these features in first-degree relatives of PCOS patients (421, 425, 426, 442). In our series of 35 families in which an adolescent girl had PCOS, 70% of the probands had a parent with metabolic syndrome; using National Cholesterol Education Program Adult Treatment Panel III (ATP-III) criteria for defining metabolic syndrome, 53% of fathers and 34% of mothers were affected (280). Using 2004 criteria for metabolic syndrome (which include assessment of glucose tolerance), 79% of fathers and 37% of mothers were affected. Excess adiposity was found in most parents; 94% of fathers and 66% of mothers were obese or overweight. Abnormal glucose tolerance was present in 84% of fathers and 49% of mothers: of these, half were diabetic (half occult) and half had IGT ("prediabetes"). Notably, this case series showed complete concordance between paternal metabolic syndrome and a polycystic ovary in affected daughters but no relationship to metabolic syndrome in these daughters, which suggested a fundamental relationship of paternal metabolic syndrome to the PCOM of PCOS. In a study that defined metabolic syndrome by ATP-III criteria and did not include GTTs, fathers were confirmed to have an increased prevalence of excess adiposity (about 85%) and metabolic syndrome (42%) (426). In another study of fasting blood sugar, fathers were also found to have a significantly higher prevalence of fasting dysglycemia than mothers; however, only maternal fasting dysglycemia was demonstrably heritable, which suggests genetic or epigenetic parent-of-origin effects (443).

5. Gene variants

Many attempts are being made to identify specific genes that underlie the intrinsic cause of PCOS. A wide variety of gene variants with linkage and/or association with PCOS have been identified by candidate gene studies (1, 417, 444, 445). Polymorphisms, linkages, and differential expression have been reported for genes encoding steroidogenic enzymes, SHBG, the androgen receptor, and gonadotropin receptors; genetic loci associated with insulin sensitivity and susceptibility to obesity, and congenital adrenal hyperplasia (418, 446–450). Dysregulation of genes involved in cell growth is suggested by the reports of upregulation of proto-oncogenic genes in endometrium (451) and of telomere shortening in leukocytes (452).

More recently, there has been widespread interest in the identification of linked genes through genome-wide association studies (GWAS), commencing with a series of studies conducted in large populations of ethnic Han Chinese in 2011 (453). The major findings in this population have been generally replicated in most other populations studied (427, 454–457). Linkages for polymorphisms in the fibrillin 3, proopiomelanocortin, and diverse signaling pathway genes have been robust in many populations (115, 453, 458). How the various linkages are functionally related to PCOS is often unclear. Variations in fibrillin 3 have been proposed to dysregulate TGF-β signaling (115) and account for ovarian stroma hyperplasia (117). The most recent and largest of the GWAS as of this writing is the report from a multicenter study of 984 PCOS cases and 2964 population controls followed by replication in 1799

PCOS cases and 1231 phenotyped reproductively normal control women (457). This was followed by a metaanalysis of the top 24 associations detected in the first and second case-control groups. Three loci reached genome-wide significance in the case-control metaanalysis of all 3 strata; 2 novel loci, chr 8p32.1 in the region of the *GATA4* transcription factor and the *NEIL2* endonuclease-encoding gene, and chr 11p14.1 in the region encoding the FSH beta subunit (FSHB), and one previously found in Chinese PCOS, chr 9q22.32 in the region of *c9orf3/FANCC* which encodes an aminopeptidase. The same chr 11p14.1 SNP, rs11031006, in the region of the FSHB gene also reached genome-wide significance in the metaanalysis of the quantitative LH levels. These findings implicate gonadotropin levels in the pathogenesis of PCOS. The GWAS approach has also been applied to ovary-specific methylation, and the findings support a role for epigenetic gene modifications in the pathogenesis of PCOS (459).

