Lubna Pal *Editor*

Polycystic Ovary Syndrome

Current and Emerging Concepts

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Springer

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To my late father, Dr. Khawaja Muhammad Mohsin Pal (Retired Major General, Army Medical Corps, Pakistan), a visionary, a philosopher, a poet, a surgeon, a friend, a passionate believer in human will and potential, a leader … and a gentleman—for his guidance and inspiration—you are missed. *To my mother, Dr. Jahanara Pal (Retired Brigadier Army Medical Corps and Professor Emeritus, Armed Forces Medical College, Pakistan), for being the role model that I strive to emulate, for the life lessons in selflessness, commitment, humility, and empathy*—you have been an inspiration at every step of the way*! To my brothers, Dr. Khawaja Muhammad Inam Pal (Associate Professor, The Agha Khan University Hospital, Karachi, Pakistan) and Shakeel Pal, for years of companionship, comradery, and support, and for the precious memories*—looking forward to spending more time catching up in years ahead! *To my husband, Dr. Sohail Kayani, for the unconditional support*—I could not have been where I am without you. *To my sons, Jehanzeb*

and Teimur, for … just being! And for their understanding and support—may your journey ahead be exciting, and the challenges surmountable and the ride "so worth it." *To the many teachers, mentors, and trainees I have had the privilege to learn from*—thank you for the invaluable lessons that have contributed to my growth as a clinician, researcher, and teacher. *To my patients*—thank you for the privilege of participating in your care and for the invaluable learning in the art of medicine.

Preface

As the most common endocrinopathy of reproductive-age women, polycystic ovary syndrome (PCOS) remains a poorly understood and yet a liberally diagnosed condition. While the exact mechanisms that underlie the chronic course of ovulatory dysfunction and hyperandrogenemia of PCOS still remain elusive, the complexity of the pathophysiological underpinnings and the diversity of recognized clinical sequelae have only expanded since the original description of this condition by Drs. Stein and Leventhal in 1935.

Heterogeneity within the entity PCOS, the somewhat overlapping phenotypic expressivity in the obese and the psychologically stressed, as well as possible racial and ethnic differences in presentation have been sources of confusion at all fronts (i.e., for the clinician caring for women with PCOS, for the researcher engaged in clinical research based on access to biological samples, and for the patients themselves). I have no doubt that the clinician reader will have encountered at least one of the following phrases during consultation with a PCOS patient: "I have trouble losing weight because of my PCOS," or "I have PCOS because of cysts on my ovaries," or "I saw four different doctors before I was diagnosed with PCOS," or "I was placed on a birth control pill to fix my PCOS." The mushrooming online blogs and PCOS support groups in recent years not only highlight the magnitude of frustration, level of unawareness, and quest for pertinent information relating to their diagnosis that is so obviously manifest in women labeled as "PCOS," but they also reflect poorly on the perspective, preparedness, and counseling offered by the providers caring for this population.

This textbook is an attempt to share with the reader (both clinician and researcher) the evolution in our understanding of this complex entity (i.e., PCOS) as regards the pathophysiology, the myriad and evolving spectrum of health implications, and the management paradigms from inception to the current "*omics*" era. The chapters are organized in distinct sections to systematically convey the following: an epidemiological snapshot and diagnostic dilemmas relating to PCOS (Section I); sequentially acquaint the reader on the relevance of genetics and disturbances in the ovary and the hypothalamo–pituitary axis and metabolic derangements as plausible players in the causation of PCOS (Section II); provide a comprehensive coverage of the

relevance of insulin sensitizers and statins, review targeted approaches to managing PCOS-related infertility and hyperandrogenism, and provide a comprehensive overview of the critical role of dietary management that must be deemed as a *first-line* approach to PCOS management (Section III). Diagnosis of PCOS in the adolescent and pregnancy in PCOS patients carry unique challenges, and surgical intervention (bariatric procedures and ovarian surgery) may be an appropriate consideration for some patients with PCOS; these concepts are comprehensively addressed in Sections IV and V. The last section of this book (VI) on the one hand crystallizes the health burden of PCOS that is recognized to extend well beyond the clinical presentation, and on the other, aims to acquaint readers with vitamin D insufficiency as a plausible pathophysiological mechanism that could impact on the endocrine and metabolic aberrations of PCOS.

It truly has been my privilege to have had this opportunity of bringing together this team *of* "*the best and the brightest*" in the field. It is my hope that this work will be deemed as equally meaningful to the clinicians who care for women with PCOS and the researchers who are attempting to understand the complexities of this disorder, and that our collective efforts will directly benefit the population of women with PCOS.

New Haven, CT, USA Lubna Pal, MBBS, MRCOG (UK), MS, FACOG

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Part I
PCOS Epidemiology and Diagnosis

Chapter 1
Diagnostic Criteria and Epidemiology of PCOS

Heather R. Burks and Robert A. Wild

Key Points

- As of 2013, the *three* recognized sets of criteria for PCOS diagnosis include the NIH criteria, the Rotterdam criteria, and the Androgen Excess and Polycystic Ovary Syndrome Society criteria.
- The prevalence of PCOS depends upon the diagnostic criteria used. Rotterdam is most inclusive, followed by the Androgen Excess Society criteria. The NIH criteria are the most strict and account for the lowest detected prevalence of PCOS
- Women with PCOS have an increased rate of many major cardiovascular risks: obesity, insulin resistance, metabolic syndrome, dyslipidemia, and type 2 diabetes. An increased risk for cardiovascular disease and events is suggested in this population.
- Acne and hirsutism may be the presenting symptoms of PCOS and should prompt a thorough evaluation.
- Women with PCOS are at an increased risk for additional chronic disorders such as depression and endometrial cancer.

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Table 1.1 Diagnostic criteria and their associated phenotypes[a]

	Potential phenotypes															
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Hyperandrogenemia	+	+	+	+	−	−	+	−	+	−	+	−	−	−	+	−
Hirsutism	+	+	−	−	+	+	+	+	−	−	+	−	−	+	−	−
Oligo-anovulation	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Polycystic ovaries	+	−	+	−	+	−	+	+	+	+	−	+	−	−	−	−
NIH 1990	√	√	√	√	√	√										
Rotterdam 2003	√	√	√	√	√	√	√	√	√	√						
AE-PCOS 2006	√	√	√	√	√	√	√	√	√							

[a]Adapted from Azziz et al. [4]

Diagnostic Criteria for Polycystic Ovary Syndrome

Since 1935, when Stein and Leventhal originally described the combination of oligo-ovulation and hyperandrogenism [1], the polycystic ovary syndrome (PCOS) has undergone multiple iterations of diagnostic criteria. Initially, description of the syndrome was based upon case reports in the literature. In the 1800s abnormal uterine bleeding was the most common symptom associated with the condition. Over time, as new and better evidence has become available, multiple efforts have been made to better characterize this syndrome to allow for better appreciation of this complex entity.

Clinicians worldwide may now choose between three major sets of *diagnostic criteria* to arrive at a diagnosis of PCOS (Table 1.1). The first set of relatively stringent criteria was outlined at the National Institutes of Health (NIH) in Bethesda, Maryland, in 1990, but has largely been replaced in clinical practice by the relatively recently proposed *Rotterdam criteria*. A task force sponsored by the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) met in Rotterdam, The Netherlands, in 2003 to review the available data and proposed a revision to the 1990 NIH diagnostic paradigm, hence the inception of the Rotterdam criteria. More recently, in 2009, the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society outlined its own set of criteria. It is important to appreciate that the subtle heterogeneities within the various diagnostic criteria utilized by investigators impacts upon the reported prevalence of PCOS in a given population.

The NIH meeting in 1990 was the first international conference on PCOS, and the guidelines that resulted from this meeting were based largely on expert opinion of the attendees, rather than the results of analytic studies [2]. The criteria set forth included (1) chronic anovulation and (2) clinical or biochemical signs of hyperandrogenism. Both criteria *must* be present, and other diagnoses *must* be excluded to allow reaching a diagnosis of PCOS. Once this initial step was taken to clearly define the syndrome, in ensuing years, better analytic studies revealed additional information that was subsequently evaluated by The Rotterdam ESHRE/ASRM-Sponsored

PCOS Consensus Workshop Group to revise the original NIH proposed set of diagnostic criteria.

The Rotterdam consensus includes three diagnostic criteria, and states that *any two of the three* must be present in order to make the diagnosis [3]. The revised criteria include (1) oligo- or anovulation, (2) clinical or biochemical signs of hyperandrogenism, and (3) polycystic appearing ovaries (PCO) on imaging. Other disorders *must*, of course, be excluded, including 21-hydroxylase deficient non-classic congenital adrenal hyperplasia (NC-CAH), Cushing's syndrome, and androgen-secreting tumors as well as commoner entities such as thyroid dysfunction and hyperprolactinemia. The addition of morphological appearance of polycystic ovaries identifies two additional phenotypes not previously included in the diagnosis: women with ovulatory dysfunction and polycystic ovaries but without hyperandrogenism, and ovulatory women with hyperandrogenism and polycystic ovaries; deeper explorations reveal that these subcategories within PCOS identified based on the Rotterdam diagnostic criteria manifest subtle but distinct hormonal and metabolic milieu when compared to cases of PCOS identified based on the more stringent NIH criteria. The stated rationale for incorporating these additional phenotypes included the recognition that PCOS does not represent a single entity, but rather occurs on a spectrum, as well as the associated long-term health risks such as of type 2 diabetes mellitus and cardiovascular disease, commonly encountered in women diagnosed with PCOS. The Rotterdam consensus statement advocated widening the inclusion criteria to avoid missing patients with the potential for these increased health risks.

The most recent set of diagnostic criteria to be released was compiled by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society in 2009 [4]. Their expert review reexamined the key recognized features of PCOS, including menstrual dysfunction, hyperandrogenemia, clinical signs of hyperandrogenism, and polycystic ovarian morphology. Each feature was examined for its appropriateness for inclusion as a defining criterion, based on a thorough review of existing literature. A slightly modified version of the criteria for the diagnosis of PCOS emerged in this process: (1) hyperandrogenism, including hirsutism and/or hyperandrogenemia, (2) ovarian dysfunction, including oligo-anovulation and/or polycystic appearing ovaries, and (3) exclusion of other androgen excess or related disorders. The AE-PCOS criteria also acknowledge that related disorders of hyperandrogenism must be excluded, but allow that the clinician may take into account the prevalence of these differential diagnoses when deciding what tests to order. Disorders to consider in the differential diagnosis of PCOS include androgen-secreting neoplasms, Cushing's syndrome, 21-hydroxylase deficient congenital adrenal hyperplasia, thyroid disorders, hyperprolactinemia, and premature ovarian failure. Similar to the NIH criteria, androgen excess is a necessary component of the diagnosis by AES criteria. Therefore, the phenotype of ovulatory dysfunction and PCO alone—permissible under Rotterdam—does not qualify for a diagnosis of the syndrome by AES criteria. The combination of menstrual dysfunction and PCO, in the absence of features of hyperandrogenism or evidence of hyperandrogenemia has, in fact, been shown to have the most similar anthropometrics,

hormonal profile, and metabolic risks to the control subjects. The AES consensus criteria for defining PCOS are thus more inclusive than the NIH version but less so than the Rotterdam criteria.

Anti-Müllerian hormone (AMH) has recently been proposed as a parameter to replace ultrasonographic assessment of PCO morphology, with specificity and sensitivity of 97.1 and 94.6 % when using the Rotterdam criteria, or 97.2 and 95.5 % using the NIH criteria [5]. Indeed, AMH levels correlate independently with both PCO morphology and androgenic profile [6]. Another parameter proposed as an adjunct to PCO morphology is an assessment of the ovarian stromal volume, measured as a ratio of the stromal area to total area of the ovary (S/A ratio). Although this S/A ratio performed well when discriminating between women with and without PCOS, and correlated with androgen levels, it has not been adopted as part of any of the existing diagnostic criteria [7, 8].

Determination of hyperandrogenism in females can be problematic, both during clinical and biochemical assessment. Laboratory assays for androgens were initially designed for detection in males and have been calibrated accordingly. For example, total testosterone assays are typically calibrated for normal male levels, the lower end of which is 250 ng/dL. The upper end of normal female total testosterone ranges between 55 and 80 ng/dL (inter-laboratory differences exist and clinicians should familiarize themselves with the assay range for the laboratories serving their patient population). Both the above specified values are well below the fifth percentile for the assay detection range, where assay results may become unreliable; notably, calibration studies have not been done to develop a commercial female assay. An additional diagnostic dilemma is that the reporting of clinical hyperandrogenism is examiner-dependent and can be subjective. While a standardized tool such as the Ferriman-Gallwey score can objectify evaluation, this method has been shown to have good intra-observer reliability but poor inter-observer reliability [9]. Furthermore, a universal application of such tools across all ethnic groups may discount the normal ethnic variability in the appearance of body hair.

Inclusion of ultrasonographic evidence of PCO morphology into the definition of PCOS is controversial. The various sets of criteria place different degrees of emphasis on an isolated phenotypic PCO component not uncommonly encountered in the general reproductive age population; the NIH criteria do not address ovarian morphology, the Rotterdam criteria in 2003 include PCO as a phenomenon distinct from menstrual irregularities, and the AES lumps ovarian morphology into an "ovarian dysfunction" category along with oligo-anovulation and requires only one or the other to suffice as a diagnostic criterion. It is important to appreciate that PCO morphology is not specific to PCOS and can be found in 20–30 % of the general population of women 20–25 years of age; isolated PCO therefore should not be considered an indication of the syndrome in the absence of menstrual irregularities, infertility, or complaints of hirsutism [10].

