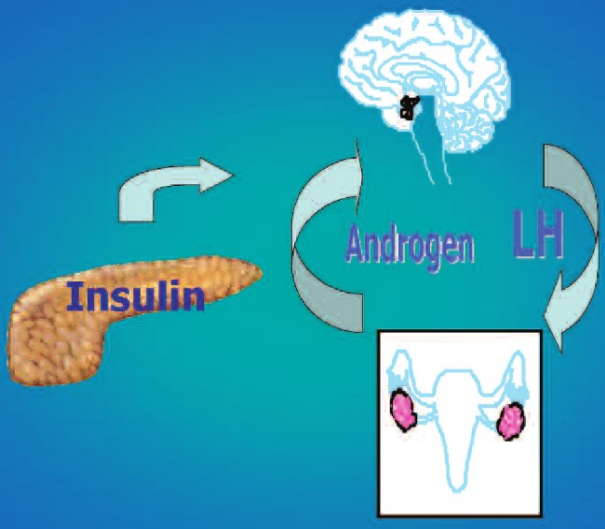
The Polycystic Ovary Syndrome

Current Concepts on Pathogenesis and Clinical Care



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THE POLYCYSTIC OVARY SYNDROME:

CURRENT CONCEPTS ON PATHOGENESIS AND CLINICAL CARE

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THE POLYCYSTIC OVARY SYNDROME:

CURRENT CONCEPTS ON PATHOGENESIS AND CLINICAL CARE

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Chapter 1

Definition, Diagnosis, and Epidemiology of the Polycystic Ovary Syndrome

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The Polycystic Ovary Syndrome (PCOS) is a heterogeneous disorder, whose principal features include androgen excess, ovulatory dysfunction, and/or polycystic ovaries, and is recognized as one of the most common endocrine/metabolic disorders of women. This syndrome was first described by Stein and Leventhal in 1935 [1], although the presence of sclerocystic ovaries had been recognized for at least 90 years before the publication of that seminal work. Following, we review the definition, diagnostic scheme, and epidemiology of PCOS as it currently stands.

1. DEFINING PCOS: BASICS

We should first note that PCOS is a functional disorder of unclear etiology, and, as such, is a diagnosis of exclusion, with other androgen excess and ovulatory disorders of clearly defined etiologies excluded. Androgen excess disorders to exclude are 21-hydroxylase deficient nonclassic adrenal hyperplasia (NCAH), adrenal or ovarian androgen-secreting tumors, disorders of generalized adrenocortical dysfunction (e.g., Cushing's disease), and use or abuse of androgenic or anabolic drugs. Although not true androgen excess, another functional disorder resulting in clinical features suggestive of androgen excess, namely idiopathic hirsutism, should be excluded. Although still controversial, many investigators also consider patients with the HyperAndrogenic–Insulin Resistant–Acanthosis Nigricans (HAIRAN) syndrome (a.k.a. type C insulin resistance syndrome [2]), as distinct from PCOS, since these women have extreme degrees of hyperinsulinism and insulin resistance far greater than the vast majority of PCOS patients

and may have other unique features, including lipodystrophy. Generally, other disorders that may result in ovulatory dysfunction, such as thyroid dysfunction and hyperprolactinemia, will also need to be excluded.

Secondly, we should recognize that PCOS is still a "syndrome," namely a collection of signs and features that characterize a disorder, where no single test is diagnostic. In essence, the whole (or global assessment) is greater than the sum of the individual features. While the disorder is relatively heterogeneous, three features are generally recognized to compose this syndrome, including androgen excess, ovulatory dysfunction, and polycystic ovaries. Androgen excess (or hyperandrogenism) is detectable either by laboratory analysis, generally measuring circulating androgen levels, or by clinical exam, primarily in the form of hirsutism. Ovulatory dysfunction is generally detectable by the presence of clinically evident oligo-amenorrhea, although about 20–30% of oligo-ovulatory women with PCOS will present with a history of apparent eumenorrhea (i.e. subclinical oligo-anovulation). Finally, while classically polycystic ovaries were diagnosed by pathologic examination, today ultrasonography is used to establish the presence of this feature. These features have been combined in various manners to arrive at specific criteria for PCOS.

2. SPECIFIC CRITERIA FOR PCOS

To date, three major criteria have been proposed, with other investigators proposing modifications of these. We will review the criteria arrived at a NIH/NICHD expert conference sponsored in 1990 [3], that proposed by an expert conference of the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) in 2003 [4], and that proposed by the Androgen Excess Society in 2006 [6].

2.1. The 1990 National Institutes of Health Criteria

The first useful definition of PCOS arose from the proceedings of an expert conference sponsored by the US National Institutes of Health (NIH) in April 1990 (Table 1). Participants were surveyed, and tabulation of the results indicated that most felt that the features of PCOS were (in order of importance): (a) hyperandrogenism and/or hyperandrogenemia, (b) chronic anovulation, and (c) exclusion of related disorders such as hyperprolactinemia, thyroid disorders, and congenital adrenal hyperplasia [3]. Polycystic ovaries were suggestive, not diagnostic, of the syndrome. We should note that these proceedings did not provide clear guidelines on how to define each criterion.

Three principal phenotypes of PCOS are recognized using the NIH 1990 criteria, including women with: (a) hirsutism, hyperandrogenemia, and oligo-ovulation, (b) hyperandrogenemia and oligo-ovulation, or (c) hirsutism

Table 1. Criteria for defining PCOS

NIH 1990 [3]
To include *all* of the following:
Clinical hyperandrogenism and/or hyperandrogenemia
Chronic anovulation
Exclusion of related disorders
ESHRE/ASRM (Rotterdam) 2003 [4,5]
To include two of the following, in addition to exclusion of related disorders:
Oligo-anovulation
Hyperandrogenism and/or hyperandrogenemia
Exclusion of related disorders
AES 2006 [6]
To include *all* of the following:
Hyperandrogenism (hirsutism and/or hyperandrogenemia)
Ovarian dysfunction (oligo-anovulation and/or polycystic ovaries)
Exclusion of related disorders

NIH is US National Institutes of Health; ESHRE is European Society for Human Reproduction and Embryology, ASRM is American Society of Reproductive Medicine, and AES is Androgen Excess Society.

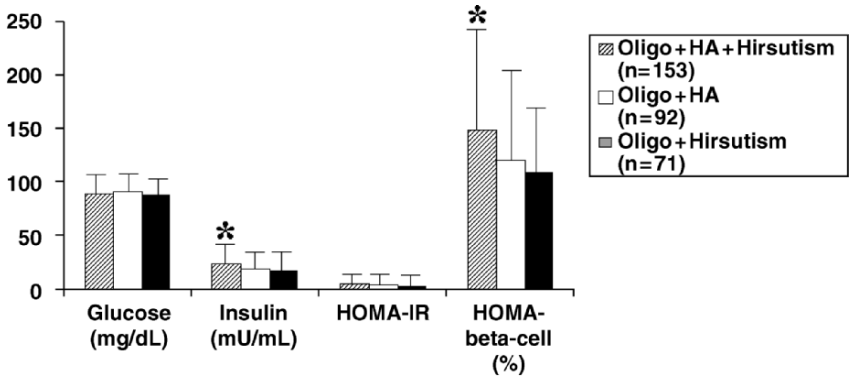

Fig. 1. Phenotypes of PCOS: relationship to degree of β-cell function but not severity of insulin resistance (adapted from Ref. [7]).

and oligo-ovulation. The overall prevalence of these phenotypes in a large study of white or black women in the US was ~50, ~30, and ~20%, respectively [7], without significant differences in mean age, body mass index (BMI), waist-to-hip ratio, racial distribution, severity of oligomenorrhea, or prevalence of family history for hyperandrogenism between the phenotypes. Alternatively, fasting insulin levels were highest in patients with hirsutism, hyperandrogenemia, and oligo-ovulation, and lowest in those women with oligo-ovulation and hirsutism only (Fig. 1). Whether different mechanisms underlie the development of these phenotypes remains to be demonstrated.

Overall, the NIH criteria have proven extremely useful to begin to define and understand, among other features, the high prevalence of the disorder [8–10], its high frequency of insulin resistance [11,12], and the considerable risk of these women for developing type 2 diabetes mellitus (DM) [13,14].

2.2. The 2003 ESHRE/ASRM (Rotterdam) Criteria

Another expert conference was organized in Rotterdam in May of 2003 (Table 1), in part sponsored by ESHRE and ASRM. The proceedings of the conference noted that PCOS could be diagnosed, after the exclusion of related disorders, by two of three features: (a) oligo- or anovulation, (b) clinical and/or biochemical signs of hyperandrogenism, or (c) polycystic ovaries [4,5]. As for the NIH 1990 criteria, other disorders should be excluded. It should be noted that these recommendations did not replace the NIH 1990 criteria; rather they expanded the definition of PCOS.

Additional phenotypes now considered as being PCOS by this criteria included: (a) women with polycystic ovaries with clinical and/or biochemical evidence of androgen excess, but no signs of ovulatory dysfunction and (b) women with polycystic ovaries and ovulatory dysfunction, but no signs of androgen excess. However, expanding the definition of PCOS without good supporting data could have significant detrimental implications for research (e.g., increased heterogeneity of the study populations), clinical practice (e.g., requiring that all these patients undergo ultrasonography), and patient quality of life (e.g., requiring long-term monitoring for the development of associated metabolic morbidities and potentially adversely affecting health care insurability).

2.3. The AES 2006 Criteria

Because of the continuing controversy regarding the definition of the PCOS, the AES, an international organization dedicated to promoting knowledge and original clinical and basic research in every aspect of androgen excess disorders, charged a Task Force to recommend an evidence-based definition for PCOS, whether already in use or not, to guide clinical diagnosis and future research. The Task Force, after review of all available published data, proposed that PCOS should be diagnosed by the presence of three features: (a) androgen excess (clinical and/or biochemical hyperandrogenism), (b) ovarian dysfunction (oligo-anovulation and/or polycystic ovarian morphology), and (c) exclusion of other androgen excess or ovulatory disorders (Table 1) [6].

The Task Force took the stand that all phenotypes (i.e., specific collection of features) that composed PCOS should be associated with a morbidity not directly part of the definition (much like diabetes is defined by those glucose levels with have been associated with the development of microvascular disease), namely insulin resistance. This definition then recognizes one

additional phenotype above that noted by the NIH 1990 criteria, namely that of women with polycystic ovaries, hyperandrogenism, and apparently normal ovulation. These patients appear to have features that approximate patients with PCOS defined by the NIH 1990 criteria, although of a milder nature. These include slight excess in circulating LH, insulin, and other markers for cardiovascular disease [15–18]. Essentially, these patients can be considered to have "mild PCOS," and, in the absence of long-term follow-up studies, may not have the same degree of reproductive or metabolic consequences as women with the full PCOS phenotype.

Alternatively, it was less clear that women with ovulatory dysfunction and polycystic ovaries, but without any evidence of hyperandrogenism, as a group have a similar morbidity to patients with PCOS (e.g., insulin resistance). For example, the prevalence of polycystic-appearing ovaries does not appear to predict abnormalities in insulin sensitivity either in women with PCOS [19] or in their sisters [20]. In addition, it is uncertain how patients with hypothalamic amenorrhea and polycystic ovaries will be differentiated from nonobese women with PCOS, an important conundrum considering the significant differences in long-term morbidity (e.g., bone loss for hypothalamic amenorrhea and increased bone mass for PCOS, increased risk of diabetes in PCOS but not hypothalamic amenorrhea patients).

Overall, the Task Force did not discard the possibility that future research could demonstrate that the subset of women with polycystic ovaries, ovulatory dysfunction, but without overt androgen excess, may actually have PCOS, but considered it prudent to withhold expanding the definition of this disorder much beyond that originally suggested by the NIH 1990 expert conference until more complete epidemiologic and long-term longitudinal data were made available.

2.4. Summary

There are strengths and weaknesses to all three criteria. The NIH 1990 criteria clearly represent the core of PCOS patients which all investigators (and practitioners) would have no doubts of defining as affected. Both the Rotterdam 2003 and the AES 2006 criteria strive to expand the NIH 1990 definition. The NIH 1990 and, to a lesser degree, the AES 2006 define populations of patients that on average (but not universally) are at higher risk for insulin resistance than the general population. Alternatively, the Rotterdam ESHRE/ASRM 2003 criteria defines a more heterogeneous group of women whose overall incidence of insulin resistance is lower than either the NIH 1990 or the AES 2006 definitions. Alternatively, by emphasizing polycystic ovarian morphology, the Rotterdam 2003 criteria identifies a group of women who may be at higher risk for ovarian hyperstimulation during ovulation induction for the treatment of ovulatory infertility.

Note also that women with PCOS demonstrate a rate of obesity higher than the general population (30–60%, depending on country of origin) [21–24], insulin resistance and hyperinsulinism (present in 50–70%) [11,13], and an LH to FSH ratio of greater than 2 or 3 (in 30–50%) [25–30]. However, these features are not included in any of the major diagnostic criteria, as they are either highly prevalent in disorders other than PCOS (e.g., obesity and insulin resistance) or are not observable in the majority of patients with routine laboratory assessments (e.g., an elevated LH:FSH ratio, because LH levels are lower in obese individuals, which accounts for a large fraction of women with PCOS).

3. DIAGNOSIS OF PCOS

The diagnosis of PCOS entails two principal steps: (a) to determine whether features suggestive of PCOS are present and (b) to exclude related androgen excess or ovulatory disorders. The exact diagnostic scheme clearly depends on what specific criteria are chosen to define PCOS. In general, we utilize the AES 2006 criteria.

3.1. Determining Whether Features of PCOS are Present

Features that may obviously suggest PCOS include: (a) long-term menstrual dysfunction or irregularity, suggestive of chronic ovulatory dysfunction, (b) dermatologic signs suggestive of hyperandrogenism, such as hirsutism, and less commonly acne or alopecia, and (c) polycystic ovarian morphology on ultrasonography.

All women with menstrual dysfunction should be evaluated closely for concomitant signs of hyperandrogenism. Any other sign or complaint of androgen excess (acne, unwanted hair growth, scalp hair shedding or loss) would warrant a more in depth evaluation for PCOS. Likewise, menstrual dysfunction accompanied by clinical signs suggestive of insulin resistance (e.g., acanthosis nigricans) would also indicate that a more thorough evaluation for PCOS (and metabolic syndrome) is needed. Finally, the presence of polycystic ovaries on ultrasonography (or at surgery) in a patient with menstrual dysfunction would likewise suggest the need to exclude PCOS. Overall, between one-quarter to one-third of all women with oligo-amenorrhea or menstrual dysfunction will have PCOS [8–10,31]. We should note that not all patients with PCOS demonstrate clinically obvious oligomenorrhea. In fact, about 20–30% of oligo-ovulatory PCOS women will present with a history of apparent eumenorrhea (i.e., subclinical oligo-anovulation) [8,21,32,33]. As such, ovulation should be confirmed (e.g., day 22–24 progesterone [P4] level) in those apparently eumenorrheic patients who demonstrate other signs of PCOS (e.g., androgen excess or polycystic ovaries).

Patients with dermatologic signs suggestive of androgen excess, principally hirsutism, should also be investigated for the presence of PCOS. Between 85 and 95% of patients with frank hirsutism (i.e., a modified Ferriman-Gallwey [mF-G] score of ≥ 6–8) will have PCOS when evaluated [21,34]. We should also note that the sole complaint of "unwanted" hair growth in the absence of frank hirsutism is a strong predictor of PCOS. In a study of 228 patients who presented with minimal unwanted hair growth and an mF-G score of 5 or less, 50% demonstrated PCOS; among patients with menstrual irregularities, 65% had an underlying androgen excess disorder, while 22% of those women who reported being eumenorrheic were so affected [35].

Alternatively, while complaints or signs of acne and scalp hair thinning are suggestive of PCOS, their predictive value for the disorder is somewhat less than is the presence of hirsutism or a complaint of unwanted hair. Between 20 and 40% of patients with persistent acne-only [36–38], and only 10% of those women with alopecia-only [39–40] will have PCOS.

Finally, patients found to have polycystic ovaries are also at risk for PCOS. Overall, it can be estimated that between 20 and 30% of patients with polycystic ovaries will have PCOS. The likelihood increases, of course, if this finding is associated with evidence of ovulatory dysfunction and/or androgen excess. We should note that the diagnosis of polycystic ovaries is relatively specific and should not be made subjectively. Dewailly and colleagues have suggested that the presence of polycystic ovaries is established when at least one ovary has either ≥12 follicles measuring 2–9 mm in diameter and/or an ovarian volume of >10 cm^3 by transvaginal ultrasonography (TV-U/S) [41]. This definition does not apply to women taking the oral contraceptive pill, since its use can modify ovarian morphology. If there is evidence of a dominant follicle (>10 mm) or a corpus luteum, the scan should be repeated during the next cycle. We should note that the definition of polycystic ovaries is still in flux. In a subsequent study, these investigators reaffirmed the use of follicle number as the best criterion, but noted that the threshold value for ovarian volume may need to be reduced to >7 cm^3 [42].

3.2. Exclusion of Other Androgen Excess or Ovulatory Disorders

As PCOS is a diagnosis of exclusion, the diagnosis can only be arrived at after other disorders have been excluded. These include 21-hydroxylase deficient NCAH (by a basal and/or stimulated 17-hydroxyprogesterone [17-HP] level, see below), androgen-secreting neoplasms (by history and clinical exam and appropriate studies in selected patients), adrenocortical hyperactivity (by clinical exam and appropriate testing), and drug-induced hyperandrogenism (by history). Overall, these disorders account for 5–10% of all women with androgen excess [21,34].

In addition, it is customary to exclude thyroid dysfunction and hyperprolactinemia by measuring a TSH, using a third-generation assay, and prolactin

level. However, we should note that recent studies suggest that the prevalence of these endocrine abnormalities in patients with apparent PCOS is relatively low, on the order of 1–3% [21,34]. Likewise, if polycystic ovaries are detected, particularly in the absence of overt signs of androgen excess, disorders that can result in this ovarian morphology need to be also excluded, such as hypothalamic amenorrhea [43,44].

3.3. Laboratory and Radiologic/Sonographic Evaluation

Patients suspected of having PCOS can be subdivided into four groups:

(a) *Women with overt long-term oligomenorrhea and hirsutism*: these women basically have PCOS, pending exclusion of related disorders. At a minimum, these women should undergo measurement of circulating TSH, prolactin, and 17-HP levels. If these values are normal, then the patient is presumed to have PCOS. Androgen levels and ovarian ultrasonography, while of some value, are not critical to establishing the diagnosis.
(b) *Women with overt long-term oligomenorrhea, but no obvious sign of androgen excess*: these women should undergo measurement of circulating androgen levels (generally total and free testosterone [T], and DHEAS) and, if elevated, assessment of TSH, prolactin, and 17-HP levels. If these latter values are normal, then the patient is presumed to have PCOS. In these women, at least according to the NIH 1990 and the AES 2006 criteria, the use of ovarian ultrasonography will not alter the diagnosis.
(c) *Women with hirsutism but apparent eumenorrhea*: these women should undergo confirmation of ovulation (most simply by measuring a P4 level in the luteal phase of the menstrual cycle, i.e., day 20–24 of the cycle) on one or two cycles. They should also undergo ovarian ultrasonography. If the patient is found to have anovulation or polycystic ovaries on ultrasonography, they should undergo measurement of TSH, prolactin, and 17-HP levels. If these values are normal, then the patient is presumed to have PCOS (either "classic PCOS" if anovulatory, or "ovulatory PCOS" if she has polycystic ovaries but normal ovulation).

A note about using 17-HP levels to screen for NCAH: 21-hydroxylase deficient NCAH affects 1–10% (depending on ethnicity) of patients with androgen excess. Approximately 90% of NCAH patients can be detected by a basal level of 17-HP > 2 ng/mL [45,46]. The blood sample should be obtained in the morning, and, most importantly, in the follicular (preovulatory) phase of the menstrual cycle. Obviously, the use of any corticosteroids will artificially suppress 17-HP levels and should not be used prior to screening. Values < 2 ng/mL virtually exclude NCAH [47]. If the screening 17-HP level is > 2 ng/mL, and the investigator is certain that the sample was not drawn in the luteal (postovulatory) phase of the cycle, an acute 30–60 min. ACTH stimulation test is performed. Values of 17-HP > 10–12 ng/mL at 30–60 min. after the IV administration of ACTH are diagnostic for NCAH.

4. PREVALENCE OF PCOS

Obviously, the prevalence of PCOS will depend to a degree on the criteria used to define this disorder. To date the prevalence of PCOS has been determined primarily using the NIH 1990 criteria. Studying 277 women seeking a preemployment physical in the southeastern US, we initially reported a prevalence of PCOS of 4.0%, not significantly different between Whites and Blacks [31]. In a subsequent, and more intensive, study of 400 unselected consecutive women ages 18–45 years in the same setting (223 Black, 166 White, and 11 of other races), the prevalence of PCOS was observed to be 6.6% [8]. The prevalence was 8.0% in Black and 4.8% in White women. While the racial difference was not statistically different, this may primarily reflect inadequate sample size (Fig. 2).

Also using the 1990 NIH criteria, a study of 192 Greek women on the island of Lesbos reported a prevalence of PCOS of 6.8%, recruited through the promise of a free medical exam [9]. Another study of 154 Caucasian blood donors in Madrid, Spain, found a similar prevalence (6.5%) [10]. Among 230 volunteers (97% White) recruited from two Oxford universities and two general practice surgeries and who agreed to participate in "a study of women's health issues," the prevalence of PCOS using the NIH 1990 criteria was 8% [48]. These data indicate that the prevalence of clinically evident PCOS using the 1990 NIH criteria in unselected women of reproductive age ranges from

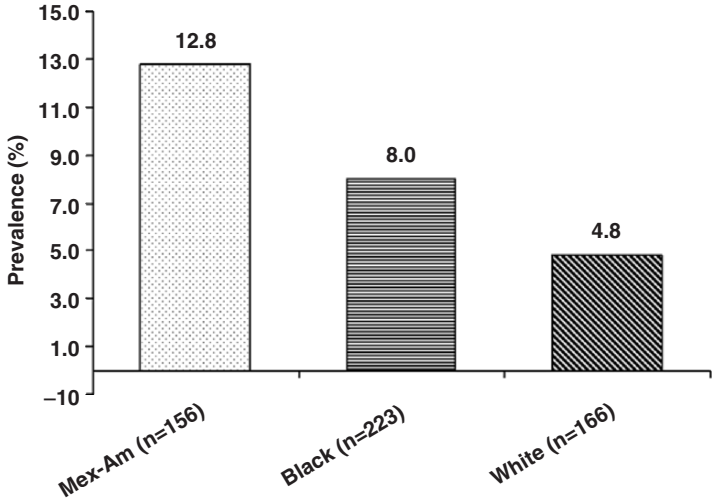

Fig. 2. Potential racial differences in the prevalence of PCOS in unselected women in the US. Whether these data are confirmed in further studies, and/or whether these differences are the product of differences in the populational prevalence of insulin resistance, remains to be confirmed (adapted from Refs. [8,59]).

6.5 to 8.0%, affecting 1 in 13–15 unselected women. This translates to at least 5 million affected women in the US and 105 million worldwide.

Alternatively, the prevalence when PCOS is defined more broadly is significantly higher. For example, in the study by Michelmore and colleagues [48] of 230 volunteers participating in a study of women's health issues in Oxford, UK, when PCOS was defined loosely as "the presence of polycystic ovaries on ultrasound plus one additional feature including: menstrual irregularity, acne, hirsutism, BMI > 25 kg/M^2, raised serum testosterone (> 3 nmol/l), or raised LH (≥10 IU/l)," 26% of the women studied had evidence of PCOS. This is threefold higher then the prevalence of PCOS (8%) that the investigators observed when using the NIH 1990 criteria. However, we should note that these features occurred frequently in women without polycystic ovaries, and 112 of the 150 women (75%) with normal ovaries had the presence of one or more of these attributes.

Recently, among 827 women with World Health Organization class II (WHO-II) oligo-ovulation (euestrogenic normo-gonadotropic ovulatory dysfunction), 456 (55%) were classified as having PCOS by the NIH 1990 criteria [49] (Fig. 3). In contrast, 754 (91%) women were noted to have PCOS according to the Rotterdam 2003 criteria, an increase of 65% and a significant difference. Of those oligo-ovulatory women classified as having PCOS by the NIH 1990 criteria, 89% also were found to have polycystic ovaries on ultrasound scan and, as such, fit all three criteria of the Rotterdam 2003 definition for PCOS (Fig. 3). The 298 additional women identified as having PCOS solely

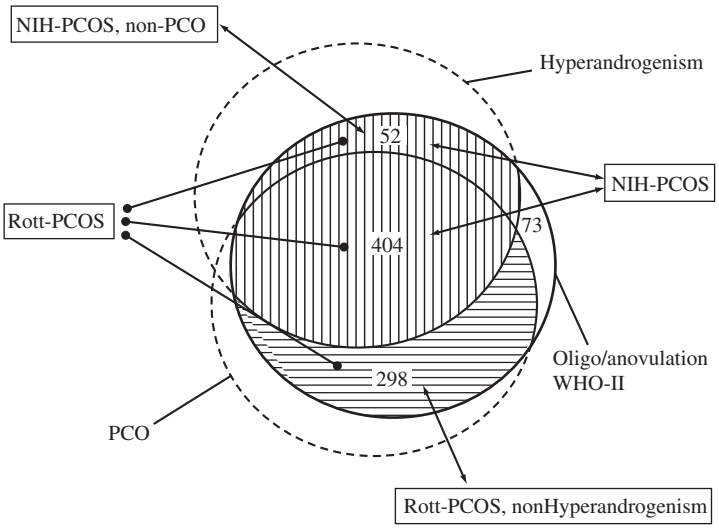

Fig. 3. Venn diagram depicting the relation between the NIH 1990 and the Rotterdam 2003 classification for PCOS among 827 women with WHO-II oligo-ovulation. The *central black circle* represents the cohort of WHO-II anovulation cases (reprinted with permission from ref. 49).

according to the Rotterdam 2003 criteria were classified based on the presence of polycystic ovaries on ultrasound (and ovulatory dysfunction), but without clinical or biochemical evidence of androgen excess (Fig. 3). Overall, among women with WHO-II ovulatory dysfunction, the prevalence of PCOS by the Rotterdam 2003 criteria appears to be over 60% larger than the group classified as PCOS by the NIH 1990 definition (91% versus 55% of the entire WHO-II cohort).

Likewise, we have preliminarily compared the prevalence of PCOS by the NIH 1990 criteria versus the Rotterdam 2003 definition among hirsute women, all evaluated endocrinologically and sonographically. In the 62 women evaluated, PCOS was diagnosed in 44 (71%) by the NIH 1990 criteria and in 57 (92%) by the Rotterdam 2003 definition [50]. We concluded that the use of TV-U/S to evaluate ovarian morphology in combination with the Rotterdam 2003 criteria led to a 20–60% overall increase in the prevalence of PCOS among hirsute or oligomenorrheic women. Further studies in larger and ethnically different populations are required to confirm this suggestion.

A number of conditions are associated with increased prevalences of PCOS, including obesity [51–53], insulin resistance [52], type 1 or type 2 DM [54–56], or oligo-ovulatory infertility [17,57,58]. The prevalence of PCOS also appears to be somewhat higher among Mexican-American than White or African-American women [59] (Fig. 1), although this observation remains to be confirmed in larger and more diverse populations. Finally, the prevalence of PCOS seems higher among populations previously experiencing premature adrenarche [60] and gestational diabetes [61,62], and, logically, in those with first-degree relatives with PCOS [63,64].

5. CONCLUSIONS

PCOS is a heterogeneous disorder of functional androgen excess, detectable either by laboratory analysis or by clinical exam, with ovulatory dysfunction and polycystic ovarian morphology also affecting a large proportion of these patients. PCOS is a diagnosis of exclusion, with other androgen excess or related disorders to be ruled out. The first broadly used definition of PCOS arose from the proceedings of an expert conference sponsored by the NIH in 1990, which noted the features of PCOS to be (in order of importance): (a) hyperandrogenism and/or hyperandrogenemia, (b) chronic anovulation, and (c) exclusion of related disorders such as hyperprolactinemia, thyroid disorders, and congenital adrenal hyperplasia. Another expert conference held in Rotterdam in 2003 expanded the NIH 1990 criteria for PCOS, noting that the disorder could be diagnosed by having two of the following three features: (a) oligo- or anovulation, (b) clinical and/or biochemical signs of hyperandrogenism, or (c) polycystic ovaries, after the exclusion of related disorders. This definition created two new phenotypes for PCOS:

(a) women with polycystic ovaries and ovulatory dysfunction but no signs of androgen excess and (b) women with polycystic ovaries with clinical and/or biochemical evidence of androgen excess, but no signs of ovulatory dysfunction.

To accommodate currently available data and arrive at an evidence-based definition for PCOS, a modification of the NIH criteria was proposed by the AES in 2006. This definition recommended that PCOS be defined by three features: (a) androgen excess (clinical and/or biochemical hyperandrogenism), (b) ovarian dysfunction (oligo-anovulation and/or polycystic ovarian morphology), and (c) exclusion of other androgen excess or ovulatory disorders. Clearly the prevalence of PCOS will depend to a degree on the criteria used to define this disorder. Using the NIH 1990 criteria, most studies have observed a 6.5–8.0% prevalence in unselected reproductive-aged women. The prevalence of PCOS is increased in the presence of obesity, insulin resistance, type 1 or type 2 DM, oligo-ovulatory infertility, premature adrenarche, prior gestational diabetes, and first-degree relatives of PCOS. It is also 20–60% higher if PCOS is defined using the Rotterdam 2003 criteria. Further investigations in larger and more ethnically diverse populations are required to more clearly establish the phenotype and epidemiology of PCOS.

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Chapter 2

Clinical Evaluation of PCOS

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Polycystic ovary syndrome (PCOS) is a common endocrinopathy in women that in its simplest form consists of unexplained hyperandrogenic chronic anovulation, which affects ~7% of the US population [1]. Because its etiology and natural history are poorly understood, there is controversy about the diagnostic criteria and clinical evaluation of the syndrome. Its origins as a named disorder track back to its original description in the 1930s by Stein and Leventhal, a pair of gynecologists from Chicago, who described a complex of signs and symptoms including oligomenorrhea, enlarged polycystic ovaries, hirsutism, and obesity, and also pioneered the treatment of wedge resection of the ovaries which resulted in more regular menses and improved fertility [2]. Since that time, there has been debate as to what the cardinal features of the syndrome are or should be, but a guiding thread of consensus stemming from this original description has been that this is an ovarian disorder of hyperandrogenism (although whether this is primary or secondary is uncertain) and is most readily diagnosed in women of reproductive age [3,4].