Among the most striking findings from GWAS to date has been the recent discovery of DENND1A (MIM *613633) as a highly significant intronic locus linked to PCOS in many populations (108). Even though the odds ratio for this association was modest (odds ratio < 2.0), a previously unsuspected protein, DENND1A, was found to play an unanticipated role in ovarian androgen formation. DENND1A.V2 isoform expression was then demonstrated to be increased in PCOS theca cells, to up-regulate cytochrome P450c17 and side chain cleavage activities, and its mRNA to be excreted in excess in urine of PCOS women, as discussed in the section V.A. Details of DENND1A pathophysiology remain to be worked out. Likewise, the genetic mechanism(s) accounting for DENND1A.V2 overexpression have yet to be determined.

There are many pieces to the puzzle of how the components of PCOS are interrelated, and the puzzle remains to be solved. It seems increasingly likely that the mechanistic pathways underlying the diverse manifestations of PCOS may involve transcription factors or other intrinsic cellular processes common to the many tissues involved. The variety of pathways involved and lack of a common thread attests to the multifactorial nature and heterogeneity of the syndrome and suggests that the syndrome may have evolved heterogeneously as diverse means of preserving anabolism and reproductive capacity in times of nutritional deprivation (460).

B. Intrauterine environment

It is becoming increasingly apparent that environmental insults during development induce persistent changes in the epigenome that lead to altered gene expression and adult disease (461, 462). Congenital virilization and intrauterine nutrition have been incriminated as risk factors for PCOS.

1. Congenital virilization

PCOS is common in females with congenital virilizing disorders, of which congenital adrenal hyperplasia is the most frequent, but not exclusive, cause (201, 463). Congenital androgenization has now been well established to lead to PCOS features in several experimental animal models (84, 431, 464, 465). Studies in the rhesus monkey exposed to androgen excess early in gestation have been particularly informative, showing characteristic PCOS features. These animals have ovarian and adrenal hyperandrogenism, oligomenorrhea, polyfollicular ovaries, and elevated LH levels associated with resistance to negative feedback inhibition of LH release by progesterone. They also have abdominal obesity, insulin resistance, IGT, and dyslipidemia. Prenatally androgenization of sheep causes increased antral follicle AMH expression similar to that in PCOS (466).

Mechanistic studies in the rat indicate that prenatal androgen programs for pubertal LH elevation and absence of the capacity to mount an LH surge via suppression of estrogen induction of hypothalamic progesterone receptors (84, 467). Studies in sheep indicate that prenatal virilization causes tissue-specific differential changes in genes that determine insulin sensitivity, with liver and muscle being insulin resistant and adipose tissue being insulin sensitive (468).

The PCOS secondary to congenital virilization indicates the potential for in utero epigenetic programming of postnatal development (462). However, the relevance of this to ordinary PCOS is unclear in view of what is known about structure-function relationships of normal human fetal ovary development.

By the beginning of the second trimester, human fetal testes have differentiated, and their secretions have begun to differentiate male from female genitalia. At this time, the normal human fetal ovary, although histologically undifferentiated, has the capacity to form and respond to androgen and estrogen, and this increases thereafter (469–473). Histologic studies indicate that primordial follicles commence organizing and growing at about 16 weeks of gestation and the oocyte population peaks at 20 weeks, at about the time that primary follicles begin appearing (472); meanwhile, follicle growth is active, and preantral follicles become developed at 24–26 weeks (471, 474). Antral follicles are first detected near term both histologically (471, 474) and ultrasonographically: pelvic ultrasonography has detected antral follicles on the seventh postnatal day in no premature infants less than 34 weeks of gestation, 5% of preterm infants (34- to 37-wk

gestation), and 30% of term infants ($P \leq .001$) (475). Corresponding to antral follicle status, premature infants have high gonadotropin levels, of the magnitude seen in ovarian insufficiency, yet do not begin estrogen and AMH production until they approach term gestational age (475, 476). This timing of ovarian acquisition of estrogen responsiveness to gonadotropins resembles that in the nonhuman primate (477) and corresponds to the late temporal appearance of FSH receptors in the primate ovary, where the developmental increase in FSH receptors reflects the estrogen-dependent increase in folliculogenesis (265). Thus, despite having the capacity for estrogen formation from the time of ovarian differentiation, ovarian estrogen production appears to be virtually unresponsive to gonadotropins until follicles become capable of responding to FSH with antral follicle growth near term gestational age.