In some ways, efforts to agree on diagnostic criteria are artifactual. There continues to be controversy and lack of complete agreement for what elements constitute optimal criteria for PCOS diagnosis, in part because of the natural clinical desire to move to discreet categorical criteria for the ease of diagnosis. In truth, there is a

Table 1.2 Relative population prevalence of PCOS (%) based on individual diagnostic criteria

	Diagnostic criteria		
	NIH[a]	Rotterdam[b]	AES[c]
March et al. [11]	8.7	17.8	12.0
Yildiz et al. [12]	6.1	19.9	15.3
Mehrabian et al. [13]	7.0	15.2	7.9

[a]National Institutes of Health international conference 1990
[b]Task force sponsored by the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), 2003
[c]Androgen Excess Society diagnostic criteria 2009

continuum of presentation from those persons minimally affected, with regular menses and only mild excess of androgens to those who have a unilateral PCO, to those who manifest more severe grades of androgen excess. Efforts to include hyperandrogenemia as diagnostic criteria will remain inadequate until the sensitivity of androgen assays is better refined because of our current inability to accurately quantify circulating androgens in women.

Prevalence of Polycystic Ovary Syndrome

The prevalence of PCOS in any specified population is dependent upon the diagnostic criteria used, but does have some regional and ethnic variation. While most reports on the prevalence of PCOS range between 2 and 20 %, the chosen diagnostic criteria are recognized to influence the determined prevalence. A retrospective birth cohort in Australia found a prevalence of 8.7 % using NIH criteria, 17.8 % using Rotterdam criteria, and 12.0 % using AES criteria [11] (Table 1.2). A similar prevalence pattern was found in Turkey, where 6.1 % met NIH criteria, 19.9 % met Rotterdam criteria, and 15.3 % met AES criteria [12]. In Iran the estimated prevalence of PCOS was 7 % based on the NIH criteria, 15.2 % using Rotterdam criteria, and 7.92 % using AES criteria [13]. In North America, most estimates of the general population in the United States range from 4 to 8 % in the literature, although most of this information comes from an unselected population of white and black women in the southeast region [14, 15]. Mexican-American women have a higher prevalence, reportedly as high as 13 % [16]. Interestingly, the estimated prevalence of PCOS among women in Mexico is 6 %, only half of that found in their counterparts in the United States [17]. These discrepancies highlight not just an ethnic diversity in the prevalence of the disorder but also the significance of lifestyle in the occurrence of PCOS.

In India, PCOS is reported among 9 % of adolescents [18]. Among Indian women 15–35 years of age evaluated at a rural gynecology clinic, 13 % presented with menstrual irregularities, half of which were found to have PCOS, estimating the prevalence to be around 6.5 % [19]. In Sri Lanka, a similar prevalence of 6.3 % was

noted among women age 15–39 [20]. In Iran, the prevalence of PCOS is reported as 8.5 % out of a sample of reproductive-aged women selected for participation in the Tehran Lipid and Glucose Study [21]. A Greek study on the island of Lesbos found a prevalence of 6.8 % [22]. The overall prevalence of PCOS among a population of urban indigenous Australian women, using NIH criteria, was 15.3 % [23]. A study in the United Kingdom found the prevalence to be 8 % using stricter NIH criteria, while 26 % of their population met Rotterdam criteria, illustrating the differences seen when using different diagnostic criteria. In Spain, a population of Caucasian women presenting spontaneously for blood donation was found to have a prevalence of 6.5 % [24]. By any measure, PCOS is one of the most prevalent endocrine disorders worldwide, with obvious regional and ethnic variation.

Excess in facial and body hair and intractable acne are common reasons for women to seek evaluation with subsequent unmasking of PCOS. Rates of hirsutism vary among ethnic groups. In the United States, the rates are similar between black and white women (around 5 %) [25], but in Kashmir, India, the prevalence is much higher at 10.5 % [26]. Among women with hirsutism, up to one-third have an underlying diagnosis of PCOS. Around 27 % of women presenting with acne were found in one study to have undiagnosed PCOS, compared to 8 % of controls [27]. Patients presenting with acne resistant to standard treatment have an even higher rate, near 50 % [28]. Among adolescents with irregular menses, after a 6-year follow-up period, 62 % continued to have irregular menses, 59 % of whom were diagnosed with PCOS. In other words, approximately one-third of the original adolescent population with irregular menses was diagnosed with PCOS within the study period [29].

Summary

PCOS is considered as the most common endocrine disorder amongst reproductive-age women and is characterized by a chronic course, with features that suggest varying combinations of reproductive functional deficits (such as ovulatory dysfunction or PCO morphology) and androgen excess (such as acne and hirsutism). The diagnosis of PCOS is based on well-defined criteria, and currently there are three major sets of diagnostic criteria available for utilization in clinical practice. Regional prevalence of PCOS can vary depending on the diagnostic criteria utilized as well as the ethnicity studied. Women with isolated symptoms of acne, hirsutism, and irregular menstrual cycles should be offered targeted screening. Beyond the symptom burden relating to PCOS that adversely impacts quality of life, and perhaps more clinically significant, is the higher prevalence of several medical comorbidities in the PCOS population that have been extensively covered in additional chapters in this text. Identifying PCOS and screening for these adjunct disorders will allow for timely institution of preventive strategies aimed at minimizing the overall health risk in this population.

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Chapter 2
Polycystic Ovarian Syndrome: A Diagnosis of Exclusion

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Key Points

- Polycystic ovary syndrome presents with features that may overlap with multiple other endocrine disorders and clinical conditions.
- These conditions must be ruled out before the diagnosis of PCOS can be established.

Introduction

Polycystic ovary syndrome (PCOS) is a common chronic condition with implications for morbidities, both in short-term (e.g., subfertility and pregnancy-related complications) and long-term risks (e.g., type 2 diabetes, cardiovascular disease, depression, poor quality of life, and overall mortality) [1]. A prompt diagnosis allows opportunities for early institution of preventive strategies for prevention of sequelae. However, diagnosing PCOS can be challenging, as the symptoms do overlap with a number of disorders that themselves require specific treatment. No single symptom,

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Table 2.1 Differential diagnoses that may mimic or coexist with PCOS

Condition	Initial screening
Hypothyroidism	TSH
NCCAH	17-Hydroxyprogesterone
Hyperprolactinemia	Prolactin
Androgen-secreting tumor	Testosterone, DHEAS
Cushing's syndrome	Midnight serum/salivary cortisol, 24-h urine free-cortisol, or dexamethasone suppression test
Exogenous androgen exposure	History-taking
Hypothalamic hypogonadism	Estradiol, FSH, LH

examination finding, or laboratory data is diagnostic of this disorder. Rather, arriving at a diagnosis requires a systematic approach aimed at excluding the differential diagnoses that could account for the patient's presentation; the diagnosis of PCOS is thus one of systematic exclusion.

Table 2.1 lists the common alternative etiologies that could mimic PCOS. On occasion, patients may experience these conditions concurrently with PCOS.

Prevalence and Epidemiology of PCOS

It is estimated that 2–18 % of women have PCOS, making PCOS the most common endocrine disorder among reproductive-aged women [2–4]. However, determination of exact prevalence is problematic owing to heterogeneity in the employed diagnostic criteria (see next section) and variability in presenting symptoms, laboratory values, and imaging studies across populations and over time.

Diagnostic Criteria for PCOS (Laboratory and Imaging)

PCOS may include hyperandrogenism, chronic anovulation, and sonographic polycystic ovarian morphology, once other etiologies have been excluded. Hyperandrogenism, either clinical or biochemical, is required for diagnosis of PCOS by the 1990 National Institutes of Health criteria and the 2006 Androgen Excess-PCOS criteria, in contrast to the 2003 Rotterdam criteria, which allows for any two of three [5–7]. Although obesity, luteinizing-hormone elevations, and insulin resistance are common among PCOS, these are not required for diagnosis [1].

Clinical hyperandrogenism can manifest with hirsutism, defined as midline distributed terminal hair growth, acne, or male-pattern balding [1]. Biochemical hyperandrogenism may include elevations in total or bioavailable testosterone or dehydroepiandrosterone sulfate (DHEAS), although the assays may not be sensitive even in the presence of clinical hyperandrogenism [1]. The presence of virilization (rapidly progressing hirsutism/acne with clitoromegaly) should raise suspicion for malignancy [1].

Chronic anovulation is suggested by oligomenorrhea with nine or fewer menstrual cycles annually, although eumenorrhea with absent ovulation is present in 20 % of PCOS patients [6]. Transvaginal ultrasound is considered the best imaging modality, with polycystic ovaries defined as having either ≥12 follicles 2–9 mm in diameter or ovarian volume >10 cm^3 in at least one ovary in the absence of ovarian lesions [5].

Etiologies that must be excluded include pregnancy, thyroid dysfunction, hyperprolactinemia, congenital adrenal hyperplasia, ovarian insufficiency, androgen-secreting neoplasm, hypothalamic amenorrhea, and Cushing's syndrome [1].

Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH), particularly nonclassical (NCAH), must be considered in any woman presenting with features of PCOS. Notably, symptoms of hirsutism, acne, alopecia, and menstrual dysfunction that are commonly attributed to PCOS can be seen in patients with CAH [8, 9].

CAH is an autosomal recessive disorder most commonly involving the mutations of the gene encoding the enzyme 21-hydroxylase and, less frequently, genes encoding 11β-hydroxylase and 3β-hydroxysteroid dehydrogenase, with phenotypes exhibiting wide variability depending on severity of mutations and number of alleles affected [9, 10]. All of these enzyme deficiencies lower production of cortisol and aldosterone by the adrenal cortex. This lowers the negative feedback of cortisol on the pituitary, prompting compensatory increased pituitary adrenocorticotropic hormone (ACTH), leading to hyperplasia of the adrenal cortex [9]. Precursor hormones proximal to the deficient enzyme accumulate that are either themselves androgenic or are converted to androgenic products and thus promote androgenization and virilization [9]. For example, 17-hydroxyprogesterone that is elevated in 21-hydroxylase and 11β-hydroxylase deficiencies may be converted to androstenedione via 17, 20 lyase, and through an alternate pathway, to dihydrotestosterone [9].

NCAH presents in later childhood to early adulthood, affecting approximately 1 in 1,000 to 2 in 1,000 [8, 9] in the general population, but may be much more prevalent in certain ethnic groups such as Hispanics, Yugoslavs, and Eastern European Ashkenazi Jews [9, 10]. Classical CAH occurs in 1 in 5,000–15,000 live births [9] and manifests as neonatal ambiguous genitalia or virilization, but diagnosis is important because 75 % of cases have associated salt-wasting [10]. Subfertility and infertility are uncommon in NCAH but more prevalent with the classical type [11]. However, NCAH has been reportedly associated with higher rates of pregnancy loss that improved with glucocorticoid therapy [11]. Twenty-seven to seventy-six percent of NCAH are carriers of a severe loss-of-function mutation, which has particular importance during pregnancy in terms of female fetus virilization [11]. If the paternal status is unknown, the mother may be treated with dexamethasone to suppress fetal ACTH and minimize excessive fetal

androgen production until prenatal diagnosis can be achieved by either chorionic villus sampling or amniocentesis [10]. This strategy is recommended, particularly if there is a history of a child or first-degree relative with classical CAH, but concerns have been raised about adverse maternal and fetal effects as a result of dexamethasone exposure [10].

Clinically, it can be difficult to distinguish NCAH from PCOS [8, 12, 13], since both present with features of androgen excess. The androgen excess associated with CAH may lead to disruption of gonadotropin release from the pituitary and ovarian cysts, producing superimposed PCOS [10]. NCAH due to 11β-hydroxylase deficiency may be distinguished from PCOS by accompanying hypertension [14]. A morning basal 17-hydroxyprogesterone level >2 ng/mL during the follicular (preovulatory) stage of the menstrual cycle has 97 % sensitivity for screening the commonest variant of NCAH that due to 21-hydroxylase insufficiency [8]. However, 17-hydroxyprogesterone will be normal in cases of 3β-hydroxysteroid dehydrogenase deficiency [15].

Once NCAH is suspected based on initial screening, patient should undergo the diagnostic ACTH Stimulation Test. Exogenous acutely administered ACTH would be expected to increase accumulation of precursors proximal to the deficient enzyme. In the case of suspected 21-hydroxylase deficiency, 17-hydroxyprogesterone (sometimes accompanied by androstenedione) is drawn at both baseline and 30–60 min following intravenous administration of 250 μg synthetic ACTH [9]. Testing should be done in the morning when endogenous ACTH is maximal [16] and during the follicular phase of the cycle to prevent false-positive progesterone elevations from ovulation. An overnight fast is preferred, given that food intake may affect the hypothalamic–pituitary–adrenal axis [17]. NCAH is diagnosed with a stimulated 17-hydroxyprogesterone level >10 ng/mL [12].

With 11β-hydroxylase deficiency, 11-deoxycortisol and 11-deoxycorticosterone increase in addition to 17-hydroxyprogesterone and androstenedione during the ACTH Stimulation Test [14]. Different hormone profiles will be seen for the rare HSD3B2 deficiency, whereby elevated 17-hydroxypregnenolone and DHEA levels are observed [15]. The clinical impression may be further refined with genotyping. CYP21A2 is encoded on the short arm of chromosome 6, and missense mutations resulting in NCAH with 20–60 % of enzyme activity preserved include P30L, V281L, R339H, and P453S [10].

Treatment of NCAH depends on the severity of symptoms and is individualized to the patient. Menstrual cyclicity can be reinstituted and hyperandrogenic features controlled through use of hormonal contraceptives and use of antiandrogens (individually or in concert). A judicious use of exogenous glucocorticoid by exerting negative feedback at the pituitary level and, hence, suppressing the ACTH-driven adrenal steroidogenic pathway, allows spontaneous resumption of ovulation without conferring additive risk of multiple pregnancy, and should be considered in anovulatory patients desirous of achieving pregnancy [9, 10]. As discussed earlier, a diagnosis of NCAH per se is not an indication for continuing glucocorticoid therapy during pregnancy [10, 18].