1. FEATURES OF PCOS

The current controversy regarding the definition and criteria for PCOS was discussed in Chap. 1. Androgen excess may be manifested by either clinical signs, most commonly by disorders of the pilosebaceous unit such as acne, hirsutism, or androgenic alopecia, or by biochemical measures, most commonly elevated circulating total or free testosterone levels. Hirsutism is defined as excess body hair in a male pattern distribution (primarily in the midline), and many patients go to great lengths to remove such hair; thus it is important to both elicit the distribution of unwanted hair on history as well on clinical exam.

Peripheral hyperandrogenism is dependent on a number of factors, most likely genetic, given the racial differences in hair distribution (for instance, Asian individuals who demonstrate little midline body hair [5]). As such, many investigators prefer a more objective measure of hyperandrogenism, i.e., circulating hyperandrogenemia. However, assessment of biochemical evidence of androgen excess can also be somewhat problematic as most testosterone assays are geared toward measuring levels in the male range (> 200 ng/dL), which is above most levels in women with PCOS, as mean levels in PCOS are in the range of 60–80 ng/dL and are unlikely to exceed 150 ng/dL [6,7]. These levels are further confounded by age and reproductive stage [8], and by medications, such as oral contraceptives, which normalize the levels of circulating androgens in women with PCOS [9]. Despite the imprecision of testosterone assays in women, a movement toward their greater standardization [10] and the recognition of the role of female hyperandrogenemia and its association with metabolic abnormalities [11,12] recommends it routinely be measured in women with suspected PCOS [13].

A history of chronic anovulation is most commonly obtained by asking how many spontaneous menstrual cycles per year the subject has. Most clinical studies identify patients at some threshold such as 6, 8, or 9 or fewer per year [14–16] or with an intermenstrual interval of more than 35–40 days. However, because anovulation with androgen excess can present with unpredictable bleeding, thought to be due to prolonged unopposed estrogen exposure, affected women may also present with a history of frequent vaginal bleeding episodes [17].

Finally there is the characteristic appearance of the polycystic ovary on ultrasonography which contains multiple (> 10–12) small follicles (~2–9 mm in diameter) tightly spaced along the periphery of the ovary, what is known as "the pearl necklace sign," with increased central stroma (Fig. 1) [18,19]. Polycystic ovaries usually present bilaterally. The name polycystic ovary is a misnomer, because there are actually an absence of "cysts," i.e., no large (> 20 mm in diameter) dominant follicle or postovulatory corpus luteum cysts are present due to the chronic anovulation [20]. The name harkens from the pathologist's view of the microscopic enlargement of these small follicles (most likely arrested or atretretic follicles [21]) as "cysts."

The designation of an ovary as "polycystic" often summons up in the patient's mind a picture of a pathologically enlarged ovary full of large symptomatic cysts. In actuality, a polycystic ovary is only modestly enlarged in terms of volume compared to a cycling ovary in the early follicular phase (prior to the development of a physiologic cyst), with a mean volume of > 10 cm^3 in PCOS versus < 8 cm^3, respectively [19]. The polycystic ovary is usually not associated with any symptoms, other than those related to hyperandrogenic chronic anovulation.

It is difficult to make the diagnosis of PCOS when a woman is on hormonal contraception, such as oral contraceptive pills (OCPs), as this will normalize circulating androgen levels and can also significantly improve (especially

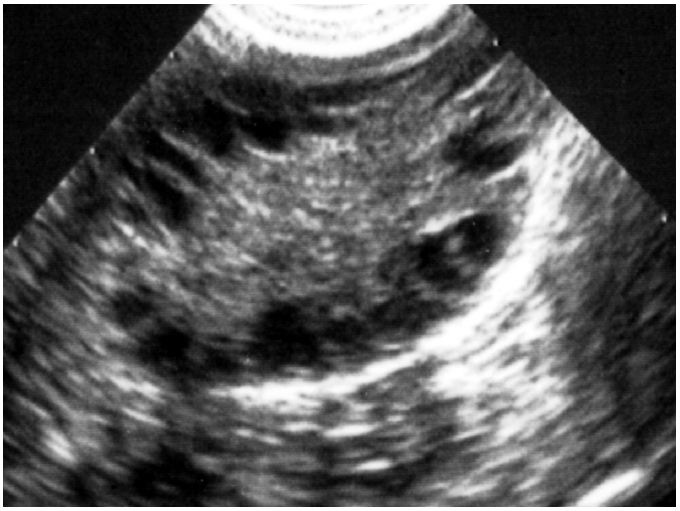

Fig. 1. Polycystic ovary on transvaginal ultrasound.

over 1–2 y periods) stigmata of hyperandrogenism, such as acne and hirsutism [22]. A recent study observed that discontinuing OCPs in women with PCOS for at least 8 weeks allows the return of all measured androgens and sex hormone-binding globulin (SHBG) levels to basal values [23].

Gonadotropins are also suppressed by hormonal contraception; however, while women with PCOS tend to display increased luteinizing hormone (LH) levels relative to follicle-stimulating hormone (FSH) [24], these are no longer included in any recommended diagnostic criteria [3,4]. There are many reasons for this. One is that the inherent pulsatility of gonadotropins [25], even with disordered secretion, can lead to a high percentage of false positives and negatives. Some investigators have recommended pooled collections to assess gonadotropins, but these are difficult to obtain in clinical practice [26]. Another confounding influence is the degree of obesity, which tends to be associated with blunted LH levels, although the same pattern of excess LH secretion can be elicited by dynamic challenge tests with GnRH [27]. Gonadotropins, therefore, have little utility in the diagnosis of PCOS, with the exception of diagnosing premature ovarian failure in which case a screening FSH would be obtained. It is worth noting that FSH levels, both basal and stimulated, tend to be normal in women with PCOS [24].

2. DIFFERENTIAL DIAGNOSIS OF PCOS

PCOS remains a diagnosis of exclusion, and it is useful to exclude other potential etiologies that can present with the triad of polycystic ovaries, hyperandrogenism, and chronic anovulation. It is important to note that the presence

of one of these signs or symptoms alone presents a much wider differential diagnosis. For instance, chronic anovulation alone may be due to failure or dysfunction of the hypothalamic–pituitary axis or to frank ovarian failure, states of steroid deficiency without androgen excess. In series of adult women presenting with amenorrhea alone, PCOS is present in about one-third of these patients [28], but rises to 70% or more when other symptoms such as hirsutism are considered [29].

Other than PCOS, other potentially serious causes of hyperandrogenism include such disorders as Cushing's syndrome and an androgen-secreting tumor [30]. These disorders are acquired and are often preceded by a period of normal menses without symptoms of hyperandrogenism. In contrast, PCOS presents in the postmenarche and tends to affect women throughout much of their reproductive life.

As Cushing's syndrome has an extremely low prevalence in the population (1–2 per million) and screening tests do not have 100% sensitivity/specificity, routine screening of all women with PCOS for Cushing's syndrome is not indicated [31]. Nonetheless, the presence of clinical signs more commonly found in Cushing's syndrome, such as ecchymoses, proximal muscle weakness, centripetal reddened striae, facial rubor and swelling, and perhaps hypertension and glucose intolerance, should signal the need for screening tests. Cortisol excess can be screened for with a 24-h urine collection for free cortisol.

Androgen secreting tumors are rare in this age group, are usually ovarian in origin, tend to have markedly elevated circulating androgen levels above the usual PCOS range, and are associated with a comparatively rapid onset of symptoms which frequently progress to frank virilization with clitoromegaly, breast atrophy, and voice changes [32,33]. Virilization is rarely, if ever, associated with PCOS, and this clinical presentation should always trigger a search for other causes, including anabolic steroid abuse.

A disorder that can present peripubertally in a similar indolent fashion as PCOS is 21-hydroxylase (21-OH) deficient nonclassic congenital adrenal hyperplasia (NCAH), also known as late-onset congenital adrenal hyperplasia. NCAH is a homozygous recessive disorder due to mutations in the *CYP21* gene, which results in an abnormal (or absent) 21-OH activity and a shift toward the overproduction of androgens. Overall, between 1 and 8% of women with androgen excess have *CYP21* deficient NCAH depending on ethnicity, with the highest rates reported in Ashkenazi Jewish populations [34]. Patients with NCAH may present with mild symptoms, many with only persistent acne or moderate degrees of hirsutism and oligoamenorrhea, and frank virilization or even severe hirsutism is relatively rare [35]. The levels of the exclusive adrenal androgen metabolite dehydroepiandrosterone sulfate (DHEAS) are not any higher in NCAH than in women with PCOS [35].

Although the frequency is relatively low, all patients with unexplained androgen excess should be screened for NCAH due to *CYP21* mutations, as

this diagnosis has a different prognosis, a different treatment regimen, and requires genetic counseling regarding the risks of congenital transmission [36,37]. The measurement of a basal 17α-hydroxyprogesterone (17-HP) in the follicular phase and in the morning can be used to screen for this disorder (normal < 2–4 ng/mL) [38]. This level is also unlikely to be affected by the concurrent use of oral contraceptives or glucocorticoids.

Other rare situations that may present with hyperandrogenic chronic anovulation are thyroid disease and hyperprolactinemia. Although the evidence linking thyroid disease to hyperandrogenism is weak, thyroid disease is common in women and merits detection. A TSH level is easily obtained, and thyroid abnormalities can readily be treated. The case for measuring prolactin is more complex. About 20–30% of women with PCOS have been reported to have mildly elevated prolactin levels [39]. In our lab, the normal prolactin level is 20 ng/mL, and we find many PCOS patients whose prolactin levels are in the range of 20–30 ng/mL. The mild elevations in prolactin probably reflect the hypothalamic–pituitary dysfunction associated with PCOS.

Polycystic ovaries on ultrasound are found in a wide variety of unrelated disorders with some syndromes having little overlap with hyperandrogenic chronic anovulation. For example, up to 30% of women with normal menses and normal circulating androgens may have polycystic ovaries [18,40,41]. There have been recent reports to suggest that polycystic ovaries per se may identify a group of women with some subtle stigmata of reproductive and metabolic abnormalities found in the endocrine syndrome of PCOS [42–44], and these data strengthened the position for incorporating the ultrasound morphology of the ovaries as part of the definition for PCOS [3,4,45]. However, clinical caution should be applied when a random ultrasound reveals polycystic ovaries in the absence of symptoms.

3. EVALUATION FOR INSULIN RESISTANCE IN PCOS

The etiology of PCOS remains unknown and the source of much speculation and research. It is the holy grail of female reproductive endocrinology, and religious-like fervor frequently accompanies the favored theories of the experts. Time and technology have shifted the focus from the ovary as the prime suspect [2] to the hypothalamic–pituitary axis [46], and currently on some primary defect in insulin action, as the primary instigator of the syndrome [47] (Fig. 2). There is clearly a vicious feedback loop in which disordered steroid feedback from the ovary (primarily androgen and weak peripherally aromatized estrogens) can lead to disordered hypothalamic–pituitary function and gonadotropin secretion (i.e., abnormal pulsatility with excess LH compared to normal FSH levels). More recently, it has been suggested that this feedback loop might have developed secondary to a systemic abnormality, such as the decreased glucose uptake in response to a given level of insulin (i.e., insulin resistant)

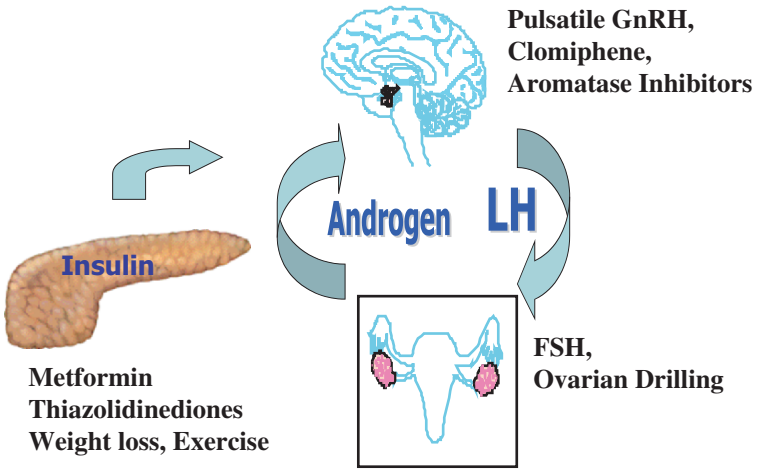

Fig. 2. Intervening in the vicious feedback cycle at many levels can restore ovulation in women with PCOS. Lowering androgens or gonadotropins, or insulin have all been reported as effective.

often observed in PCOS women [47]. Adding to the dilemma is that treatment of each one of these putative mechanisms can restore ovulation in many women with PCOS (Fig. 2).

This reconfiguration of PCOS as a metabolic syndrome with reproductive implications has led to extensive study of these women for signs and stigmata of insulin resistance. Women with PCOS appear to have a level of peripheral insulin resistance comparable to that of women with type 2 diabetes mellitus (DM) [48]. However, women with PCOS tend to demonstrate normal fasting glucose and normal glycohemoglobin levels, but tend to be glucose intolerant after glucose challenge. Consequently, about 40% of women with PCOS display impaired glucose tolerance or a 2-h glucose level ≥ 140 mg/dL after a standard 75 g oral glucose challenge after an overnight fast [49–51]. Women with PCOS often display both fasting and glucose challenged hyperinsulinemia, evidence for beta-cell compensation in response to the peripheral insulin resistance. However, the degree of compensation is inadequate for the degree of peripheral insulin resistance present, suggesting they are well on the road to developing type 2 DM [52,53].

Based on the prevalence of glucose intolerance in the larger population of US women ages 20–44 years (i.e., 7.8% impaired glucose tolerance and 1.0% undiagnosed diabetes) [54], the prevalence of glucose intolerance in PCOS (~40%) [49–51], and the prevalence of PCOS (~7%) [1], it can be extrapolatedthat PCOS contributes to approximately 30% of impaired glucose tolerance and 40–45% of type 2 DM among reproductive-aged women in the US. Risk factors for glucose intolerance in women with PCOS include a family history of diabetes, age, obesity, and especially, centripetal (android) body fat distribution [49–51].

In the US, obesity frequently accompanies PCOS. In the most comprehensive study of the prevalence of PCOS in an unselected population (i.e., women applying for work at a university hospital in Alabama), 24% were overweight (body mass index [BMI] 25.0–29.9 kg/M^2), and 42% were obese (BMI > 30 kg/M^2) [1]. Obesity further exacerbates metabolic and reproductive abnormalities in women with PCOS, worsening insulin resistance and the degree of hyperinsulinemia, and stimulates the expression of the PCOS phenotype in susceptible individuals as family studies suggest [55].

Overall, insulin resistance results in hyperinsulinemia, which, in turn, stimulates androgen secretion by theca cells [56,57] and suppresses the hepatic production of SHBG [58,59]. Thus, both obesity and insulin resistance lead to lower SHBG levels

Table 1. Suggested diagnostic evaluation for PCOS

Physical

(1) Blood pressure

(2) Body mass index (weight in kg divided by height in M^2) (BMI 25–30 = overweight, BMI > 30 = obese)

(3) Waist: measure to determine body fat distribution (value > 88 cm = abnormal)

(4) Presence of stigmata of hyperandrogenism/insulin resistance. Acne, hirsutism, androgenic alopecia, acanthosis nigricans

Laboratory

(1) Documentation of biochemical hyperandrogenemia. Total testosterone and/or bioavailable/free testosterone

(2) Exclusion of other causes of hyperandrogenism
- TSH (thyroid dysfunction)
- Prolactin (hyperprolactinemia)
- 17-Hydroxyprogesterone (nonclassical congential adrenal hyperplasia due to 21 hydroxylase deficiency), random normal: <3–4 ng/mL or fasting am <2 ng/mL
- Consider screening for Cushing's syndrome and other rare disorders such as acromegaly

(3) Evaluation for metabolic abnormalities
- 2-h OGTT (fasting glucose [<110 mg/dL = normal, 110–125 mg/dL = impaired fasting glucose, >126 mg/dL = type 2 diabetes) followed by 75 g oral glucose ingestion and then 2 h glucose level (<140 mg/dL = normal glucose tolerance, 140–199 mg/dL = impaired glucose tolerance, >200 mg/dL = type 2 diabetes)
- Fasting lipid and lipoprotein level (total cholesterol, HDL-C, triglycerides [LDL usually calculated by Friedewald equation])

(4) Optional tests to consider
- Ultrasound of ovaries for baseline evaluation/morphology prior to ovulation induction or in cases of virilization or rapid conversion to an androgen excess state
- Gonadotropin determinations to determine cause of amenorrhea
- Fasting insulin in younger women, those with severe stigmata of insulin resistance and hyperandrogenism, or those undergoing ovulation induction
- 24-Urine test for urinary free cortisol with late onset of PCOS symptoms or stigmata of Cushing's syndrome

OGTT is oral glucose tolerance test, 75 g.

and higher bioavailable levels of androgens [60]. In fact, SHBG may become a measure in the future that reflects both abnormalities in ovarian production and insulin resistance [61].

4. SUMMARY

PCOS is a disorder associated with hyperandrogenism, polycystic ovaries, and ovulatory dysfunction. The clinical evaluation discussed in this chapter has been summarized in Table 1. Because PCOS is a diagnosis of exclusion, other disorders should be excluded. Women with PCOS should be evaluated for both reproductive and metabolic abnormalities. Like the heterogeneity in the combination of reproductive signs and symptoms that characterize PCOS, metabolic risk factors are variably present in women with PCOS. Alternatively, there may be a publication bias toward linking PCOS with metabolic adversity. Further study of the long-term sequelae and the predictive role of surrogate markers will greatly aid the clinical evaluation of women with PCOS (see Chap. 8).

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Chapter 3

Genetics of PCOS

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1. THE INHERITED BASIS OF PCOS AND ITS COMPONENT PHENOTYPES

Polycystic ovary syndrome (PCOS) is considered a common, complex genetic disorder, as are conditions such as schizophrenia, asthma, and type 2 diabetes. Such common diseases, including PCOS, appear to have a complex, multifactorial etiology, wherein a variety of predisposing genes, not just one gene, interact with environmental and lifestyle factors to produce disease. Studies in families demonstrated the heritable nature of PCOS itself as well as the component phenotypes of PCOS. This has led to a large number of population studies attempting to discover genes that influence PCOS using the candidate gene approach.

1.1. Heritability of PCOS and Hyperandrogenemia

Family studies demonstrate that PCOS is significantly more prevalent among family members than in the general population. A recent large study of Dutch twins revealed a heritability for PCOS of 0.79 (1.0 indicating a trait completely determined by genetics), suggesting genetic factors contribute highly to development of the syndrome [1]. Among first-degree female relatives (on no hormonal therapy) of 93 patients with PCOS, 35% of premenopausal mothers and 40% of sisters were also affected with the disorder [2]. These affection rates are significantly higher than the 4–6% observed in the general population. In another study, 115 sisters of 80 women with PCOS were evaluated; of these, 22% met criteria for PCOS [3]. An additional 24% of these sisters had hyperandrogenemia with normal menses. Total and free testosterone levels

were similar between the sisters with hyperandrogenemia only and the sisters and probands with PCOS. A bimodal distribution of testosterone levels in the sisters of women with PCOS was observed, suggesting a major genetic component to hyperandrogenemia [3]. Brothers of women with PCOS also have elevated levels of androgens such as dehydroepiandrosterone sulfate [4].

1.2. Heritability of Insulin Sensitivity and Secretion in PCOS

Not only is PCOS itself a heritable condition, but within PCOS, insulin resistance and insulin secretion appear to be under significant genetic control. Among sisters of women with PCOS, those who had PCOS or hyperandrogenemia with regular menses had lower insulin sensitivity than unaffected sisters, assessed by fasting insulin and glucose measurements [5]. Likewise, in families of Australian patients with PCOS, hyperinsulinemia was found to occur in ~70% of all family members, suggesting that this trait was inherited [6]. In studies of families of women with PCOS, insulin secretion levels, quantified directly by the frequently sampled intravenous glucose tolerance test, displayed significant heritability, suggesting a genetic component to beta-cell dysfunction in PCOS [7].

2. THE CANDIDATE GENE APPROACH AS APPLIED TO PCOS GENETICS

2.1. Genetic Epidemiology and the Candidate Gene Approach

Given that investigators often start with no knowledge of which genetic variants may lead to disease, they must take advantage of chromosomal markers. Markers, such as microsatellites and single nucleotide polymorphisms (SNPs), are polymorphic variants interspersed throughout the genome. Microsatellites are tandem repeats of short nucleotide sequences, occurring with variable numbers of the repeated unit; SNPs are changes at a single genomic base pair, comprising two possible alleles. These markers are used as tags to track disease-causing variants or mutations. The underlying principle is that markers that are close to disease-causing variants tend to be inherited on the same chromosomes. Linkage refers to the situation wherein markers in a region of the genome are inherited in a non-random fashion in relation to a particular phenotype. Association refers to the situation wherein a particular allele of a marker is found with greater frequency in those with a particular phenotype. Candidate gene association studies have been the main method applied to PCOS genetics. In the candidate gene approach, common polymorphic genetic markers within a gene of interest, selected based on its hypothesized role in the disease, are evaluated to determine whether the polymorphisms are associated with the phenotype in populations or in families.

Inherent to the candidate gene approach are assumptions regarding the underlying pathophysiology of the disease under study. This is a particular problem in PCOS, as the underlying causes are still fundamentally unknown.

Candidate genes evaluated so far were selected from pathways affecting components of PCOS (see below). Genes coding for transcription factors or signaling pathway components that may globally affect the organs involved in PCOS (pituitary, ovaries, adrenals, pancreatic beta cells, insulin-responsive tissues) are unlikely to be selected as candidate genes by this approach.

2.2. Challenges in PCOS Genetics

To date, efforts to identify genes that influence PCOS susceptibility have largely utilized the candidate gene approach, resulting in over 155 publications over the past decade. Despite repeated attempts to identify the putative gene or genes responsible for this disorder, the PCOS gene(s) remain elusive. Despite many positive results, no gene or genes have clearly emerged as most important in PCOS, and many positive results were not confirmed in subsequent studies. Studies of the genetic etiology of PCOS have been hampered by various limitations, including: (a) only one or two variants genotyped in each gene; (b) incomplete or inaccurate characterization of the phenotype in cases or controls, (c) inability to assign a PCOS phenotype to prepubertal girls, postmenopausal women, and men; (d) possible inclusion of patients with nonclassic adrenal hyperplasia (NCAH); (e) lack of appropriate controls; (f) unclear ethnic/racial composition; (g) varying criteria used to diagnose PCOS in different studies (in part due to the lack of universally accepted diagnostic criteria); (h) small numbers of subjects in most studies.

(a) *Only one variant genotyped*: almost every candidate gene study in PCOS assessed the effect of one or two variants in each gene. This provides only partial information on whether a gene is associated with PCOS. There is increasing evidence that genetic variation is best described by groups of associated polymorphisms (inherited together on the same chromosome) referred to as haplotypes. Haplotypes reflect global gene structure, encompassing chromosomal blocks that have remained unbroken by recombination during the population history of the gene. Identification of a haplotype associated with increased or decreased disease risk should facilitate identification of the actual functional variant that affects disease risk, because this variant should lie on chromosomes identified by that haplotype. Haplotypes carry more information than the individual SNPs that comprise them. Haplotypes capture the majority of common variation in a gene; consequently, the use of haplotypes is more likely to identify gene variations than is the use of random SNPs.

The human genome is organized in haplotype blocks (most of which are longer than 10 kb), with three to five commonly occurring (> 5%) haplotypes per block [8]. Only six to eight variants are sufficient to define the most common haplotypes in each block. Thus, a manageable number of appropriately chosen SNPs (termed haplotype-tagging SNPs, or htSNPs) can be genotyped to identify the most common haplotypes in a population, providing critical tools for association studies. The goal of the International HapMap Project

is to delineate haplotype-tagging SNPs in all human genes [9], which will greatly facilitate future haplotype-based association studies.

As discussed above, prior candidate gene studies in PCOS genotyped only one or a few SNPs per gene, indicating only incomplete coverage of each candidate gene. This is particularly true for larger genes that may contain multiple haplotype blocks. As an example, consider the gene for PPAR gamma coactivator-1 (*PGC1*), a logical candidate gene for PCOS, given the role of the PPAR gamma system in insulin sensitivity and adipogenesis. This large gene (98 kb) contains several haplotype blocks, revealed by the International Hapmap Project, which genotyped 157 SNPs in the gene; the linkage disequilibrium structure in Caucasians is displayed in Fig. 1. Eight haplotype blocks are present in the gene. The one study that evaluated *PGC1* as a candidate gene for PCOS was a negative study that examined only one variant, Gly482Ser (NCBI dbSNP rs8192678) [10]. As indicated in Fig. 1, this variant is present in the first haplotype block; therefore, it gives no information on the remainder of the gene lying outside of this block. Thus, consideration of only one variant provided incomplete coverage of the gene, leaving open the possibility that a relevant

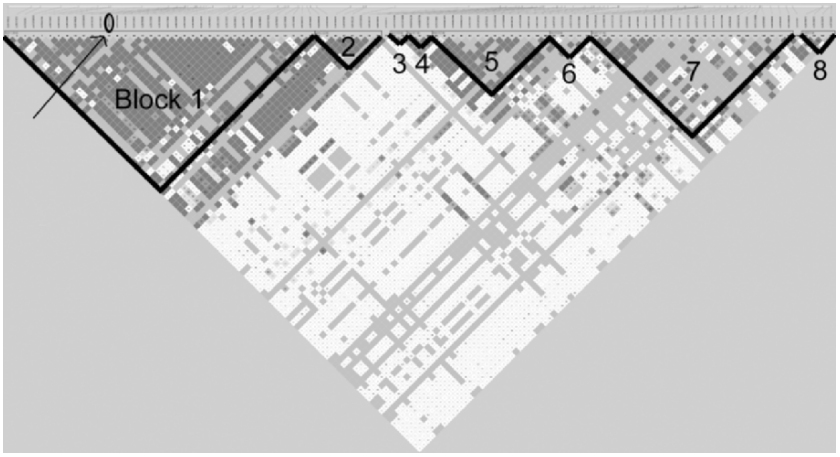

Fig. 1. Linkage disequilibrium plot for *PGC1*. Linkage disequilibrium (LD) among *PGC1* single nucleotide polymorphisms (SNPs) was determined by the International Hapmap study of the gene in Caucasians. SNPs in the gene are arranged at the top. The linkage disequilibrium plot displays D' values (%) for each pair of SNPs in the box at the intersection of the diagonals from each SNP. The *dark solid blocks* indicate $D' = 1$ (100%) for the corresponding pair of variants. The *lighter solid boxes* also indicate $D' = 1$, but with a low confidence score. Haplotype blocks are indicated by the *black triangles* and are numbered 1–8. Haplotypes were determined by the solid spine of LD algorithm of the program Haploview (http://www.broad.mit.edu/mpg/haploview/). The variant genotyped by Wang et al [10] is indicated by the *arrow* and *circle*. This variant is not informative for the portions of the gene outside of block 1.

functional variant was missed in the remainder of the gene. Unfortunately, negative published studies tend to discourage other investigators from replication attempts.

Application of the haplotype approach to PCOS genetics, particularly for genes wherein functional variants are unknown, should reduce the number of false negative studies and may allow more positive findings to be replicated. Studies of the calpain-10 gene in PCOS utilized haplotypes, based on the htSNPs from the original report associating calpain-10 with type 2 diabetes [11]. A few other PCOS studies reporting haplotypes constructed haplotypes from only two variants, unlikely to fully characterize haplotype blocks. Only a few other genes, including those for calpain-5 [12], aromatase [13], and the steroid 5-alpha reductase genes [14] have undergone detailed haplotype analysis in PCOS.

(b) *Difficulties in phenotype assignment*: PCOS presents unique challenges to the genetic epidemiologist. Foremost is the lack of consensus on diagnostic criteria. Thus, different genetic studies have classified individuals as having PCOS based on many different diagnostic schemas. Some definitions depend on ultrasound documentation of polycystic ovaries (alone or in combination with hormonal/ovulation criteria), others utilize the 1990 NIH consensus conference criteria, others the 2003 Rotterdam criteria, and others have required biochemical hyperandrogenemia be present. Additionally, phenotypic criteria even for component phenotypes of PCOS are not agreed upon. For example, criteria to classify a woman's ovaries as "polycystic" on ultrasound have changed over time. These issues surely have contributed to the conflicting results in the literature on PCOS genetics. Furthermore, some studies may not have completely evaluated controls, carefully ruling out any personal or family history of endocrinopathy. Other studies may have inappropriately included within PCOS cases women with other disorders that phenotypically resemble PCOS, such as adult-onset NCAH.

Thus, phenotypic heterogeneity leads to subtle differences in study populations between studies. Given that PCOS may represent the final phenotypic expression of more than one underlying pathology (and thus different underlying genes), it is immediately apparent how this is a major challenge in PCOS genetics.

(c) *Small numbers of subjects*: many of the studies in Tables 1–3 report results on less than 100 women with PCOS. Therefore, it is likely that many underpowered studies resulted in false negative reports and that several small studies produced false positive results. The power issue is particularly relevant to common disease genetics. Validated genetic determinants of type 2 diabetes, such as the Pro12Ala variant of the *PPARG* gene and the Glu23Lys variant of the Kir6.2 pancreatic potassium channel, only modestly alter risk for type 2 diabetes, on the order of 10–20%. If genes with similar magnitude of effect influence PCOS risk, then many of the studies to date were seriously underpowered and inadequate to detect genetic variants predisposing to PCOS.