Androgen production by the primate fetal ovary seems to parallel this quiescent fetal ovarian pattern of estrogen production and contrasts with that of testosterone in the male fetus (472, 477, 478); fetal male serum testosterone peaks above female levels at 16–20 weeks of gestation as fetal pituitary LH secretion peaks and CG levels are waning (471, 479, 480). Therefore, it would be surprising if the PCOS ovary were found to be hyperfunctioning in utero. Nevertheless, some have postulated that to be the case (462).

The most direct way of demonstrating fetal hyperandrogenism, if it exists, would seem to be measurement of amniotic fluid testosterone levels at 12–24 weeks. (A methodological note: direct assays of androgens are often problematic [45]; the steroid output of the fetal adrenal-placental unit generates a steroid milieu that is unique to pregnancy [481]; this pregnancy-related steroid pattern interferes with direct testosterone assays that are accurate for children and adults, so high-specificity assays after preliminary chromatography are necessary for the accurate assay of pregnancy-related samples [482]; whether any specific postchromatographic RIA or mass spectrometry method is superior to another in this setting remains to be directly tested.) In normal pregnancies a distinct sex difference in amniotic fluid testosterone has been shown by postchromatographic RIA and mass spectrometry (483–485). This contrasts with the absence of fetal sex-dependent differences in normal maternal serum androgen levels (484, 486) or umbilical cord androgen levels among normal and PCOS offspring (486, 487).

In view of all the reciprocal paracrine interactions that seem necessary for normal postnatal theca cell function, it seems unlikely that PCOS theca cell dysfunction could emerge and initiate developmental programming during the window of time in midgestation (16–24 wk) after sexual differentiation is complete and before fetal gonadal endocrine production is suppressed by high adrenoplacental unit estrogen production and the coincident fall in fetal pituitary and CG levels (471, 479, 480). Furthermore, it is unlikely that endogenous ovarian androgen overproduction could exceed the high capacity of placental aromatase to protect the fetus from androgen excess (488, 489), as is the case in different-sex twin pregnancies. Nevertheless, it has been proposed that the midgestational uterine environment causes excessive fetal androgenization (462). This notion has received some support that is of questionable significance. Term PCOS placentas have changes that favor increased androgen production: they possess significantly more (40%) 3βHSD1 activity and less (30%) aromatase activity than controls (489); the relationship of this biochemical change to the significant histoanatomic evidence of hypoxic change of such placentas is unclear (490). However, no clear picture of placental aromatase deficiency has emerged from measurement of umbilical cord blood sex steroid concentrations: estriol has been reported to be higher (489), and estradiol, estrone, and androstenedione have variably been reported to be lower (486, 487). PCOS women bearing a girl had second trimester amniotic fluid testosterone concentrations that were intermediate between those of controls bearing a girl ($P = .019$) and controls bearing a boy ($P = .10$) (491); however, these data have a substantial element of nonspecificity, because they were obtained by a direct RIA that yielded absolute values averaging 80%–500% higher for amniotic fluid obtained from female-bearing women than reported for the postchromatographic assays noted above (483–485). PCOS women bearing a girl had significantly higher serum testosterone and androstenedione levels by postchromatographic mass spectrometry at 20 weeks of gestation and at delivery than did comparable controls, with a similar trend seen in PCOS women bearing a boy (486). This is both intriguing and unexpected; if confirmed, this would seem more likely to arise from maternal ovaries than from PCOS fetal ovaries secreting more androgen into the maternal circulation than normal fetal testes.