Tumors

In women, androgen-secreting tumors can originate from the ovary or the adrenal gland. Rapidity of onset and symptom progression and evidence of virilization should alert one to the possibility of an androgen-secreting tumor as a mechanism for the PCO-like clinical picture. Tumors vary in the potency of androgens secreted, although weaker androgens may be converted into potent hormones in peripheral sites. Serum total testosterone >200 ng/dL has been used as a threshold for alerting clinicians regarding the possibility of an underlying androgen-secreting tumor, and warrants further evaluation to rule out the possibility of an ovarian and/or adrenal tumor [19, 20]. At this specified threshold, the test has a high sensitivity and negative predictive value. However, because of the relatively low overall prevalence of androgen-secreting tumor/s, the positive predictive value of total testosterone for diagnosing an androgen-secreting ovarian or adrenal tumor is poor [20]. It is important to appreciate that total testosterone levels at ≤200 ng/dL do not necessarily exclude the possibility of an underlying tumor; additionally, tumor interpretation can be challenging because a standardized testosterone assay is not available [1]. Virilizing tumors of the adrenal glands are also extremely rare. Measurement of DHEAS has been recommended for screening of virilizing adrenal tumors given its exclusive production by the adrenal, with further workup at levels >600–700 μg/dL, but it is difficult to assess positive predictive value because the prevalence of tumor is so low [20]. Levels that suppress to normal with dexamethasone administration suggest a non-neoplastic process [19].

Ultrasound is generally regarded as the initial imaging choice for evaluating the ovaries. However, sex cord-stromal tumors can be difficult to visualize by ultrasound given that a majority are isoechoic to the ovary [21]. When ultrasound imaging of the ovaries is inconclusive, additional imaging is warranted, preferably by magnetic resonance imaging (MRI) rather than computer tomography (CT). Even with advanced imaging, however, virilizing ovarian tumors can be difficult to localize [22]. When this occurs, the ovarian veins can be sampled bilaterally and testosterone levels can be measured to identify the laterality of ovarian source of excess androgen [22]. The ovarian vein with abnormally high androgen levels can identify on which side the tumor exists [22].

The treatment of androgen-secreting ovarian tumors depends on the stage at the time of diagnosis. Most are stage I, meaning that they are unilateral and localized to the ovary, and are treated with surgery [23, 24]. Surgery may involve unilateral or bilateral oophorectomy accompanied by hysterectomy, depending on the patient's fertility desires, along with staging [23, 24]. In contrast to the ovary, CT is a reasonable initial imaging modality for virilizing adrenal imaging tumors [19]. In the event of a suspected focal adrenal source of androgen excess, patients should be promptly referred for definitive management that will likely entail a surgical approach [19].

Cushing's Syndrome

Cushing's syndrome is an uncommon but potentially life-threatening condition that can mimic PCOS. Caused by excessive exposure to cortisol, with an incidence of 0.7–2.4 per million population per year [25], the clinical presentation commonly overlaps that of PCOS, including progressive weight gain, menstrual abnormalities, and features of hyperandrogenism (acne and hirsutism) [25, 26]. Clinical stigmata include evidence of central adiposity, dorsal cervical fat accumulation, proximal muscle weakness, violaceous cutaneous striae, depression, hypertension, osteopenia, and glucose intolerance [25, 26]. Anterior pituitary corticotroph tumors leading to excessive ACTH production are responsible for 70 % of the cases of Cushing's syndrome, with the remainder being due to ectopic ACTH production [25]. Cushing's syndrome may be the presenting feature of an occult cancer presenting as a paraneoplastic syndrome, or it may result secondary to cortisol-secreting tumors or even follow exogenous exposure to glucocorticoids [25].

Screening for Cushing's syndrome may be accomplished by sampling cortisol in serum, saliva, or urine, either alone or in combination. Timing of sample takes into consideration diurnal changes of ACTH and cortisol, which peak in the morning and nadir at night [16]. Salivary cortisol assessed between 2,300 and midnight is used as a surrogate for midnight serum cortisol [26]. Falsely positive screening may be encountered in cases of depression, alcoholism, anorexia nervosa, and use of estrogen-containing contraceptives (within 6 weeks of testing), rifampin, antiepileptic drugs, or late pregnancy [25, 27]. Contraceptive use increases cortisol binding globulin and, thereby, total serum or salivary cortisol, but it does not affect free-cortisol levels in urine [27]. Renal dysfunction or severe illness may produce false-negative results [25, 27]. An abnormal result for 24 h urine free-cortisol is considered as >4 times normal (<150 μg/24 h, depending on the assay) [26–28]. A salivary cortisol level >8.6 nmol/L (0.31 μg/dL) or repeatedly >4.3 nmol/L (0.15 μg/dL) is highly suggestive of Cushing's syndrome, with sensitivity of 90–95 % and specificity of 90–100 %, but results may be compromised in patients with depression or for occupations with disrupted sleep–wake cycles [26, 27].

Suppression of normal pituitary ACTH release follows exposure to an exogenous glucocorticoid, thereby decreasing serum cortisol level. As a screening strategy for diagnosing overproduction of cortisol, the overnight dexamethasone suppression test (DST) utilizes administration of 1 mg dexamethasone at 2300 hours, followed by serum cortisol measurement in early morning, between 0800 and 0900 hours; serum cortisol level of <50 nmol/L (<1.8 μg/dL) is taken to reflect adequate adrenal suppression [25, 26]. An overnight fast is preferred, as serum cortisol levels during the DST may be affected by food intake [17]. Less commonly, a total of 4 mg dexamethasone is administered in divided doses of 0.5 mg every 6 h over 48 h, and serum cortisol is measured at 0900 hours at the start and end of the test [25]. The false-negative rate of the DST is reported to be 8 % [26].

Once a diagnosis of CS has been made, the patient should be referred to an endocrinologist for further management. While definitive management is beyond

the scope of reproductive endocrinology, the next steps focus on unraveling the underlying mechanism(s) for cortisol overproduction. Serum ACTH levels, if abnormally elevated, should prompt imaging studies to identify focal pituitary pathology and/or metastatic lesions that may account for ACTH overproduction. Alternatively, non-suppressible excess cortisol in the setting of low ACTH levels should direct one to imaging of the adrenals for evidence of an adrenal mass or hyperplasia. In the absence of evident adrenal asymmetry or mass, adrenal vein sampling may help establish a diagnosis of adrenal overproduction of cortisol; definitive surgical intervention can then follow, if indicated [25].

Exogenous Androgens

Prior to making a diagnosis of PCOS, a hyperandrogenic patient should be queried as to exposure to agents with androgenic activity such as danazol, synthetic derivatives of testosterone for "performance enhancing," and exogenous use of testosterone by the patient or her partner (symptoms of androgen excess have been reported from passive transfer through physical contact of transdermal testosterone gel being used by partner) [29]. Once exposure is discontinued, symptoms typically improve, although virilization with clitoromegaly, male-pattern balding, voice deepening, and hirsutism may persist [30].

Idiopathic Hyperandrogenism

At least in one study, idiopathic hyperandrogenism accounted for approximately 15 % of cases of presumed androgen excess in premenopausal women of Italian origin [31]. The diagnosis is based on clinical and biochemical assessment. Ovulatory menstrual cycles, sonographically normal ovaries, and a normal androgen milieu despite clinical evidence of hyperandrogenism identify these cases as distinct from PCOS [31].

Hypothyroidism

While menstrual disturbances are commonly encountered in the setting of thyroid dysfunction, the prevalence of menstrual dysfunction is much higher with hypothyroidism compared to hyperthyroidism [32]. Evaluation of thyroid function must be undertaken in any woman presenting with menstrual irregularities. Thyroid evaluation is typically performed by obtaining a serum thyroid-stimulating hormone (TSH) level, preferably in the morning when the level is at its nadir [33]. If the TSH is elevated, a thyroxine (T4) serum level can be obtained. In the case of overt

hypothyroidism, laboratory findings would demonstrate an elevated TSH level and a decreased free-T4 level [33]. Elevated TSH in the setting of normal free-T4 levels is consistent with subclinical hypothyroidism, which can progress to overt disease over time [33]. Less commonly, free T4 is low but TSH is normal or low, which suggests hypothalamic or pituitary dysfunction [33]. Patients having severe hypothyroidism with TSH >10 mIU/mL have significant menstrual disturbances (34 % vs. 23 % in controls), including irregular cycles, oligo-, poly-, and amenorrhea, but those with mild-moderate hypothyroidism were not significantly different [32]. The prevalence of TSH >4.5 mIU/mL in the United States among women age 12–49 is 3.1 % [34] but increases to 31.5 % at TSH of 4–4.5 mIU/mL [35]. Other laboratory findings of hypothyroidism include elevated low-density lipoprotein (LDL), high triglycerides, hyponatremia, normocytic anemia, proteinuria, hyperprolactinemia, and elevated C-reactive protein levels [33].

Patients with overt hypothyroidism are encouraged to start thyroid replacement therapy, which is typically continued for life [33]. This has particular importance for women of reproductive age, although evidence to support therapy in the setting of subclinical hypothyroidism is debated [36]. The thyroid gland makes thyroxine (T4) and the more biologically active triiodothyronine (T3) [33]. Twenty percent of T3 is synthesized from the thyroid gland, with the remainder derived from peripheral conversion of T4 by deiodinase enzymes [33]. T3 preparations have short half-lives and require more frequent daily dosing, so T4 in the form of levothyroxine is preferred [33]. After initiating pharmacologic treatment of hypothyroidism, TSH levels are followed until normalized [33].

Hashimoto's disease is the most common cause of hypothyroidism in the United States and is the result of autoimmune thyroid damage [33]. Other relatively uncommon etiologies for hypothyroidism include iodine deficiency, radioablation or surgery to treat hyperthyroidism, radiation to the neck, central dysfunction from a cranial mass, radiation to the head, or ischemia [33]. Certain medications can also result in hypothyroidism, such as lithium, amiodorone, interferon-alpha, and interleukin-2 [33].

Hyperprolactinemia

Hyperprolactinemia is a relatively common endocrinopathy with an estimated prevalence at 15 % in women with isolated anovulation, and 43 % in those manifesting a combination of galactorrhea with anovulation [37]. While hyperprolactinemia may be asymptomatic, common clinical features include galactorrhea, oligo-amenorrhea, reduced libido, and subfertility [38].

Prolactin is synthesized and released by lactotroph cells in the anterior pituitary and is inhibited by dopamine from the hypothalamus via the pituitary portal circulation [37–39]. Diurnal variations in circulating levels of prolactin are evident, with the levels being highest during sleep and lowest during waking hours [39]. Normal serum levels of prolactin are 10–28 μg/L [37–39].

A spectrum of physiological, pathological, and pharmacological etiologies may underlie hyperprolactinemia. Physiologic causes of prolactin excess include pregnancy, lactation, breast stimulation, stress, sexual intercourse, and exercise [37–39]. Pathologic contributors to prolactin excess include pituitary and non-pituitary tumors, of which the most common are tumors of lactotrophs (prolactinoma) that account for about 30 % of all pituitary adenomas [37–39]. Iatrogenic causes of hyperprolactinemia are not uncommon; commonly used pharmacological agents that can cause hyperprolactinemia include verapamil, metoclopramide, methyldopa, phenothiazine, and reserpine [37–39]. Estrogen, thyrotropin-releasing hormone (TRH), epidermal growth factor, and dopamine receptor antagonists promote synthesis and secretion of prolactin [37–39]. In primary hypothyroidism, in which thyroid hormone secretion is low, compensatory increased TRH would increase both TSH and prolactin secretion [39].

Per the Endocrine Society, serum prolactin levels are accurate for diagnosing hyperprolactinemia, and dynamic testing (TRH, L-dopa, nomifensine, and domperidone) is not superior or necessary [38]. Despite diurnal variations [39], prolactin can be drawn at any time of day [38]. It is best to measure prolactin at least 1 h after awakening or eating [40]. It is preferable to draw prolactin levels prior to performing breast or pelvic examinations, though one study did not find that breast examinations affected prolactin levels in euprolactinemic women [41]. Prolactin levels persistently >25–28 μg/mL need further evaluation. If the patient with moderately elevated prolactin level <150 μg/mL is asymptomatic or has less-typical presenting symptoms such as decreased libido in the setting of eumenorrhea, polyethylene glycol precipitation to identify macroprolactin forms, which have low bioactivity, can avoid further workup [38, 40]. Symptomatic patients should receive a gadolinium-enhanced MRI to evaluate the pituitary for an adenoma, particularly a macroadenoma with size >10 mm [38, 40]. Although macroadenomas are associated with prolactin levels >250 μg/mL, tumor size does not always correlate with serum levels, so imaging is warranted even with low degrees of prolactin elevations [38, 40]. Symptoms of headaches, visual changes, features suggestive of cranial nerve palsy, and perturbations in pituitary hormones in addition to prolactin excess (such as concomitant thyroid and adrenal dysfunction) are concerning as mass effect phenomena secondary to a pituitary tumor, and merit expeditious evaluation with brain MRI and visual field testing [38].

If the patient with hyperprolactinemia is asymptomatic, does not have amenorrhea, and does not have a macroprolactinoma, treatment is not needed [38]. Treatment of hyperprolactinemia depends on the underlying mechanism and the individual patient's clinical profile. In medication-induced hyperprolactinemia, discontinuation of the offending agent may restore gonadal function, but may come at the expense of exacerbating other medical conditions, particularly psychiatric [38]. Restoration of functioning of the hypothalamic–pituitary–ovarian (HPO) axis through use of dopamine agonists allows resumption of spontaneous ovulation; this strategy is particularly meaningful for those desirous of fertility, and in those diagnosed with a pituitary macroadenoma wherein tumor shrinkage can be achieved in more than 90 % of patients through lactotroph suppression [37–39].