Table 1. Candidate genes for which the balance of the evidence is against a role in PCOS

ACTR1	Activin receptor 1
ACTR2A	Activin receptor 2A
ACTR2B	Activin receptor 2B
ADRB3	Beta 3 adrenergic receptor
APOE	Apolipoprotein E
CYP11A	Cholesterol side-chain cleavage enzyme
CYP21	21-Hydroxylase
DAX1	Dosage sensitive sex reversal
DRD3	Dopamine D3 receptor
F2	Prothrombin
FOXC2	Forkhead box C2
FSHB	Follicle stimulating hormone beta subunit
FSHR	Follicle stimulating hormone receptor
FST	Follistatin
GCR	Glucocorticoid receptor
GDF9	Growth differentiation factor-9
GDF9B/BMP15	Bone morphogenetic protein 15
GNRHR	Gonadotropin releasing hormone receptor
GYS1	Glycogen synthase
H6PD	Hexose-6-phosphate dehydrogenase
HLA-C	Major histocompatibility complex, class I, C
HSD11B1	11-Beta hydroxysteroid dehydrogenase type 1
HSD17B1	17-Beta hydroxysteroid dehydrogenase, type I
HSD17B2	17-Beta hydroxysteroid dehydrogenase, type II
HSD17B3	17-Beta hydroxysteroid dehydrogenase, type III
HSD3B1	3-Beta hydroxysteroid dehydrogenase type I
HSD3B2	3-Beta hydroxysteroid dehydrogenase type II
IGF1	Insulin-like growth factor 1
IGF1R	Insulin-like growth factor 1 receptor
IGF2R	Insulin-like growth factor 2 receptor
IGFBP1	Insulin-like growth factor binding protein 1
IGFBP3	Insulin-like growth factor binding protein 3
IL1B	Interleukin 1 beta
IL1RA	Interleukin 1 receptor antagonist
INHA	Inhibin A
INHBA	Inhibin beta-A
INHBB	Inhibin beta-B
INHC	Inhibin C
INS	Insulin
INSL3	Leydig insulin-like protein 3
LHB	Luteinizing hormone beta subunit
LHR	Luteinizing hormone receptor
MTHFR	Methylenetetrahydrofolate reductase
OB	Leptin
OBR	Leptin receptor
PGC1	Peroxisome proliferator-activated receptor, gamma, coactivator-1, alpha
POMC	Pro-opiomelanocortin

Table 1. Candidate genes for which the balance of the evidence is against a role in PCOS—cont'd

PTP1B	Protein tyrosine phosphatase, non-receptor type 1
SF1	Nuclear receptor subfamily 5, group A, member 1
SORBS1	Sorbin and SH3 domain containing 1
STAR	Steroidogenic acute regulator
TNFA	Tumor necrosis factor alpha
UCP2	Uncoupling protein 2
UCP3	Uncoupling protein 3
UGT2B15	UDP glucuronosyltransferase 2 family, polypeptide B15

Table 2. Candidate genes with conflicting evidence for a role in PCOS

ADIPOQ	Adiponectin
AR	Androgen receptor
CAPN10	Calpain-10
CYP17	17α-hydroxylase/17,20-lyase
F5	Coagulation factor V
INSR	Insulin receptor
IRS2	Insulin receptor substrate 2
MC4R	Melanocortin 4 receptor
PPARG	Peroxisome proliferator-activated receptor gamma
RETN	Resistin
SERPINE1	Plasminogen activator inhibitor-1

To illustrate the pitfalls of small studies, consider the gene for hexose-6-phosphate dehydrogenase (*H6PD*). The Arg453Gln variant (NCBI dbSNP rs6688832) in *H6PD* has been implicated in cortisone reductase deficiency, a hyperandrogenic disorder resembling PCOS; thus, *H6PD* was considered a candidate gene for PCOS. An initial study of 116 PCOS cases and 76 controls found differences in Arg453Gln allele frequency between Spanish PCOS and controls [15]. Subsequently, a United Kingdom study composed of 256 nuclear PCOS families, 213 unrelated PCOS cases, and 549 controls found no association of this variant with PCOS [16]. A subset of the Dallas Heart Study evaluated for PCOS (85 cases, 597 controls) also found no association of the Arg453Gln variant with PCOS [17]. The following are a few of the possible explanations for the conflicting results: (a) *H6PD* variation influences PCOS risk in Spanish individuals but not United Kingdom or American individuals; (b) the Spanish study result was a false positive related to small sample size; (c) differences in how PCOS was diagnosed explains the different results. This kind of uncertainly plagues the field of PCOS genetics.

(d) *Lack of replication of positive results*: when an initial report describes an association of a genetic variant with a disease, often subsequent reports

Table 3. Candidate genes for which current evidence suggests a role in PCOS[a]

ADRB2[b]	Beta 2 adrenergic receptor
AGT[b]	Angiotensinogen
CAPN5[b]	Calpain 5
CYP11B2[b]	Aldosterone synthase
CYP19	Aromatase
CYP1A1[b]	Cytochrome P450, family 1, subfamily A, polypeptide 1
D19S884	Chromosome 19 microsatellite (in gene for fibrillin-3)
EPHX[b]	Microsomal epoxide hydrolase
FEM1A[b]	Fem-1 homolog a
GSTM1[b]	Glutathione S-transferase M1
GSTT1[b]	Glutathione S-transferase theta 1
HLA-A[b]	Major histocompatibility complex, class I, A
HLA-B[b]	Major histocompatibility complex, class I, B
HLA-DRB1[b]	Major histocompatibility complex, class II, DR beta 1
HSD17B5[b]	17-Beta hydroxysteroid dehydrogenase, type V
IGF2[b]	Insulin-like growth factor 2
IL1A[b]	Interleukin 1 alpha
IL6	Interleukin 6
IL6R[b]	Interleukin 6 receptor
IL6ST[b]	Interleukin 6 signal transducer
IRS1	Insulin receptor substrate 1
MMP1[b]	Matrix metalloproteinase 1
PC1[b]	Plasma cell membrane glycoprotein 1
PON1[b]	Paraoxonase
PPP1R3A[b]	Protein phosphatase 1, regulatory (inhibitor) subunit 3A
SHBG	Sex hormone binding globulin
SRD5A1[b]	Steroid 5-alpha reductase type 1
SRD5A2[b]	Steroid 5-alpha reductase type 2
TNFR2[b]	Tumor necrosis factor receptor 2

[a]All studies require confirmation.
[b]Single study or studies from one institution only.

attempt to replicate the initial result. Such replication is necessary to establish whether a gene truly plays a role in disease.

PCOS genetics is often criticized because positive reports of association were usually not subsequently confirmed by others. Besides false positive and false negative studies, such lack of replication may be due to the study of different ethnic groups: a certain genetic variant may interact with other variants and local environmental influences such that it alters phenotype only in a particular group. Thus, ideally replication studies should first be carried out in the same ethnic group, with the goal of validating the initial result. Subsequent replication attempts in other populations would serve to determine whether the particular genetic variant universally affects disease susceptibility. However, even when ostensibly the same ethnic group is studied, subtle differences in the history of the population may lead to ethnic differences such that two nominally

similar cohorts are sufficiently different in genetic background as to limit replication. Unfortunately, given the international interest in PCOS genetics, replication efforts have usually occurred in different ethnic groups.

2.3. Current State of Investigation on the Genetics of PCOS

Candidate gene studies in PCOS have generally targeted genes regulating several areas: (a) steroid biosynthesis and action; (b) gonadotropic action; (c) weight and energy regulation, and (d) insulin action and production (Tables 1–3). Most recently, candidate genes that may affect cardiovascular disease via inflammation, hypercoagulability, or blood pressure have also been examined in PCOS. Several provocative genetic associations with PCOS have been reported that are slowly starting to illuminate the underlying causes of PCOS.

Table 1 lists candidate genes for which the balance of the evidence is against a role in the development or phenotype of PCOS. Several of these genes (e.g. *CYP21*, *HSD3B2*) have been the subject of multiple studies, most of which have been negative. A very few of them (e.g., *CYP11A1*, *INS*) have been convincingly ruled out as candidate genes for PCOS by analyses of a large number of subjects. On the other hand, several other genes (e.g. *HSD17B3*, *UGT2B15*, *FOXC2*) were examined in only one study. As noted above, the issue of underpowered studies of small sample size raises the possibility that a true genetic association was missed in many instances. It is possible that a gene that exerts a modest effect on PCOS risk may unfortunately be listed in Table 1.

Table 2 lists candidate genes whose role in PCOS is still controversial. For most of these genes, there have been positive association studies as well as negative association studies. Given the issues outlined above, it is difficult to determine which studies are valid and which are not. It is also possible that a particular gene does influence PCOS in one ethnic group but not in another, such that an association study in the former but not the latter is positive. Given that multiple groups around the world have conducted PCOS genetic studies, this is a likely explanation, in part, for the nonreplication of many results. However, the small sample size issue is likely to be a more important contributor to the confusion in the literature. For the genes in Table 2, studies with very large sample sizes will be needed to provide convincing evidence for or against these genes as etiologic in PCOS.

A review of the publications concerning the androgen receptor gene (*AR*) in PCOS will illustrate the typical situation wherein the evidence is conflicting regarding a gene's role in PCOS. Regarding the androgen receptor, the literature has focused on a polymorphic CAG repeat in exon 1, which codes for a polyglutamine tract in the N terminus of the androgen receptor protein (number of repeats normally ranging from 11 to 31, usually around 20, with stable inheritance). In vitro, increasing repeat length appears to modestly impair androgen receptor transactivation function. Given that PCOS is a hyperandrogenic disorder, the possibility that this variant could modulate risk of PCOS or the

severity of hyperandrogenism in affected individuals led to its study as a candidate gene variant. The first published study included only 34 women with hyperandrogenic hirsutism (presumably PCOS) and 15 healthy controls (all Caucasians from Spain) and found no differences in the number of CAG repeats between cases and controls [18]. Later in the same year, another group found no difference in CAG repeat length between 91 PCOS cases and 112 controls (from Singapore), but that cases with lower testosterone levels tended to have shorter repeats, suggesting that androgen receptors with higher functionality allow women with lower testosterone levels to manifest PCOS [19].

Subsequently, an Australian study of 205 PCOS cases and 831 controls (the latter originally recruited for a separate study) found that the cases had longer CAG repeat lengths [20]. A Finnish study of 106 cases and 112 controls showed no association of the CAG repeat with PCOS [21]. Recently, a German study of 63 PCOS women found that the CAG repeat length modified the relationship between free testosterone levels and insulin resistance [22]. The largest study (in terms of cases), consisting of 313 PCOS cases and 277 controls from the Southeastern United States (74% White, 21% Black), found that shorter CAG length was associated with PCOS [23]. Immediately apparent is the fact that each study was conducted in a different population. Also, different diagnostic criteria for PCOS were used, some studies requiring ultrasound appearance of polycystic ovaries [19], while others did not [21,23].

Table 3 lists candidate genes that may play a role in PCOS but require confirmation. Multiple studies on four of these genes (*CYP19*, *IRS1*, *IL6*, *SHBG*) and one microsatellite (D19S884) have been published, with most but not all supporting a role in PCOS. The literature on D19S884 will illustrate one of the more promising results in PCOS genetics. Suggestive association of this marker with PCOS was first identified in a cohort of 150 PCOS families [24]; this marker was examined because it is on chromosome 19, ~1 Mb from the insulin receptor. Subsequently, association of D19S884 with PCOS was found in a group of 85 Caucasian PCOS patients and 87 matched controls [25]. A study in 108 Caucasian women with PCOS and 66 controls (roughly half from Spain, half from Italy) found no association of D19S884 with PCOS in either group separately or both groups combined [26]. The investigators that originally described the D19S884 association have subsequently replicated association of D19S884 with PCOS first in an additional 217 families [27], then in another 98 independent families [28], using robust family based testing of linkage and association. They have also genotyped numerous additional markers in the region and still find strongest evidence with D19S884, particularly the allele comprising 17 CA repeats (termed allele 8). These studies provide strong evidence for a PCOS susceptibility locus mapping to chromosome 19p13.2, at or near the dinucleotide repeat marker D19S884. This marker lies in an intron of the fibrillin-3 gene, which must now be considered a candidate for PCOS. It is also possible that the region of this marker contains a regulatory element

that controls expression of gene(s) elsewhere on chromosome 19. Of note, chromosome 19 contains a number of potential PCOS candidate genes.

The majority of the genes listed in Table 3 have been reported in only one publication or by only one group of investigators. Therefore, while promising, these genetic associations must be considered tentative until replicated by other groups. Of note, many of these candidate genes are related to inflammation or cardiovascular risk, reflecting a recent focus on these processes in PCOS.

3. EPIGENETICS: A NEW AVENUE IN PCOS GENETICS

Epigenetics refers to a set of reversible heritable changes in gene expression or function that occur without a change in DNA sequence (genotype). Epigenetic processes include, among others, imprinting, gene silencing, X chromosome inactivation, and gene regulation by histone modification. Epigenetics has been scarcely considered in the genetics of common, complex diseases; however, it has recently been shown to be a possible factor in PCOS genetics.

Investigation of the CAG repeat in the androgen receptor on the X chromosome has also led to analyses of X chromosome inactivation. Fortuitously, the most common method of analyzing X chromosome inactivation involves analysis of the methylation state of a restriction fragment length polymorphism in the androgen receptor gene itself, combined with assessment of CAG repeat length. When this *Hpa*II site is methylated, the restriction enzyme does not cut; when it is not methylated, the restriction enzyme can cut. Methylation occurs on the inactivated X chromosome; in individuals heterozygous at the CAG repeat, it can be determined which allele is inactive. Theoretically, X chromosome inactivation should be random, with 50% of paternal alleles inactivated and 50% of maternal alleles inactivated within each subject. Skewed inactivation in cases but not controls, wherein one allele is preferentially methylated, would indicate a possible role for this epigenetic phenomenon in pathogenesis of the disease. One small study detected skewed X chromosome inactivation in 5 of 34 women with hyperandrogenic hirsutism, wherein the shorter allele was often preferentially inactivated [18]. Similarly, a larger study documented preferential expression of longer CAG repeat alleles in PCOS; nonrandom X inactivation occurred more frequently in PCOS than in control women [20]. In another study by the same investigators, 40 women with PCOS and their sisters were evaluated. Discordance between the sister pairs in terms of diagnosis (one with PCOS and one without) was correlated with different X inactivation pattern between the sisters [29]. These few studies suggest epigenetics may play a role in modulating the effect of the androgen receptor gene (and other X chromosome genes) in PCOS pathogenesis. Whether skewed inactivation of other chromosomes plays a role in PCOS is currently unknown.

The results of a study examining genomic instability in PCOS hint at more global epigenetic phenomena in PCOS [30]. In this study of 19 women with PCOS and 19 well-matched controls, the frequency of micronuclei in cultured peripheral lymphocytes was used as a marker of genomic instability. Micronuclei were three times more common in PCOS than controls, suggesting higher genomic instability in PCOS. It is possible that this instability reflects differences in epigenetic changes in PCOS.

It is anticipated that future genetic studies of PCOS will also take into account epigenetic phenomena. Perhaps the lack of consideration of epigenetics has contributed to the lack of a breakthrough discovery in PCOS genetics to date.

4. CONCLUSIONS

The inherited nature of PCOS has been firmly established. Unfortunately, most candidate gene studies have been in small cohorts. Additional issues such as only one or two variants genotyped per gene have confounded PCOS genetics. As a result, despite a large number of positive reports, no particular gene is universally recognized as importantly contributing to PCOS risk. However, significant progress has been made and a number of potential candidate genes have been identified. Future efforts should focus on confirming these genes as well as considering epigenetics in the pathogenesis of PCOS.

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Chapter 4

Insulin Action in Polycystic Ovary Syndrome: In Vivo and In Vitro

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1. INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common disorder affecting 6–10% of women of childbearing age [1–3]. It is the most frequent endocrine disorder among young women and the principal medical cause of female infertility in North America. It is defined by the presence of hyperandrogenism, chronic anovulation, and/or polycystic ovaries (see Chap. 1 for a fuller discussion of the definition of PCOS), after exclusion of all secondary causes. However, most experts in the field agree that hyperandrogenemia is the central feature of PCOS and probably results from the same ovarian dysfunction that causes oligoanovulation and infertility. Therefore, in this chapter, we will focus on the mechanisms of hyperandrogenemia in PCOS, as this was the most studied characteristic of PCOS in the literature, particularly with respect to insulin actions.

PCOS is also a major health issue for young women [1]. Indeed, the prevalence of metabolic syndrome (46%), impaired glucose tolerance (16–35%), and type 2 diabetes mellitus (DM) (2.5–17.7%) are much more frequent in PCOS women as compared to normal women of similar age (23, 7.8, and 1, respectively). Women with PCOS are also characterized by hypertension, dyslipidemia, procoagulant state, proinflammatory state, and endothelial dysfunction. Therefore, women with PCOS have a higher prevalence of risk factors for cardiovascular diseases, disorders that have been demonstrated in cohort studies to be more frequent in PCOS women [1].

PCOS is also a common and well-defined clinical model of insulin resistance and prediabetic state. Indeed, women with PCOS demonstrate insulin

resistance and a compensatory hyperinsulinemia [1], which appears to play a critical role in the syndrome's pathogenesis [1–3]. However, not every woman with insulin resistance and hyperinsulinemia develops PCOS, and there is evidence that a subgroup of women with typical PCOS is neither insulin resistant nor hyperinsulinemic [4,5]. Many questions remain unanswered regarding both the nature of insulin resistance in PCOS and the mechanisms by which insulin resistance or insulin produces hyperandrogenemia.

2. INSULIN ACTION IN PCOS

2.1. In Vivo Characteristics

Dunaif et al. [6] demonstrated that obese PCOS women were more insulin resistant than their lean counterparts and that obese and lean women with PCOS were both more insulin resistant than BMI-matched normal controls. These findings suggested that, in PCOS, insulin resistance results from an intrinsic form of resistance to insulin in addition to insulin resistance due to obesity. Morin-Papunen et al. [7] confirmed these findings in obese women, but were unable to demonstrate reduced glucose insulin sensitivity in lean PCOS women. Furthermore, Vrbikova et al. did not find any difference in glucose sensitivity to insulin between lean PCOS women and matched controls [8]. This apparent contradiction might be explained by the finding that lean PCOS women with normal insulin levels have normal glucose insulin sensitivity compared to controls, in contrast to lean PCOS women with hyperinsulinemia [9]. Therefore, the proportion of normo-versus hyperinsulinemic lean women in the studied populations might explain these discrepancies.

Dunaif et al. also demonstrated that resistance to insulin is not due to hyperandrogenemia in PCOS. Indeed, normalization of testosterone levels in these women after treatment with a long-acting gonadotropin-releasing hormone (GnRH) agonist for 12 weeks did not change insulin sensitivity or hepatic glucose production in women with PCOS [10]. These findings were confirmed in more recent studies by other groups.

Taken together, these results demonstrate that some women with PCOS are neither insulin resistant nor hyperinsulinemic. Thus, insulin resistance and compensatory hyperinsulinemia are not necessary to develop PCOS. Insulin resistance to glucose metabolism might just favor the clinical expression of PCOS and result from the same genetic, metabolic, and/or environmental factors as in the general population. Most women with PCOS might just be more likely to be insulin resistant because this latter abnormality favors the development of hyperandrogenemia. This would explain why PCOS women are more insulin resistant on average in most studies and why many of them are obese, since obesity almost always causes some degree of insulin resistance.

It must be clarified that in most scientific articles insulin sensitivity is defined as the capacity of insulin to stimulate glucose metabolism. Indeed, the gold standard technique to assess insulin sensitivity is the insulin–glucose clamp, which directly measures insulin-stimulated glucose disappearance. This measure reflects mainly glucose transport in peripheral insulin-sensitive tissue, i.e., in muscles to a large degree. However, insulin has many other actions beside glucose metabolism, such as regulating lipid and amino acids metabolism, protein synthesis, and cellular growth and differentiation [11]. As discussed below, it is important to keep in mind that sensitivity to insulin actions on glucose metabolism might differ from other insulin actions.

2.2. In Vitro Molecular Defects

The mechanisms of metabolic insulin resistance in women with PCOS remain largely unknown. There is not enough evidence to actually discern whether PCOS is a consequence of a specific defect in insulin action or results from adaptive hyperinsulinemia associated with any condition of insulin resistance. Overall, there are few studies specifically assessing the metabolic actions of insulin in PCOS.

Studies in adipocytes from women with PCOS have shown that the number and affinity of insulin receptors are not obviously decreased [12]. However, it was demonstrated that the maximal rate of glucose uptake, the abundance of GLUT4 glucose transporters, and the inhibition of lipolysis stimulated by insulin were all decreased in adipocytes from women with PCOS. This adipocytes insulin resistance was also shown to occur at an early step in insulin signaling [12]. These findings were also described in PCOS women without obesity, glucose intolerance, or increased waist-to-hip ratio, suggesting that they might be intrinsic to the syndrome [13].

Dunaif and colleagues also did not find that the number or affinity of receptors of insulin were affected in perpetuated cultured skin fibroblasts [14], but observed that autophosphorylation of these receptors after binding with insulin was decreased in approximately 50% of PCOS subjects. These receptors were characterized by a constitutive increase in the phosphorylation of their serine residues and a decrease in the insulin-stimulated phosphorylation of their tyrosine residues. They were also less capable of phosphorylating insulin receptors substrates (IRSs), suggesting that exaggerated serine phosphorylation of insulin receptors impairs their activity [14]. The high level of constitutive serine phosphorylation of insulin receptors was observed to be independent of the presence of obesity and type 2 DM, suggesting that this defect affects an early step in insulin signaling which might be unique to PCOS. Moreover, purification studies and reversal by inhibitors of serine kinase activity suggested that this defect was due to a putative serine phosphorylation factor [15].

Finally, Dunaif et al. [16] also found that muscle biopsies obtained during insulin–glucose clamp protocols from women with PCOS were characterized

by impaired insulin-stimulated association of insulin receptor substrate-1 (IRS-1) with phosphatidylinositol 3-kinase (PI-3K), along with in vivo decrease in glucose transport. Again, this defect appeared to affect an early step in insulin signaling and was independent of obesity and type 2 DM, suggesting a unique defect. However, most of the defects found in other common insulin resistant conditions (e.g., type 2 DM, metabolic syndrome, obesity, etc.) have also been described in PCOS. Thus, there is no clear evidence to date that the insulin resistance found in PCOS is specific to this condition.

3. ANDROGENIC ACTIONS OF INSULIN IN PCOS

3.1. In Vivo Insulin Actions

3.1.1. PCOS and Hyperandrogenemia. Increased ovarian androgen responsiveness was demonstrated in vivo in women with PCOS using GnRH agonists or human chorionic gonadotropin (hCG) stimulation tests [17]. Chronic stimulation by luteinizing hormone (LH) or insulin has been suggested as the cause of this ovarian androgen hyperresponsiveness. In order to determine the role of chronic stimulation by LH, normal and obese PCOS women were challenged with hCG before and 4 weeks after LH suppression with a long-acting analogue of GnRH [17]. This study demonstrated that suppression of LH for 4 weeks did not alter the exaggerated 17-hydroxyprogesterone response to hCG, suggesting that LH was not implicated in the androgenic hyperresponsiveness of PCOS. Conversely, numerous studies have demonstrated that any treatment aimed at improving insulin resistance in lean and obese women with PCOS (e.g., weight loss, metformin, D-*chiro*-inositol, and peroxisome proliferator-activated receptor gamma [PPARγ] agonists) results in lower androgen levels [1]. Importantly, the exaggerated steroidogenic response to LH stimulation tests also improved [18,19], suggesting a normalization of androgen hyperresponsiveness due to correction of chronic hyperinsulinemia or to direct effects on the androgenic pathway(s) of insulin signaling, or both.

Hyperandrogenemia is also improved in chronically hyperinsulinemic PCOS women after interventions that only decrease their insulin levels. Testosterone and nonsex hormone-binding globulin (SHBG)-bound testosterone levels decreased significantly in obese PCOS women after 10 days of treatment with diazoxide, which decreases insulin levels by directly suppressing insulin secretion from β-cells [20]. Moreover, a recent randomized-controlled trial demonstrated that reduction of insulinemia for 6 months with acarbose also reduced serum testosterone levels in obese PCOS women [21], and results were confirmed in a 3-month prospective study in hyperinsulinemic PCOS women [22]. Acarbose directly inhibits small intestine α-glucosidase enzymes, which slows down glucose absorption and decreases glucose-stimulated

insulin secretion. Thus, these studies underscore the importance of insulin in the pathogenesis of PCOS hyperandrogenemia.

Low levels of SHBG contribute significantly to PCOS hyperandrogenemia because it reduces testosterone binding, resulting in an increase in free testosterone levels. Since high free testosterone levels have little negative feedback on ovarian or adrenal androgen production in women, testosterone binding is an important contributor to androgenemia. Many studies have shown that SHBG levels are inversely correlated with the circulating levels of insulin or with the degree of insulin resistance in women with or without PCOS. In type 1 diabetic patients, portal insulin concentrations, rather than insulin sensitivity, were found to be related to SHBG levels [23]. Furthermore, diazoxide-induced insulin reduction increased SHBG levels in obese women with PCOS [24]. This finding was also confirmed after lowering insulin concentrations for 3 or 6 months with acarbose [21,22]. Thus, insulin stimulation rather than insulin resistance is the cause of low SHBG levels in obese PCOS women and contributes to PCOS hyperandrogenemia also by this mechanism.

We conducted a randomized-controlled trial using two insulin-sensitizing drugs (metformin and rosiglitazone, a PPARγ agonist) in 100 nonobese women with PCOS and normal insulin levels, both during fasting and an oral glucose tolerance test (OGTT) [4]. Our results demonstrate normalization of testosterone levels (Fig. 1a) in actively treated groups comparatively to placebo. Despite normo-insulinemia at baseline, metformin significantly reduced insulin levels, but not rosiglitazone (Fig. 1b). Therefore, metformin might improve hyperandrogenemia in these women in part by decreasing insulin levels, which suggests that their hyperandrogenemia is indeed related to insulin action and might result from *increased* androgenic sensitivity to insulin. However, rosiglitazone did not improve hyperandrogenemia by decreasing insulin levels, suggesting that PPARγ agonists might directly improve this androgenic hyperresponsiveness to insulin, restoring the normal relationship between insulin levels and androgen production. PPARγ agonists enhance insulin-stimulated glucose metabolism in adipose, muscle, and hepatic tissues, and improve compensatory hyperinsulinemia. But insulin levels remain unchanged in subjects with normal glucose insulin sensitivity treated with PPARγ agonists [25], as in our trial.

Finally, it is possible to increase insulin levels while maintaining normal glucose concentrations using the euglycemic-hyperinsulinemic clamp. Experimental hyperinsulinemia for 22 hours in normal female rats did not increase testosterone or androstenedione levels as compared to control rats infused with an identical volume of vehicle [26]. Similarly, nonobese normal women did not increase their androgen levels during a 2-hour euglycemic-hyperinsulinemic clamp [27]. However, similar elevation of exogenous insulin levels for 2 hours significantly increased testosterone and androstenedione levels in obese and nonobese women with PCOS as compared to weight-matched ovulating normal women [28]. Testosterone levels were also increased at the end of a 270-min insulin infusion,

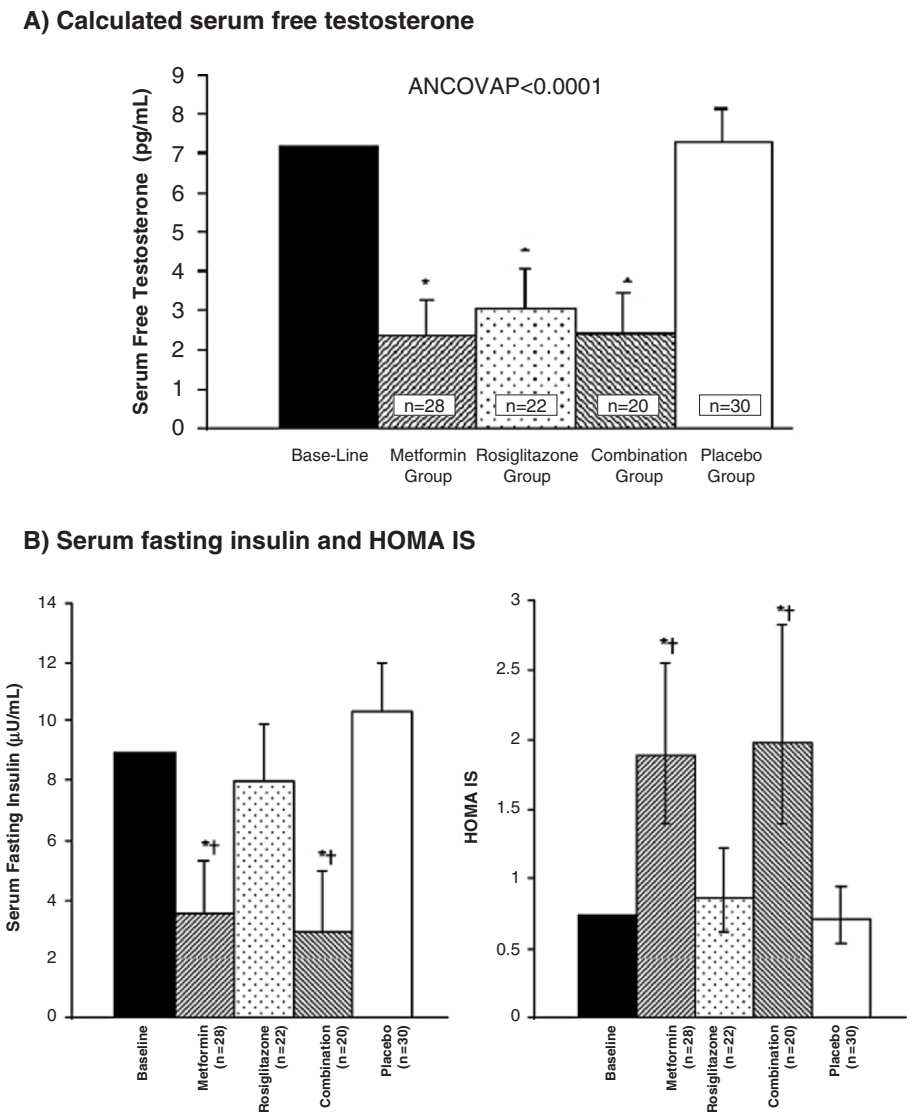

Fig. 1. Serum free testosterone concentrations (**a**), and fasting insulin levels and insulin sensitivity estimated by the homeostasis model assessment (HOMA IS) calculation (**b**), in women with the PCOS and normal insulin levels, before and after the administration of insulin-sensitizing drugs or placebo for 6 months. Values are the mean and 95% confidence interval.* indicates $P < 0.05$ for comparison with the group given placebo and $^{\dagger}P < 0.05$ for comparison with the group given rosiglitazone, using Tukey HSD tests after ANCOVA analysis (adapted from Baillargeon et al. [4], with permission).

with clamped glucose levels, in six women with PCOS [29]. Therefore, in vivo stimulation with exogenous insulin stimulates androgen production only in women with PCOS, supporting again the hypothesis that PCOS is characterized by increased androgenic sensitivity to insulin.

The cause of hyperandrogenemia in women with PCOS might also arise from dysfunction of the hypothalamic-pituitary axis. In vivo insulin actions on this axis will be discussed later in the section reviewing insulin action in PCOS ovulatory dysfunction.

3.1.2. In Vivo Androgenic Hypersensitivity to Insulin. So far, we have presented many evidences suggesting that insulin action is pivotal to the pathophysiology of PCOS, both in hyperinsulinemic and normo-insulinemic women. We cannot exclude the possibility that metabolic insulin resistance with compensatory hyperinsulinemia chronically increases the androgenic response to insulin by, for example, up-regulating ovarian steroidogenic enzyme activities, effectors of the LH signaling pathway, or type 1 insulin-like growth factor (IGF) receptors, or by decreasing IGF binding globulin-1 (IGFBP-1) in the ovaries, without involving specifically the androgenic insulin signaling pathways. However, only a minority of woman with insulin resistance and hyperinsulinemia develop PCOS, and there is evidence that a subgroup of women with typical PCOS is neither insulin resistant nor hyperinsulinemic [4,7–9]. Therefore, all these observations suggest that the pathogenesis of PCOS involves a defect that predisposes women to insulin-induced hyperandrogenemia.