Because of the difficulty of safely measuring indexes of fetal hyperandrogenism, postnatal markers of in utero androgen action have been explored. Congenital androgen excess of a degree sufficient to affect genital differentiation is known to be significantly disruptive to the development of normal female gender satisfaction (492, 493). Reversed sexual orientation is unusual in PCOS (494), and there is no evidence of excess gender dissatisfaction aside from that resulting from body image perception. The ratio of second and fourth digit lengths has been evaluated because it is slightly but significantly lower in men than women and masculinized (lengthened) in prenatally androgenized vs

control primates (462, 495); however, in 3/4 reports, this ratio was not supportive of a fetal masculinizing effect in PCOS (462). This ratio in normal females, but not males, at birth was found to correlate with amniotic fluid testosterone (484). An alternative approach has been to search for biochemical markers of prenatal programming. A preliminary report has appeared of infant daughters of women with PCOS having urinary steroid metabolite evidence of increased global 5αRD activity compared with control infants, a finding compatible with congenital androgen programming (496).

2. Disturbed fetal nutrition

There is good evidence that fetal undernutrition programs for metabolic syndrome and related cardiovascular disease in adulthood (497, 498). It has been proposed that low birth weight is likewise a marker for a fetal origin of PCOS (499); this proposal is more controversial.

Several studies support an association between inadequate fetal nutrition and subsequent development of PCOS. First, a retrospective study evaluating Catalan girls with premature pubarche found that it was associated with intrauterine growth restriction and postmenarcheal PCOS and insulin resistance (499): this led to the hypothesis that low birth weight was a marker for a fetal origin of PCOS (and an even more sensitive marker of premature adrenarche). Probes of birth records have supported the concept that low birth weight is associated with PCOS. An Italian neonatal registry-based study showed low birth weight, irrespective of gestational age, to be associated with PCOS features and insulin resistance (500). A similar Brazilian birth cohort follow-up (43 small for gestational age, 122 appropriate for gestational age) examined at an average age of 29 years showed small for gestational age to confer a 2.4-fold risk for PCOS but none for hyperinsulinemia (501). An Australian follow-up of a birth cohort of 2199 women showed that the weight-for-length at birth was significantly inversely associated with increased risk for PCOS (502). A converse association between birth weight and PCOS was suggested by 2 studies of adults in which PCOM and other PCOS features were associated with a relatively high birth weight (503, 504).

In contrast to these studies, larger longitudinal or retrospective studies in Northern Europe demonstrated no relationship between birth weight and PCOS symptoms (505, 506). Similarly, birth weight by recall was similar to that of controls and/or population data among 854 United States and 170 Italian adults and adolescents with PCOS (507, 508), only 9%–10% recalled low birth weight.

C. Postnatal environment

Postnatal environmental risk factors for PCOS can be viewed as precipitating latent, congenitally programmed susceptibility traits to become manifest.

1. Insulin resistance

All extreme insulin-resistant states are associated with PCOS. The compensatory hyperinsulinemia of insulin resistance is closely associated with the anovulation of PCOS. Ovulatory patients with PCOS are less insulin-resistant than anovulatory patients with PCOS (509). All treatments that lower insulin levels improve ovarian dysfunction and ovulation (510–512).

Ordinary obesity is the most common cause of insulin resistance, and we are in the midst of a worldwide obesity epidemic (513), which is one reason why PCOS may be recognized more often than in decades past. Weight loss sufficient to improve indexes of insulin sensitivity in PCOS improves menstrual cyclicity and ovulation (210, 288, 308, 514–516).

Two syndromes of intractable obesity in childhood, pseudo-Cushing's syndrome and pseudoacromegaly, herald PCOS in adolescence (52, 303). These syndromes are characterized by moderately severe insulin resistance.

It is possible that the transient physiologic insulin resistance of puberty may contribute to physiologic anovulation or the development of PCOS during adolescence. Insulin resistance and compensatory hyperinsulinemia normally peak in midpuberty (517–520). The waning insulin resistance as puberty progresses generally parallels improvement in menstrual regularity, but the nature of this association remains to be investigated.

2. Hyperandrogenism

Postnatal androgen excess causes ovarian hyperandrogenism in some animal models (461), as does androgen excess in girls with poorly controlled congenital adrenal virilizing disorders (201). In some species, adipogenesis is also stimulated and glucose metabolism deteriorates (461, 521).