Dopamine agonists (such as bromocriptine, cabergoline, and quinagolide) are the mainstay of pharmacologic treatment in clinical practice; acting on the D2-type receptors on the pituitary lactotrophs, these agents decrease the synthesis and cellular release of prolactin [37–39]. Treatment with dopamine agonists restores ovulation in about 90 % of women with anovulatory infertility secondary to hyperprolactinemia [37–39]. Cabergoline is preferred for macroprolactinomas over bromocriptine, with magnitude of tumor shrinkage 90 % vs. 50 %, respectively [38].

Decreased bone mineral density and predisposition to skeletal fragility should be considered as a long-term risk relating to chronic hyperprolactinemia and premenopausal hypogonadism [38]. Dopamine agonist therapy should be considered as a strategy to normalize ovarian function in patients deemed at a lifetime risk for skeletal fragility, as correction of hypogonadism allows an opportunity for optimizing bone mass accrual [38].

However, dopamine agonist therapy may exacerbate psychiatric illness, and, therefore, may not be appropriate for some patients [38, 40]. Estrogen-containing hormonal contraceptives may be considered in hypogonadal women with a microprolactinoma not desiring pregnancy or those who are not good candidates for dopamine agonist therapy [38, 40].

Hypothalamic Hypogonadism

Hypothalamic hypogonadism (HH) is the most common cause for menstrual irregularities in adolescents and must be considered in the differential diagnosis when evaluating for PCOS [42]. While oligo-amenorrhea dominates the clinical picture, subtle features suggestive of hyperandrogenemia may be apparent (e.g., acne and excess villous hair). Circulating levels of androgens are typically within normal range, although mild excess in free testosterone may be seen and can be attributed to suppressed serum sex hormone binding levels (a sequel to protracted hypoestrogenemia). Laboratory testing typically indicates low estradiol, while gonadotropin levels may be low or normal, and other etiologies relating to thyroid or prolactin disorders are excluded [42].

HH is categorized as functional, implying a correctable cause, and nonfunctional. In functional HH, pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) responsiveness to hypothalamic gonadotropin release hormone (GnRH) is intact, but the pulsatility of GnRH secretion is altered. Potentially reversal causes of functional HH include psychologic stress, nutrition deficit, and exercise excess [42]. Stress (psychological as well as physical) is a recognized contributor to HH; stress-induced release of hypothalamic corticotropin release hormone (CRH) disrupts the pulsatile release of GnRH, thereby resulting in dysfunction of the pituitary gonadotrophs [42]. Anorexia nervosa (AN) is classically associated with HH. Combinations of extreme nutritional limitations, low percentage body fat mass, and concomitant leptin deficiency as well as ongoing psychological stress are

recognized as contributory to HH of patients diagnosed with AN; the latter explains the inconsistent recovery of the hypothalamic–pituitary–ovarian axis function, despite fat mass being regained through intervention in some patients diagnosed with AN [42]. Athletes endure combinations of extreme physical and psychological stresses as well as dietary restrictions that predispose them to HH [42]. Nonfunctional hypothalamic states as causes for HH are relatively uncommon and merit consideration in appropriate clinical settings; these include idiopathic HH, Kallmann's syndrome, infection, and chronic illness [42]. Idiopathic HH presents as primary amenorrhea and absent pubertal development and is estimated at 1 in 50,000 females [43]. Kallmann's syndrome is a rare condition caused by a genetic mutation and additionally presents with anosmia (apparent or covert) due to failure of olfactory neuron migration during embryonic development [42]. Infectious etiologies include tuberculosis, sarcoidosis, and syphilis [42].

Management considerations for HH are guided by underlying mechanisms and the patient's unique clinical profile. Of particular concern is the long-term risk for skeletal fragility given that HH is a protracted state of hypoestrogenemia. For patients with HH not desirous of pregnancy, estrogen therapy (either as a hormonal contraceptive formulation or as menopausal hormonal regimen) offers skeletal benefit for all except those with AN [44]. For the latter, some success with normalization of the HPO-axis function, and skeletal protection may be achieved through combinations of targeted psychological support and therapy and through deliberate weight gain [42]. Additionally, bisphosphonate risedronate and low-dose testosterone, both used off-label, improved bone density in a trial involving premenopausal women with AN [44]. Exogenous leptin therapy has demonstrated therapeutic success in achieving normalization of the HPO functioning, albeit in research settings [45].

For patients with HH seeking fertility, correction of the underlying condition may restore GnRH pulsatility; more realistically, however, successful ovulation can be achieved either through use of exogenous gonadotropins (albeit at the cost of an increased risk for multiple pregnancy) or where access to pharmacological GnRH is available with use of exogenous GnRH administered in a physiological paradigm through a transcutaneous pump (a physiological approach that holds minimal risk for multiple ovulation) [42].

Hyperandrogenic Insulin Resistance with Acanthosis Nigricans (HAIR-AN) Syndrome

Hyperandrogenic insulin resistance with acanthosis nigricans, or HAIR-AN, syndrome represents an extreme state of insulin resistance and can be identified in 1–3 % of women presenting with clinical features of hyperandrogenism [46]. Although there is no gold-standard test, a diagnosis is suggested based on markedly elevated basal or glucose-stimulated insulin levels (more than 3–5 times higher than the upper limit of normal range) [46]. It is quite plausible that rather than being a distinct entity, HAIR-AN may represent a more metabolically severe form

of PCOS [46, 47]. Despite the severe hyperinsulinemia, pancreatic β cells appear to be dysfunctional, such that the severity of hyperinsulinemia is disproportionate to the glycemic profile of these patients [47]. High circulating levels of insulin are recognized to drive androgen synthesis by the ovarian theca cells and, thus, are of pathophysiological relevance to the overall clinical picture. Management strategies aim at improving insulin sensitivity through a combination of lifestyle modifications and pharmacological therapy [46, 47].

Hyperreactio Luteinalis

Hyperreactio luteinalis is an uncommon condition that occurs during pregnancy in the second or third trimester and produces severe virilization [48]. The etiology is unknown but may be due to increased ovarian sensitivity to hCG, thereby increasing androgen production by theca cells and causing large cystic ovaries [48]. The risk is increased in gestational trophoblastic disease [48]. Although the condition is unlikely to be mistaken for PCOS during pregnancy, it is possible that a provider may see the patient for the first time during the postpartum period.

Coexisting with PCOS

Although related endocrinologic disorders are typically ruled out prior to establishing the diagnosis of PCOS, they may coexist. Hyperprolactinemia was present in 7 % of Spanish women with hyperandrogenism [49]. As half of those cases were associated with macroprolactin, which would point more toward PCOS as the primary etiology for the hyperandrogenism, it is unclear whether the association is coincidental and independent or somehow related [49].

Autoimmune hypothyroidism and thyroiditis are more prevalent among PCOS patients compared to controls, and may relate to increased humoral immunity in the setting of unopposed estrogen [50, 51]. Subclinical hypothyroidism (TSH 4.5–10 mIU/mL) was present in 11 % of Brazilian women with PCOS and was associated with higher LDL cholesterol and prolactin levels than those with normal TSH [52]. Women with PCOS and subclinical hypothyroidism also have higher triglycerides than euthyroid PCOS women [53]. This relationship is likely bidirectional, as metformin therapy for women with both PCOS and hypothyroidism reduced TSH levels [54], while thyroid hormone therapy for hypothyroid patients with polycystic ovaries decreased both ovarian size and serum androgen levels [55].

Superimposed PCOS can occur due to the hyperandrogenic state from NCAH [10], with the two coexisting in 10 % of NCAH-afflicted Greek women [13].

Among patients with functional hypothalamic amenorrhea, 30–50 % have sonographic polycystic ovary morphology [56]. Although these patients are not

Table 2.2 Diagnostic paradigms for PCOS[a]

	Society consensus criteria		
	NIH 1990	Rotterdam 2003	AES 2006
Signs/symptoms	Oligomenorrhea + androgen excess	Any 2	Androgen excess + either anovulation or PCO
Oligomenorrhea or anovulation	+	+/–	+/–
Hyperandrogenism	+/–	+/–	+/–
Hyperandrogenemia	+/–	+/–	+/–
PCO on ultrasound	–	+/–	+/–

[a]Reprinted from Fertility and Sterility, 81/1, revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome, 19–25, Copyright 2004, with permission from Elsevier

Table 2.3 Suggested workup for evaluation of PCOS

TSH
Prolactin
Androgens
DHEAS
Testosterone (total and free)
17-Hydroxy progesterone
Pelvic ultrasound
Miscellaneous
Glucose tolerance test or hemoglobin a1c
Lipids
24-h Urine-free cortisol

hyperandrogenic initially, they have higher stimulated serum androstenedione and testosterone levels than controls [57]. This exaggerated ovarian response to gonadotropins is similar to that found in PCOS; a normal unstimulated androgen status in a subgroup of women with HH may be a reflection of ovarian quiescence due to basal hypothalamic suppression, and recovery of HPO axis in this population may unmask a PCOS phenotype [57].

Summary

PCOS is the most common cause of hyperandrogenic anovulation among reproductive-aged women. Early diagnosis allows the opportunity of timely interventions aimed at reducing long-term morbidity in this population. A variety of potentially serious disorders can, however, mimic the clinical presentation of PCOS. Despite a patient meeting well-defined clinical criteria (Table 2.2), the diagnosis of PCOS is one of exclusion after systematic evaluation to rule out common endocrinopathies that can masquerade as PCOS (Table 2.3).

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Part II
Pathophysiology of PCOS

Chapter 3
Recent Advances in the Genetics of Polycystic Ovary Syndrome

Michelle R. Jones, Ning Xu, and Mark O. Goodarzi

Key Points

- Genome-wide association studies in large sample sizes have identified, with high confidence, a number of susceptibility loci for PCOS.
- Epigenetics is an emerging field that has recently been applied to PCOS, which potentially may explain its developmental origins.
- Identification of PCOS susceptibility genes will expand our understanding of pathways and processes implicated in the syndrome's etiology, allowing development of new diagnostic and treatment modalities.

The Heritable Basis of PCOS

Familial Aggregation Studies

The genetic basis of polycystic ovary syndrome (PCOS) was recognized over 40 years ago [1]. Early studies that focused on the prevalence of PCOS-related traits in the siblings of PCOS probands suggested an autosomal dominant model of

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inheritance [2, 3]. Several small family studies of prevalence of PCOS or related phenotypes supported this hypothesis, with prevalence ranging from 51 to 66 % in first-degree relatives of women with PCOS [4, 5]. The rate of polycystic ovarian morphology or male pattern baldness in first-degree relatives of PCOS subjects was reported as 51 %, and it was subsequently suggested that a single gene might be responsible for not only the oligomenorrhea and hirsutism in women with PCOS but also the male pattern baldness in male family members of PCOS subjects [5]. A large study utilizing 250 consecutive PCOS probands and their families found that 75 % of the probands reported at least one family member with either hirsutism alone or PCOS including hirsutism [6]. In this report, 50 % of mothers or sisters, 25 % of aunts, and 20 % of grandmothers had hirsutism or hirsutism with oligomenorrhea, suggesting a high heritability for PCOS [6]. Subsequent reports established a prevalence of PCOS in the premenopausal mothers and sisters of probands at 35 % and 40 %, respectively [7].

A study of hyperandrogenemia in sisters of PCOS probands found that while 22 % of sisters had PCOS themselves, another 24 % had evidence of hyperandrogenemia when tested, with regular menstrual cycles; the bimodal distribution of testosterone levels in the sisters led the authors to propose control by a single autosomal locus [8]. The presence of a key component phenotype of PCOS without the full manifestation of the syndrome suggested that phenotypic heterogeneity may have a genetic basis, whereby some sisters of PCOS probands had inherited some but not all the genetic risk factors for PCOS, thus resulting in a partial phenotype. Following the initial reports, however, systematic genetic investigations failed to support an autosomal dominant mode of inheritance; rather, PCOS appears to be inherited as a common complex trait, with multiple susceptibility loci.

Twin Studies

Study of a small cohort of both monozygotic and dizygotic twin pairs initially suggested that PCOS was unlikely to be an autosomal dominant disorder due to the high rate of discordance in the phenotype, but rather a polygenic or X-linked disorder [9, 10]. A subsequent questionnaire-based study of over 3,100 Dutch twins identified only 92 subjects with PCOS (prevalence of 2.97 %) [11], a lower prevalence than typically reported in population-based studies. This large twin study reported a monozygotic correlation for PCOS (r^2) of 0.72, and a dizygotic correlation for PCOS of 0.38. The authors concluded that 72 % of variance in risk of PCOS has a genetic basis, strongly indicating a significant genetic component to the disorder [11]. There is no increased risk to the female member of opposite-sex twin pairs, suggesting that the genetic risk for PCOS significantly outweighs any risk that may result from prenatal androgen exposure that may arise in opposite-sex twin pairs [12].

The Candidate-Gene Era

Challenges of the Candidate Approach

The genetics of PCOS has largely been pursued using the candidate-gene approach, which focuses on genes of interest selected on their hypothesized role in the disease under study. This approach has not proven particularly fruitful in furthering our understanding of genetics of complex diseases for a number of reasons [13]. Until recently, these studies have generally been performed in small- to moderate-sized cohorts (typically considerably less than 500 cases and 500 controls) and only investigated a single, or very few, markers in a single gene. Such small studies are susceptible to both false-negative and false-positive results. Another challenge of candidate-based gene discovery is inherent to the genetic architecture of complex diseases. Many genes each contribute a small amount of susceptibility risk to the disease, meaning that often thousands of samples need to be studied to detect their effects. Sample sizes of this magnitude have not been available in PCOS studies until very recently.