We recently assessed the effect of insulin on androgen levels in lean PCOS women with normal insulin levels and metabolic insulin sensitivity (measured by the insulin–glucose clamp technique) [5]. Reduction of insulin secretion with diazoxide in these women significantly decreases levels of free testosterone and androstenedione, and increases SHBG levels (Fig. 2). Since androstenedione is not bound to SHBG, its significant decrease confirms that androgen biosynthesis was improved. Importantly, suppression of insulin secretion with diazoxide did not alter testosterone or SHBG levels in healthy, nonobese women.

The significant improvement of free testosterone and SHBG levels observed after treatment with diazoxide were in contrast to the absence of change observed after treatment with the long-acting GnRH agonist leuprolide acetate (Fig. 2), despite near total suppression of LH levels. Therefore, in lean normo-insulinemic PCOS women, it appears that LH suppression is more effective than insulin reduction to decrease androgen biosynthesis, as assessed by androstenedione levels; but insulin lowering is more effective to reduce hyperandrogenemia, as assessed by free testosterone levels, because it improves both androgenesis and SHBG levels. Therefore, our novel findings further support that insulin contributes to hyperandrogenemia even in PCOS women with normal metabolic insulin sensitivity and insulin levels, possibly due to increased insulin sensitivity of their androgenic insulin pathway(s).

3.2. In Vitro Molecular Mechanisms

Since hyperandrogenemia in women with PCOS might arise from either ovarian theca cells, liver production of SHBG, or hypothalamic-pituitary dysfunction, we will now review in vitro molecular mechanisms of insulin actions on

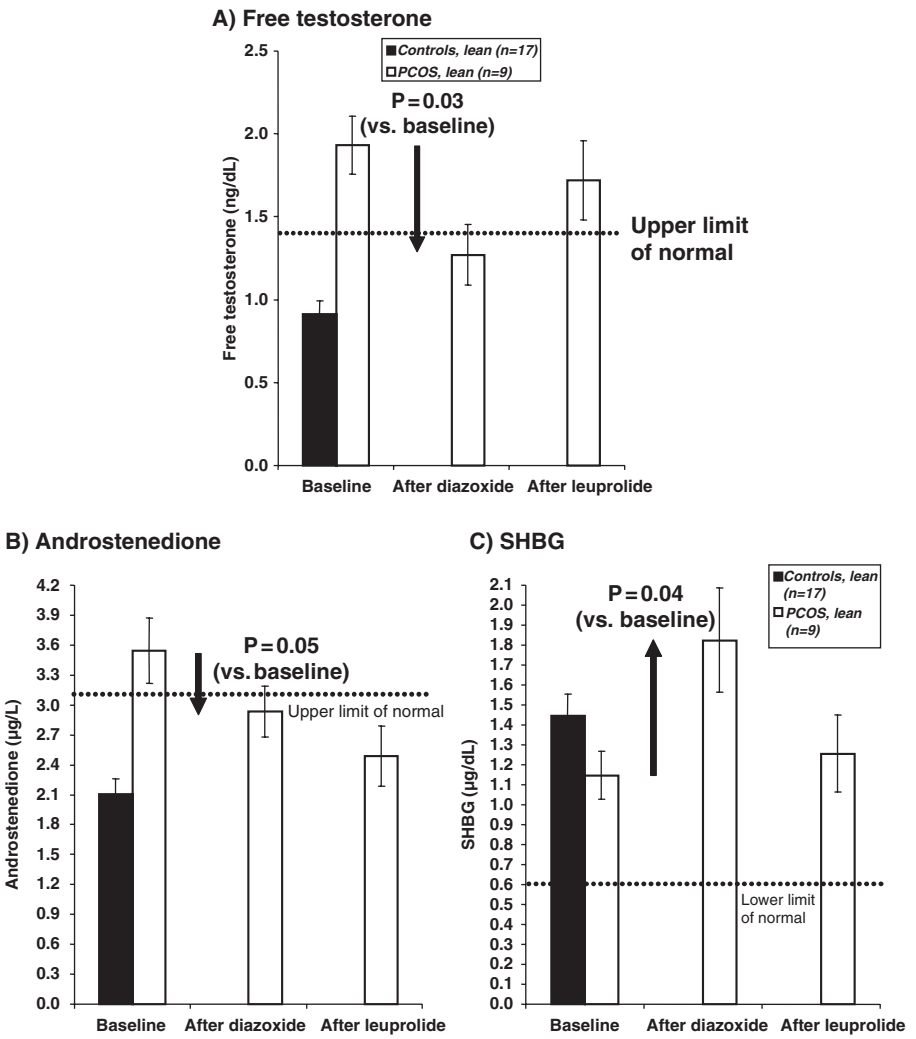

Fig. 2. Selected laboratory results at baseline, after diazoxide, and after leuprolide acetate in lean PCOS and lean normal women. Free testosterone was calculated by the method of Sodergard et al. (adapted from Baillargeon et al. [5], with permission).

theca cells and hepatocytes. Molecular mechanisms of insulin action on hypothalamic and pituitary cells will be discussed in the section reviewing insulin actions and PCOS ovulatory dysfunction.

3.2.1. Ovarian Theca Cells. Multiple studies demonstrate that insulin stimulates ovarian steroidogenesis in vitro (Table 1). The ovarian androgen response to insulin is markedly increased in cultured ovarian cells from PCOS

women [30–32] as compared to normal ovaries, suggesting that PCOS theca cells are also characterized by androgenic hyperresponsiveness to insulin. Indeed, physiologic concentrations of insulin are enough to stimulate androgen biosynthesis in PCOS theca cells, but supra-physiologic levels are usually needed in normal models. Furthermore, combined stimulation with LH and insulin at physiological concentrations synergistically increases androgen biosynthesis in normal (Table 1) and PCOS ovarian tissues [31,32], suggesting important cross talks between both signaling pathways.

Studies in human or mammalian normal theca cells have also shown that insulin stimulates basal and LH-induced P450c17 activity in vitro (Table 1), which is the required enzyme for trafficking steroidogenesis toward androgen biosynthesis. Basal and LH-induced progesterone production was stimulated by insulin in many studies (Table 1), but not all. This effect is probably observed mainly in thecal cells that have undergone a higher level of luteinization, due to culture techniques or sources of the cells (e.g., in vitro fertilization protocols involving high doses of gonadotropins). Likewise, insulin alone did not stimulate estradiol or progesterone production in theca cells from a woman with hyperandrogenism and insulin resistance [33]. There is also important evidence that insulin stimulates the proliferation of theca cells and decreases their apoptosis in vitro (Table 1). This effect of insulin probably explains the observation of increased ovarian stoma volume in women with PCOS. Finally, insulin has been shown to increase the expression of high density lipoprotein (HDL) receptors in theca cells, which favors cholesterol uptake and increases intracellular levels of the cholesterol needed for androgen biosynthesis.

LH enhances theca-cell steroidogenesis principally through the cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA) pathway. The molecular mechanisms by which insulin regulates steroidogenesis are not well understood. In classical insulin-responsive tissues, insulin actions are mediated via two major pathways involving the phosphorylation of IRSs: the PI-3K

Table 1. In vitro insulin actions in ovarian *theca cells*, based on studies in normal mammalian ovarian cells, published and indexed in medline

Effects	Change	References
Basal androgen production	↑	[30, 34, 35, 73–80]
LH-induced androgen production	↑	[33–35, 39, 74, 77–87]
Basal P450c17 activation	↑	[88, 89]
LH-induced P450c17 activation	↑	[38, 39, 82, 89]
Basal progesterone production	↑	[73–75, 80, 86]
LH-induced progesterone production	↑	[80, 83, 86, 90, 91]
Cellular proliferation	↑	[75, 76, 83, 92–94]
Apoptosis	↓	[95]
HDL receptor expression and intracellular cholesterol	↑	[96]

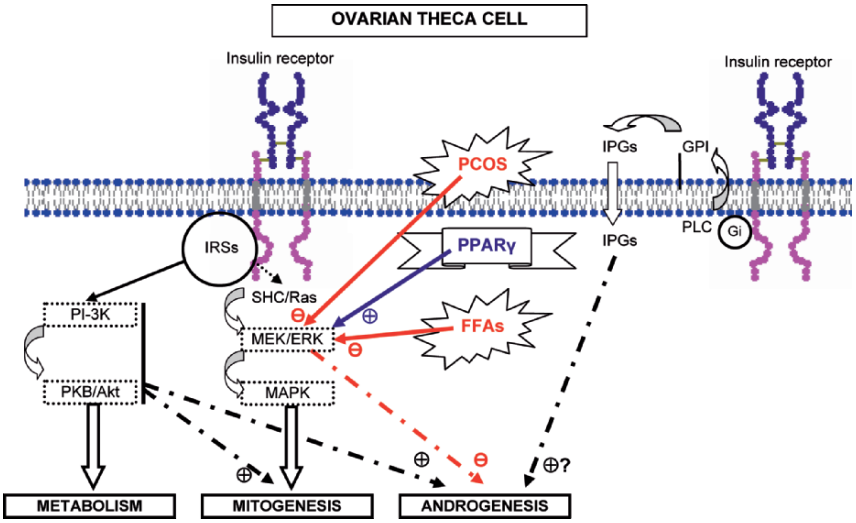

Fig. 3. Possible molecular mechanisms involved in the actions of insulin on ovarian theca cells. *Abbreviations*: ERK: extracellular signal-regulated kinases; FFAs: free fatty acids; GPI: glycosyl-phosphoinositol; IPGs: inositol-phosphoglycans; IRSs: insulin receptor substrates; MAPK: mitogen-activated protein kinase; MEK: MAPK/ERK kinase; PCOS: polycystic ovary syndrome; PPARγ: peroxisome proliferator-activated receptor gamma; PI-3K: phosphoinositide 3-kinase; PKB: protein kinase B; PLC: phospholipase C; Ras: rat sarcoma virus; and SHC: Src homologous and collagen-like.

pathway, implicated in the metabolic effects of insulin, and the mitogen-activated protein kinase (MAPK) pathway, responsible for the mitogenic effects of insulin [11] (illustrated in Fig. 3).

Both in normal [34] and PCOS [30] ovarian theca cells, it has been shown that insulin acts through its own receptor. Because LH and insulin act physiologically via distinct intracellular signaling mechanisms, their synergistic enhancement of theca-cell steroidogenesis likely entails important interactions between these two respective pathways. Indeed, it has been shown that insulin significantly increases LH-driven cAMP accumulation in cultured porcine theca cells [35]. The insulin-stimulated increase of cAMP is probably induced through PI-3K or protein kinase C (PKC), since the activation of adenyl cyclase by insulin was blocked with the use of nonspecific inhibitors of PI-3K, PKC, and Gi-protein in rat muscle tissues [36]. On the other hand, LH was shown to induce rapid activation of Janus kinase 2 (JAK2) in whole ovary of normal rats, which then activated IRS-1 and the PI-3K as well as the MAPK pathways [37]. Simultaneous stimulation with LH and insulin induced higher phosphorylation levels of these proteins compared with each hormone alone. Therefore, important cross talks exist between the insulin and LH signaling pathways, such that increased activity of one pathway might also increase the responsiveness of the other pathway.

A study on normal human theca cells demonstrated that specific blockade of PI-3K, but not the specific inhibition of MAPK/ERK kinase (MEK), markedly inhibits the combined insulin and LH stimulation of ovarian P450c17 activity [38]. However, P450c17 activity increased after inhibition of MEK. A recent study confirmed these results in normal and PCOS theca cells and found constitutively reduced levels of phosphorylated MEK and extracellular signal-regulated kinases (ERK) in PCOS cells. These constitutive defects were correlated with increased androgen production, irrespective of the presence of insulin [32]. Infection with dominant-negative MEK1 increased P450c17 mRNA, whereas constitutively active MEK1 reduced P450c17 mRNA abundance. This suggests that alterations in the MAP kinase pathway might cause androgen hyperresponsiveness to insulin in PCOS ovaries (Fig. 3).

It was also shown that insulin-stimulated theca cells proliferation was inhibited by specific inhibitors of both MEK and PI-3K in rats. Finally, Nestler et al. [30] provided evidence that insulin also stimulates testosterone biosynthesis by PCOS theca cells through inositolglycan mediators. In this study, the use of a synthetic inositolglycan mediator stimulated PCOS theca cells androgen production to the same extent as insulin.

PPARγ nuclear receptors were found in theca ovarian cells, and ligands of these receptors significantly decreased LH and insulin-stimulated testosterone production in theca cells [39]. To our knowledge, the molecular mechanisms underlying these observations have not been studied. However, it has been found that PPARγ agonists can increase the levels of activated ERK in adipose, liver, and muscle cell systems [40]. Therefore, these findings suggest that PPARγ receptors are directly implicated in insulin-stimulated ovarian androgen production and might improve some of the molecular defects associated with increased androgen responsiveness to insulin in PCOS, such as ERK activity (Fig. 3).

3.2.2. Hepatocyte Production of SHBG. In an *in vitro* model of human hepatoma cells, insulin was shown to equipotently inhibit SHBG and IGFBP-1 production [41]. Insulin also directly inhibited in vitro SHBG production by human hepatoblastoma-derived cells [42]. These results are in agreement with in vivo observations and suggest that insulin directly inhibits hepatic production of SHBG, which in turn will decrease testosterone binding and increase free testosterone in women.

3.2.3. Selective Defects of Insulin Actions. Clinical examples of selective defects in the metabolic pathway of insulin action were reported in pseudoacromegaly and in diabetic patients with strong family history of type 2 DM. Resistance to insulin action on carbohydrate metabolism with preserved insulin stimulation of mitogenesis was demonstrated in cultured skin fibroblasts from such patients [43]. Book and Dunaif also demonstrated that a

selective defect in insulin action in PCOS fibroblasts affects metabolic, but not mitogenic, signaling pathways [44].

Inhibition of the metabolic branch of insulin signaling, by blocking the PI-3K pathway, leads to enhanced mitogenic action of insulin in endothelial cells, suggesting significant cross talk between the different pathways of insulin signaling. The studies of King et al. have led to the hypothesis that the cardiovascular complications of DM, for example, may result from diminution of the PI-3K pathway activity, which causes a decrease in the antiatherogenic effects of insulin, along with an increased activation of the MAPK pathway due to hyperinsulinemia and/or PKC-induced up-regulation, which can result in an increase in the atherogenic effects of insulin [45].

Finally, a study in ovarian luteinizing granulosa cells from women with PCOS [46] highlighted a selective defect in insulin activity, e.g., resistance in the metabolic pathway associated with an *increase* in mitogenic activity. Since the mitogenic pathway of insulin implicates mainly the MAP kinase pathway [11] (Fig. 3), as suggested for the androgenic pathway of insulin (see above), the activity of the androgenic insulin pathway might be increased as well. Thus, the observation that insulin-signaling pathways may express differential, and even *divergent*, activity levels under various circumstances supports the possibility that such selective defect also exists for the insulin androgenic pathway(s) in PCOS women. Moreover, the study demonstrated that troglitazone, a PPARγ agonist, corrects this hypersensitivity of the mitogenic insulin-signaling pathway along with improvement of the insulin resistant metabolic pathway. These results suggest that PPARγ agonists may also directly improve a possible hypersensitivity of the androgenic insulin-signaling pathway (Fig. 3).

4. INSULIN ACTIONS AND OVULATORY DYSFUNCTION IN PCOS

4.1. In Vivo Insulin Actions

4.1.1. Ovulation. Many randomized and controlled studies have demonstrated that insulin-sensitizing therapies, including lifestyle modifications and drugs, improve ovulatory function both in lean and obese women with PCOS [1]. Indeed, two recent meta-analyses of pertinent randomized-controlled trials concluded that metformin significantly increases ovulation rates in PCOS women, either alone or in combination with clomiphene citrate [47,48] (see also Chap. 7). But it is not possible with these studies to determine whether the improvement in ovulatory function with insulin-sensitizing treatments is due to the decrease in insulin levels, following improved insulin resistance, or to the direct correction of a defect in insulin action, or both. However, two randomized-controlled trials have indicated that the sole reduction of insulin levels for 3 or 6 months with acarbose, which does not alter

insulin signaling, significantly improves ovulation rates in hyperinsulinemic women with PCOS [21,22]. Thus, these results suggest that insulin directly alters ovulatory function in women with PCOS.

As previously mentioned, we conducted a randomized-controlled trial in 100 nonobese women with PCOS and normal insulin levels [4]. The use of two insulin-sensitizing drugs in these PCOS women, i.e., metformin and rosiglitazone, significantly improved their ovulation rates comparatively to placebo. Since their insulin levels were normal, these women were presumably normally sensitive to the action of insulin on glucose metabolism. Thus, the observed benefit was probably not due to direct correction of metabolic insulin resistance.

However, the benefit of insulin sensitizers on ovulatory function might result from reduction of insulin levels below baseline concentrations, as observed with the use of metformin (Fig. 1b). Indeed, if these women were hypersensitive to the deleterious effects of insulin on ovulatory function, insulin lowering would explain the improvement in ovulatory rates. Another possibility is that these insulin-sensitizing drugs directly improve the defect causing impaired insulin actions on ovulatory function. Indeed, rosiglitazone improved ovulation rates without affecting insulin levels in our PCOS women (Fig. 1b). Therefore, the results of our trial in nonobese PCOS women with normal insulin levels suggest that PCOS might be characterized by increased sensitivity to the actions of insulin on ovulatory function.

Women with PCOS were also subjected to a 10-h two steps hyperinsulinemic–euglycemic clamps, at low and very high insulin levels, before and after treatment with pioglitazone for 5 months [49]. Before treatment, estradiol responses to recombinant human follicle-stimulating hormone (rhFSH) were unaltered during both low- and high-dose insulin infusions as compared to baseline. As expected, pioglitazone significantly improved insulin sensitivity and insulin levels in these PCOS women. After pioglitazone treatment, estradiol responses to rhFSH remained unchanged during low-dose insulin infusion but were significantly increased with the high-dose insulin infusion. These results suggested that PCOS granulosa cells might be resistant to insulin-stimulated estradiol biosynthesis, which is reversed by pioglitazone. However, it has been shown that incubation of normal bovine granulosa cells with high levels of insulin inhibits FSH-induced P450 aromatase enzyme, as opposed to low insulin levels (see also below) [50]. Therefore, it is also possible that chronic hyperinsulinemia in PCOS cause a resistance to FSH-induced estradiol production, which is improved by reducing insulin levels or by direct actions of PPARγ agonists.

4.1.2. Hypothalamic-Pituitary Axis. Hyperinsulinemia maintained for 22 days in normal female rats did not change GnRH-stimulated levels of FSH and LH [26]. In addition, infusions of exogenous insulin do not alter LH secretion in PCOS women [51]. Four-week treatment of obese PCOS women with

metformin did not slow their LH pulse frequency despite significant improvements in insulin levels [52]. Similarly, 16- and 20-week treatment with the thiazolidinedione pioglitazone in obese PCOS women did not alter LH pulse patterns, despite improvement in insulin sensitivity [51,53]. However, rosiglitazone was shown to significantly decrease LH levels in lean and overweight women with PCOS after 12 weeks [54]. In this last study, the average weights of women with PCOS were lower as compared to PCOS subjects in the pioglitazone studies.

Furthermore, LH levels appear to be negatively correlated with obesity in women with PCOS [55], despite the fact that higher weight is associated with higher insulin levels. Indeed, PCOS women with normal weight and insulin levels tend to have higher levels of LH than obese hyperinsulinemic PCOS women [56]. Another study, however, found similarly elevated plasma LH levels, LH pulse amplitude, and integrated LH responses to GnRH in obese and nonobese PCOS women as compared to the normal group, despite increased insulin levels in the obese PCOS group [57]. Together, these findings suggest that the hyperinsulinemia associated with PCOS does not directly result in neuroendocrine abnormalities. However, we cannot exclude the possibility that women with PCOS have increased sensitivity to insulin-stimulated LH production, which is attenuated in obese PCOS women due to other factors, such as leptin resistance, other adipokines, higher testosterone levels, etc.

Lean PCOS women were challenged with a GnRH agonist before and 4–6 weeks after treatment with metformin [18]. The results showed a significant decrease in basal and GnRH-stimulated LH levels with metformin, but not with placebo, along with significant reduction in insulin levels. Furthermore, we recently assessed the effect of insulin in lean PCOS women with normal insulin levels and metabolic insulin sensitivity, as above mentioned [5]. Our PCOS subjects ($n = 9$) were characterized by very high LH levels as compared to normal women ($n = 17$), i.e., 13.3 ± 1.5 versus 5.8 ± 1.1 mIU/mL, respectively. These high levels of LH decreased nonsignificantly after pure reduction of insulin levels with diazoxide, from 13.3 ± 1.5 to 10.5 ± 1.4 mIU/mL. However, diazoxide-induced insulin reduction in obese PCOS women did not alter LH levels and LH pulse frequency or amplitude [20]. Thus, these findings suggest that lean PCOS women might display hypersensitivity to insulin-induced LH release, which might be attenuated or counteracted by other factors in obese PCOS women.

4.2. In Vitro Molecular Mechanisms

Molecular mechanisms of ovulatory dysfunction might implicate ovarian theca cells, ovarian granulosa cells, and hypothalamic and/or pituitary cells. Since the actions of insulin in theca cells as well as potential dysfunction in PCOS have been previously discussed, we will now review in vitro molecular mechanisms of insulin action in granulosa cells as well as hypothalamic and pituitary cells.

Table 2. In vitro insulin actions in ovarian *granulosa cells*, based on studies of normal mammalian ovarian cells

Effects	Change	References
Basal progesterone production	↑	[31, 59, 66–68, 97–104]
FSH-induced progesterone production	↑	[31, 58, 102, 103, 105, 106]
LH-induced progesterone production	↑	[31, 58, 60, 90, 104, 107]
Basal estradiol production	↑	[31, 59, 75, 97, 100, 108]
FSH-induced estradiol production	↑	[58, 87, 101, 106, 109]
LH-induced estradiol production	↑	[31, 58]
Basal P450 aromatase activity	↑	[50, 108, 110]
FSH-induced P450 aromatase activity	↑	[109]
IGFBP-1 synthesis (via insulin receptor)	↓	[66, 99, 111–113]
Cellular proliferation	↑	[75, 97, 101, 114, 115]
LH-induced LDL receptor expression	↑	[107]
Glucose transport or activation of glycogen synthase	↑	[46, 116]
Intracellular free fatty acids accumulation	↑	[111]

4.2.1. Ovarian Granulosa Cells. Table 2 enumerates the actions of insulin that are described in normal ovarian granulosa cells from human or mammalian in vitro models. Insulin has been shown to stimulate basal and FSH- or LH-induced progesterone and estradiol production. Insulin is also capable of stimulating basal and FSH-induced P450 aromatase activity, which is the obligatory step in order to convert androgens and progesterone to estradiol. However, in bovine granulosa cells incubated with high dose of insulin, FSH failed to stimulate aromatase activity [50]. Thus, high insulin levels might favor progesterone and androgen production over estradiol biosynthesis.

In granulosa cells insulin also inhibits IGFBP-1 synthesis, which causes an increase in IGF-1 bioavailability and increases its actions on granulosa and/or theca cells. IGF-1 has been shown to stimulate ovarian cell proliferation and androgen production. Insulin can also directly stimulate granulosa cells proliferation via its own receptor. Finally, insulin increases LH-induced low-density lipoprotein (LDL) receptor expression, glucose transport, glycogen synthase activity, and intracellular free fatty acids (FFAs) accumulation. Increased accumulation of FFAs might exaggerate their potential deleterious effects on insulin action (see Sect. 6 below). Therefore, increased actions of insulin in granulosa cells might alter the hormonally induced selection of the dominant follicle, by changing the progesterone-to-estradiol ratio, and might impair normal follicular atresia, by promoting cellular proliferation. These effects could induce anovulation and the development of polycystic ovaries.

Indeed, granulosa cells from PCOS women have been demonstrated to produce more estradiol under basal conditions, and after FSH and insulin stimulation, and more progesterone following FSH, LH, and insulin stimulation, as compared to controls [58]. Other investigators confirmed that FSH-induced

estradiol synthesis was higher following stimulation with insulin in PCOS compared to normal granulosa cells [31]. However, highly luteinized granulosa cells collected from women with PCOS during in vitro fertilization protocols did not show increased progesterone production after stimulation with FSH, LH, or insulin compared to controls, but demonstrated higher stimulation of estradiol synthesis by FSH, LH, and insulin [59]. Therefore, PCOS granulosa cells seem hypersensitive to the actions of insulin that are observable in normal granulosa cells, particularly regarding estradiol production.

The molecular mechanisms of insulin action in granulosa cells are illustrated in Fig. 4. As depicted, studies have demonstrated that the metabolic actions of insulin are mediated by the PI-3K pathway, similarly to classical insulin-sensitive tissues [60]. Insulin stimulates granulosa cells proliferation through the MAPK pathway, which is in accordance with the mitogenic actions of insulin in other tissues [61].

The specific inhibition of MAPK in vitro resulted in higher FSH-induced production of progesterone [62] and lower FSH-induced synthesis of estradiol [62]. Similarly, specific inhibition of MEK or ERK decreased FSH- or LH-induced progesterone synthesis [63]. Also, activation of MAPK by the hepatocyte

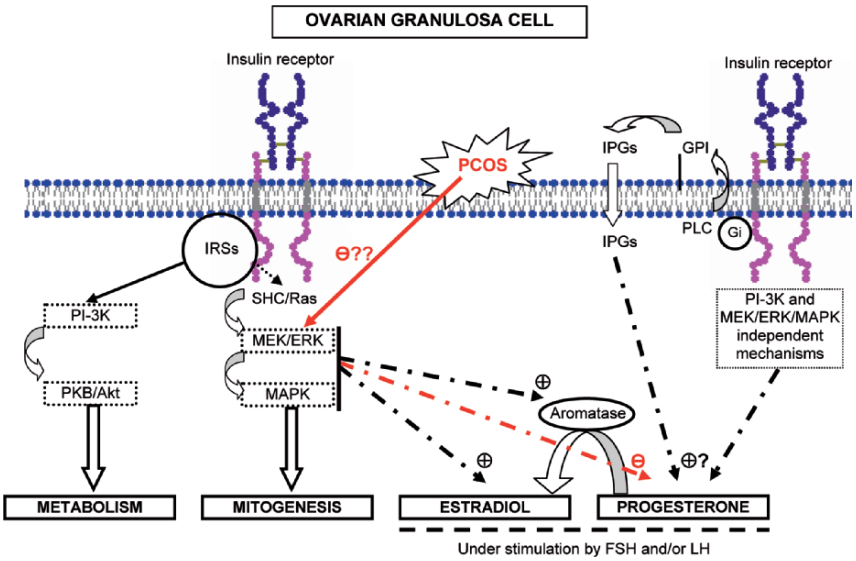

Fig. 4. Possible molecular mechanisms involved in the actions of insulin on ovarian granulosa cells. *Abbreviations*: ERK: extracellular signal-regulated kinases; FFAs: free fatty acids; GPI: glycosyl-phosphoinositol; IPGs: inositol-phosphoglycans; IRSs: insulin receptor substrates; MAPK: mitogen-activated protein kinase; MEK: MAPK/ERK kinase; PCOS: polycystic ovary syndrome; PPARγ: peroxisome proliferator-activated receptor gamma; PI-3K: phosphoinositide 3-kinase; PKB: protein kinase B; PLC: phospholipase C; Ras: rat sarcoma virus; and SHC: Src homologous and collagen-like.

growth factor inhibited progesterone production and stimulated cells proliferation in granulosa cells [64], and prostaglandin F2α-induced activation of MEK decreased LH-stimulated progesterone synthesis [65]. Inhibition of the MAPK pathway was also shown to decrease the activity of P450 aromatose and FSH-stimulated estradiol production [62]. This might be of importance in PCOS because the activation of MEK/ERK appears to be constitutively reduced in PCOS theca cells (as previously discussed) [32]. If such defect also exists in granulosa cells, it would explain intracellular accumulation of progesterone and reduction in estradiol levels, which would impair the selection of a dominant follicle and ovulation.

Since insulin directly stimulates progesterone production in cultured granulosa cells, this action is probably mediated through mechanisms other than the activation of the MAPK pathway. Furthermore, a group using mixed ovarian culture showed that inhibition of PI-3K fails to abolish stimulatory effect of insulin on progesterone production, suggesting the presence of PI-3K-independent insulin signaling pathway(s) in human [66]. Since IGFBP-1 has been shown to be reduced after stimulation of granulosa cells by insulin [66], it is possible that an increase in IGF-1 bioavailability explains insulin-mediated progesterone biosynthesis. Indeed, it was shown that IGF-1 stimulates progesterone production in human granulosa cells [67]. Finally, Romero et al. [68] have demonstrated that specific blockade of the inositolphosphoglycan putative mediators of insulin action abolishes insulin-mediated progesterone synthesis in granulosa cells. Thus, it appears that some intracellular pathways mediate insulin-stimulated progesterone production, which is in turn inhibited by activation of the MAPK pathway.

4.2.2. Hypothalamic and Pituitary Cells. In a model of hypothalamic cells, i.e., immortalized neurons expressing GnRH, insulin-stimulated GnRH secretion, which was mediated by ERK1/2, but not PI-3K [69]. Insulin also stimulated GnRH promoter activity in mouse-derived GnRH-expressing neurons through activation of MEK, another effector of the MAPK pathway. In vitro stimulation with insulin increases basal and GnRH-stimulated LH and FSH mRNA expression or secretion from cultured rat pituitary cells [70] and pituitary-derived LbetaT2 cells [71]. It was also shown in LbetaT2 cells that inhibition of MEK decreases LHβ gene expression [72]. Therefore, increased insulin levels or activity could dysregulate the hypothalamic–pituitary control of ovulation and could increase LH levels, which might contribute to hyperandrogenemia.