3. Other precipitants and risk factors

Excessive LH stimulation at puberty may play a role in the pathogenesis of PCOS ("hyperpuberty") (522). The best support for this theory is in the setting of congenital virilization, in which there is prenatal programming for LH excess at puberty (see section VI.B.1).

Most uncontrolled follow-up studies of idiopathic central precocious puberty have not been consistent with an increased incidence of PCOS (523). However, a controlled study of girls with early puberty reported a higher prevalence of PCOS in 25 adolescents who chose treatment

with GnRHag, as compared with a similar group of 55 girls who declined treatment (36% vs 15%) (524). The treated group had significant elevation of androstenedione in association with menstrual dysfunction or polycystic ovaries. In this study, PCOS was defined using AE-PCOS criteria (phenotypes 1 through 3), which are broader and less specific than the modified NIH criteria now accepted for the diagnosis of adolescent PCOS (phenotypes 1 and 2) (Table 1). Confirmation in a randomized trial using highly specific androgen assays and more stringent PCOS diagnostic criteria is needed before considering GnRHag treatment to be a risk factor for PCOS.

Premature adrenarche may pose a moderately increased risk for PCOS/FOH (431, 525). These individuals overall appear to carry approximately a 2-fold (15%–20% risk of developing PCOS), although the risk may vary with ethnic group. Some studies suggest that this risk is related to low birth weight (499), others do not (431). The association of premature adenarche with the subsequent development of PCOS may indicate that premature adrenarche is sometimes an early manifestation of steroidogenic dysregulation (431).

Adolescents with epilepsy appear particularly susceptible to develop PCOS when treated with valproic acid, an antiepileptic drug that augments the transcription of P450c17 and other steroidogenic enzymes (526, 527).

Endocrine disruptors have been suspected of aggravating PCOS (445, 528, 529). Serum bisphenol A levels are elevated in PCOS, giving reason to believe that it may play a role in the pathogenesis of the syndrome.

D. Implications for evolutionary origin of PCOS

PCOS presents an evolutionary paradox; it is very common across populations, although it is an infertility disorder. The classical theories postulate that PCOS developed through natural selection as a spectrum of independent, diverse genetic adaptations that evolved to preserve anabolism and reproductive capacity via increased androgen and insulin production in ancient times of nutritional deprivation, although in current times of plenty this phenotype is disadvantageous (460, 530, 531). An alternate mechanism may be "intralocus sexual conflict," that is, some PCOS-related genotypes may not disappear because they improve the reproductive fitness of the human male (eg, by promoting male hyperandrogenism) and thus compensate for reduced female fertility (531).

E. Summary

PCOS seems to arise as a complex trait that results from the interaction of diverse genetic and environmental factors that usually first becomes manifest at puberty. At its simplest, this is a "2-hit" hypothesis that can be thought of because of a congenitally programmed predisposition ("first hit") that becomes manifest upon exposure to a provocative environmental factor ("second hit") (Table 5). There is evidence for the congenital hit being genetic or acquired, with diverse causes of each. The provocative factor is postnatal and usually seems to be insulin-resistant hyperinsulinemism, which may have been programmed congenitally either on a hereditary or acquired basis or acquired postnatally due to simple (exogenous) obesity.

VII. Conclusions and Implications for Future Research

This review indicates that research to date is consistent with the concept that most PCOS is due to FOH that arises from dysregulation of steroidogenesis that sensitizes ovarian steroidogenesis to LH. In our experience, FOH is the common denominator in approximately 90% of the hyperandrogenic anovulation cases. Typical FOH accounts for two-thirds of FOH: it is characterized by 17OHP hyperresponsiveness to gonadotropin stimulation. This secretory abnormality resembles the biochemical dysfunction that is a constitutive characteristic of theca cells of classic PCOS and seems to indicate an intrinsic abnormality in the normal mechanism for down-regulation of the steroidogenic response to LH. Similar dysregulation of adrenocortical steroidogenesis seems to account for the associated FAH found in about one-quarter of cases. The pathophysiologic and biochemical basis of functionally atypical FOH is unclear. Typical FOH has more severe hyperandrogenism and a higher prevalence of PCOM than atypical FOH. It is also associated with a significantly higher prevalence of glucose intolerance in the presence of similarly increased insulin resistance, which suggests a relationship of the intrinsic ovarian abnormality to pancreatic β-cell failure.