More than 100 candidate genes have been examined, but only a handful of genes have been found to be associated with PCOS or its component traits with replication in independent cohorts. These genes are from a number of pathways that include steroidogenesis, insulin and glucose metabolism, inflammation, and obesity. The application of improved study design, including haplotype and tagging approaches that capture the majority of common (frequency >5 %) variation in the gene of interest (using the concept of linkage disequilibrium), have helped confirm [14] or clearly disprove [15] a role for several proposed candidate genes.

In this chapter, we will focus on recent candidate genes that have positive evidence for replication in the literature. We will not exhaustively discuss the many genes that have been studied, as this has been done in several reviews [13, 16–19]. Of note, the limited number of genes discussed below reflects in part the fact that many genes have been reported only once, with no attempt at independent replication [20, 21].

Successes from the Candidate-Gene Era

One of the most convincing candidate loci for PCOS is a 1 cM region on chromosome 19p13.2 that includes the *INSR* gene, which codes for the insulin receptor. Initial reports described both linkage and association between the dinucleotide repeat microsatellite marker D19S884 (allele 8, 17 units of the repeat motif, is the most significantly associated allele), which lies within intron 55 of the fibrillin-3 gene [22]. *FBN3* is a member of the fibrillin family of extracellular matrix proteins. The fact that D19S884 is 1.2 cM from the *INSR* gene indicates that this locus might harbor multiple susceptibility genes. Replication of association between this marker and

PCOS in independent cohorts was subsequently reported by the original authors, with larger sample sizes gleaning increasingly significant *P* values [23–25]. In a total of 465 families, the authors reported an association between allele 8 of the microsatellite and PCOS with $P < 7 \times 10^{-5}$ [24]. Allele 8 of D19S884 was also correlated with circulating levels of transforming growth factor β (beta) 2 (TGF-β2), inhibin B, and aldosterone [26]. Associations have also been reported between this microsatellite and metabolic phenotypes such as increased fasting insulin [27]. The function of this intronic microsatellite marker is unknown; however, recent studies have suggested promoter activity at the sequence encompassing D19S884 [24]. There has not been any indication of allele-specific promoter activity [24], and the expression level of the *FBN3* gene itself has been reported to be extremely low in a number of ovarian cell types [28], indicating that the D19S884 association signal may be an index signal for causal variants in other gene(s) elsewhere in the region [28].

There are a number of other viable candidate genes within this region that are worth considering, including *RETN*, which codes for the adipokine resistin. A single promoter SNP (*RETN*-420C/G) was not associated with PCOS [29, 30]. Because a comprehensive tag SNP approach to *RETN* has not been published to date, a role for this gene in PCOS cannot be fully ruled out. Two other as yet uninvestigated genes that may influence susceptibility for PCOS are *ELAVL1* and *ANGPTL4*, which act in steroid and metabolic pathways, respectively.

The initial studies did not support a role for *FBN3* in the PCOS ovary [28]. The initial inclusion of D19S884 was to investigate variants surrounding the *INSR* gene [22]. In early studies of *INSR*, investigators used exon sequencing in an effort to identify novel functional variants in PCOS cases [31–33]. These studies were not able to identify exonic variants associated with PCOS; however, they were hampered by small sample sizes, likely a function of the costly methods available for sequencing at the time. After the initial reports of both linkage and association at D19S884, the *INSR* gene became a target for a number of additional studies. A minority of studies were able to find associations between SNPs in the *INSR* gene and PCOS risk, many of which are novel SNPs (Table 3.1) [14, 34–42].

There have also been several reports failing to find association between the *INSR* gene and PCOS; however, these studies used small sample sizes [42], assayed a single marker [38], or used a family-based approach in a modest number of families [41], which may have impacted these findings. To date the most extensive investigation of the *INSR* gene in PCOS covered the entire gene with a tagging approach and included a discovery cohort and a replication cohort [14]. In the discovery cohort, 5 of 30 SNPs across the *INSR* gene were associated with PCOS (rs10401628, $P = 0.004$; rs12459488, $P = 0.007$; rs12971499, $P = 0.009$; rs2252673, $P = 0.011$; rs6510949, $P = 0.028$), 4 of which were carried forward to a replication study. The SNP rs2252673 was confirmed to be associated with PCOS in the large replication cohort, independent of body mass index (BMI-adjusted *P* values: $P_{\text{discovery}} = 0.008$, $P_{\text{replication}} = 0.006$, $P_{\text{meta-analysis}} = 0.0006$). Because this variant is located in intron 11 of the *INSR* gene, it is unclear how it might affect *INSR* expression or function to influence the PCOS phenotype; as with D19S884, rs2252673 may be a proxy marker for other functional, causal variants in the region. The most important validation of the *INSR* locus as etiologic in PCOS is the discovery of an association signal at the

Table 3.1 Association studies of variants in the *INSR* gene and PCOS[a]

Study author	Population	Sample size[b]	Analysis	Marker	Marker location	Uncorrected *P* value
Siegel et al. [35]	Caucasian	99/136	χ^2	His1058His	Exon 17	NS
Chen et al. [36]	Chinese	120/40	χ^2	His1058His	Exon 17	NS
Jin et al. [37]	Chinese	109/107	χ^2	Cys1008Arg	Exon 17	NS
Lee et al. [40]	Korean	174/93	χ^2	His1058His	Exon 17	NS
Lee et al. [38]	Korean	134/100	χ^2	+176477 C>T	Intron 21	0.0019
Mukherjee et al. [39]	Indian	180/144	χ^2	His1058His	Exon 17	NS
Goodarzi et al. [14]	Caucasian	275/173 and 526/3,585	Logistic regression	rs2252673	Intron 11	0.00058
Xu et al. [41]	Chinese	260 trios	TDT	rs10500204	Intron 3	NS
Xu et al. [41]	Chinese	260 trios	TDT	rs8108622	Intron 3	NS
Xu et al. [41]	Chinese	260 trios	TDT	rs2059807	Intron 7	NS
Xu et al. [41]	Chinese	260 trios	TDT	rs1799817	Exon 17	NS
Unsal et al. [42]	Turkish	44/50	χ^2 and logistic regression	rs1799817, rs2229334, rs2229430, and others	Intron 12 exon 12, exon 17	NS

[a]Cohort included a total of 150 nuclear families: 148 Caucasian families and 2 Caribbean families

[b]Sample sizes are given as case/control unless otherwise described

INSR gene in a large, well-designed case/control genome-wide association study (GWAS) [43], discussed in greater detail later in this chapter.

A number of genes, although not studied as extensively as *INSR*, have been identified as candidates and successfully replicated in independent cohorts, which remains the gold standard, even in light of GWAS approach, for validation of association signals. The steroidogenic genes *FEM1B*, *HSD17B6*, and *SGTA* have all been implicated as PCOS-risk genes and replicated in independent association studies. The FEM family of genes are homologs of the *Caenorhabditis elegans fem1a* gene, a masculinizing gene in the nematode [44]. *FEM1A* was chosen as a candidate for PCOS based on its location at chromosome 19p13.3, near the linkage and association signal at 19p13.2, its role in gender determination in the nematode, and the discovery of a novel variant (H500Y) in a woman with PCOS [45]. Two SNPs in *FEM1A* (rs8111933 and rs12460989) and two SNPs (rs1015240 and rs6494730) in *FEM1B*, which is located at 15q22 and encodes fem-1 homolog b, were associated with PCOS status [46]. Association of *FEM1B* variation with PCOS was later replicated in a family-based approach [25]. A single SNP (rs898611) in the 17β (beta)-hydroxysteroid dehydrogenase type 6 gene (*HSD17B6*), at 12q13.3, was associated with increased BMI, increased homeostasis model assessment of insulin resistance (HOMA-IR), increased fasting insulin, and decreased fasting glucose to insulin ratio in two independent European-origin PCOS cohorts [47, 48] and with BMI in a cohort of Chinese women with PCOS [49]. The small glutamine-rich tetratricopeptide repeat-containing protein alpha (*SGTA*) gene at 19p13 has also been studied

and replicated as a PCOS locus, with an SNP (rs1640262) identified as protective against PCOS [25, 50]. *SGTA* encodes a member of the androgen receptor chaperone–co-chaperone complex that may modulate androgen signaling.

A large proportion of PCOS patients suffer obesity, insulin resistance, hyperinsulinemia, and lipid abnormalities [51], which translate into increased risk of type 2 diabetes and possibly cardiovascular disease [52], making these pathways good targets for candidate gene exploration. An intronic SNP in the proopiomelanocortin gene (*POMC*), which encodes a polypeptide that is cleaved to yield hormones involved in steroidogenesis, energy metabolism, obesity, and lipolysis, was initially reported by Urbanek et al. and replicated in an independent cohort by the same group [22, 25]. A number of SNPs in the fat mass- and obesity-associated gene (*FTO*) are associated with BMI in PCOS [53–56]. *FTO* is located on chromosome 16q12.2; its product acts to remove methyl groups from specific residues on both DNA and RNA. It is one of the most widely replicated obesity loci and has been found to affect BMI in a number of populations [57, 58]. It remains largely unclear how this gene acts to affect obesity traits despite extensive work to determine its function [59]. The role of *FTO* in obesity in PCOS was recently estimated in a meta-analysis of published studies (Table 3.2) and supports the candidate-based studies demonstrating a larger effect of *FTO* variants on BMI than in the general population [60].

A novel approach to gene discovery in PCOS has been utilization of expression data to guide candidate gene selection. Gene expression profiling has been performed in a number of tissues, including ovary, omental fat, lymphocytes, and subcutaneous adipose tissue [61–64]. These gene expression data sets were mined to identify both *DKK1* and *DNAJB1* as PCOS-risk candidates [65]. *DKK1* was underexpressed in PCOS omental tissue [62] but overexpressed in PCOS ovarian theca cells [61] and implicated in cell cycle regulation [66]. An intronic variant (rs1569198) was associated with total testosterone levels in one cohort and with dehydroepiandrosterone sulfate levels in a second cohort, supporting a role for *DKK1* in the androgen pathway in PCOS women [65]. *DNAJB1*, the second candidate examined in this study, is a molecular chaperone known to act in the androgen pathway and is also transcriptionally regulated by insulin [67]. *DNAJB1* had reduced expression in the PCOS ovary [68] and is located in the 19p13.2 susceptibility region. A synonymous variant (rs3962158) in exon 1 of the *DNAJB1* gene was associated with insulin-related traits in two independent cohorts [65]. Gene expression studies of subcutaneous adipose tissue indicated that *FOS* was highly underexpressed in PCOS [64]. Silencing of *FOS*, a member of the AP-1 transcription factor complex, has been shown to increase *CYP17* mRNA in both theca and granulosa cells of the ovary [69], consistent with reduced *FOS* expression in PCOS. Three intronic SNPs were associated with PCOS (rs8006998, $P=0.0031$; rs8013918, $P=0.0006$; rs8013942, $P=0.0087$) in a discovery cohort, with replication of the association of rs8006998 with PCOS in an independent cohort [70]. In a larger sample set of subcutaneous adipose tissue, underexpression of *FOS* in PCOS was confirmed; *JUNB*, another member of the AP-1 regulatory complex, was also underexpressed [70].

Table 3.2 Meta-analyses of PCOS genetic studies

Locus	Variant	Study	Number of studies	Cases	Controls	Result
ACE	287 bp Alu InDel	Jia [167]	6	1,451	773	NS
ADIPOQ	T45G: rs2241766	Gao [168]	8	792	1,322	$P=0.002–0.01$
		Xian [169]	11	920	960	<0.05
	G276T: rs1501299	Gao [168]	5	511	587	$P=0.03–0.04$
		Xian [169]	8	895	1,024	<0.05
AR	CAG repeat	Wang [170]	17	2,068	2,192	NS
CYP17	–34 T/C: rs743572	Li [171]	10	1,321	1,017	NS
FTO	rs9939609	Wojciechowski [60]	7	2,548	–	Increased BMI with risk allele, $P=2.26\times10^{-11}$
INSR	1058C/T[a]	Ioannidis [172]	8	795	576	NS
IRS1	Gly972Arg: rs1801278	Ioannidis [172]	11	889	1,303	$P<0.05$
		Ruan [173]	15	1,358	1,561	$P<0.001–0.09$
IRS2	Gly1057Asp: rs1805097	Ruan [173]	5	519	883	NS
MTHFR	C677T: rs1801278	Bagos [174]	6	223	392	NS
PAI1	4G/5G: rs1799889	Bagos [174]	7	1,538	710	$P<0.05$
PPARG	Pro12Ala: rs1801282	San Millan [175]	9	1,199	2,674	$P=0.02$
		Tang [176]	13	1,598	1,881	NS
		Zhang [177]	17	2,176	2,373	$P=0.003$
		He [88]	17	2,149	2,124	$P=0.002$

[a]Current genome builds do not include a variant labeled 1058C/T or His1058His. His1085His (C/T variant rs1799817) is the most likely current label for this variant

The plethora of small studies published over the years led to much uncertainty in the field of PCOS genetics. Several promising candidate genes have had numerous positive association reports as well as several negative reports. To attempt to clarify the role of such genes, investigators have conducted a number of meta-analyses, in the hope that the aggregate increased sample size would be able to more conclusively rule in or rule out the candidate genes examined. An example wherein this approach has brought clarity is the *ADIPOQ* locus. Combining information and samples across studies in meta-analysis has been done for 11 genes in PCOS (see Table 3.2), and although they typically focus on a single marker, they provide increased certainty of reported results from the literature.