5. CONCLUSIONS

The key points discussed concerning the in vivo and in vitro actions of insulin in PCOS are depicted in Table 3. The in vitro and in vivo evidences currently available support the direct effect of insulin in stimulating androgen, estradiol,

Table 3. Insulin Action and PCOS – Key Points

- PCOS women are characterized by androgen hyperresponsiveness to insulin in vivo and in vitro, even in those with normal insulin levels and sensitivity
- Insulin-sensitizing and pure insulin-lowering therapies of PCOS women, but not suppression of LH, improve hyperandrogenemia and/or hyperresponsiveness to LH stimulation, suggesting that they are related to insulin action in vivo
- Increased androgenic responsiveness to insulin might be explained by reduced activity of the MAP kinase pathway in PCOS theca cells
- PPARγ agonist insulin-sensitizing agents increase MAP kinase activity in insulin-sensitive tissues and decrease androgenic response to insulin in theca cells
- Insulin-sensitizing therapies and pure insulin lowering with acarbose improve ovulatory function in both hyperinsulinemic and normo-insulinemic PCOS women, suggesting that PCOS anovulation is directly caused by increased insulin actions
- In lean PCOS women, insulin directly stimulates in vitro GnRH and LH release, and metformin decreases GnRH-stimulated LH production, suggesting a role of insulin action in the hypothalamic-pituitary dysregulation of PCOS women
- Insulin-stimulated estradiol production and cellular proliferation are mediated by the MAP kinase pathway in granulosa cells, and inhibition of this pathway increases progesterone synthesis, which is otherwise stimulated by insulin through MAP kinase and PI-3K independent pathways

and progesterone biosynthesis, in decreasing SHBG production, in inducing theca cell proliferation, in inhibiting granulosa cells apoptosis, and in affecting GnRH and gonadotropin regulation. These findings suggest potential mechanisms by which high insulin levels or increased insulin actions could cause hyperandrogenemia and ovulatory dysfunction, which characterize PCOS. Thus, insulin action, rather than metabolic insulin resistance, is probably central to the pathogenesis of PCOS. Furthermore, there is some in vivo evidence, in PCOS women with normal insulin levels and metabolic insulin sensitivity, suggesting that insulin directly results in hyperandrogenemia and possibly ovulatory dysfunction.

Therefore, these findings suggest that PCOS might result from hypersensitivity to the effects of insulin on androgen biosynthesis and/or ovulatory function. In a minority of women, this defect could be sufficiently severe to cause typical PCOS in the absence of metabolic evidence of insulin resistance and hyperinsulinemia. However, in most women with PCOS, concomitant development of insulin resistance and hyperinsulinemia would be necessary for expression of the syndrome. This hypothesis could explain why most obese women do not develop PCOS, because they lack this predisposing defect, and why some women with typical PCOS are not insulin resistant, at least in regards to glucose homeostasis.

6. FUTURE AVENUES OF INVESTIGATION

Future investigations should probably focus on the mechanisms and the causes of this hypothesized androgenic insulin hypersensitivity of PCOS. Such studies might result in a better understanding of the pathogenesis of PCOS and provide evidence for the selection or development of novel therapies for this condition. Improved understanding of those mechanisms underlying the increased insulin activity associated with metabolic insulin resistance might also be pertinent to other conditions associated with insulin resistance, such as the proliferative complications of type 2 DM or the development of metabolic syndrome.

As mentioned, there is evidence suggesting that increased insulin action in PCOS might be directly improved by PPARγ agonists. Although the mechanisms by which PPARγ agonists improve in vitro insulin action in theca cells is still unknown, a possibility is through the activation of MEK or ERK, which have been shown to be constitutively inhibited in PCOS theca cells. Therefore, future studies assessing the cellular mechanisms by which PPARγ agonists improve PCOS hyperandrogenemia might lead to the discovery of processes implicated in the reversal of the defects in insulin action in PCOS.

Furthermore, such a defect might not be directly genetically determined, but rather induced by the interaction between genetic and environmental factors. An interesting candidate factor is increased exposition of insulin-sensitive tissues to FFAs, due to high dietary fat intake or altered FFAs metabolism. Indeed, a recent controlled, randomized, crossover trial demonstrated that FFAs increase the production of adrenal androgen precursors in vivo in men. It was also shown that prolonged experimental elevation of plasma FFAs reduces muscle and hepatic glucose insulin sensitivity in humans.

Plasma FFAs levels are increased in obese PCOS women, compared to controls, and correlate with resistance to insulin-stimulated glucose metabolism. Moreover, Usui et al. demonstrated that the FFA palmitate decreases MAPK activity in vitro in rat fibroblasts and could therefore induce the molecular defects observed in PCOS theca cells (Fig. 3). Finally, PPARγ agonists prevent FFAs-induced hepatic, peripheral, and adipose tissue insulin resistance. As mentioned, they also increase MAPK pathway activity. Therefore, increased exposure to FFAs might explain both the metabolic insulin resistance and the hyperandrogenemia of women with PCOS. Since PPARγ agonists reduce tissue exposure to FFAs and prevent FFAs-induced alterations of insulin actions, they might also improve hyperandrogenemia through these mechanisms. Thus, future studies should assess the impact of increasing FFA levels in vivo or exposing ovarian cells to FFAs in vitro on hyperandrogenemia and androgenic hypersensitivity to insulin, in normal and PCOS women.

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Chapter 5

Ovarian Steroidogenic Abnormalities in PCOS

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Polycystic ovary syndrome (PCOS) is a common, clinically heterogeneous disorder that affects approximately 6–10% of premenopausal women [1,2]. Hyperandrogenemia is the biochemical hallmark of PCOS. Reproductive and endocrine abnormalities include disordered gonadotropin secretion, oligomenorrhea and anovulatory infertility, and endometrial hyperplasia. Obesity, hirsutism, acne, and alopecia are often associated with PCOS. The metabolic consequences of PCOS include insulin resistance, lipid abnormalities, and possibly an increased risk of cardiovascular disease [3].

The familial aggregation of PCOS has been recognized for many years, supporting a genetic contribution to its etiology. Recently, investigators have reported a strong association between PCOS and an allele on 19p13.2 [3]. However, at present, there is no general agreement on a mode of inheritance. Even though more than 50 candidate genes have been considered or studied to no avail, most investigators believe that PCOS will prove to be an oligogenic syndrome that involves genes governing steroid hormone biosynthesis as well as insulin/glucose homeostasis. Limited progress in identifying the genetic basis for PCOS has resulted, in part, from the difficulties arising from the analysis of a complex genetic disease, which includes the heterogeneity of the PCOS phenotype, the likely contribution of multiple genes, and the uncertain contribution of the environment to the PCOS phenotype [3].

1. OVARIAN ANDROGEN AND ESTROGEN BIOSYNTHESIS

In the human ovarian follicle, androgen biosynthesis takes place primarily in theca interna cells in response to the pituitary gonadotropin, luteinizing hormone (LH) [2]. Theca-derived androgens then diffuse into ovarian granulosa cells where estrogen biosynthesis takes place in response to follicle stimulating hormone (FSH) in the nonluteinized follicle and in response to LH in the luteinized follicle. Both FSH and LH act predominately via cyclic AMP/protein kinase A mediated post-receptor signaling. A large array of paracrine and autocrine signals including hormones, growth factors, and cytokines have also been reported to regulate estrogen and androgen biosynthesis.

Theca cells express a cytochrome P450 with 17α-hydroxylase and 17,20-lyase activity (P450c17; encoded by the *CYP17* gene), which is the rate-limiting enzyme required for androgen biosynthesis. P450c17, a single enzyme with both 17α-hydroxylase and 17,20-lyase activities, is necessary for the conversion of pregnenolone (P5) to 17α-hydroxypregnenolone (17OHP5), dehydroepiandrosterone (DHEA), and Δ5-androstenediol, and for the conversion of progesterone (P4) to 17α-hydroxyprogesterone (17OHP4) [2]. Granulosa cells express cytochrome P450 aromatase (P450arom, encoded by the *CYP19* gene), which aromatizes the A ring of C19 androgens to the phenolic A ring of Δ4-androstendione (Δ4-A) and testosterone (T) to estrone (E1) and estradiol (E2), respectively [4].

Both granulosa and theca cells express steroidogenic acute regulatory protein (StAR), encoded by the *STAR* gene, which promotes the translocation of cholesterol to the inner mitochondrial membrane, as well as cytochrome P450 cholesterol side chain cleavage enzyme (P450scc; encoded by the *CYP11A1* gene). Both cell types also express 3β-hydroxysteroid dehydrogenase type II (3β-HSD; encoded by the *HSD3B2* gene), and a variety of 17β-hydroxysteroid dehydrogenase (17β-HSD) isoforms (encoded by aldo-ketoreductase [*AKR*] genes) each of which is required for androgen biosynthesis [2]. In human theca cells, the 17β-HSD type V (17β-HSDV, encoded by *AKR1C3*) reduces Δ4-A to T, rather than the type III, which catalyzes the formation of T in Leydig cells, or the type I, an estrogenic enzyme that catalyzes the formation of E2 in granulosa cells [2].

In human theca cells, androgen biosynthesis (Fig. 1) proceeds through the Δ5 steroid pathway and metabolism of P5 to 17OHP5, DHEA and Δ5-androstenediol by the combined action of 17α-hydroxylase/17,20-lyase; and conversion of DHEA to Δ5-androstenediol by 17β-HSDV or 20α-hydroxysteroid dehydrogenase (20α-HSD, encoded by the *AKR1C1* gene) [2]. Both DHEA and Δ5-androstenediol can be further converted to Δ4-A and T by 3β-HSD in either the granulosa or theca cell compartment. Human theca cells have the capacity to convert P5 to P4 and 17OHP4. However, human 17,20-lyase completely lacks the

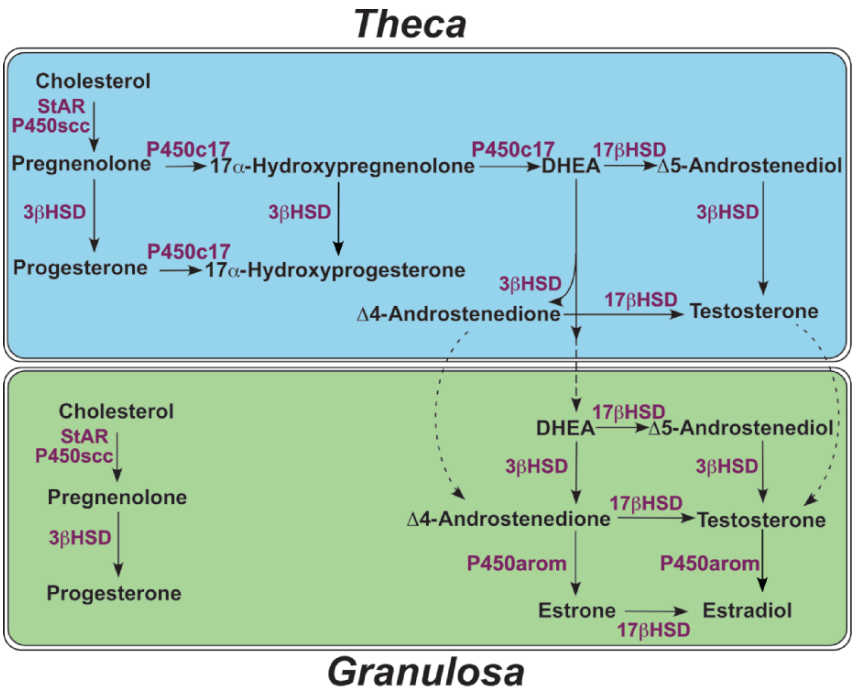

Fig. 1. A schematic diagram of the human ovarian steroid biosynthetic pathway. In the human ovarian follicle, theca cells produce androgens that are converted to estrogens by granulosa cells. Theca and granulosa cells express steroidogenic acute regulatory protein (StAR), encoded by the *STAR* gene, which promotes the translocation of cholesterol to the inner mitochondrial membrane. P450 cholesterol side chain cleavage enzyme (P450scc) encoded by the *CYP11A1* gene, which converts cholesterol to pregnenolone (P5), is also expressed in both cell types. For androgen biosynthesis, theca cells express cytochrome P450 17α-hydroxylase (P450c17), a single enzyme with 17α-hydroxylase and C17,20 lyase activities, which are necessary for the conversion of P5 to 17α-hydroxypregnenolone (17OHP5), dehydroepiandrosterone (DHEA), and for the conversion of progesterone (P4) to 17α-hydroxyprogesterone (17OHP4). In the human ovary, as in the adrenal, androgen biosynthesis proceeds through the Δ5 steroid pathway, whereby P5 is metabolized to 17OHP5 and DHEA by the combined action of 17α-hydroxylase /17,20-lyase (i.e., P450c17); this is followed by subsequent conversion of DHEA to Δ5-androstenediol by 17β-hydroxysteroid dehydrogenase (17β-HSD). P5, DHEA, and Δ5-androstenediol can be converted to P4, Δ4-androstenedione (Δ4-A, and testosterone (T) by 3β-HSD in granulosa or theca cells. In granulosa cells, androgen substrates (i.e., Δ4-A, DHEA, and T) can be further converted to estrogens by cytochrome P450 aromatase (P450arom), encoded by the *CYP19* gene. In granulosa cells, 17β-HSD types I, II, and IV are expressed, whereas type V is expressed in the theca.

ability to convert 17OHP4 to Δ4-A. In contrast to the rodent and bovine ovary, studies examining steroid metabolism in normal and PCOS theca cells have demonstrated that DHEA and Δ5-androstenediol, rather than Δ4-A, are the major products of P5 metabolism. Therefore, Δ5 steroids are the primary source of

substrate for androgen biosynthesis in the human ovary and the most suitable markers for examining the regulation and dysregulation of androgen production in the PCOS ovary.

The ovarian phenotype of PCOS is characterized by an increased number of small antral follicles, with a hypertrophied thecal wall, aligned under a thickened capsule. The PCOS ovary maintains increased steroidogenesis in association with abnormal and arrested follicle growth [4]. The increased number of follicles in polycystic ovaries is likely to result from an extended period of follicle growth, which results in abnormal folliculogenesis at all stages [4]. These follicles are arrested in development at the stage where selection of a dominant follicle would normally occur. Compared with follicles of the same size in cycling ovaries, these growth arrested follicles contain an increased number of steroidogenic cells in the theca interna with increased CYP17 and CYP11A1 mRNA [5] and a decreased number of granulosa cells.

During normal follicle development, the granulosa cells begin to express *CYP19* mRNA when the follicle grows to approximately 6–7 mm in diameter [6]. In the PCOS ovary, follicles arrest at 5–8 mm in diameter, and granulosa cells express lower levels of *CYP19* mRNA independent of the follicle size, even though there are normal concentrations of FSH in the follicular fluid [6]. In addition, both theca and granulosa cells appear to be luteinized as they express LH receptors and have increased *CYP11A1* mRNA abundance [5]. Therefore, it is clear that there are abnormalities of proliferation and differentiation in both the theca and granulosa compartments of the polycystic ovary in vivo, which are discussed further below and summarized in Table 1.

2. DYSREGULATED ESTROGEN BIOSYNTHESIS IN PCOS

Follicular fluid from PCOS follicles contain elevated levels of androgens and lower amounts of E2 [7]. This elevated androgen to estrogen ratio results from an increase in thecal *CYP17* gene expression and androgen production

Table 1. Abnormalities of the PCOS ovary "in vivo"

- Follicular androgen to estrogen ratio is increased
- The number of small (5–8 mm) follicles, resulting from growth arrest at primary follicular stage, is increased
- Thecal CYP17 and STAR expression are elevated
- Granulosa *CYP19* expression is reduced
- Both theca and granulosa *CYP11A1* and LH receptor mRNA are increased
- Follicular 5α-reductase activity is enhanced
- Granulosa cell sensitivity to FSH is enhanced

concomitant with a reduction in *CYP19* gene expression and decrease in the conversion of androgens to estrogens in granulosa cells [2,6]. Even though excess thecal androgen substrate is provided to the PCOS granulosa cell, these androgens are not aromatized to estrogens, and follicular fluid levels of E2 are reduced. This loss of aromatase and E2 biosynthesis "in vivo" has been proposed to involve dysregulation of autocrine and paracrine signaling within the follicle. These findings are substantiated by the observation that in contrast to PCOS granulosa cells "in vivo" where *CYP19* gene expression and aromatase enzyme activity and E2 production are suppressed, PCOS granulosa cells both in short- and long-term culture "in vitro" have both markedly increased aromatase activity and P4 production compared to normal granulosa cells [8,9]. 5α-Reductase activity has also been reported to be elevated in the granulosa cells of PCOS ovaries, leading to markedly increased metabolism of Δ4-A to 5α-androstanedione, a competitive inhibitor of aromatase activity [10].

Women with PCOS also exhibit an exaggerated serum E2 response to recombinant human FSH compared with similarly treated normal women. This enhanced granulosa cell responsiveness is consistent with excessive follicular development following gonadotropin therapy and the corresponding risk of ovarian hyperstimulation syndrome. Reports also suggest that inhibin B and E2 induction by FSH is significantly enhanced in PCOS women, occurring more rapidly and robustly than in normal controls. In contrast, the induction of inhibin A was similar in normal and PCOS women. In women with PCOS, the increased response to FSH does not appear to be the result of elevated circulating androgens, since treatment with the antiandrogen flutamide does not alter FSH responsiveness [11].

3. DYSREGULATED ANDROGEN BIOSYNTHESIS IN PCOS

There is convincing evidence to support the concept that excess androgen production in PCOS results from a primary abnormality in ovarian thecal steroid production. Immunohistochemical studies have demonstrated that there is greater immunoreactive *CYP17* protein in the theca interna cell layer of small, large, and atretic follicles from PCOS ovaries that is independent of the thickness of the theca interna layer. In 1994, Gilling-Smith et al. [12] published the first studies demonstrating that androgen and progestin production are elevated on a per cell basis in PCOS theca cells in primary culture. Subsequent studies by these investigators demonstrated that androgen production is inhibited following complete GnRH suppression of pituitary LH production in patients with PCOS, further establishing that ovarian theca cells are the primary source of excess androgen biosynthesis in PCOS [12]. In 1999, a comparison of the steroid biosynthetic capacity of normal and PCOS theca

cells grown for multiple population doublings verified that in PCOS theca cells, basal and cAMP-stimulated DHEA, 17OHP4, T, P4, and Δ4-A production was increased compared to cells propagated from normal cycling women [13]. The latter studies were of significant importance because propagating the cells for successive population doublings under long-term culture conditions should negate the effect of their prior "in vivo" hormonal environment.

The finding that increased androgen and progestin biosynthesis is a stable characteristic of propagated PCOS theca cells maintained in long-term culture has provided new opportunities to examine the molecular and cellular basis for increased ovarian androgen biosynthesis in PCOS cells under conditions where the cells are distant from their in vivo paracrine and endocrine milieu. Studies of propagated PCOS cells have revealed that augmented steroid biosynthesis is due to increased steroidogenic activity and transcription of genes encoding steroidogenic enzymes, which is reflected by increased levels of steroidogenic enzyme mRNAs as well as enhanced promoter activities [2].

The biochemical phenotype displayed by PCOS theca cells is consistent with the findings from family studies indicating genetic control of androgen production. Steroidogenic abnormalities associated with PCOS theca cells in long-term culture are presented in Table 2, and include increased 3β-HSD, P450c17, and 20α-HSD enzyme activities, and increased P450scc, 3β-HSD, P450c17, and 20α-HSD mRNA accumulation [2]. In contrast, similar levels of STAR and 17β-HSDV (*AKR1C3*) mRNA were observed in normal and PCOS theca cells in long-term culture [2].

Table 2. Steroidogenic abnormalities of PCOS theca and granulosa cells "in vitro"

	Activity	mRNA	Transcription	mRNA stability
Theca cells[a]				
StAR		–	–	–
P450scc		↑	↑	↑
3βHSD	↑	↑		
P450c17	↑	↑	↑	↑
20α-HSD	↑	↑		
17β-HSDV	–	–		
Granulosa cells[b]		|	|	|
P450arom	↑	↑		

(↑) increase; (–) no significant difference.

[a]Increased 17α-hydroxyprogesterone, DHEA, and progesterone biosynthesis.

[b]Increased Progesterone biosynthesis.

4. INCREASES IN *CYP17* AND *CYP11A1* GENE TRANSCRIPTION AND mRNA STABILITY IN PCOS

In PCOS theca cells maintained in long-term culture, increased basal and cAMP-stimulated androgen and P4 biosynthesis has been reported to result from increased *CYP17* and *CYP11A1* mRNA accumulation, gene transcription, and mRNA stability in PCOS [2]. Significant progress has been made examining the molecular basis for increased transcriptional regulation of *CYP17* and *CYP11A1* gene expression in PCOS theca cells. Analysis of the *CYP17* promoter in normal and PCOS theca cells has demonstrated that a 16 bp sequence, spanning −174 to −158 bp from the start site of transcription, is required for increased *CYP17* promoter function in PCOS theca cells [2,14]. Mutation of specific base pairs within this sequence was found to ablate increased promoter activity in PCOS. Moreover, these mutations resulted in the loss of binding of the transcription factor nuclear factor 1C (NF-1C).

A comparison of NF-1C binding in nuclear extracts isolated from normal and PCOS theca cells demonstrated that NF-1C binding was reduced in PCOS cells. In addition, NF-1C was observed to repress *CYP17* promoter function, suggesting that reduced NF-1C-dependent repression may be associated with increased *CYP17* promoter activity and gene expression in PCOS theca cells. In Fig. 2, an illustration is presented of the sequence between −188 and −140 bp of the 5′ flanking sequence of the human *CYP17* promoter showing the binding sites of factors that are known to regulate the *CYP17* promoter in

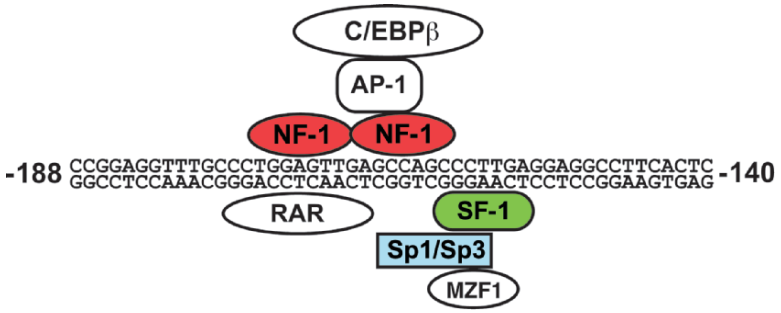

Fig. 2. The −188 to −140 bp region of the *CYP17* promoter and associated regulatory factors. A schematic diagram of the −188 to −140 bp region of the 5′-flanking sequence of the human *CYP17* gene, which confers increased transcription of the *CYP17* promoter in PCOS theca cells. Regulatory factors that are known to bind this region are shown in color, including Sp1 and Sp3 factors, nuclear factor-1 (NF-1), and steroidogenic factor-1 (SF-1). Putative binding elements for CCAT-enhancer binding protein-β (C/EBP-β), activating protein-1 (AP-1), retinoic acid receptor (RAR), and myeloid zinc finger-1 (MZF-1) have also been included based on similarities to consensus binding sites, however, the association of these factors with the *CYP17* promoter has not been confirmed.

theca cells, as well as the approximate binding sites of putative regulatory (derived from database analysis).

It is interesting to note that a common feature of these putative *CYP17* regulatory factors (i.e., AP-1, NF-1, SF-1, C/EBPβ, MZF-1, RAR, and Sp1), is that they have been reported to be regulated by intracellular cell signaling pathways including the mitogen activated protein kinase (MAPK) pathways ERK, p38, and JNK [2]. Recent supershift analysis has indicated that the transcription factors NF-1C, SF-1, Sp1, and Sp3 from normal and PCOS theca cell nuclear extracts bind to the −188/−140 *CYP17* promoter sequence [14]. Whereas NF-1C represses the *CYP17* promoter, steroidogenic factor-1 (SF-1) potently stimulates the *CYP17* promoter and contributes to cAMP-dependent responsiveness. Sp1 and Sp3 have also been shown to activate the *CYP17* promoter in adrenocortical H295 cells, however, the role of Sp factors in theca cell *CYP17* transcription is still unknown.

Analysis of the *CYP11A1* promoter in normal and PCOS theca cells has demonstrated that augmented promoter function in PCOS theca cells results from preferentially increased basal regulation conferred by sequences between −160 and −90 bp of the transcriptional start site. We have analyzed the −160/−90 bp sequence of the human *CYP11A1* promoter and have found regions of homology to the minimal −188/−140 bp *CYP17* promoter sequence that confers increased regulation in PCOS theca cells. As expected, transcription factor database analysis of the homologous regions indicates putative recognition sequences similar to those presented in Fig. 2, including SF-1, NF-1, Sp1/Sp3, C/EBPβ, RAR, and MZF-1. NF-1C was observed to repress not only *CYP17* promoter function but also *CYP11A1* promoter function, suggesting that diminished NF-1C-dependent repression may be associated with the coordinate increase in *CYP17* and *CYP11A1* promoter activity and gene expression in PCOS theca cells.

With respect to the increase in *CYP17* and *CYP11A1* mRNA stability in PCOS theca cells, there is a growing body of literature demonstrating that RNA degradation is not a default process. Sequences that control mRNA decay can be found in the 5′ untranslated region (UTR), coding region, and/or 3′UTR of a specific mRNA. Regulation of transcript stability is accomplished through the association of proteins that bind to the 5′UTR alone or in association with proteins that bind with the 3′UTR. Although the transcriptional regulation of genes encoding steroidogenic enzymes has been widely studied, the regulation of mRNA abundance by posttranscriptional mechanisms, i.e., alterations in mRNA stability, has been largely unexamined. Cumulative data from in vitro degradation assays and transient transfection analyses have demonstrated that increased *CYP17* and *CYP11A1* mRNA abundance in PCOS theca cells results not only from increased mRNA synthesis, but also from a coordinate decrease in the degradation of *CYP17* and *CYP11A1* mRNA [2,15]. This enhanced mRNA stability in PCOS has recently been shown to

require the 5′UTRs of the *CYP17* and *CYP11A1* mRNAs [2], however, the factors involved in altered mRNA stability in PCOS have not yet been identified.

5. INSULIN SENSITIVITY AND THE PCOS OVARY

There is controversy about whether the hyperandrogenism associated with PCOS results from alterations in the intracellular signaling cascades linked with insulin resistance. Although investigators initially proposed that hyperinsulinemia resulting from the insulin resistant state produces ovarian hyperandrogenism by spillover occupancy and activation of the insulin-like growth factor-1 (IGF-1) receptors [16], studies have shown no differences in the ED_{50} of insulin-stimulated androgen biosynthesis in propagated theca cells [13] or estrogen biosynthesis in freshly isolated granulosa cells obtained from normal and PCOS ovaries [17].

PCOS theca and granulosa cells are not insulin resistant and are, in fact, equally sensitive to insulin. In contrast, subsequent reports have shown that insulin-dependent lactate production in granulosa-lutein cells from PCOS was reduced compared with normal ovulatory women, suggesting that insulin-stimulated glucose uptake and utilization are impaired in PCOS [18,19]. In patients with PCOS and insulin resistance, there are reports to suggest that D-chiro-inositol signaling is altered [2,20].

Total insulin receptor mRNA expression, but not intrafollicular insulin levels, was observed to be elevated in follicular aspirates of PCOS in vitro fertilization patients [2]. Differences in insulin receptor substrate (IRS) signaling molecules in normal and PCOS theca cells, but not granulosa cells have been reported [2]. Furthermore, reports demonstrating increased IRS-2 expression in PCOS theca cells, as well as studies demonstrating inhibition of thecal androgen biosynthesis by insulin sensitizing drugs such as metformin, have suggested that insulin-dependent signaling may in fact be disrupted in the PCOS ovary [2].

6. MITOGEN ACTIVATED PROTEIN KINASE SIGNALING AND ALTERED STEROID BIOSYNTHESIS IN PCOS

MAPKs are all proline directed, serine-threonine kinases, that are phosphorylated (i.e., activated) on threonine and tyrosine in response to a wide variety of stimuli, including cytokines, growth factors (i.e., insulin), hormones, cellular stress, and cell adherence [21]. The Ras/MEK/ERK pathway is an important signaling cascade involved in the control of cell proliferation and differentiation. While some investigators have suggested that cAMP-stimulated activation of the MEK/ERK signaling cascade augments ovarian steroid biosynthesis, others

have demonstrated that inhibition of the MEK/ERK signaling cascade is associated with increased steroid biosynthesis. In human adrenocortical H295 cells, a reduction in the activation state of the ERK1/2 has also been associated with increased *CYP17* gene expression [21].

Similarly, a comparison of the phosphorylation states of MEK1/2 and ERK1/2 in normal and PCOS theca cells propagated in long-term culture revealed a gross reduction in the tone of MEK/ERK signaling in PCOS cells. Infection of normal theca cells with a dominant negative MEK1 adenovirus resulted in increased DHEA production, *CYP17* mRNA accumulation, and 17α-hydroxylase enzyme activity, and treatment with the pharmacological MEK1 inhibitor, PD98059, augmented *CYP17* gene transcription [21]. In contrast, infection with a constitutively active MEK1 inhibited both DHEA synthesis and *CYP17* mRNA accumulation. The observed lack of a response of PCOS theca cells to PD98059 at the level of transcription further supports the idea that the suppression of the MEK/ERK signaling pathway plays a pivotal role in regulating androgen synthesis in the PCOS.

Given the controversial role of insulin in regulating androgen biosynthesis in normal and PCOS theca cells, experiments were performed to assess the effects of insulin on ERK phosphorylation and overall androgen biosynthesis. Results from these experiments demonstrated that insulin treatment did not significantly affect the phosphorylation state of ERK1/2 in normal or PCOS theca cells. Moreover, in the absence of insulin treatment, CYP17 mRNA abundance and DHEA accumulation remains increased in PCOS theca cells as compared to normal theca cells [21]. These data suggest that alterations in MEK/ERK signaling, CYP17 mRNA accumulation, and androgen biosynthesis do not appear to be directly associated with insulin action [21]. Nonetheless, the conundrum still remains that insulin does in fact modulate androgen and CYP17 mRNA accumulation and other insulin-mediated signaling pathways differentially in normal and PCOS theca cells.

7. GENE EXPRESSION PROFILING AND CANDIDATE GENE ANALYSIS IN PCOS

Global gene expression profiling of normal and PCOS theca cells using subtractive suppressive hybridization and oligonucleotide microarray has provided data to suggest that dysregulation of androgen biosynthesis is associated with selective differences in several gene networks that are involved in steroid hormone biosynthesis as well as insulin and glucose homeostasis [2]. These analyses demonstrated that approximately 2% of genes expressed in the theca cell exhibit altered mRNA abundance in PCOS. Characterization of these genes revealed that specific enzymes involved in retinoic acid synthesis and metabolism are altered in the PCOS theca cell.