Insulin-resistant hyperinsulinism is often an important aggravating factor in PCOS pathogenesis. About half of PCOS women have an abnormal degree of insulin resistance for BMI. The insulin resistance of PCOS is independent of obesity and thus to some extent constitutive. It

Table 5. PCOS Etiology as a Complex Trait Involving 2 Hits

A. Congenital hit
• Gene variants affecting ovarian function
• Congenital virilization
• Disturbed fetal nutrition
B. Provocative hit
• Insulin-resistant hyperinsulinemia
- Type 2 mellitus-related gene variants
- Postnatal obesity
• Hyperpuberty

selectively affects tissue-specific metabolic actions of insulin with the result that the compensatory hyperinsulinemia sensitizes ovarian theca cells to LH stimulation. Stimulation of adipogenesis and lipogenesis and inhibition of lipolysis by insulin excess also appear to contribute to the obesity of PCOS.

Currently, specific testing for FOH has little clinical utility beyond possibly identifying a subpopulation of PCOS patients whose androgen excess arises from simple obesity, and so would be expected to be reversible by weight loss, or distinguishing adolescents with PCOS from those with physiologic anovulation. The main place of biochemical phenotyping of PCOS would seem to lie in the investigation of phenotype-genotype correlations. Ultimately, the goal of research into PCOS pathophysiology is to understand the biochemical and genetic subtypes of PCOS as well as we currently understand the biochemical and genetic subtypes of congenital adrenal hyperplasia.

The etiology of FOH is multifactorial. FOH usually seems to develop as a complex trait from interactions between predisposing congenital factor(s) and provocative environmental factor(s). The most common provocative factors seem to be obesity and insulin resistance, which occur in about half of cases and which themselves have heritable components. Obesity up-regulates ovarian androgen production primarily via insulin-resistant hyperinsulinemia and to some extent via inflammatory cytokines.

Improved comprehension of the cause of PCOS will be necessary to facilitate diagnosis and treatment of the disorder. The current diagnostic criteria are very broad. Although this approach facilitates diagnosis, it obscures the recognition that obesity alone appears capable of causing hyperandrogenic anovulation. Thus, the elucidation of simple specific biochemical markers for the syndrome as distinct from such features as PCOM would be desirable. Accomplishing this would also enable the identification of specific genetic predisposing factors, the search for which is hampered by the somewhat nonspecific nature of the phenotypic criteria in current use.

Knowledge about the nature of the congenital predisposing factors is in in its infancy. Although congenital androgenization is the most well-established mechanism for experimentally reproducing the PCOS phenotype, it is unlikely to cause ordinary PCOS. In the search for causes of PCOS, this seems most likely to serve as a model for exploring epigenetic programming. The promise of molecular genetic approaches to understanding the cause of PCOS is illustrated by the recent identification by genome-wide association screening of a previously unrecognized protein variant in androgen-producing cells, DENND1A.V2, as a facilitator of steroidogenesis.

A complementary approach to understanding the pathogenesis of PCOS would involve developing untransformed human cell lines for all the tissues that are dysfunctional in PCOS: theca, granulosa, adrenocortical zona reticularis, and preadipocyte cells. This would facilitate knowledge about the normal and abnormal regulation of ovarian steroidogenesis, homologous desensitization to LH, and folliculogenesis as well as potentially identify commonality among the mechanisms underpinning the associated abnormalities seen in PCOS. One of the great mysteries about PCOS is what the common denominator may be that links ovarian hyperandrogenism, obesity, and insulin resistance. Considerable basic research will be necessary to discern this.

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