The candidate-gene era has provided several promising susceptibility genes for PCOS, although only few of them have been widely replicated or widely accepted as risk genes. This experience was shared in a number of other complex diseases, such as type 2 diabetes, where the candidate-gene era yielded only two clear susceptibility genes, *PPARG* and *KCNJ11* [71, 72]. As targets of the antidiabetic thiazolidinediones and sulfonylureas, respectively, these genes were logical candidates

for study. Since the GWAS era commenced in 2007, the number of validated susceptibility loci for type 2 diabetes has grown to over 50 [73]. The functional relevance of most of these genes is unknown; therefore, they would not have been selected for study in the candidate-gene approach. The hypothesis-free GWAS approach has allowed the discovery of dozens of novel loci in several other common complex diseases and traits. As described below, GWAS has finally been applied in PCOS genetics.

New Frontiers in PCOS Genetics

Genome-Wide Association Studies in PCOS

With the introduction of genotyping platforms capable of assaying hundreds of thousands, even millions, of SNPs in a single experiment, a new case/control association approach has been applied to many common multigenic diseases with great success. These platforms allow a systematic scanning of variation in the genome using a *hypothesis-free* approach to assay many of the genes in the genome without knowing their biological function or relevance to the disease/s being studied. PCOS genetics has only recently moved into high-throughput genotyping approaches [74], with two GWAS being reported in independent cohorts by a single group [43, 75]. In early 2011, the first GWAS in PCOS by Chen et al. reported three susceptibility loci identified in a large Chinese cohort (4,082 cases and 6,687 controls) [75]. The study design utilized a very large replication cohort to provide confirmation of the most significant results found in the initial GWAS. This enabled a meta-analysis to then be performed combining the discovery and replication cohorts, providing highly significant association results. Three distinct loci were identified by this group, 2p16.3 (top signal: rs13405728, meta-analysis $P = 7.55 \times 10^{-21}$), 2p21 (top signal: rs13429458, meta-analysis $P = 1.73 \times 10^{-23}$), and 9q33.3 (top signal: rs2479106, meta-analysis $P = 8.12 \times 10^{-19}$), each containing promising genes for PCOS: *LHCGR* (luteinizing hormone/choriogonadotropin receptor), *THADA* (thyroid adenoma associated), and *DENND1A* (DENN/MADD domain containing 1A), respectively.

There are two very clear candidate genes adjacent to the association signal detected at 2p16.3, *LHCGR* and *FSHR*, which encode the luteinizing hormone/choriogonadotropin receptor and the follicle-stimulating hormone receptor, which play important roles in hormone signaling in the gonads, making them very plausible susceptibility genes for PCOS. The association signal at the index SNP (rs13405728) was not initially replicated in Caucasian cohorts [76–78], but a study examining ~100 SNPs across the entire locus in Caucasians recently reported association between two linked SNPs (rs7562215, $P = 0.0037$; rs10495960, $P = 0.0046$) and PCOS [79]. This study utilized an SNP tagging approach (to capture the majority of common variants in *LHCGR* and *FSHR*) and found that the top SNP in the Chinese GWAS [75] was not informative in Caucasians due to its low minor allele frequency.

The newly identified risk SNPs reported in this large Caucasian replication effort both map to intron 1 of the *LHCGR* gene and intron 11 of the *STON1-GTF1A1L* read-through gene fusion product that is encoded on the reverse strand. While *LHCGR* seems the most likely causative gene at this locus, little is known about the function of the *STON1-GTF2A1L* gene read-through product, so it cannot be entirely excluded as a possible causative gene. This study also claimed to have identified an independent association signal in the *FSHR* gene (rs1922476, $P = 0.0053$); however, as multiple testing corrections were not employed in the PCOS association analyses, these results require confirmation.

The susceptibility locus at 2p21 encompasses a number of genes, including *ZFP36L2*, *THADA*, and *PLEKHH2*, all with largely unknown functions; however, *THADA* is a well-established type 2 diabetes locus [80]. There are also two dense gene family clusters adjacent to this locus, the *DYNC2* gene family and the *ABCG* gene family, a susceptibility locus for cholesterol [81]. Five studies have attempted to replicate the association signal at this locus, with two reporting a positive association between SNPs in *THADA* and PCOS [77, 82]. Three studies did not find an association between PCOS and the index SNP rs13429458 [76, 78, 83]. Both had modestly sized cohorts and may have been underpowered to detect the association in Caucasians, as the minor allele frequency of rs13429458 is considerably greater in Chinese individuals than Caucasians (0.23 in CHB (Chinese Han from Beijing) versus 0.09 in CEU (Central Europeans in Utah), two reference populations from the Human Genome Diversity Project). Not all studies analyzed the same SNPs reported by Chen et al. [75], but this locus-driven replication does appear to confirm a role for *THADA* or surrounding genes in PCOS risk. A comprehensive tagging approach is needed for this locus.

The 9q33.3 locus identified in the initial PCOS GWAS has been the most widely replicated in studies utilizing Caucasian cohorts [76–78, 83], strongly implicating the *DENND1A* locus as a PCOS-risk gene in multiple ethnicities. It has been suggested previously that PCOS is likely to be an ancient disorder; the finding that *DENND1A* variation consistently affects PCOS risk across ethnicities is consistent with the concept that PCOS may have arisen early in the history of humans [84]. Little is currently known of the function of *DENND1A*. It belongs to the connecdenn family and is implicated in clathrin-mediated endocytosis. Expression of this gene has been reported in testes, ovarian theca cells, and adrenal carcinoma [85]. The locus also contains the genes *CRB2* and *LHX2*, of unclear function, and a microRNA, miR601.

The relationships between quantitative traits and risk SNPs identified in the initial GWAS report from *LHCGR*, *THADA*, and *DENND1A* have been examined in a number of studies, including a large Chinese cohort (1,731 cases and 4,964 controls) [86]. This report found a significant association between rs13429458 from the *THADA* gene and increased luteinizing hormone, testosterone and luteinizing hormone to follicle-stimulating hormone ratio [86]. A second risk SNP from the *THADA* gene, rs12478601, was significantly associated with increased low-density lipoprotein levels. A single SNP in *DENND1A*, rs2479106, was associated with increased 2-h oral glucose tolerance test insulin levels [86]. These markers were not

Table 3.3 Newly discovered PCOS-risk markers in the second Chinese GWAS

Marker	Chr.	Gene	*P* value
rs2268361	2p16.3	*FSHR*	9.89×10^{-13}
rs2349415	2p16.3	*FSHR*	2.35×10^{-12}
rs4385527	9q22.32	*C9orf3*	5.87×10^{-9}
rs3802457	9q22.32	*C9orf3*	5.28×10^{-14}
rs1894116	11q22.1	*YAP1*	1.08×10^{-22}
rs705702	12q13.2	*RAB5B*/*SUOX*	8.64×10^{-26}
rs2272046	12q14.3	*HMGA2*	1.95×10^{-21}
rs4784165	16q12.1	*TOX3*	3.64×10^{-11}
rs2059807	19p13.3	*INSR*	1.09×10^{-8}
rs6022786	20q13.2	*SUMO1P1*	1.83×10^{-9}

found to be associated with BMI or HOMA-IR [86], consistent with observations from the initial GWAS [75]. The association between rs13429458 and testosterone was not observed in the initial GWAS study, despite a large overlap of subjects used in the two studies [75, 86]. The reason for this heterogeneity in results remains unclear. Follow-up studies in European-origin cohorts also yielded inconsistent results, with no quantitative trait associations in two studies [77, 83], association of *THADA* SNP rs12468394 with testosterone levels in one study [78] and associations of *LHCGR* SNP rs13405729 with measures of glucose metabolism and *DENND1A* SNP rs2479106 with lipid and obesity measures in another study [76]. Quantitative trait association studies of GWAS loci are intended to shed light on the mechanism whereby the risk SNPs may influence the disease process. At this time, a clear picture has not emerged for the PCOS loci.

Subsequent to the initial GWAS report, the same group reported an additional and independent GWAS, which expanded the list of PCOS susceptibility loci (Table 3.3) [43]. The *LHCGR*/*FSHR* locus was found to harbor two independent association signals, potentially implicating both *LHCGR* and *FSHR* as independent risk genes [43]. Interestingly, both *FSHR* and C9orf3 are risk loci for erectile dysfunction [87], as well as PCOS [43], suggesting a role for these genes in reproductive function in both men and women. Novel risk loci at the *YAP1*, *TOX3*, and *SUMO1P1* genes were also reported, but the role for these genes in PCOS etiology remains unclear. Additional association signals include those at *RAB5B*/*SUOX*, a known type 1 diabetes locus [88], and *HMGA2*, a well-described locus for height and type 2 diabetes [89, 90]. The extensively studied candidate locus *INSR* was reported as a PCOS-risk gene in this second GWAS. Located approximately 1.2 cM from the D19S884 marker discussed earlier in this chapter, it remains unclear whether *INSR* is the only risk gene at this locus, or if the previous association at D19S884 represents an independent signal. Many of these newly reported PCOS-risk genes are from insulin and glucose metabolism pathways, and confirm the importance of insulin resistance and metabolic disturbance in PCOS [91]. This second GWAS has increased the list of PCOS-risk genes to 11 loci, and replication efforts in other ethnicities are sure to follow. GWAS in European-origin cohorts are underway at this time.

Epigenetics in PCOS

Epigenetics is the study of heritable changes in gene expression that are not encoded directly within the DNA sequence of the genes [92]. Affected by a variety of influences including fetal development, environmental chemicals, aging, diet, and drugs, epigenetic mechanisms include DNA methylation, histone modifications (acetylation, methylation, phosphorylation, sumoylation, and ubiquitylation), and other changes in chromatin factors and structures [93–95].

Epigenetic mediators regulate different cellular functions, from gene expression, to genome stability, X-chromosome inactivation (XCI), imprinting, and developmental reprogramming of non-imprinted genes [96]. Aberrant epigenetic changes such as abnormal DNA methylation are implicated in human diseases with fetal origins, such as cancer, cardiovascular disease, metabolic syndrome, and others [96, 97]. It has been hypothesized that PCOS is such a disease with epigenetic contributions to its pathogenesis [98].

Fetal origins of PCOS have been hypothesized based on several animal models. PCOS-like traits can be induced by prenatal (or perinatal) androgen excess (via administration of testosterone or dihydrotestosterone) in mammals such as rhesus monkeys, sheep, rats, and mice [99–102]. Female fetuses exposed to elevated androgens in utero develop PCOS-like phenotypes as adults, including hyperandrogenism, oligomenorrhea, multifollicular ovaries, insulin resistance, and impaired insulin secretion [99, 103]. During fetal development, prenatal androgen exposure may lead to permanent abnormalities in reproductive and/or metabolic systems, resulting in the development of PCOS in adulthood [98].

Studies of common adult diseases with fetal origins such as obesity, diabetes, and cancer suggest that intrauterine environmental exposures can reprogram development via epigenetic mechanisms [104–113], suggesting epigenetic modifications may also underlie the fetal origins of PCOS. Epigenetic alterations may be an important link between intrauterine events and adult diseases [114, 115]. Androgen excess during early life may represent an environmental hormonal insult that disturbs the fetal epigenome, causing developmental programming of PCOS [116]. It has been reported that abnormal estrogen exposure in utero can lead to epigenetic alterations with adverse consequences such as cancer later in life [111, 112, 117]. Similar to estrogen effects, androgens may modify the epigenome as endocrine disruptors, with resulting effects on gene expression, cell proliferation, apoptosis, survival, and differentiation [118, 119].

Investigators have found that epigenomic disturbances may contribute to the development of PCOS. XCI was first studied in PCOS because the androgen receptor gene (*AR*) is located on the X-chromosome. Studies have utilized a polymorphic CAG repeat in exon 1 of the androgen receptor as a marker in XCI analysis; in subjects heterozygous for this marker, nonrandom or skewed XCI can be assessed. Longer lengths of this repeat, which codes for a polyglutamine tract in the transactivation domain, have been found to correlate with impaired androgen receptor function [120–122]. Different studies have yielded conflicting results.

No significantly different patterns of XCI compared to controls were found among women with hyperandrogenic or idiopathic hirsutism [123], women with PCOS [124, 125], or daughters of PCOS women [126]. However, preferential expression of longer CAG repeat alleles was found in a study of infertile women with PCOS [127]. In a subsequent family-based study, the same group identified that different patterns of XCI were much more likely in sister pairs discordant for PCOS diagnosis as compared to sister pairs concordant for diagnosis [128]. A larger sample size study showed overall XCI was not different between PCOS and controls, but shorter alleles were preferentially active among the women with nonrandom XCI [129], consistent with findings of a subsequent study [130]. Biologically, preferential activation of chromosomes with shorter alleles, not longer alleles, would be expected to lead to increased androgen sensitivity and increased PCOS risk.

In recent years, an increasing number of studies in PCOS have examined DNA methylation, one of the best understood and most readily measured epigenetic markers. Cytosine at genomic CpG sites can be methylated to 5-methylcytosine. CpG islands are regions of the genome that have a higher concentration of CpG sites (CpG-rich regions >500 bp in length containing a GC content >55 %), located in 70 % of human promoters [131]. Methylation of cytosine at CpG islands is thought to regulate gene expression. Usually, hypomethylation is associated with gene expression, and hypermethylation with gene silencing. Gene function may be regulated by DNA methylation in gene bodies as well as in promoters [132]. Non-CpG-methylated sites (CpA, CpT) have been detected in human stem cells [133–135]. Differential DNA methylation may correlate with gene expression more strongly at CpG island shores (2 kb up/downstream of CpG islands) than within CpG islands [136, 137]. Epigenetic alterations are tissue specific. The NIH Roadmap Reference Epigenome Mapping Consortium is developing reference epigenomic maps for a wide range of human tissues and cells. Currently, over 120 unique human primary cells, tissues, and pluripotent cells are included in the database [138]. This will facilitate future epigenetic research.