Further examination of the effects of retinoids on theca cells demonstrated that retinoid treatment differentially stimulated androgen biosynthesis and *CYP17* gene expression in normal and PCOS theca. The transcription factor GATA6 was also increased in PCOS as compared to normal theca cells. GATA6 is expressed in both the gonads and the adrenal and has been shown to activate the *CYP17*, *CYP11A1*, *STAR*, and cytochrome b5 promoters. Furthermore, both GATA6 transcription and mRNA stability were observed to be altered in PCOS theca cells. Additional data from array analysis have substantiated that cross talk between several signaling pathways, including the MAPK pathway, may be dysregulated in the PCOS ovary. For instance, there are data to suggest that the Wnt signaling pathway is altered. Tribble 3 (TRB3), which inhibits Akt/PKB phosphorylation, exhibits decreased gene expression in PCOS theca cells, and cAMP-GEFII, which augments Akt/PKB phosphorylation, exhibits increased gene expression in PCOS theca cells [22]. Thus, global gene expression profiling has identified potential pathways that may determine the PCOS theca cell phenotype. These observations reinforce the notion that PCOS theca cells have a unique molecular fingerprint suggestive of a genetic alteration or a stable epigenetic imprint. Additional investigation and characterization of these signaling networks in normal and PCOS theca cells could elucidate the molecular mechanisms underlying PCOS and provide insight regarding new modes of therapeutic treatment.

Genetic linkage studies have identified a candidate PCOS susceptibility locus D19S844 on chromosome 19p13.2 [3]. D19S884 is located in a nonconserved intronic sequence between exons 55 and 56 of fibrillin 3 (*FBN3*) [3]. The identity of the affected gene(s) or putative gene regulatory element associated with the PCOS phenotype is presently unknown. However, there are several known candidate genes in this region that are currently under investigation including FBN3, MAP2K7, and RETN [3]. FBN3 is a component of the microfibrils of the extracellular matrix. At first glance, there is little to suggest a connection between an extracellular matrix component and the morphological and metabolic phenotypes of PCOS. However, the family of fibrillin genes also regulates the action of the transforming growth factor beta (TGFβ) superfamily of signaling molecules, which include activin, inhibin, and the bone morphogenic proteins (BMPs), molecules that have known roles in controlling ovarian function.

MAP2K7, encodes mitogen activated protein kinase kinase 7 (MKK7), a mitogen-activated serine/threonine kinase that activates c-Jun N-terminal kinase in response to activation by growth factors, cytokines and stress. MKK7 phosphorylation has been observed to be increased in propagated PCOS theca cells, and infection of human theca cells with a MKK7 adenovirus increases CYP17 mRNA and DHEA accumulation [15]. The resistin (RETN) gene is also localized to this region. No evidence has been found for the association of variants in the *RETN* gene with hyperandrogenemia, obesity,

or insulin resistance in PCOS families [3]. However, serum resistin concentrations are elevated 40% in PCOS women as compared to control women and are indicative of body mass index and T levels, but not insulin resistance in women with PCOS. Moreover, since resistin has been shown to augment cAMP-dependent thecal P450c17 activity in vitro, abnormal resistin secretion in the PCOS ovary may contribute to abnormal androgen biosynthesis [23].

8. PARACRINE AND AUTOCRINE REGULATORS THAT MAY IMPACT OVARIAN STEROID ABNORMALITIES AND FOLLICULAR GROWTH IN THE PCOS OVARY

Paracrine and autocrine regulation in and between granulosa and theca cells is known to involve a variety of signals including hormones (androgens, progestins, ApoE), growth factors (inhibins, EGF, TGFα, TGFβ IGFs, KGF, BMPs, relaxins, INSL factors, endothelin, VEGF), cytokines (interleukins, TNF), and extracellular matrix components (fibronectin, laminin, collagen) [24–26]. However, as of yet, there are only a handful of factors that have been casually related to altered androgen biosynthesis and PCOS. For example, recent findings suggest that the steroidogenic capability of the developing follicle and corpus luteum is regulated by neovascularization and paracrine modulation by endothelial cells [27]. Specifically, steroidogenic cells produce and respond to angiogenic factors and vasoactive peptides.

Endocrine gland-derived vascular endothelial growth factor (EG-VEGF), an endothelial cell mitogen within steroidogenic tissues, is strongly expressed in the theca interna and stroma of the PCOS ovary. Vascular endothelial growth factor (VEGF) is mainly localized within the granulosa. Furthermore, cell and stage-specific expression of these two factors in PCOS ovaries suggests that they may coordinate angiogenesis and possibly ovarian cyst formation.

Another endothelial factor capable of regulating steroid biosynthesis in the PCOS ovary is endothelin. Elevated levels of endothelin have been observed in PCOS women, irrespective of BMI. Interestingly, endothelin levels are returned to normal following metformin therapy [28]. Endothelin receptors are expressed on human luteinizing granulosa and endothelin-1 inhibits P4 and estrogen production purportedly by decreasing both cAMP- and FSH-responsiveness [28]. Furthermore, endothelin-converting enzyme, an endopeptidase which converts big endothelin-1 to endothelin-1, is expressed in granulosa and theca cells of growing and preovulatory follicles. Examination of the role these endothelial factors play in the normal and PCOS ovary has been limited and further investigation is required.

Anti-Mullerian hormone (AMH), a member of the TGFβ superfamily, is produced by both granulosa and theca cells and reflects follicular development. PCOS

women have been reported to have increased serum levels of AMH which have been shown to be associated with an excessive number of growing antral follicles. Recent studies also indicate that prepubertal daughters of women with PCOS have increased serum AMH levels both during infancy and childhood, suggesting that follicular development may also be altered during infancy and childhood [29]. In contrast, lower amounts of AMH have been reported in PCOS granulosa cells of primordial and transitional follicles [30]. These findings indicate that a relative deficiency of AMH in primordial and transitional follicles may contribute to disordered early follicle development and annovulation in PCOS.

There are data to suggest that the action of other members of the TGFβ superfamily could be disrupted in the PCOS ovary. For instance, inhibin A and inhibin B concentrations are significantly reduced in the follicular fluid of women with PCOS compared with those in the follicular fluid of size-matched follicles from normal women, which is consistent with evidence that the mRNA expression of specific inhibin subunits is decreased in PCOS granulosa cells [7,31,32]. The finding that the TGFβ superfamily member, growth differentiation factor-9 (GDF-9), has reduced expression in PCOS oocytes is also interesting, in view of the observation that GDF-9 inhibits androgen biosynthesis in human ovarian theca cells maintained in long-term culture [32]. In addition, the BMPs have been shown to modulate follicle growth and development by influencing granulosa cell sensitivity to FSH and IGF and inhibiting thecal androgen production [33]. Theca cells produce BMP-4 and -7, which act in an autocrine/paracrine fashion upon both theca cells and neighboring granulosa cells to regulate steroidogenesis, cell proliferation, and peptide hormone secretion [33].

In luteinized granulosa cells from women with PCOS the matrix metalloproteinase to tissue inhibitor of metalloproteinase (MMP:TIMP) balance is shifted toward greater MMP activity. Cultured luteinized granulosa cells obtained from PCOS patients secrete higher levels of MMP-9 and MMP-2 compared to granulosa cells from normal ovulatory patients whereas the secreted basal level of TIMP-1 was similar in both types of granulosa cells. It is therefore reasonable to speculate that inappropriate extracellular matrix remodeling may contribute to dysregulated follicular development and atresia in the PCOS ovary [34].

In addition to the findings presented above, immunohistochemical and biochemical approaches have been utilized to identify differences in the expression and localization of a variety of potential regulators of ovarian follicular development and steroidogenesis in the normal and PCOS ovary. Differential expression of leptin in the PCOS ovary has been reported, whereas no differences in plasminogen activator inhibitor-1, and apoptotic-inducing factors Fas and Fas ligand were observed [32]. There are also data to suggest that the EGF receptor is upregulated in PCOS granulosa cells. A variety of other autocrine and paracrine factors have also been examined and implicated in the etiology of PCOS. Leukemia inhibitory factor, a cytokine expressed

in endometrial and trophoblast cells and required for implantation, is decreased in follicular fluid in PCOS patients and has been reported to be associated with early pregnancy loss in PCOS [32]. In addition, circulating levels of tumor necrosis factor α have been reported to be elevated in women with PCOS [32]. However, the collective impact of these findings on ovarian androgen biosynthesis is presently unknown.

9. SUMMARY

Although we are learning significantly more about the dysregulation of androgen and estrogen biosynthesis in the PCOS ovary, it is evident that there are a wide array of studies that successfully determine the genetic and/or molecular and cellular basis for PCOS. To reach this objective, clinical and basic science investigators will need to continue to combine forces and design new collaborative and complementary studies utilizing both in vivo and in vitro approaches to further examine the basis of altered steroid biosynthesis and folliculogenesis in the PCOS ovary.

Unfortunately, one of the major obstacles for investigators examining biochemical and molecular processes in the PCOS ovary, without question, is the increasing difficulty in obtaining fresh ovarian specimens, as well as other tissue, from well-characterized PCOS and normal patients. For in vitro studies, there are distinct benefits to performing experiments with ovarian cells in primary culture as well as long-term culture, and investigators will need to make coordinated efforts to work together and perform studies using both systems. Comparable studies focusing on alterations in steroid biosynthesis, cellular function, and gene expression in PCOS granulosa, luteal, stromal cells, as well as epithelial cells and oocytes, are necessary.

Given that PCOS is a genetic disorder, future studies examining alterations in endometrial, testicular, and adrenal steroid biosynthesis in PCOS are warranted to fully understand the common mechanisms underlying defects in androgen biosynthesis in PCOS. Moreover, investigators examining the ever-increasing array of network signaling cascades in various tissues affected in women with PCOS (i.e., adipose, muscle, ovary, endometrium, skin), should also work together with similar and overlapping scientific approaches to more fully understand the primary and/or full array of signaling defect(s) in PCOS.

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Chapter 6

Role of Obesity and Adiposity in PCOS

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The polycystic ovary syndrome (PCOS), one of the most common causes of hyperandrogenism and chronic oligo-anovulation, affects 4–7% of women [1]. The clinical features of PCOS are heterogeneous and may change throughout the lifespan, starting from adolescence to postmenopausal age. Among other factors, this is largely dependent on the influence of obesity and metabolic alterations, including an insulin resistant state and the metabolic syndrome, which consistently affect most women with PCOS [2].

This represents an important factor in the evaluation of PCOS throughout life and implies that PCOS, by itself, may not be a hyperandrogenic disorder exclusively restricted and relevant to young and fertile-aged women, but may also have some health implications later in life. Whereas in young women with PCOS, hyperandrogenism and menstrual irregularities are the major complaints, symptoms related to androgen excess, oligomenorrhea or amenorrhea, and consequent infertility, represent the major complaints of adult PCOS women during the reproductive age.

Obesity has an important impact on the progression and severity of these manifestations in proportion to its degree, particularly in the presence of the abdominal type of obesity [2]. Obesity also renders affected women more susceptible to develop type 2 diabetes mellitus (T2DM), although there are some differences in the prevalence rates between countries and ethnicities, and potentially favors the development of cardiovascular disease (CVD). While the pathophysiology of PCOS has a genetic component (see Chap. 3), it is likely that the main factors responsible for the increasing prevalence of PCOS are related to the influence of the environment, including dietary habits and other still undefined factors [1].

This chapter focuses on the prevalence of obesity in women with PCOS and its pathophysiologic role in the development of the PCOS phenotype, with specific reference to clinical and biochemical hyperandrogenism, menstrual

abnormalities, and infertility. Metabolic alterations, including insulin resistance, the metabolic syndrome, and states of glucose intolerance will also be discussed.

1. PREVALENCE OF OBESITY IN PCOS

The prevalence of PCOS in the general population is approximately 6% according to the major clinical studies published so far [3] (see Chap. 1). These studies have been performed in several European and North-American populations, with a total of 1,253 women investigated [3]. Notably, the populations included in these studies were different in ethnicity (Caucasians, European, Black, and White Americans), as were the recruitment criteria for the studies (preemployment evaluation, blood donors, or women accepting an invitation to participate in a free-medical examination), whereas the prevalence rates proved remarkably similar, at least in four studies. Overall, these studies have several limitations, the most evident of which is that none of them was performed according to the classical epidemiological rule, which is the random selection of a representative large cohort of women living in a well-defined geographical area or a specific country. Although the finding of a similar prevalence rate in most of the available studies renders this methodological flaw less unacceptable, nonetheless it is quite clear that well-designed epidemiological studies need to be performed in order to achieve more accurate estimates worldwide. Notably, an increased prevalence of PCOS has been reported for women who present with gestational diabetes and T2DM.

We are facing a worldwide public health emergency due to the increasing epidemic of obesity and related disorders. The problem of obesity has achieved global recognition only in the past 10–15 years. Recent estimates of the prevalence of obesity, based on the body mass index (BMI) measurement in appropriate population samples, demonstrate that its increasing prevalence is recognized worldwide, with few exceptions. The International Obesity Task Force estimates that at least 1.1 billion adults are currently overweight worldwide, including 312 million who are obese [4]. Furthermore, with the use of new Asian BMI criteria, the number of affected individuals may be even higher. Most importantly, there is emerging evidence that obesity is increasing not only in adults, but also in children, where prevalence rates of more than 10% have been reported, particularly in Western countries. This may be particularly relevant for PCOS, whose clinical manifestations generally first appear at the time of adolescence, as obesity may unfavorably affect the natural history of the hormonal (androgen secretion) and metabolic changes characterizing the transition from prepuberty to adulthood [5].

It is possible that the increasing epidemic of obesity may be one of the factors responsible for the worldwide increased incidence of young women attending endocrinologic or gynecological clinics because of menstrual irregularities, clinical hyperandrogenism, and infertility, features consistent

with the PCOS phenotype. The association between obesity and alterations of reproductive function in women was recognized long ago. In Stein and Leventhal's original description, obesity, together with hirsutism and infertility, represented one of the characteristics of the syndrome that eventually bore their names. The association between obesity and signs of androgen excess, menstrual alterations, and infertility, was subsequently confirmed in epidemiological and clinical studies (reviewed in [2]).

The prevalence of obesity in PCOS appears to be much greater than that expected in the general population. Although the cause of this association remains unknown, a recent comprehensive review by Ehrmann [1] noted obesity in more than 30% of PCOS patients and in some series, a prevalence of as high as 75%. In the few epidemiological articles cited above, the prevalence of obesity among PCOS women ranged from 40 to 60%. In our large cohort of PCOS women, we observed that 18% were overweight (BMI 25–29.9 kg M^{-2}) and 43% were obese (BMI ≥ 30 kg M^{-2}) [3].

2. PATTERN AND IMPLICATIONS OF BODY FAT DISTRIBUTION IN WOMEN WITH PCOS

Obesity tends to be abdominal in its distribution in PCOS women, and even lean affected women may have a fat distribution favoring visceral depots, particularly in the abdomen [2]. This is likely due to the action of androgens on the regulation of fat metabolism, differentiation, and morphology, through specific receptors whose distribution and characteristics vary according to the different fat depots [6]. In fact, stimulation with androgens appears to upregulate the expression of their own receptors in fat [6]. Androgens stimulate lipolysis in adipose tissue and, when administered chronically, they induce an antiadipogenic effect, at least in primary cultured preadipocytes [6]. In isolated cultured differentiated adipocytes from omental and abdominal subcutaneous fat obtained from overweight or obese individuals, testosterone in physiological concentrations caused a depot-specific reduction in catecholamine-stimulated lipolysis in subcutaneous fat cells. This effect may be result from reduced protein expression of β3-adrenoreceptors and hormone sensitive lipase (HSL), the principal regulatory factors of the lipolytic pathways (see [7]).

The action of androgens on adipose tissue is part of the biology of human sex differences. Insulin acts to inhibit lipolysis, potentially stimulating fat deposition. Alternatively, in men, testosterone acts as a lipolytic hormone, therefore acting to somewhat counteract the insulin and dietary-induced increase in visceral fat depots. The obese male phenotype, which is associated with a reduction in testosterone blood levels [8], as well as classic hypogonadal men, is in fact typically associated with increased abdominal fat distribution and an enlargement in visceral fat depots [8].

In contrast, in normal weight and normal insulin sensitive women, adipocytes release limited amounts of free fatty acids (FFAs) while demonstrating normal amounts of lipoprotein lipase (LPL); normal testosterone levels support the action of insulin in suppressing FFA release (i.e., antilipolytic effect) from adipocytes [8]. Obese women actually demonstrate increased production of FFAs and inhibition of LPL secretion, due to the presence of hyperinsulinemia secondary to their prevalent insulin resistance [8]. In this setting, increased androgens further aggravate the detrimental effects of insulin on FFA release from adipocytes.

This gender difference in the effect of testosterone on adipocyte lipolysis may relate, in part, to differences in the hormonal milieu. While testosterone would be expected to reduce abdominal obesity in women, as it does in men, this does not occur because of the protective effect of estrogens. In fact, the androgen receptors in female adipose tissue seem to have the same characteristics as those found in male adipose tissue, but estrogens down-regulate the density of these receptors [8]. Furthermore, the addition of androgens may further increase the pool of circulating estrogens via aromatization. As such, it appears clear that testosterone increases visceral fat deposition in women [8]. Female-to-male transsexuals treated with testosterone do in fact have an increase in visceral fat only after being oophorectomized [8]. In addition, administration of androgens to postmenopausal women has been documented to increase visceral fat content, while reducing subcutaneous fat [8].

Testosterone may therefore favor the development of the abdominal fat distribution pattern in hyperandrogenic states. In a series of 121 consecutive women with PCOS, we demonstrated that approximately 60% had an abdominal fat distribution, regardless of BMI [6]. A high prevalence of the abdominal phenotype has been observed even in normal weight PCOS patients [6]. These data imply that abdominal adiposity may be regarded as a common feature of hyperandrogenic states and consequently may have some, albeit still poorly defined, role in the pathophysiology of the metabolic abnormalities associated with the abdominal obesity phenotype.

3. PATHOPHYSIOLOGY OF OBESITY ON THE HYPERANDROGENIC, OVULATORY, AND METABOLIC ALTERATIONS OF PCOS

The high prevalence of obesity in women with PCOS has profound effects on both the pathophysiology and the clinical manifestations of the disorder. The pathophysiologic mechanisms by which obesity influences the expression of PCOS are complex and not completely understood (see [2,6] for recent reviews). In the following sections, we will focus on the role of obesity in the

development of hyperandrogenism and associated metabolic alterations, principally insulin resistance and the metabolic syndrome.

3.1. Hyperandrogenism, and Ovulatory and Menstrual Dysfunction

Obesity has profound effects on the clinical, hormonal, and metabolic features of PCOS, which largely depend on the degree of excess body fat and on the pattern of fat distribution. Various studies have evaluated the impact of obesity on the hyperandrogenic state in women with PCOS. They have uniformly demonstrated that obese PCOS women are characterized by significantly lower sex hormone-binding globulin (SHBG) plasma levels and more severe hyperandrogemia, in comparison to their normal-weight counterparts. In addition, a negative correlation has been reported between body fat mass and circulating androgens in PCOS. It has also been repeatedly reported that a higher proportion of obese PCOS women complain of hirsutism and menstrual disorders compared to normal-weight patients. There is, therefore, consistent evidence that increasing body weight may favor a more severe form of hyperandrogenism in women with PCOS [2].

Menstrual abnormalities and chronic oligo-anovulation are dependent, among other factors, on the exaggerated ovarian androgen production. They are more frequent in overweight and obese subjects than in normal-weight PCOS women. There is also evidence that blunted responsiveness to pharmacological treatments for induction of ovulation, such as clomiphene citrate or gonadotropin administration, may be more common in obese PCOS women [6]. In addition, compared to normal-weight women, obese PCOS women may have lower ovulatory responses to pulsatile gonadotropin-releasing hormone (GnRH) analog administration and lower pregnancy rates after gonadotropins, including low-dose human menopausal gonadotropin (hMG) or pure FSH administration [6]. Altered spontaneous or stimulated ovulation are, however, also dependent on insulin resistance and hyperinsulinemia, which are much more marked in obese PCOS women, particularly those with the abdominal phenotype [3,6]. In fact, administration of insulin-sensitizing agents (metformin and thiazolidinediones) has been repeatedly associated with improved ovulatory and menstrual cyclicity [9] and clomiphene-induced ovulation rates [10], even if androgen levels are not significantly modified.

The pathophysiologic mechanisms underlying the relationship between obesity and PCOS, and the mechanisms involved in determining hyperandrogenism and associated infertility, have been extensively reviewed [6]. It is likely that the mechanisms by which excess androgen production occurs in PCOS women differ in part depending on whether the patients are normal weight, overweight, or obese. Briefly, the main factors responsible for the differences include gonadotropins, insulin, estrogens, the growth hormone (GH) insulin-like growth factor 1 (IGF-1) axis, the hypothalamic–pituitary– adrenal axis, opioids, leptin, and the insulin-like factor 3 (INSL3). These are discussed as follows.

3.1.1. The Gonadotropic Axis. An increase in circulating luteinizing hormone (LH) levels, as a result of a GnRH-mediated increase in the amplitude and frequency of pulsatile LH secretory pattern, is inconsistently found in PCOS women [11,12]. The occurrence of spontaneous ovulation is associated with normalization of LH secretion in PCOS women. However, the gonadotropin secretion is markedly affected by the presence of obesity in PCOS. In fact, LH concentrations are inversely related to body weight in PCOS women, associated with decreased LH pulse amplitude and a decreased LH response to GnRH stimulation. In contrast, increased LH concentrations are a common finding in normal-weight women with PCOS. Obesity, therefore, attenuates the role of altered gonadotropin secretion in the pathogenesis of hyperandrogenism in PCOS women.

In females, insulin acts as a true gonadotropic hormone [13,14]. At the ovarian level, acting through its own and the IGF-I receptor, insulin synergizes LH action and stimulates ovarian steroidogenesis in both granulosa and theca cells. In addition, insulin appears to increase pituitary sensitivity to GnRH action [4]. Notably, a large number of PCOS women demonstrate insulin resistance and compensatory hyperinsulinemia, and ovarian androgen production can, in this way, be over-stimulated. Interestingly, it is not completely clear why this over-responsiveness of ovarian androgen secretion does not occur in insulin-resistant hyperinsulinemic women without PCOS. Estrogens may be involved, since obesity is associated with increased production of estrogens in the adipose tissue. Excess estrogens may exert positive feedback regulation on gonadotropin release, in turn stimulating a rise in ovarian androgen production [15].

3.1.2. The GH/IGF-1 Axis. Alterations of the GH/IGF-1 system activity may also play a role in favoring altered ovarian androgen secretion and granulosa cell function in PCOS [14]. The bioavailability of IGF-1 is increased in normal-weight PCOS women, probably because of the insulin-induced suppression of hepatic and ovarian IGF-binding protein-1 (IGFBP1) and the GH-induced stimulation of hepatic IGF-1. Alternatively, IGF-1 bioavailability appears to be reduced in obese PCOS women, due to the obesity-related reduction in basal and stimulated GH concentrations and the inhibitory effect of high circulating insulin on IGF-1. Given the close interaction between insulin and IGF-1 in stimulating ovarian steroidogenesis, it is likely that insulin excess, rather than IGF-1, has the major responsibility in stimulating androgen production in obese PCOS women. By contrast, high IGF-1 action at ovarian level may be more relevant in normal weight PCOS women.

3.1.3. The Adrenal Cortex. The role of the adrenal glands in determining androgen excess in PCOS has been debated for a long time, since increased levels of the adrenal androgen metabolite dehydroepiandrosterone sulfate (DHEAS), which is predominantly of adrenal origin, are frequently

observed in these patients. Recent data suggest that hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis may occur in obese individuals, particularly those with abdominal obesity [16], and may be in part responsible for increased testosterone levels in women with obesity and PCOS. An increased response of adrenal androgens, and adrenocorticotropic hormone (ACTH) and cortisol, to corticotrophin releasing hormone (CRH) administration is observed in a subset of obese PCOS women [7].

Conditions of hypercortisolism, such as Cushing's syndrome, are good examples of how the HPA axis may differently regulate gonadal function according to gender. In men, Cushing's syndrome is associated with reduced gonadotropin levels and low testosterone concentrations, regardless of the extent of hypercortisolism. Alternatively, women with Cushing's syndrome and mild hypercortisolism may present with androgen excess of both adrenal and ovarian origin, and polycystic ovaries; however, when the hypercortisolism is severe the gonadal axis is inhibited, similar to what is observed in men with Cushing's syndrome. A recent study [17] has observed a positive relationship between the HPA axis activity (measured by the ACTH and cortisol response to a combined stimulation with human CRH plus arginine–vasopressin [AVP]) and free testosterone levels in obese females; in contrast, obese men demonstrated a negative relationship between the activity of the HPA axis and free testosterone levels. These findings suggest that, at least in PCOS women with abdominal obesity, increased HPA axis activity may play a role in determining the higher testosterone levels.

3.1.4. The Opioid System. As in obesity, PCOS women are characterized by increased opioid system activity and increased levels of plasma immunoreactive β-endorphin (reviewed in [2]). In humans, β-endorphin administration increases insulin secretion from the pancreatic β-cells [2]. Inhibition of the opioid tone may decrease the degree of hyperinsulinemia in PCOS women, secondary to reduced insulin secretion and improved hepatic clearance. The administration of β-endorphin has been found to reduce LH release in normal but not PCOS women, suggesting a condition of β-endorphin resistance in this disorder. Alternatively, the infusion of physiological doses of β-endorphin has been observed to induce a significant increase in insulin concentrations in obese, but not in normal-weight subjects, suggesting β-cell hypersensitivity to opioids in obesity. Moreover, administration of opioid antagonists has been found to suppress basal and glucose-stimulated insulin blood levels in obese women, particularly in those with the abdominal phenotype, but not in normal-weight controls [2]. Finally, increased β-endorphin responsiveness to acute CRH administration has also been observed in women with abdominal obesity [2]. These data suggest that increased opioid tone would be expected to play a role in the hyperinsulinemia of obese PCOS women, although no studies confirming this hypothesis are available as yet.

3.1.5. Leptin. Leptin, a product of the *OB* gene, is an adipose-derived messenger to the brain reflecting the amount of energy stored and is one of the most important orexigenic hormones acting at the central neuroendocrine nuclei to control food intake and energy balance [18]. Obesity is characterized by increased leptin concentrations, and hyperleptinemia is thought to be indicative of leptin resistance at central levels, thereby explaining the lack of reduced feeding in the presence of excess leptin concentrations. Leptin, however, is also a crucial hormone for gonadal function and reproduction [19], regulating the gonadal axis at both central and peripheral levels. In fact, leptin regulates GnRH and gonadotropin secretion, leptin receptors being highly expressed in the hypothalamus. In addition, high leptin concentrations in the ovary may participate in the regulation of theca cell function and interfere with the development of dominant follicles and oocyte maturation [18]. In addition, leptin appears to directly stimulate ovarian 17α-hydroxylase activity and is involved in both ovarian and adrenal steroidogenesis. To date, contradictory results have been reported on leptin levels in women with PCOS, and either higher levels than expected for BMI or normal concentrations have been detected [6]. Whether high leptin levels play a role in determining increased ovarian androgen production in PCOS has not been adequately investigated as yet.

3.1.6. Insulin-Like Factor 3. INSL3 is a member of the relaxin-insulin family and is produced by the Leydig cells in the testis and, at reduced levels, by ovarian theca interna cells of antral follicles, the corpora lutea, and the ovarian stroma [20]. Among the factors potentially involved in the stimulation of the gonadal expression of INSL3, recent data obtained in rats suggests an important role for LH [21]. The ovaries from most women affected by PCOS are characterized by hyperplasia of the theca interna and of the cortical stroma and by an increased number of small antral follicles. The majority of women with PCOS, particularly normal-weight subjects, also have LH levels that are above the normal range. In a recent study [22], we measured INSL3 circulating levels in a group of women with PCOS, both normal-weight and overweight/obese, and compared them to appropriate age and BMI-matched controls. In PCOS women, we also investigated the association of INSL3 with the gonadotropin and androgen pattern and with the ovarian morphology. We found that INSL3 serum concentrations were significantly higher in normal-weight PCOS women, but not in overweight/obese affected patients, compared to matched controls. Moreover, INSL3 serum concentrations were significantly and positively correlated with androgen levels and LH concentrations not only in PCOS, but in all women. Finally, in PCOS women, INSL3 levels were significantly correlated with ovarian follicle number, but not with ovarian volume. These data are consistent with the possibility that LH-mediated ovarian androgen hypersecretion is mediated by INSL3, at least in normal-weight PCOS. Accordingly, these findings also introduce new

concepts in the pathophysiology of ovarian hyperandrogenism in PCOS women, which may partially differ according to the different phenotypes.

Other potential factors involved in favoring excess androgen production in obese PCOS women, but not discussed, include diet, ghrelin, and the endocannabinoid system [2,6]. A simplified overview of the various factors influencing ovarian androgen production in PCOS women, according to their adiposity phenotype, is depicted in Fig. 1.

3.2. Hyperinsulinemia, Insulin Resistance and the Metabolic Syndrome

PCOS women are characterized by a high prevalence of several metabolic abnormalities that are strongly influenced by the presence of obesity. Adequate confirmation of the role of obesity in determining hyperinsulinemia and insulin resistance in women with PCOS derives from studies

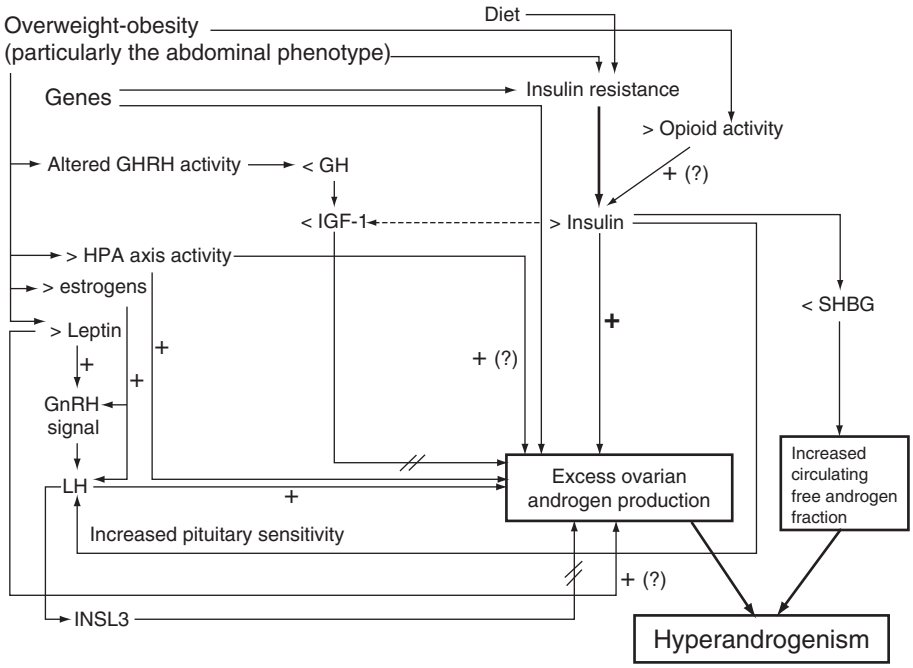

Fig. 1. A schematic representation of the balance of factors determining hyperandrogenism in obese PCOS women. The genetic background can influence excess androgen production directly or through hyperinsulinism. Dietary factor may have an additional, albeit still undefined, role in favoring insulin resistance and hyperandrogenemia. **Abbreviations:** GH is growth hormone; GHRH is growth hormone–releasing hormone; IGF-1 is insulin-like growth factor 1; GnRH is gonadotropin-releasing hormone; HPA is hypothalamic–pituitary– adrenal axis; INSL3 is insulin-like factor 3; LH is luteinizing hormone; SHBG is sex hormone-binding globulin; and \\, no effect (although the factor may play a role in normal weight PCOS women).

comparing groups of normal-weight and obese PCOS women. Both fasting and glucose-stimulated insulin concentrations are significantly higher in obese than in nonobese PCOS subgroups [2,11]. Studies using the euglycemic–hyperinsulinemic clamp technique, the frequently sampled intravenous glucose tolerance test (FSIVGTT), or the intravenous insulin tolerance test have clearly demonstrated that obese PCOS women have significantly lower insulin sensitivity than their nonobese PCOS counterparts and, therefore, a more severe insulin resistant state (reviewed in [5]). The percentage of women affected by PCOS and obesity who present with glucose intolerance is rather high, ranging from 20 to ~50 [11]; this prevalence rate is higher than that reported in population-based studies of premenopausal women. In contrast, glucose intolerance in normal-weight PCOS women is uncommon [11]. Collectively, these data suggest that obesity per se plays an important role in altering the insulin–glucose system in PCOS women.