A pilot study compared the percent global methylation levels of peripheral blood DNA between 20 PCOS women and 20 age- and BMI-matched healthy controls. The total methylated fraction in DNA was recognized by a 5-methylcytosine antibody. Although no significant difference was found, this work highlights a need to study DNA methylation in key tissues and specific target genes or regions [139].

DNA methylation states of key candidate genes, including *AR*, *LHCGR*, *FSHR*, and the imprinted gene *H19* (imprinted maternally expressed transcript) were studied in a dehydroepiandrosterone (DHEA)-induced mouse model of PCOS. In this mouse model, female prepubertal mice injected with DHEA daily for 20 consecutive days developed PCOS-like features. Methylation of *LHCGR* was lost in the ovaries of these mice, suggesting aberrant methylation of *LHCGR* may play a role in the pathogenesis of PCOS [140]. This is consistent with the clinical observation that LH is frequently elevated in women with PCOS; furthermore, variation in *LHCGR* was associated with PCOS in the first published GWAS of this disorder [75]. Because DHEA can be converted to androstenedione, testosterone, and dihydrotestosterone, and testosterone can be aromatized to estradiol, whether

DHEA demethylated *LHCGR* directly or indirectly via its derivatives deserves further investigation.

Another study explored epigenetic alterations of the genes peroxisome proliferator-activated receptor gamma 1 (*PPARG1*), histone deacetylase 3 (*HDAC3*), and nuclear corepressor 1 (*NCOR1*) in granulosa cells of PCOS women with hyperandrogenism. Hypermethylated CpG sites in the *PPARG1* promoter and hypomethylated CpG sites in the *NCOR1* promoter were observed, and expression levels of these two genes were altered consistently with their methylation changes (decreased and increased expression, respectively). Their methylation and expression levels were also studied in a rat model of PCOS, in which PCOS traits were established by subcutaneous injection of testosterone. *PPARG1* and *NCOR1* showed the same direction of methylation and expression changes in rat and human granulosa cells. This study suggested hyperandrogenism may induce epigenetic changes of these genes and alter their expression in granulosa cells, which may result in ovarian dysfunction in PCOS women with hyperandrogenism [141].

A recent study measured methylation levels in the follistatin gene (*FST*) promoter region among women with and without PCOS [142]. Out of ~90 CpG sites analyzed, only one site displayed a small but significant methylation difference in peripheral blood. Neither the methylation level of this site nor the expression of *FST* was significantly different in endometrial tissues between PCOS and controls, leading the authors to conclude that *FST* promoter methylation does not associate with PCOS. A drawback of this study was that the cases and controls were not matched for BMI, which itself can be associated with epigenetic changes [143].

With the development of high-throughput technology, genome-scale methylation microarrays have been used to identify genes or CpG sites with differential methylation in specific tissues. The first large-scale methylation analysis in the PCOS field was performed on visceral adipose tissue from prenatally androgenized (PA) rhesus monkeys, a well-studied PCOS model [144]. PA female fetuses exposed to elevated androgens in utero often develop PCOS-like phenotypes in adulthood [99, 145]. Visceral adipose tissue was first studied due to its close correlation with metabolic diseases. Methylation levels at a total of 27,578 CpG sites were measured in 10 controls and 15 PA female monkeys (infants and adults) that were exposed to elevated androgens in early gestation. A Bayesian statistical analysis was performed to test all probes simultaneously in a single test, avoiding multiple testing problems that often occur when analyzing large-scale array data. Significantly differentially methylated CpG sites were identified, 163 sites from infant tissue and 325 sites from adult tissue. Pathway analysis found that many of these genes participate in TGF-β (beta) signaling. Abnormal levels or function of members of the TGF-β superfamily such as anti-Müllerian hormone (AMH), inhibin B, activin A, follistatin, and fibrillin 3 have previously been implicated in PCOS [22, 26, 146, 147]. Altered methylation patterns of genes in the same pathway in these PCOS-like prenatally androgenized monkeys further suggest that dysfunctional TGF-β signaling participates in the pathogenesis of PCOS.

In addition to DNA methylation, small, noncoding microRNAs (miRNA) are another epigenetic modulator. MiRNAs are endogenous small RNAs of

approximately 22 nucleotides. In mammals, as posttranscriptional regulators, miRNAs integrate with the RNA-induced silencing complex (RISC), then bind to imperfect complementary sites of their mRNA targets, forming double-stranded RNA, resulting in gene silencing via translational repression [148, 149]. MiRNAs may regulate specific gene expression in oocyte maturation and ovarian follicular development in mice; in humans, miRNAs modulate gene expression in granulosa cells and contribute to the progression of ovarian cancer [150]. MiRNAs play important roles in regulation of ovarian development and function and are critical for female fertility [151]. Given these observations, miRNAs may participate in the development of PCOS, via targeting specific genes in the ovary. Testosterone has been found to affect miRNA expression [152]. In the prenatally androgenized sheep model, maternal testosterone altered miRNA expression in fetal ovaries, and some of the miRNAs with differential expression target either key genes in gonadal development or genes involved in insulin signaling and metabolism [153]. More miRNAs need to be investigated to reveal their roles in the pathogenesis of PCOS. Of interest, a specific miRNA, miR601, which has a role in apoptosis and represses NF-κ (kappa)B transcription factor-dependent expression [154], is located in the *DENND1A* region, the best-validated PCOS susceptibility locus to date [75].

PCOS patients also manifest increased genomic instability, DNA damage, and micronucleus formation [155–158], likely reflecting epigenetic alterations, because genomic instability has been associated with hypomethylation of CpG islands [159]. Frequencies of micronuclei were significantly increased in exfoliated buccal cells of PCOS patients, and significantly more damage was observed in leukocyte DNA compared with controls [155, 158].

Histone modifications have also been studied in PCOS. Valproate, an antiepileptic drug, can induce a PCOS-like picture via stimulation of theca cell androgen synthesis. Valproate effects on theca cells were correlated with increased histone acetylation, leading to altered expression of target genes, such as increased expression of *CYP17* and *CYP11A*. The gene expression profiles of valproate-treated normal theca cells and untreated PCOS theca cells were similar, suggesting valproate-induced histone acetylation may resemble a possible underlying mechanism for PCOS [160]. The investigators who found alterations in DNA methylation status of *PPARG1* and *NCOR1* also studied histone acetylation. HDAC3 is responsible for deacetylation of histone H3 at lysine 9 (H3-K9). Global acetylation levels of H3-K9 were reduced in the ovaries of PCOS model rats. Similar alterations in acetylation and *HDAC3* expression were also found in human granulosa cells isolated from preovulatory follicles of controls and then cultured and treated with dihydrotestosterone [141]. Thus, androgen-induced changes in histone modification might represent an additional epigenetic mechanism in the pathogenesis or perpetuation of PCOS.

Epigenetic findings may have clinical applications. Increasing numbers of DNA methylation markers have been identified and used for diagnosis, early detection, and risk assessment for breast cancer, lung cancer, and cardiovascular diseases [161–164]. New technologies drive the discovery of biomarkers and have enabled rapid progress in biomarker evaluation and validation [69]. Pharmacologically,

chemical agents such as azacitidine have been used to inhibit DNA methylation and reactivate tumor suppressor genes in myelodysplastic syndromes [165, 166]. Findings from epigenetic studies may potentially direct therapeutic development in PCOS in the future.

Summary and Future Directions

The recent advances in many genetic technologies have provided unprecedented insight into the genetics of many common disorders. Many of these technologies are yet to be applied to PCOS, but as their cost decreases and success in other diseases becomes apparent, it seems inevitable that they will also be used to aid in identifying what is likely to be a long list of susceptibility genes contributing to PCOS risk. The ability to survey other forms of genetic variation such as copy number variants, rare variants with large effects and changes in methylation status of genes will increase our ability to understand the complex pathways involved in PCOS susceptibility. In light of the progress made to date, particularly with the surge of risk genes identified by GWAS, additional studies are critically needed to more extensively replicate the identified loci, particularly across different ethnicities. The causal or functional variants also need to identified, as the tagging SNPs used to capture variation in high-throughput genotyping platforms are seldom the functional variants. Fine-mapping studies providing dense coverage across these newly identified risk loci may help identify functional variants and more clearly indicate the gene responsible for each risk-association signal. Gene identification is only the first step in the enormous task of understanding their role, and the role of the variants within them, in the biology of PCOS. Although challenging, these studies will provide deep insight into the disease pathophysiology and aid in diagnosis, drug development, and perhaps prevention.

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Chapter 4
Ovarian Dysfunction in Polycystic Ovary Syndrome

Helen D. Mason, Nafi Dilaver, and Suman Rice

Key Points

- Polycystic ovaries (PCO) are enlarged and contain many follicles that are increased in number from the earliest growing stages.
- The theca cells of PCO intrinsically overproduce androgens owing to changes in mRNA expression and promoter stimulation.
- Granulosa cells in PCO are not lacking in responsiveness to FSH but appear to be prematurely luteinized.
- Resistance in the insulin-stimulated glucose uptake pathway in the ovaries of insulin resistant women may reduce energy availability for the growing follicle and the maturing oocyte, contributing to defects in folliculogenesis.

Introduction

On a diagnostic pelvic ultrasound scan, there are two features of polycystic ovaries (PCO) that are most evident: the increase in size and density of the ovarian stroma and the large number of subcapsular antral follicles. Although the multiple small size follicles in PCO appear to retain a degree of functionality, there are well-documented defects in both granulosa and theca cell function in ovaries of women with polycystic ovary syndrome (PCOS). While many women with the isolated PCO morphology are ovulatory [1–3], a failure of selection of a dominant follicle and sequelae of anovulation and oligo- or amenorrhoea are commonly encountered in women with PCOS. Indeed, PCOS is the most common cause of anovulatory infertility [4]. In clinical research, it is the presence of the symptoms of the syndrome

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in the group with PCOS that is most important; however, in ovarian studies differentiation is made between PCO that are ovulatory or anovulatory. It is likely that the manifestation of morphological changes in the PCO is genetic, whereas the subsequent ovulatory or anovulatory status is largely environmental, and for this reason, ovarian studies are divided along these lines. For many years, it was assumed that the lack of follicle growth would be due to overproduction of a local inhibitory factor [5–8], but until recently convincing evidence was lacking. Increasingly, it appears that the PCO may have prenatal origins, and the most representative animal models are now being produced in animals that have been exposed to increased levels of androgens in utero [9–11]. This chapter will address in detail each of the above aspects of disordered ovarian function in PCOS.

Morphological Ovarian Changes: Polycystic Ovary

Increased Preantral Follicles

The first extensive morphological study to address the issue of an increase in preantral follicle number in PCO was performed in the 1960s and 1970s by a pathologist [12] who counted follicles at all developmental stages in a large number of histological sections of normal and polycystic ovaries. The investigator observed similar numbers of *primordial follicles*; the cumulative number of growing and atretic follicles in PCO, however, was almost twice that of normal ovaries. Atresia is the fate of all follicles that are not selected to ovulate; thus, by definition the vast majority of ovarian follicles are destined to undergo atresia [13, 14]. Hughesden hypothesized that an over-recruitment of primordial follicles into the growing phases, and an increase in follicle turnover was the likely mechanism to explain the appearance of PCO. Premature exhaustion of the oocyte pool and, hence, early menopause are likely sequelae to an accelerated follicular recruitment; given that early menopause is not a feature of PCOS, a plausibility that follicular over-recruitment and accelerated turnover are principal determinants of ovarian dysfunction of PCOS remains arguable.

Two further studies have improved our understanding of the precise nature of the ovarian defect in PCOS. Webber et al. counted follicles in ovarian biopsies and found a dramatic sixfold increase in the median density of preantral follicles in PCOs of anovulatory women [15]. In particular, there was a decrease in the percentage of primordial follicles, which was reflected in a specific increase in growing follicles of the primary stage. Theoretically, accrual of primary follicles accompanied by a delay in progression of folliculogenesis should predispose women with PCOS to delayed menopause; given that the latter is not proven in women with PCOS, this led the authors to conclude that the excess of small growing follicles must be due either to an increased follicle pool established prenatally, or be secondary to a reduction in the rates of follicular atresia. In the second study, follicles were counted in sections of fixed archived ovaries [16]. This group found no change in the number of primordial follicles, but again showed a specific increase in primary

follicles compared to normal ovaries that was on the order of fivefold and that they described as "stockpiling" in PCO.

There are a limited number of possible combinations of events in early folliculogenesis that could cause the number of growing follicles to be increased in PCOS without impacting on the age at menopause in this population. Webber et al. addressed the possibility that in PCO, follicles undergo atresia less frequently than in normal ovaries. On culturing ovarian follicles obtained from normal and PCO for up to 15 days, the investigators found that whereas 80 % of follicles in normal ovaries underwent atresia during this time, only 53 % of those in PCO were atretic.

Functional Underpinnings in Polycystic Ovaries

A number of groups attribute androgens with a role in altering early folliculogenesis [17]. The obvious way to examine this would be to expose cultured ovarian follicles to androgens in vitro; however, preantral follicles are notoriously difficult to culture with high rates of atresia and activation of growth of most of the primordial follicles [18, 19]. In order to overcome these experimental limitations, we grew pieces of lamb ovarian cortex in fertilized chicken eggs. In this model, the tissue is supplied by the embryonic chick circulation, optimizing the growth environment for growing follicles; indeed the majority of follicles remain healthy. We found a significant reduction in follicle atresia within ovarian cortical pieces exposed to androgen "in ovo," with primary follicles being the class most affected. These results support a role for androgens in prolonging the life of early follicles in PCO and contributing to the altered morphology [20]. That androgens might be able to exert such an effect on early follicles is dependent upon the expression of the androgen receptor (AR). Investigation of the presence of AR mRNA by nested polymerase chain reaction (PCR) of individual human follicles revealed that although not present in the resting primordial follicle, there was an increase in the number of AR-positive follicles with progressive follicular development [21]. It must be assumed, therefore, that a "stockpile" of primary follicles forms the basis of PCO, feeding an increased pool of preantral and subsequently antral follicles to give the PCO its characteristic appearance.