Various studies in American, Asian, and Italian cohorts have indicated that women with PCOS have a tendency toward the early development of T2DM and that its prevalence was higher in these women when compared to the general population, regardless of ethnicity and geographic area [3]. Interestingly, in all these studies it was also found that T2DM occurred almost always in women who were obese and very rarely in their nonobese counterparts. Obesity therefore seems to favor the development of T2DM in PCOS women.

Several recent studies have identified defects of insulin secretion in obese women with PCOS. Using the FSIVGTT, Dunaif and colleagues reported that obese PCOS women mount an inadequate insulin secretory response to compensate for their peripheral insulin resistance, suggesting relative β-cell dysfunction [11]. However, regardless of the degree of alteration in insulin secretion, in a 10-year follow-up study, we found that both fasting and glucose-stimulated insulin and C-peptide levels tended to increase spontaneously and significantly in PCOS women with age, suggesting a worsening insulin resistant state over time [23]. In the same study, we also found that several women developed impaired glucose tolerance. Longitudinal data are therefore warranted to investigate which factors, namely progressive insulin resistance and/or subtle alterations of insulin secretion, can predict the susceptibility of obese PCOS women toward the development of T2DM. Although PCOS per se may be associated with alterations of both lipid and lipoprotein metabolism, the coexistence of obesity usually leads to a more atherogenetic lipoprotein pattern. A greater reduction in high-density lipoprotein (HDL), together with higher levels of both triglycerides and total cholesterol levels, have been observed in obese PCOS women compared to their normal-weight counterparts (reviewed in [2]).

Many women with PCOS may present with features characteristic of the metabolic syndrome. Defining the metabolic syndrome is a difficult task, and, over the past few years, many definitions have been proposed, focusing on the association between obesity, abdominal fat distribution, dyslipidemia, and

other cardiovascular risk factors. One of the most commonly used definitions is the one proposed by the National Cholesterol Education Program Expert Panel (NCEP/ATPIII) in the US [24]. The prevalence of the metabolic syndrome is very high in the general population, with significant variability according to the prevalence of various environmental factors, ethnicity, and geographic distribution; regardless, available data suggest that the prevalence of the metabolic syndrome is significantly higher in women with PCOS, ranging from 35 to 50% [3]. As expected, the majority of these women are obese, and most of them are characterized by the presence of the abdominal phenotype. However, the metabolic syndrome may also be present in apparently normal-weight PCOS women, although to a much lesser extent.

There are several reasons to suggest that insulin resistance and the metabolic syndrome should be considered separate entities. Very few studies describing the relationship between reliable measurements of insulin resistance and all of the components of each cluster of features used to define the metabolic syndrome have been reported. In a large group of healthy volunteers, Cheal et al [25] found that, although insulin resistance and the presence of the metabolic syndrome were significantly associated, the sensitivity and positive predictive values only equaled 46 and 76%; the presence of overweightness, high triglycerides, low HDL-cholesterol, or elevated blood pressure were the most common factors included in the diagnosis of the metabolic syndrome itself.

In a study performed in a cohort of 289 PCOS women with a wide range of BMIs, comparing them to age-matched normal weight healthy control women, we determined the prevalence of insulin resistance (measured by simple mathematical tests and insulin concentrations). We also determined how many women with PCOS and the metabolic syndrome, according to the NCEP/ATP III criteria, were insulin-resistant compared to PCOS women without the metabolic syndrome [6]. We found that 55% of PCOS women had fasting hyperinsulinemia, 37% had higher values of insulin resistance determined by the homeostasis model assessment (HOMA-IR), and 49.5% had a higher insulin sensitivity index (ISI), i.e., higher insulin resistance, as determined by the response to an oral glucose tolerance test [6]. These data indicate that 40–50% of our PCOS subjects were insulin resistant. Moreover, we found that in those PCOS women with the metabolic syndrome, 87.3% had hyperinsulinemia, 74.6% had higher HOMA-IR values, and 79.4% had higher ISI values, compared to 54.7, 32.8 and 56.7%, respectively, in those without the metabolic syndrome, a significant difference. Notably, the mean BMI was significantly higher in the PCOS women with the metabolic syndrome than without. Overall, it appears that insulin resistance is present in at least 70–85% of women with PCOS and the metabolic syndrome and that obesity plays a major role in determining which women will develop the metabolic syndrome.

There is considerable debate as to whether women with PCOS are susceptible to an increased risk of CVD [26]. Recently, a growing amount of data have

indicated that states of insulin resistance, such as T2DM, obesity (particularly the abdominal phenotype), and PCOS, are characterized by evidence of impaired coagulation and fibrinolysis, anatomical and functional endothelial injury, vascular dysfunction, and chronic subclinical inflammation, all of which represent independent risk factors for CVD. Despite the fact that obese PCOS women have been found to demonstrate a worse profile in these pro-atherosclerotic risk factors, it nonetheless remains controversial whether this increased risk is primarily related to the obesity and the consequent insulin resistant state or to PCOS per se.

4. SUMMARY AND PERSPECTIVES

Obesity is a costly and increasingly prevalent condition in Western societies. Among other comorbidities, it is frequently associated with reduced fertility and signs and symptoms of androgen excess. In women with PCOS, obesity is very common, although its prevalence in this disorder has not been estimated on a strictly epidemiologic basis. Intriguingly, obesity has an important pathophysiologic impact on PCOS, and obese PCOS women are characterized by a worsened endocrine and metabolic profile and poorer fertility. Moreover, there is some evidence that the pathogenetic factors involved in determining the hyperandrogenism and metabolic abnormalities may differ somewhat between overweight/obese and lean PCOS women; some of these mechanisms may be amplified or reduced according to the presence of excess body weight. These findings emphasize the concept that PCOS is a heterogeneous disorder potentially involving different pathophysiologic mechanisms, according to the degree of obesity and the distribution of the adiposity.

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Chapter 7

Strategies for Ovulation Induction in the Management of Anovulatory Polycystic Ovary Syndrome

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The management of anovulatory infertility in the polycystic ovary syndrome (PCOS) has traditionally involved the use of clomiphene citrate (CC) and then gonadotropin therapy or laparoscopic ovarian surgery, in those who are clomiphene resistant. There is no clear role for insulin sensitizing and insulin lowering drugs, and algorithms for their place in therapy are still to be agreed upon. Newer therapeutic approaches include aromatase inhibitors and the potential use of in vitro maturation (IVM) of oocytes collected from unstimulated (or minimally stimulated) polycystic ovaries. There has been an unfortunate shift away from monofollicular ovulation induction to the use of in vitro fertilization treatment (IVF), based on a false premise of greater cumulative conception rates and appropriate concerns about multiple pregnancy. Superovulation for IVF presents significant risks for women with polycystic ovaries, namely the potentially life-threatening complication of ovarian hyperstimulation syndrome (OHSS). Carefully conducted and monitored ovulation induction can achieve good cumulative conception rates, and, furthermore, multiple pregnancy rates can be minimized with strict adherence to criteria that limit the number of follicles that are permitted to ovulate.

1. PCOS AND ANOVULATORY INFERTILITY: THE BASICS

The PCOS accounts for approximately 80% of women with anovulatory infertility. Various factors influence ovarian function, and fertility is adversely affected by an individual being overweight or having elevated serum concentrations of LH. The principles of therapy are first to optimize health before commencing

therapy and then induce regular unifollicular ovulation, while minimizing the risks of OHSS and multiple pregnancy. Weight loss, in those who are overweight, improves the endocrine profile, the likelihood of ovulation and a healthy pregnancy, and the response to every type of ovulation induction therapy.

Strategies to induce ovulation include first weight loss, then drugs to induce ovulation with conventional first line therapy being oral antiestrogens (principally CC), parenteral gonadotropin therapy, and laparoscopic ovarian surgery. There have been no adequately powered randomized studies to determine which of these therapies provides the best overall chance of an ongoing pregnancy.

Appropriate pretreatment investigations are required including a semen analysis of the male partner and an assessment of tubal patency. There are some who consider the latter unnecessarily invasive in those who are at low risk of tubal damage. We consider, however, that it is important to exclude a covert tubal obstruction before committing to the time, expense, and risks associated with ovarian stimulation.

Normal ovarian function relies upon the selection of a follicle, which responds to an appropriate signal (follicle stimulating hormone [FSH]) in order to grow, become "dominant," and ovulate. This mechanism is disturbed in women with PCOS, resulting in multiple small cysts, most of which contain potentially viable oocytes but within dysfunctional follicles. Hypersecretion of luteinizing hormone (LH) is found in 40% of women with PCOS and is associated with a reduced chance of conception and an increased risk of miscarriage, possibly through an adverse effect of LH on oocyte maturation [1]. The finding of a persistently elevated early to midfollicular phase LH concentration in a woman who is trying to conceive suggests the need to suppress LH levels by either pituitary desensitization, with a gonadotropin-releasing hormone agonist, or laparoscopic ovarian diathermy (LOD). There are, however, no large prospectively randomized trials that demonstrate a therapeutic benefit from a reduction in serum LH concentrations during ovulation induction protocols. The assessment of serum LH concentration in the midfollicular stage of the stimulated cycle is helpful in predicting the likelihood of a successful outcome – particularly in the context of CC therapy (see below).

Elevated serum concentrations of insulin are more common in both lean and obese women with PCOS than weight-matched controls with normal ovaries. Indeed, it is hyperinsulinemia that many feel is the key to the pathogenesis of the syndrome, as insulin stimulates androgen secretion by the ovarian stroma and appears to affect the normal development of ovarian follicles, both by the adverse effects of androgens on follicular growth and possibly also by suppressing apoptosis and permitting the survival of follicles otherwise destined to disappear.

2. LIFESTYLE AND WEIGHT LOSS IN PCOS

The patient's body mass index (BMI) correlates with both hyperinsulinemia and an increased rate of cycle disturbance and infertility [2,3]. The greater the interval between menstrual periods, the greater the disturbance in insulin metabolism [4]. Even moderate obesity, such as a BMI > 27 kg m^{-2}, is associated with a reduced chance of spontaneous ovulation [5] or response to ovulation induction therapy [6]. A body fat distribution leading to an increased waist:hip ratio (WHR) appears to have a more detrimental effect than body weight alone, due to the metabolic activity of visceral fat [7]. Monitoring treatment is also harder in obese women because their ovaries are more difficult to see on ultrasound scans, thus raising the risk of missing multiple ovulation and multiple pregnancy. National guidelines in the United Kingdom for managing overweight women with PCOS advise weight loss, preferably to a BMI of less than 30 kg m^{-2}, before commencing drugs for ovarian stimulation [8].

Clark and colleagues [9] studied the effect of a weight loss and exercise program on women with a BMI > 30 kg m^{-2} and anovulatory infertility who were CC-resistant. The emphasis of the study was a realistic exercise schedule combined with positive reinforcement of a suitable eating program over 6 months. Thirteen out of the 18 women enrolled completed the study, reinforcing the difficulties some individuals have in sustaining even moderate changes in lifestyle. Weight loss had a significant effect on endocrine function, ovulation, and the chance of pregnancy. Fasting insulin and serum testosterone concentrations fell, and 12 of the 13 subjects resumed ovulation; 11 becoming pregnant – five spontaneously and the remainder were now responsive to CC.

Thus, with appropriate support, patients with PCOS may ovulate spontaneously without medical therapy. An extension of this study, in women with a variety of diagnoses, demonstrated that in 60 out of 67 PCOS subjects, weight loss resulted in spontaneous ovulation with lower than anticipated rates of miscarriage and a significant saving in the cost of treatment [10]. Even a modest loss of 5% of total body weight can achieve a reduction of central fat, an improvement in insulin sensitivity, and restoration of ovulation. Lifestyle modification is clearly a key component for the improvement of reproductive function in overweight women with anovulation and PCOS [8].

In a recent editorial we argued that the considerable risks in pregnancy associated with obesity are not usually appreciated when patients with PCOS attend clinics and request fertility treatment [11], and we posed the question as to whether it is appropriate to offer treatment or to insist on weight loss. Or does any overweight woman have the right to receive treatment, irrespective of the possible outcome?

It is well known that pregnancy carries considerable risks for women who are obese, including increased rates of congenital anomalies (neural tube and cardiac

defects), miscarriage, gestational diabetes, hypertension, and problems during delivery [12,13]. In addition, women with PCOS and obesity have an increased risk of gestational diabetes because of the additional insulin resistance caused by pregnancy itself [14]. Increasingly many women with PCOS have type 2 diabetes mellitus (DM) prior to conception. The outcomes of pregnancy in women with DM are much worse than in the general population and are at least equivalent to, if not slightly worse than, in women with type 1 diabetes [15]. Overweight mothers are also more likely than others to have hypertension and thromboembolism, leading to a higher risk of maternal mortality [16].

The use of insulin lowering or sensitizing agents has excited much interest in the management of PCOS, but even metformin is less effective for women with anovulation and extreme obesity, although perhaps a higher dose is required than currently prescribed [17]. Many obese women who wish to conceive are now prescribed metformin, often at body weights greater than would be permissible for treatment to induce ovulation. Those who ovulate and conceive while remaining obese will have to face considerable additional risks during pregnancy. Is it appropriate to treat these women with metformin unless they have already lost weight? At the very least, the risks of the pregnancy to mother and child should be explained, understood, and actively managed before embarking on treatment. The importance of encouraging and achieving weight loss as first line treatment cannot be overestimated.

We suggest that women with obesity and PCOS should try to attain a BMI of less than 30 kg m^{-2} prior to commencing ovulation induction and defer even treatment with metformin until they reach a target BMI of 35 kg m^{-2} or less. Consideration of age is of course important, yet ultimately the main consideration should be for the potential health of the pregnancy and any children born.

3. CC THERAPY

Antiestrogen therapy with CC or tamoxifen has traditionally been used as first line therapy for anovulatory PCOS [18]. Clomiphene citrate has been available for many years, and its use has tended not to have been closely monitored.

Antiestrogen therapy is usually commenced on day 2 of the cycle and given for 5 days. If the patient has oligo/amenorrhea, it is necessary to exclude pregnancy and then induce a withdrawal bleed with a short course of a progestogen, such as medroxyprogesterone acetate 5–20 mg/day for 5–10 days. The starting dose of CC is 50 mg/day, for 5 days beginning on days 3–5 of the menstrual cycle (the first day of bleeding is considered day one of the cycle). If the patient has not menstruated by day 35 and she is not pregnant, a progestogen-induced withdrawal bleed should be initiated. The dose of CC should only be increased if there is no response after three cycles, as of those

women who will respond to 50 mg/day, only two-thirds will do so in the first cycle. Doses of 150 mg/day or more appear not to be of benefit. If there is an exuberant response to 50 mg/day, as in some women with PCOS, the dose can be decreased to 25 mg/day. Discontinuation of CC therapy should be considered if the patient is anovulatory after the dose has been increased up to 100 mg/day. If the patient is ovulating, conception is expected to occur at a rate determined by factors such as the patient's age.

Clomiphene citrate may cause an exaggeration in the hypersecretion of LH and have antiestrogenic effects on the endometrium and cervical mucus. All women who are prescribed CC should be carefully monitored with a combination of endocrine and ultrasonographic assessment of follicular growth and ovulation because of the risk of multiple pregnancies, which is approximately 10%. Clomiphene therapy should therefore be prescribed and managed by specialists in reproductive medicine.

An ovulatory trigger in the form of parenteral administration of human chorionic gonadotropin (hCG) is very rarely required and should only be given if there has been repeated evidence of an unruptured follicle, by ultrasound and serum progesterone monitoring. Women with PCOS should have LH measured on day 8 in a cycle that follows an ovulatory cycle; if the LH is >10 IU/L, the chance of conception is reduced and risk of miscarriage is elevated. In this case, the options include LOD or gonadotropin therapy.

3.1. Results of CC Therapy

Clomiphene citrate induces ovulation in approximately 70–85% of patients, although only 40–50% conceive [19]. It is recommended that at least the first cycle of treatment, if not all cycles, should be monitored with a combination of serial ultrasound scans and serum endocrinology [20]. Kousta and colleagues [21] reported treatment of 167 patients with CC in whom there was a cumulative conception rate of 67.3% over 6 months in women who had no other subfertility factors, which continued to rise up to 12 cycles of therapy. These investigators reported a multiple pregnancy rate of 11%, similar to that described in other series, and a miscarriage rate of 23.6%, with those who miscarried tending to have a higher serum LH concentration immediately after CC administration. If a pregnancy has not occurred after 10–12 normal ovulatory cycles, it is then appropriate to offer the couple assisted conception.

Shoham and colleagues [22] studied the hormonal profiles in a series of 41 women treated with CC, of which 28 ovulated. In those who ovulated, 17 exhibited normal patterns of hormone secretion and five conceived, while 11 exhibited an abnormal response, characterized by significantly elevated serum concentrations of LH from day 9 until the LH surge, together with premature luteinization and higher E2 levels throughout the cycle. None of the patients

with this abnormal response conceived. This strengthens the argument for careful monitoring of therapy and discontinuation if the response is abnormal.

Patients with anovulatory infertility who are resistant to antiestrogens may be prescribed metformin combined with CC, parenteral gonadotropin therapy, or laparoscopic ovarian surgery. The term "clomiphene-resistance" strictly speaking refers to a failure to ovulate rather than failure to conceive despite ovulation, which should be termed "clomiphene-failure."

4. AROMATASE INHIBITORS

Aromatase inhibitors have been proposed as an alternative treatment to CC therapy, as the discrepancy between ovulation and pregnancy rates with CC has been attributed to its antiestrogenic action and estrogen receptor depletion. The aromatase inhibitors suppress estrogen production and thereby mimic the central reduction of negative feedback through which CC works. Letrozole, the most widely used antiaromatase for this indication, has been shown to be effective, in early trials, in inducing ovulation and pregnancy in women with anovulatory PCOS and inadequate CC response and improving ovarian response to FSH in poor responders [23]. Anastrozole is currently being examined as a possible alternative. Evidence from larger trials is still awaited, but some encouragement may be taken from the solidity of the working hypothesis and the success of the preliminary results. The role of aromatase inhibitors in an algorithm for ovulation induction has yet to be agreed upon. Furthermore, the possible teratogenicity of aromatase inhibitors has to be fully evaluated, and manufacturers currently do not advise its use for ovulation induction.

5. GONADOTROPIN THERAPY

Gonadotropin therapy is indicated for women with anovulatory PCOS who have been treated with antiestrogens and have either failed to ovulate or, if they responded to CC, developed other issues reducing their chance of conception (e.g., persistent hypersecretion of LH). In order to prevent the risks of overstimulation and multiple pregnancy with gonadotropin therapy, the traditional standard step-up regimens (when 75–150 IU/day are increased by 75 IU/day every 3–5 days [24]), have been replaced by either "low-dose step-up" regimens [25] or "low-dose step-down" regimens [26].

The low-dose step-up regimen employs a starting daily dose of 0.5–0.75 of an ampule (37.5–50 IU/day), which is only increased after 14 days if there is no response and then only by half an ampule every 7 days [27]. Treatment cycles using this approach can be quite long – up to 28–35 days – but the risk of multiple follicular growth is lower than with conventional step-up regimens. The initiation of follicular growth requires a 10–30% increment in the dose of exogenous FSH, and the threshold changes with follicular growth

due to an increased number of FSH receptors, so that the concentration of FSH required to maintain growth is less than that required to initiate it. In ovulation induction protocols, stimulation with gonadotropins does not require a background of pituitary desensitization. To date, there is no difference in efficacy between the different gonadotropin preparations [28].

It can be very challenging to stimulate the development of a single dominant follicle in women with PCOS. While attempts have been made to predict a multifollicular response by determining midfollicular endocrine profiles and numbers of small follicles, it is harder to do so prior to commencing ovarian stimulation and hence determine the required starting dose of gonadotropin. In order to prevent multiple pregnancy, strict criteria are required before the administration of hCG with no more than two follicles ≥ 14 mm, with the largest > 17 mm.

White and colleagues [29] reported their extensive experience of the low-dose regimen in 225 women, with over 934 cycles of treatment and resulting in 109 pregnancies in 102 women (45%). Seventy-two percent of the cycles were ovulatory (fewer than 5% of patients failed to ovulate) and 77% of these uniovulatory. The multiple pregnancy rate was 6%. Despite using a low-dose protocol, 18% of cycles were abandoned because more than three large follicles developed – a further reminder of the sensitivity of the polycystic ovary even when attempts are made to reduce the response. The only factor that influenced the outcome significantly was the patient's BMI; those women with a BMI > 25 kg/M^2 had a higher rate of abandoned cycles (31% versus 15% in those of normal weight), a lower cumulative conception rate over six cycles (46.8% versus 57% for the whole group), and a miscarriage rate of 31%. Another series reported the cumulative conception and live birth rates in 103 women with CC-resistant PCOS [30], with the cumulative conception and live birth rates after 6 months being 62% and 54%, respectively, and after 12 months 73% and 62%, respectively.

6. SURGICAL OVULATION INDUCTION

An alternative to gonadotropin therapy for CC-resistant PCOS is laparoscopic ovarian surgery, which has replaced the more invasive and damaging technique of ovarian wedge resection. Laparoscopic ovarian surgery is free of the risks of multiple pregnancy and ovarian hyperstimulation and does not require intensive ultrasound monitoring. Furthermore, LOD appears to be as effective as routine gonadotropin therapy in the treatment of CC-insensitive PCOS [31]. In addition, laparoscopic ovarian surgery is a useful therapy for anovulatory women with PCOS who fail to respond to CC and who persistently hypersecrete LH, need a laparoscopic assessment of their pelvis, or who live too far away from the hospital to be able to attend for the intensive monitoring required of gonadotropin therapy. Surgery does of course carry its own risks and must be performed only by fully trained laparoscopic surgeons.

After laparoscopic ovarian surgery, with restoration of ovarian activity, serum concentrations of LH and testosterone fall. Whether patients respond to LOD appears to depend on their pretreatment characteristics, with patients with high basal LH concentrations having a better clinical and endocrine response. We performed a small prospective study in which we randomized women to receiving either unilateral or bilateral LOD [32]. We found that unilateral diathermy restored bilateral ovarian activity, with the contralateral, untreated ovary often being the first to ovulate after the diathermy treatment. We also found that the only significant difference between the responders and nonresponders was a postdiathermy fall in serum LH concentration.

Commonly employed methods for laparoscopic surgery include monopolar electrocautery (diathermy) [33] and laser [34], while multiple biopsy alone is less commonly used. The greater the amount of damage to the surface of the ovary, the greater the risk of periovarian adhesion formation. This led Armar to develop a strategy of minimizing the number of diathermy points to four per ovary for 4 s at 40 W [35]. Wedge resection of the ovaries resulted in significant adhesions – in 100% of cases in some published series. The risk of adhesion formation is far less after LOD (10–20% of cases), and the adhesions that do form are usually fine and of limited clinical significance. We advise instilling 1,000 mls of Adept® solution into the pouch of Douglas, which, by cooling the ovaries, prevents heat injury to adjacent tissues and reduces the adhesion formation.

Ovarian diathermy appears to be as effective as routine gonadotropin therapy in the treatment of CC-insensitive PCOS, and the Cochrane database concludes that, while there is insufficient evidence to demonstrate a difference between 6 and 12 months follow up after LOD and 3–6 cycles of ovulation induction with gonadotropins, multiple pregnancy rates are considerably reduced with LOD [31]. The largest randomized controlled trial (RCT) to date is the multicenter study performed in the Netherlands in which 168 patients resistant to CC were randomized to either LOD (n = 83) or ovulation induction with recombinant FSH (rFSH, n = 65) [36]. The initial cumulative pregnancy rate after 6 months was 34% in the LOD arm versus 67% with rFSH. Those who did not ovulate in response to LOD were then given first CC and then rFSH, so by 12 months the cumulative pregnancy rate was similar in each group at 67% (Fig. 1). Thus, those treated with LOD took longer to conceive and 54% required additional medical ovulation induction therapy.

It has been suggested that, to demonstrate a 20% increase in pregnancy rate over 6 months from 50 to 70% with an 80% power, at least 235 patients would be required in each arm of a study to compare LOD with gonadotropin therapy. The current meta-analysis in the Cochrane database includes a total of only 303 women [31]. The ongoing pregnancy rate following ovarian drilling compared with gonadotropins differed according to the length of follow up. Overall, the pooled odds ration for all studies was not statistically significant

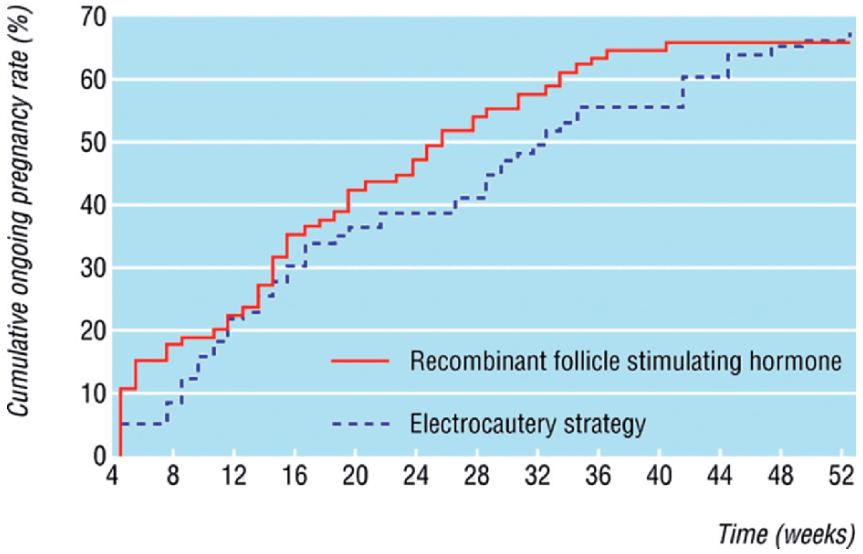

Fig. 1. Cumulative ongoing pregnancy rate over time from electrocautery or ovulation induction with recombinant follicle stimulating hormone (reprinted with permission from [36]).

(OR 1.27, 95% CI 0.77, 1.98). Multiple pregnancy rates were reduced in the ovarian drilling arms of the four trials where there was a direct comparison with gonadotropin therapy (OR 0.16, 95% CI 0.03, 0.98). There was no difference in miscarriage rates in the drilling group when compared with gonadotropin in these trials (OR 0.61, 955% 0.17, 2.16).

7. THE ROLE OF METFORMIN AND OTHER INSULIN SENSITIZERS

It is logical to assume that therapy that achieves a fall in serum insulin concentrations should improve the symptoms of PCOS. The biguanide metformin both inhibits the production of hepatic glucose, thereby decreasing insulin secretion, and also enhances insulin sensitivity at the cellular level. The efficacy of metformin in PCOS was first described by Velazquez and colleagues [37], and, in the last decade, many studies have been carried out to evaluate the reproductive effects of metformin in patients with PCOS. Most of the initial studies, however, were observational, and any randomized studies published involved a small number of participants. Indeed, two systematic reviews published in 2003 revealed that the majority of the published studies on the effects of metformin alone on the menstrual cycle in women with PCOS had a sample size of less than 30 women [38,39].

Metformin ameliorates hyperandrogenism and abnormalities of gonadotropin secretion in women with PCOS and can also restore menstrual cyclicity. Metformin appears to be less effective in those who are significantly obese (BMI > 35 kg m^{-2}) [40,41], and there are still no agreed algorithms for its use. Furthermore, there is no agreement on predictors for response or the appropriate dose or whether dose should be adjusted for body weight or other factors.

Initial studies appeared to be promising, suggesting that metformin could improve fertility in women with PCOS [39]. Additional data on the use of metformin together with CC has indicated striking results with a 90% pregnancy rate compared with only 8% in those who received placebo with CC [45]; however, the number of patients was small. More recent large RCTs have observed that the beneficial effects of metformin first line therapy for the treatment of the anovulatory patient with PCOS is significantly less than CC. In a multicenter trial of 20 Dutch hospitals, 228 women with PCOS were treated either with CC plus metformin or CC plus placebo [42]. The ovulation rate in the metformin group was 64% compared with 72% in the placebo group, a nonsignificant difference (Table 1). There were no significant differences in either rate of ongoing pregnancy (40% versus 46%) or rate of spontaneous abortion (12% versus 11%). A significantly larger proportion of women in the metformin group discontinued treatment because of side effects (16% verssus 5%). The investigators concluded that metformin is not an effective addition to CC as the primary method of inducing ovulation in women with PCOS.

Table 1. Randomized double blind trial of clomiphene citrate (CC) plus metformin versus CC plus placebo for induction of ovulation in women with newly diagnosed PCOS: rates of ovulation, pregnancy, and spontaneous abortion

	Clomiphene citrate + metformin (n = 78)	Clomiphene citrate + placebo (n = 84)	Risk difference % (95% CI)	Relative risk (95% CI)
Ovulation	61 (78%)	68 (81%)	–3 (–15 to 10)	0.97 (0.8 to 1.1)
Ongoing pregnancy	44 (56%)	51 (61%)	–4 (–19 to 11)	0.93 (0.7 to 1.2)
Spontaneous abortion	13 (17%)	12 (14%)	2 (–9 to 14)	1.17 (0.6 to 2.4)

Figures are numbers (percentages in parentheses) of women.
Reprinted with permission from [42].

The pregnancy in polycystic ovary syndrome (PPCOS) trial sponsored by the US National Institutes of Health (NIH) noted that, as first line therapy for the treatment of anovulatory infertile PCOS women, metformin alone was significantly less effective than CC alone and that the addition of metformin to CC produces only marginal benefits [43]. This multicenter study enrolled 676 infertile PCOS women (diagnosed by an elevated testosterone level and oligomenorrhea, ≤ 8 spontaneous menses/year, after exclusion of secondary causes of hyperandrogenemia) who were seeking pregnancy. All were off confounding medications and in otherwise good health, ages 18–39 years, and had no other obvious infertility factors, with at least one patent fallopian tube, normal uterine cavity, and partner with sperm concentration of 20 million/mL in at least one ejaculate. After progestin withdrawal, these women were equally randomized to three different treatment arms for a total of six cycles or 30 weeks: (a) metformin 1,000 mg twice daily plus placebo, (b) CC 50 mg/day for 5 days (day 3–7 of cycle) plus placebo, or (c) combined metformin 1,000 mg twice daily plus CC 50 mg /day for 5 days (day 3–7). Overall, live birth rates were 7.2% (5/208), 22.5% (47/209), and 26.8% (56/209), respectively, with the metformin alone group being significantly lower than the other two groups. Pregnancy loss rates tended to also be higher in the metformin alone group (40.0% versus 22.6% and 25.5%, respectively).

We set out to evaluate the combined effects of life-style modification and metformin on obese anovulatory women (BMI > 30 kg/M^2) with PCOS [41] in a prospective, randomized, double blind, placebo-controlled, multicenter study. All the patients had an individualized assessment by a research dietitian in order to set a realistic goal which could be sustained for a long period of time with an average reduction of energy intake of 500 kcal/day. As a result, both the metformin-treated and placebo groups managed to lose weight, but the amount of weight reduction did not differ between the two groups. An increase in menstrual cyclicity was observed in those who lost weight but again did not differ between the two arms of the study [41].

The very variable findings from the published studies on the use of metformin reflect the large differences in study populations, particularly with respect to body weight. Insulin sensitivity decreases (or insulin resistance increases) with BMI. It has been known that nonobese women with PCOS respond better to metformin than obese women to metformin [40,44].

We have shown a dramatic increase in ongoing pregnancy rates in women with polycystic ovaries treated with metformin for only 4 weeks during an IVF cycle. It therefore appears that metformin may have a direct effect on ovarian function and enhances the outcome of some fertility therapies in some women – probably those with relatively mild metabolic dysfunction. There has been a tendency to discontinue metformin once a pregnancy has been achieved, although a number of studies have confirmed its apparent safety,

with lack of teratogenicity and potential for reducing the risk of miscarriage and gestational diabetes, although large RCTs are awaited.

An interesting study of 120 CC-citrate-resistant women found no significant difference in rates of ovulation when LOD was compared with metformin therapy (approximately 55% in each group), yet those treated with metformin had higher pregnancy rates (18.6% versus 13.4%) and live birth rates (82.1% versus 64.5%) [47], although the differences were not significant [48].

The insulin-sensitizing agent troglitazone also appeared to significantly improve the metabolic and reproductive abnormalities in PCOS [49], although this product has been withdrawn because of reports of fatal liver damage. The new generation of thiazolidinediones (rosiglitazone and pioglitazone) may be of benefit to the older woman with PCOS but should not be prescribed to women wishing to conceive, because of an uncertain safety profile in pregnancy. Newer insulin sensitizing agents are currently being evaluated as is the phosphoglycan containing drug d-*chiro*-inositol [50].

8. IVF IN WOMEN WITH POLYCYSTIC OVARIES

In vitro fertilization is not the first line treatment for PCOS, but many patients with the syndrome may be referred for IVF, either because there is another reason for their infertility or because they fail to conceive despite ovulating (whether spontaneously or with assistance) – that is their infertility remains unexplained. Furthermore, approximately 30% of women have polycystic ovaries as detected by ultrasound scan. Many will have little in the way of symptoms and may present for assisted conception treatment because of other reasons (for example tubal factor or male factor). When stimulated, these women with asymptomatic polycystic ovaries have a tendency to respond sensitively and are at increased risk of developing OHSS.

The response of the polycystic ovary to stimulation in the context of ovulation induction aimed at the development of unifollicular ovulation is well documented and differs significantly from that of normal ovaries. The response tends to be slow, with a significant risk of ovarian hyperstimulation. Conventional IVF depends on inducing multifollicular recruitment, and again the response of the polycystic ovary differs from the normal, with a potentially "explosive" response based on the presence of many partially developed follicles present in the polycystic ovary. Thecal hyperplasia (in some cases with raised levels of LH and/or insulin) provides large amounts of androstenedione and testosterone, which act as substrates for estrogen production. Granulosa cell aromatase, although deficient in the "resting" polycystic ovary, is readily stimulated by FSH. Therefore, normal quantities of FSH act on large amounts of substrate (testosterone and androstenedione) to produce large amounts of intraovarian estrogen. Ovarian follicles, of which there are too many in polycystic

ovaries, are increasingly sensitive to FSH (receptors which are stimulated by high local concentrations of androgens and estrogen) and, as a result, there is multiple follicular development associated with very high levels of circulating estrogen. In some cases, this may result in OHSS, to which patients with polycystic ovaries are particularly prone.

In addition, insulin acts as a cogonadotropin and augments theca cell production of androgens in response to stimulation by LH and granulosa cell production of estrogen in response to stimulation by FSH. Also, there is widespread expression of vascular endothelial growth factor (VEGF) in polycystic ovaries. VEGF is an endothelial cell mitogen that stimulates vascular permeability, hence its involvement in the pathophysiology of OHSS. VEGF is normally confined in the ovary to the blood vessels and is responsible there for invasion of the relatively avascular Graafian follicle by blood vessels after ovulation. The increase of LH at midcycle leads to expression of VEGF, which has recently been shown to be an obligatory intermediate in the formation of the corpus luteum. It has been shown that, compared with women with normal ovaries, women with polycystic ovaries or PCOS have increased serum VEGF [51].

The above data serve to remind us of the close relationship of polycystic ovaries with OHSS and also provide a possible explanation for the multifollicular response of the polycystic ovary to gonadotropin stimulation. One of the mechanisms that underpins the unifollicular response of the normal ovary is diversion of blood flow within the ovaries, first from the nondominant to the dominant ovary and, second, from cohort follicles to the dominant follicle. This results in diversion of FSH away from the cohort follicles and permits them to undergo atresia. The widespread distribution of VEGF in polycystic ovaries may prevent this diversion of blood flow, leaving a substantial number of small and intermediate sized follicles in "suspended animation" and ready to respond to gonadotropin stimulation. The distribution of VEGF in the polycystic ovary therefore helps to explain one of the fundamental features of the polycystic ovary, namely the loss of the intraovarian autoregulatory mechanism that permits unifollicular ovulation to occur.

Case–control study of the outcome of IVF in women with polycystic ovaries as compared with control patients with normal ovaries has consistently shown the development of more follicles, higher serum estradiol concentrations, and more eggs but often lower fertilization rates [52,53]. Rates of OHSS are significantly higher than controls at 10% compared with the expected rate of 1%.

A long running debate in ovulation induction for women with PCOS is whether the use of FSH alone has any benefit over human menopausal gonadotropins (hMG) – is the hypersecretion of LH responsible for the exaggerated response to stimulation of the polycystic ovary? Does minimizing circulating LH levels by giving FSH alone improve outcome? The consensus from a combination of meta-analyses suggests that there is no difference in outcome whether hMG, urinary-FSH, or recombinant-FSH is used [54,55].

The recent introduction of schedules of gonadotropin stimulation that incorporate treatment with GnRH antagonists holds promise for patients with polycystic ovaries and PCOS. Gonadotropin-releasing hormone (GnRH) antagonists do not activate the GnRH receptors and produce a rapid suppression of gonadotropin secretion within hours. A systematic review in the Cochrane Database showed that there is a trend of reduction of OHSS in the GnRH antagonist treatment groups with the combined odds ratio of 0.47 (95% CI: 0.18, 1.25) [56]. A dramatic reduction in the rate of OHSS has also been shown with the use of metformin for the first four weeks of an IVF treatment cycle [46].

9. IVM OF OOCYTES

In recent years, IVM has attracted a lot of interest as a new assisted reproductive technique. The immature oocytes are retrieved from antral follicles of unstimulated (or minimally stimulated) ovaries via the trans-vaginal approach. The oocytes are subsequently matured in vitro in a special formulated culture medium for 24–48 h. The mature oocytes are fertilized, usually by intra-cytoplasmic sperm injection (ICSI), and the selected embryos are transferred to the uterus 2–3 days later. Although IVM is labor-intensive compared with conventional IVF treatment, there are a number of clinical advantages by the avoidance of large doses of exogenous gonadotropins, most importantly by avoiding the risk of OHSS. Since patients with PCOS have more antral follicles and a higher risk of developing OHSS compared with those without, IVM may be a promising alternative to conventional IVF.

Significantly more immature oocytes are retrieved from polycystic ovaries than from normal ovaries, and the overall oocyte maturation and fertilization rates are similar among the three groups. The subsequent pregnancy and live birth rates per transfer are then significantly higher in patients with polycystic ovaries because of a greater choice in the embryos selected for transfer. IVM yields significantly fewer mature oocytes than IVF cycles and therefore fewer embryos per retrieval, and implantation rates are still lower in IVM compared with IVF cycles, which may be due to a reduced oocyte potential or a reduced endometrial receptivity [57]. Continuous improvements in the culture medium and synchrony between endometrial and embryonic development will hopefully result in better IVM success rates in the future.

10. SUMMARY

The key principle in achieving ovulation induction for women with PCOS is to achieve unifollicular ovulation and thereby avoid the significant risks of multiple pregnancy and OHSS. Clomiphene citrate still remains the first line medical therapy for anovulatory PCOS; however, it may be time to rethink current strategies, particularly with the promising early experience of metformin.

Further studies are currently underway comparing gonadotropin therapy with CC as first line treatment.

Compared with medical ovulation induction with gonadotropins for the CC-resistant patient, the advantage of LOD is that it need only be performed once and intensive monitoring is not required, as there is no danger of multiple ovulation or ovarian hyperstimulation. Gonadotropin therapy appears to provide similar long term cumulative conception rates as LOD, although time to pregnancy is quicker. In the future, gonadotropin therapy may be made easier by the use of long-acting FSH preparations and orally active agents.

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Chapter 8

Long-Term Morbidity of PCOS

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1. INTRODUCTION

The polycystic ovary syndrome (PCOS) results in a number of immediate and long-term morbidities that are associated with a significant impact on quality of life and on economic costs. Immediate morbidities include menstrual dysfunction and abnormal uterine bleeding, subfertility and infertility, and androgen excess-related dermatologic abnormalities including hirsutism, acne, and androgenic alopecia, and an increased risk of obstetrical complications such as pregnancy-induced hypertension and gestational diabetes. However, PCOS is also associated with an increased risk of various other long-term complications or morbidities including cancer, type 2 diabetes mellitus (DM), the metabolic syndrome (MS), and possibly cardiovascular disease (CVD).

2. PREVALENCE OF PRINCIPAL MORBIDITIES ASSOCIATED WITH PCOS

2.1. Cancer

Because PCOS is associated with oligo-anovulation in the face of continued, and generally unopposed, hyperestrogenemia, obesity, and hyperinsulinism, the risk of estrogen-sensitive neoplasias may increase, including endometrial

and breast cancer. In turn, as excess androgen action has been implicated in the etiology of ovarian cancer [1], it is possible that the hyperandrogenism of PCOS may result in an increased risk of ovarian malignancy. In the following sections, we review the currently available epidemiologic data to support or refute these suppositions.

2.1.1. Endometrial Hyperplasia and Carcinoma. In addition to menstrual dysfunction and infertility, women with PCOS may be at increased risk for endometrial hyperplasia [2,3] and carcinoma [4,5]. However, most of these studies have actually investigated groups of women with endometrial neoplasia and ascertained the prevalence of polycystic ovaries. In a small study, Cheung evaluated 36 consecutive patients with PCOS by endometrial biopsy and observed that 35.7% had endometrial hyperplasia and 25% of these had cytological atypia [2]. However, today most women with menstrual dysfunction or irregularity are placed readily on some form of progestin, either cyclic or more commonly in the form of an oral contraceptive. Since these drugs decrease the prevalence of endometrial carcinoma to close to the background rate [6], it is possible that the risk of frank endometrial cancer in PCOS patients today is relatively low. Overall, the prevalence of endometrial abnormalities in PCOS is still unclear due to the paucity of large-scale screening studies and the small number of patients actually identified with carcinoma [7].

2.1.2. Breast Cancer. Data concerning the risk of breast cancer in PCOS are conflicting and insufficient [8–13]. In a long-term follow-up study of 786 women diagnosed with PCOS in the UK between 1930 and 1979 and followed for an average of 30 years, Pierpoint and colleagues reported that the standardized mortality ratio (SMR; the ratio of observed to expected deaths) was 1.48 (95th percentile confidence interval [CI] 0.79–2.54) for breast cancer; breast cancer was also reported to be the leading cause of death in their population [10]. However, in a subsequent report, these same researchers stated that the 31-year-follow-up did not demonstrate a significantly increased risk of mortality and morbidity from breast cancer in women with PCOS [11].

In agreement, in a US cohort study of 34,835 women, 833 of which developed breast cancer during the follow-up, subjects with PCOS were not more likely to have a breast cancer (relative risk [RR] 1.2; 95th CI 0.7–2); adjustment for age at menarche, age at menopause, parity, oral contraceptive use, body mass index (BMI), waist/hip ratio, and family history of breast cancer further lowered this RR to 1.0 (95th CI 0.6–1.9) [9]. A multicenter, population-based, case–control study including 4,730 women with breast cancer and 4,688 controls aged 20–54 years revealed an age-adjusted odds ratio (OR) for breast cancer of 0.52 (95th CI 0.32–0.87) among women with a self-reported history of physician-diagnosed PCOS [8].

A family history of breast cancer may also be another indicator of the risk these individuals may have. Atiomo and colleagues performed a survey

of 107 women with (n = 41) and without (n = 66) PCOS and reported a higher prevalence of a positive family history of breast cancer among women with PCOS compared to controls (20% versus 5%, $p < 0.05$) [12]. Alternatively, in a 12-year-follow-up study of 240 women (116 cases and 124 controls), Soran and colleagues found similar percentages of women with a family history of breast cancer in PCOS compared to controls (23.3% versus 21.8%) [13]. Overall, current data suggests that PCOS patients do not have a significantly higher risk for breast cancer than do matched controls.

2.1.3. Ovarian Cancer. Using whole-organ multiple ovarian sections from 200 hysterectomy and bilateral salpingo-oophorectomy specimens, Resta and colleagues [14] found a high frequency of hyperplastic and metaplastic changes on the surface epithelium or in the inclusion cysts of ovaries of PCOS patients compared to patients without PCOS (68% versus 22%, respectively). These epithelial changes were considered possible morphological precursors of common epithelial tumors. Analyzing data from the Cancer and Steroid Hormone Study (CASH), Schildkraut et al. [15] identified 476 women with epithelial ovarian cancer and assessed 4,081 controls ascertained via random-digit telephone dialing. The risk of ovarian cancer was found to be 2.5-fold (95th CI 1.1–5.9) higher among women who reported that they had been diagnosed with PCOS before the study period. However, we should note that this OR was calculated using only seven women with ovarian cancer (1.5%) and 24 controls (0.6%) who reported that they had been diagnosed with PCOS.

In a long-term follow-up study of 786 women diagnosed with PCOS in the UK between 1930 and 1979, traced from hospital records and followed for an average of 30 years, the SMR relative to the national rate for ovarian cancer among PCOS women was 0.39 (95th CI 0.01–2.17) [10]. In agreement, a cross-sectional questionnaire survey of 217 women with and without PCOS failed to detect a positive association between PCOS and a family history of ovarian cancer [12].

Overall, it would appear that PCOS is not associated with an increased risk of ovarian cancer when compared to matched controls, although prospective studies of large populations of PCOS and matched controls are still needed.

2.2. Glucose Intolerance

Among women with PCOS in the US, with average of 28–30 years, the prevalence of type 2 DM ranges from 4% to 10% [16–19]. In a study of 11,035 women with PCOS identified in an integrated health care delivery system, using health plan databases indicated that these women were more likely than those without PCOS to be diagnosed with diabetes (OR 2.45, 95th CI 2.16–2.79), even after adjusting for BMI and known confounders [19]. Overall, women with PCOS are at a 2- to 6-fold higher risk of developing type 2 DM compared to age-matched

average women, who have a prevalence of type 2 DM of 1.6%–2.0% [20,21]. As expected, the risk of impaired glucose tolerance (IGT) is also higher, with 20–30% of PCOS subjects affected [16–18]. This risk is higher in those PCOS women with a positive family history of diabetes, although there is no significant association between race and glucose tolerance status [22].

The prevalence of diabetes appears to be also higher among first-degree relatives of women with PCOS [18,23,24], suggesting that the risk of type 2 DM among women with PCOS is, in part, determined by heritability. Sir-Petermann and colleagues reported that insulin sensitivity was significantly lower and the prevalence of type 2 DM was 1.89-fold higher in the parents of PCOS women compared with the parents of controls, even after adjustment for sex, age, and BMI [24]. Yildiz et al. observed type 2 DM and IGT in 16% and 30% of mothers and in 27% and 31% of fathers, respectively, of women with PCOS [23]. In addition, IGT was found in 5% of PCOS sisters. We observed that type 2 DM in a first-degree relative was evident in 44% of PCOS women with diabetes and 39% of those with IGT [18]. In contrast, significantly fewer (21%) of normal glucose-tolerant women with PCOS had a diabetic first-degree relative. These data suggests that the risk of type 2 DM in PCOS is, at least in part, determined by inherited factors.

We should note that the increased risk for type 2 DM observed in PCOS women reflects, to a significant degree, the high prevalence of obesity observed in these patients. For example, 90% of diabetic PCOS patients diagnosed in the study by Legro and colleagues, who assessed 254 patients diagnosed either at the Pennsylvania State University College of Medicine or at Mount Sinai School of Medicine, had a BMI of 30 kg/M^2 or greater [16]. When the risk of type 2 DM in PCOS was adjusted for BMI, the increased prevalence of diabetes was no longer significantly different than controls, at least in one study assessing 319 women with PCOS and 1,060 controls [25].

2.3. Metabolic Syndrome

It is likely that PCOS is present in a significant fraction of women with the MS. As women with PCOS are frequently obese, and abdominal obesity is an important feature of MS, most of these patients will already have at least one of the features of this latter syndrome. Furthermore, in PCOS, obesity is associated with greater degrees of insulin resistance [26] and dyslipidemia, particularly lower levels of high-density lipoprotein (HDL)-cholesterol [27], increasing the probability that these patients will exhibit MS. Alternatively, it is less clear that nonobese women with PCOS are at increased risk for the MS.

In one study of 106 women with PCOS, the prevalence of MS was not increased in those women whose BMI was < 25 kg/M^2 and were less than 30 years old; alternatively, patients with a BMI > 25 kg/M^2 and/or who were 30 years or older had a higher prevalence of MS when compared to age-matched controls [28]. The importance of obesity, and possibly diet, on the prevalence

of MS is further highlighted by the relatively low prevalence of the syndrome in non-US PCOS patients [29,30] compared to those patients residing in the US [28,31,32]. In general, PCOS women in the US are significantly more obese than their non-US counterparts [33].

We studied 368 nondiabetic PCOS women who were screened for participation in a multicenter trial to evaluate the effects of troglitazone on ovulation and hirsutism [22]. The prevalence for individual components comprising MS were (a) waist circumference > 88 cm (80%), (b) HDL-cholesterol < 50 mg/dL (66%), (c) triglycerides ≥ 150 mg/dL (32%), (d) blood pressure ≥ 130/85 mm Hg (21%), and (e) fasting glucose levels ≥ 110 mg/dL (5%). Three or more of these individual criteria, defining the presence of MS, were present in 123 (33.4%) subjects overall. This prevalence is markedly higher than the 6.7% prevalence of MS reported in women between the ages of 20 and 30 years, and the 15% prevalence reported in women between ages 30 and 40 years., from the Third National Health and Nutrition Examination Survey (NHANES III) using the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III) definition for MS [34]. The prevalence of MS did not differ significantly between racial/ethnic groups but increased with the free testosterone level; the prevalence of MS was 19.8, 31.3, 46.9, and 35.0%, from lowest to highest quartile of free testosterone concentration, respectively, after adjustment for BMI. Women in the top BMI quartile were 13.7 times more likely (95th CI 5.7–33.0) to have MS compared with those in the lowest quartile, and none of the 52 women with a BMI < 27.0 kg/M^2 had MS.

The prevalence of MS in PCOS is, at least in part, a matter of definition. Diagnostic criteria for both disorders are multiple and in flux, with at least three different criteria in use for PCOS [35] and six for MS [36]. As such, the definitions used will have an important impact on the specific prevalence observed. For example, Vural and colleagues evaluated 43 women with PCOS and 43 age-matched controls in Turkey [30], using the WHO criteria, and 11.6% of PCOS women were diagnosed as having MS, significantly greater than controls (0%). Alternatively, if the ATP III criteria were used only 2.3% of PCOS were affected with MS, a nonsignificant difference from controls. In addition, this study included PCOS women diagnosed by the Rotterdam 2003 criteria, which has the potential of including less androgenized and insulin resistant patients, possibly partially accounting for the lower frequency of MS observed in this population.

Overall, MS and its individual components are common in PCOS, particularly among women with the highest BMI, and insulin and androgen levels. The prevalence of MS increases with age [34], and likewise the prevalence in PCOS rises with age [32]. Nonetheless, of the patients studied by Dokras and colleagues, ~24 and 46% of the patients aged less than 30 years or 30–39 years, respectively, had MS [32]. These data clearly denote the importance of screening

all women with PCOS for MS, regardless of age. Finally, we should note that the prevalence of MS among women in the US, defined by the ATP III criteria, is ~23% among all women and ~15% among those aged 20–49 years [34]. Since PCOS, defined by the NIH 1990 criteria, affects 6.6% of reproductive-aged women [37], it is possible that this disorder is present in 10% of all women with MS and in 20% of those of reproductive age. Consequently, because PCOS has other important reproductive and quality of life implications, women with MS should also be investigated for the presence of PCOS.

2.4. Cardiovascular Disease

CVD, including coronary heart disease (CHD), stroke, and peripheral artery disease, is a leading cause of death, affecting 6% of the overall population, at least in the US. Patients with PCOS demonstrate a higher prevalence of CVD risk factors, and possibly selected CVD events, than comparable controls.

2.4.1. Risk Factors for CVD in PCOS. Patients with PCOS demonstrate a higher prevalence of insulin resistance, hyperandrogenemia, type 2 DM, MS, and total and abdominal obesity, compared to controls, important risk factors for the development of CVD. A higher prevalence of dyslipidemia has also been reported among PCOS patients [19,38–40].

In a study including 195 women with PCOS and 62 controls, Legro and colleagues reported that the prevalence of borderline high total cholesterol (TC; ≥ 200 mg/dL) was higher among PCOS than controls (48% versus 22%, respectively), although the prevalence of abnormally low levels of high density lipoprotein (HDL)-cholesterol (< 35 mg/dL) was similar in both groups (48% versus 45%, respectively) [39]. Lo and colleagues, in their study of 11,035 women with PCOS, observed that women diagnosed with PCOS were more likely than those without PCOS to also be diagnosed with dyslipidemia (OR 1.53, 95th CI 1.39–1.68), even after adjusting for BMI and known confounders [19]. Overall, it appears that women with PCOS are 1.5–2.0 more likely to have dyslipidemia than matched controls. However, we should also note that evidence supporting an increased prevalence of dyslipidemia in PCOS is not uniform. For example, in a study of 398 women with PCOS screened for inclusion in a trial evaluating the effectiveness of troglitazone, we [41] reported that the prevalence of abnormally high TC levels in this population was similar to, and the prevalence of low HDL-cholesterol levels was actually less than, that observed among women aged 20–39 in NHANES IIII, i.e., 7.6% and 25.6%, respectively [42].

The prevalence of hypertension in PCOS ranges from 10 to 39%, depending on age, representing a 1.4- to 3.5-fold increase above controls [9,25,43–46]. However, we should note that the prevalence of hypertension in PCOS is significantly greater in obese patients [45] and, in one study, when the prevalence of hypertension was adjusted for BMI, the difference in risk between PCOS and age-matched controls was no longer significant [25].

Finally, other risk factors for CVD, including evidence of impaired fibrinolysis (e.g., increased plasminogen activator inhibitor-1 [47–49] and homocysteine levels [50,51]) and chronic subclinical inflammation (e.g., increased levels of white blood cells [52], soluble intercellular adhesion molecule-1 and soluble endothelial leukocyte adhesion molecule-1 [53], and C-reactive protein [53–58]) are more prevalent in PCOS than controls. Overall, patients with PCOS demonstrate an increased prevalence of conditions that are associated with an increased risk for CVD. The beneficial effect of insulin sensitizers on these parameters has been documented [47,53,55,59,60].

2.4.2. Clinically Evident CVD in PCOS. In agreement with the above findings, there is evidence of subclinical CVD including morphologic alterations (e.g. left ventricular hypertrophy [61], increased carotid intimal media thickness [62–65], and coronary artery and aortic calcification [40,66]), and functional and endothelial abnormalities (e.g., decreased diastolic filling [50,61], left ventricular ejection fraction [61,67], leg blood flow responses to graded intrafemoral artery infusions of methacholine chloride [68], and brachial flow-mediated dilation [57,64], and higher pulse wave velocity across the aorta and brachial artery [54], resistance to the vasodilating action of insulin [68], and endothelin-1 levels [59,64]). Evidence of cardiovascular dysfunction is present even in young normal-weight women with PCOS [49,52,58,61,64,69].

Notwithstanding, evidence for clinically apparent CVD is much less clear. Legro [70] reviewed the available evidence for an association between PCOS and CVD, and noted that while existing data suggested that PCOS may adversely affect or accelerate the development of an adverse cardiovascular risk profile, and even of subclinical signs of atherosclerosis, it did not appear to lower the age of clinical presentation to a premenopausal age group. A number of populational studies support the apparently modest role of PCOS in premature CHD [25,45]. It is possible that the lack of a significant association of PCOS with CVD or CHD-related events or mortality in these studies may be due to the short length of follow-up of the studies or the relatively young age of the PCOS patients at the time of follow-up.

To address, we prospectively studied 390 postmenopausal women enrolled in the NIH-NHLBI sponsored Women's Ischemia Syndrome Evaluation (WISE) study, not currently taking hormone replacement or oral contraceptive therapy [71], PCOS was observed in 104 women (defined by a history of irregular menses accompanied by biochemical evidence of hyperandrogenemia, i.e. the top quartile of either androstenedione, total or free testosterone, or free androgen index). Women with evidence of PCOS had a 2.6-fold (95th CI 1.5–4.5) higher risk of CVD or myocardial infarction (MI), compared to their counterparts. This relationship was maintained in a risk-adjusted model controlling for diabetes, hypertension, and angiographic coronary artery disease severity. Furthermore, a study of 319 PCOS women surveyed a mean of 31 years after

diagnosis observed a significant increase in the incidence of cerebrovascular disease in PCOS compared to controls (3.1% versus 1.2%, respectively) [25], although this association remains to be confirmed by other investigators.

Overall, although the long-term risk of hypertension and cerebrovascular disease appears to be increased in PCOS, these risks remain to be confirmed, and the extent to which the prevalence of CHD events are increased in the disorder is unclear. Most importantly, the incidence of CVD-associated events or mortality does not appear to be grossly increased in reproductive-aged PCOS patients compared to age-matched controls, although it is possible that these women may demonstrate an increased incidence of CVD as they age.

3. ESTIMATING THE ECONOMIC BURDEN OF PCOS

We have previously calculated the healthcare-related economic burden in PCOS based on the above prevalence of disease [72]. Our estimate of the health-care-related economic burden of premenopausal women with PCOS, which did not include CVD, exceeded $4 billion annually in the US alone. The calculated economic burden of PCOS patients during their reproductive years is about threefold that of hepatitis C ($1 billion in 1998) [73] and about one-third that of morbid obesity ($11 billion in 2000) [74]. Approximately 40% of the calculated burden was due to the increased prevalence of type 2 DM associated with PCOS. The recognition that MS, and possibly its CVD consequences, could affect as many as 50% of women with PCOS could easily triple the estimated health care burden of these patients. Notably, the costs of the diagnostic evaluation on all patients accounted for a relatively small portion of the calculated economic burden, about 2%.

4. CONCLUSIONS

Women with PCOS are at significantly higher risk for glucose intolerance and type 2 DM (Table 1). In addition, while they demonstrate a higher prevalence of markers for CVD and of cardiovascular dysfunction during the reproductive age, PCOS women may demonstrate a higher prevalence of CV, including MI and cerebrovascular accidents, but only in the menopause. While women with PCOS appear to be at increased risk for endometrial cancer, the extent of this risk is unclear. Overall, the risk of these women for ovarian or breast cancer does not appear to be grossly increased. Nonetheless, it is clear that large well designed epidemiologic and longitudinal studies are required to determine the true prevalence and risk of PCOS patients for long-term morbidities. These studies are critically needed, particularly in view of the high prevalence of PCOS, which appears to affect at least 7% of unselected repro-

Table 1. Long-term morbidities of patients with PCOS

Cancer	
Endometrial	Increased, extent not certain
Breast	Not increased
Ovarian	Not increased[a]
Glucose intolerance	
IGT	Increased, affects 20–30% of PCOS[b]
Type 2 DM	Increased, affects 4–10% of PCOS (2–6 fold controls)[c]
Metabolic Syndrome	Increased, affects 10–50% of PCOS[c]
CVD	
Dyslipidemia	Increased, 15–50%, 1.5–2.0 fold of controls[c]
Hypertension	Increased, affects 10–40% of PCOS (1.4- to 3.5-fold of controls)
Cerebrovascular events	Increased in postmenopause, affects ~3% of PCOS[a]
CHD/MI events	Increased in postmenopause, 1.5–4.5 fold of controls[a]

CVD, cardiovascular disease; CHD, cardiac heart disease; MI, myocardial infarction; DM, diabetes mellitus; IGT, impaired glucose tolerance.
[a]Additional studies needed.
[b]Risk is higher in women with obesity or a family history of type 2 DM.
[c]Less in those women with normal weight or who are less than 30 years old.

ductive aged women. Finally, we should note that costs of the diagnostic evaluation accounts for a relatively minor part of the total economic burden of the disorder. This suggests that more widespread screening for the disorder is potentially a cost-effective strategy, leading to earlier diagnosis and intervention and possibly the amelioration and prevention of serious sequelae.

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