The clearest support for androgens having a role in this regard comes from models of prenatal androgenisation; prenatal exposure to androgens is observed to disrupt subsequent adult ovarian phenotype. Specifically, in prenatal androgen exposure models in rodents and primates, the ovaries contain multiple antral follicles [22] that appear to have prolonged survival, when tracked by ultrasound scanning [23]. At the preantral stages, a specific increase in primary follicles [24] is seen; however, unlike the human PCO, prenatal androgen exposure models demonstrate evidence of compromised ovarian reserve, as reflected in lower anti-Müllerian hormone (AMH) levels, and predisposition to early ovarian failure, caused by a reduced number of primordial follicles [22, 24, 25]. It is thus apparent that prenatal androgen exposure does not entirely mimic PCOS.

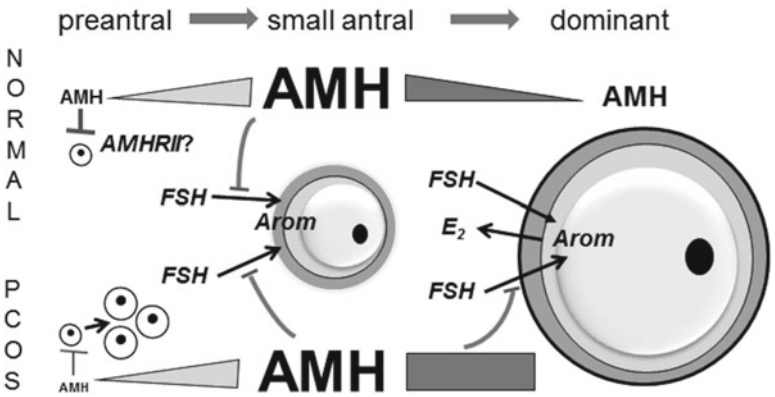

Fig. 4.1 In the normal ovary, AMH is expressed in small preantral follicles at low amounts. At this stage, AMH probably acts to limit the number of follicles undergoing initiation and, therefore, to preserve the follicle pool. In follicles from PCOS, AMH production in preantral follicles was low, suggesting that the AMH "brake" on follicle initiation might be reduced, resulting in accelerated initiation and the subsequent development of multiple follicles. However, only a very small number of preantral follicles were AMHR-positive, limiting the likelihood of AMH having a role in increased follicle number in PCOS. In normal ovaries, AMH concentrations peak in small antral follicles, and at this stage, AMH inhibits FSH-stimulated aromatase and FSH receptor expression, consequently inhibiting growth. Following selection, AMH production ceases, removing the block to FSH-stimulated follicle development. The factor responsible for this cessation is unknown. In the polycystic ovary, AMH levels in small antral follicles are high and do not reduce, preventing this release from inhibition of FSH-stimulated growth and possibly contributing to anovulation

Anti-Müllerian hormone has been proposed to play a role in establishing the polycystic ovarian phenotype. A paracrine factor identified for its male fetal differentiative role, is expressed by the ovarian granulosa cells from 36 weeks gestation onwards with levels peaking in small antral follicles (Fig. 4.1) [26]. It acts as an inhibitor of follicle growth, as demonstrated by the AMH knockout mouse model [27]. It is proposed that AMH expression in preantral and small antral follicles in this model acts locally to inhibit the initiation of growth of the primordial pool. Although granulosa cells in antral follicles of PCO overexpress AMH [28], interestingly, AMH was expressed in a lower percentage of preantral follicles in PCO than in normal ovaries, emulating the AMH knockout model and suggesting a mechanism that permitted an accelerated follicular initiation, as seen in PCOS [29]. A number of factors argue against this, however. In the AMH knockout mouse, the reproductive lifespan is reduced [26, 27], which is not the case in PCOS. Furthermore, we were unable to find many AMHR II-positive preantral follicles in normal human ovaries [21]. It is possible, therefore, that the reduced AMH production in small preantral follicles in PCO is a marker rather than the cause of altered follicle growth.

Genetic underpinnings of PCOS are recognized, and the search for *the gene* or determinant genes has been facilitated in recent years by the introduction of robust methodologies. In one such study, an association was found between PCOS and the dinucleotide repeat microsatellite marker D19S884, which is located in intron 55 of

fibrillin-3 (FBN-3) gene [30]. FBN-3 is part of the family of fibrillins that includes -1 and -2, and these interact with latent transforming growth factor beta (TGFβ) binding proteins and control the bioactivity of members of the TGFβ family in tissues: many members of this family have recognized roles in ovarian function [31]. Comparison of expression levels of FBN-3 in the adult ovary demonstrated low and comparable levels of expression both in the normal and PCO [32]. In the fetal ovary, however, the picture was different, and FBN-3 was found to be highly expressed and to be localized between nests of developing follicles [33]. This offers an intriguing prospect that in PCOS, the altered expression of FBN-3, possibly via an effect on TGFβ action, alters the development of ovarian follicles at the earliest stages.

Finally, the search for successful methods of culturing preantral primate follicles has unexpectedly revealed a further reason why follicles may survive better in PCO, both in vivo and in vitro. Follicles naturally initiate growth in the dense outer cortical layer of the ovary; therefore, it was considered that imitation of the rigid cortical environment as a three-dimensional framework of matrix, would allow a more physiological milieu for follicular growth [34]. Intriguingly, follicular survival was directly related to the degree of rigidity of the matrix. Anyone who has dissected or operated on a PCO is aware that the outer tunica and the ovarian cortex are excessively dense compared to the normal ovaries; rigidity of the ovarian tissue in PCO may itself, therefore, be another key to reduced follicular atresia, as is apparent in ovaries of women with PCOS.

Theca Dysfunction in PCOS

Hyperandrogensim is one of the defining features of PCOS, and it has been shown that the majority of the excess androgen in women with PCOS is of ovarian origin [35]. Within the ovary, the theca cell layer converts cholesterol to androgens through a succession of intermediary steps, the primary ovarian androgenic output being androstenedione. Evidence of exaggerated steroidogenesis in theca cells of PCOS came from culture of isolated cells in the 1990s, which showed in decreasing order, an overproduction of androstenedione, progesterone, 17α-hydroxyprogesterone, and dihydroepiandrosterone [36, 37]. These findings led to the concept of an *intrinsic defect* in PCO and directed the search for "PCO genes" towards the steroid synthesis pathway; however, although linkage was found to a number of genes encoding enzymes in the steroidogeneic pathway in a small number of individuals, a definitive genetic mechanism for aberrant ovarian steroidogenesis in PCOS remains elusive [38].

In addition to an inherent overproduction of androgens by ovarian theca, excessive responsiveness of the theca cells to luteinizing hormone (LH) is suggested as an alternative mechanism to explain the ovarian androgen excess of PCOS. Indeed, levels of LH are often raised in PCOS patients [37, 39, 40]. While the pace of advancements in our understanding of ovarian biology of PCOS was retarded by the lack of readily available theca cells from both normal and PCO, the development of

a system permitting long-term culture and passaging of theca cells in which steroidogenic capacity was retained has added impetus to progress in this area. Interestingly, it was found that even in long-term culture, cells from PCO retained their hyperandrogenic phenotype [41]. Molecular studies consequently focused on P450 17α-hydroxylase, encoded for by the *CYP17* gene, which has both 17α(alpha)-hydroxylase and 17,20 lyase activities and therefore catalyzes the key steps of conversion from pregnenolone to 17α-hydroxypregnenolone and thence to dehydroepiandrosterone (DHEA) before subsequent conversion to androstenedione and testosterone. An increased expression of the *CYP17* gene, and increased stability of *CYP17* mRNA have been described in theca cells of ovaries from women with PCOS [42]. Overexpression of 3β(beta) HSD (hydroxysteroid dehydrogenase), the enzyme responsible for conversion of DHEA to androstenedione, is further described in PCOS. An investigation of the *CYP17* gene revealed increased gene promoter function, which was determined to be due to a mutation in a region between −174 and −158 base pairs from the transcription start site. Within this site, there is a binding region for the transcription factor nuclear factor -1C (NF-1C); in theca cells from PCO, this site was mutated in a way that reduced binding. NF-1C was subsequently shown to repress *CYP17* promoter function and also, interestingly, that of *CYP11A1*, providing an explanation for the increased promoter function of both [43]. It is apparent, therefore, that defective repression of promotor function of these key genes in the steroid synthesis pathway contribute to the drive to increased androgen production. The steroid synthesis pathway in the ovary is shown in Fig. 4.2 with those enzymes found to be differentially expressed in theca from PCO highlighted.

Differences in gene expression between theca from normal and PCO were revealed by microarray analyses [44]. The first family of genes shown to be differentially expressed in PCO compared to normal ovaries were those involved in retinoic acid synthesis and action. Retinol (vitamin A) is essential for reproduction and had previously been shown to affect steroidogenesis by cells in culture [45, 46]. Interestingly, *CYP17* expression was stimulated by retinol in theca cells from PCO, whereas cells from normal ovaries did not respond [42], pointing to a possible role for the retinol family in ovarian hyperandrogenism of PCOS. The microarray analyses also revealed that the mRNA for *GATA6*, a member of the GATA family of zinc finger transcription factors that has also been shown to regulate steroid production, was also overexpressed in theca from PCO. Subsequent analysis revealed that the stability of GATA6 mRNA was also increased in women with PCOS [47]. Despite the observed differences, however, no sequence variations were found in the GATA6 gene locus in PCOS, thus ruling this out as a candidate "PCO gene." Later the same group refocused on CYP11A1, demonstrating that as seen with *CYP17*, both the basal and stimulated CYP11A1mRNA abundance, mRNA stability and promoter activity were increased in long-term cultured theca cells from PCO compared to theca cells from normal ovaries. Furthermore, as for *CYP17*, it was found that an area in the *CYP11A1* promoter that bound nuclear factor 1C2, a transcription factor, was altered in theca cells of PCO. In the basal state, therefore, ovarian theca cells of women with PCOS overexpress CYP11A1 owing to a combination of increased promoter activity and prolonged mRNA half-life [48].

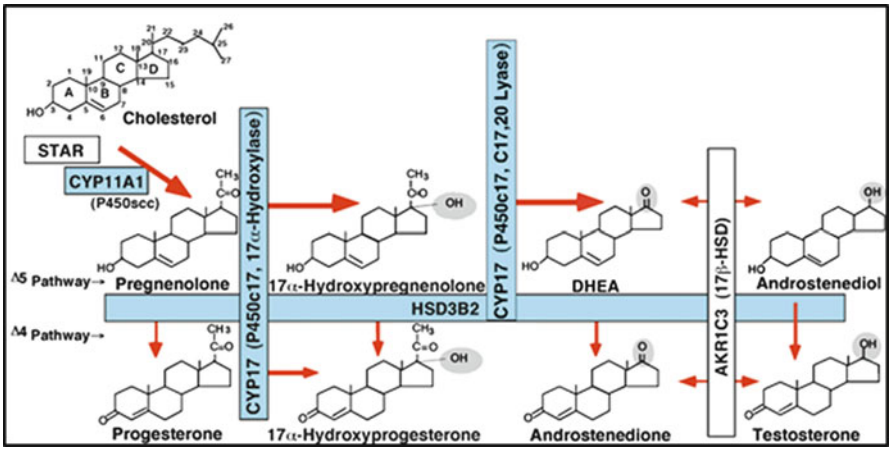

Fig. 4.2 Steroidogenic pathway in human theca cells. Androgen biosynthesis results from sequential loss of carbon from cholesterol catalyzed by a series of enzymes shown in boxes. Pregnenolone is converted to androstenediol via the Δ(delta)-5 steroid pathway and to progesterone via the activity of 3β(beta)-hydroxysteroid dehydrogenase (3β(beta)-HSD) and to testosterone via the Δ(delta)-4 pathway. Theca cells express steroidogenic acute regulatory protein (StAR), which promotes translocation of cholesterol to the inner mitochondrial membrane. Here the initial conversion to pregnenolone is catalyzed by P450 cholesterol side chain cleavage enzyme (P450scc) encoded for by the *CYP11A* gene. Theca also expresses the *CYP17* gene, which encodes cytochrome p450 17α(alpha)-hydroxylase (P450c17), which has both 17α(alpha)-hydroxylase and C17,20 lyase activities. It converts pregnenolone to 17α(alpha)-hydroxypregnenolone and dehydroepiandrosterone (DHEA) and progesterone to 17α(alpha)-hydroxyprogesterone and androstenedione (A). CYP11A, CYP17, and 3β(beta)-HSD expression (shaded) have all been shown to be increased in theca cells from women with PCOS. DHEA and A are finally converted to androstenediol and testosterone by the action of 17β(beta)-hydroxysteroid dehydrogenase (17β-HSD), which is encoded by the *AKR1C3* gene. Reprinted from Trends in Endocrinology and Metabolism, 17/2, Wickenheisser JK, Nelson-DeGrave VL, McAllister JM, Human ovarian theca cells in culture, 65–71, Copyright 2006, with permission from Elsevier

Throughout the history of investigation of hyperandrogenism in PCOS, one question has until recently remained unanswered: Is the androgen excess the result of a primary defect in the ovary, and a cause for disturbed follicular dynamics of PCOS, or is androgen excess merely a consequence of disordered ovarian follicle growth? A study of prenatal androgen exposure in sheep as a model may shed some light on this. In these experiments, increased androgen production by the ovarian theca occurred *prior* to any changes in ovarian morphology, providing the strongest evidence that androgens may be the cause of PCO phenotype [10].

Finally, it should be noted that in addition to an intrinsic theca cell defect, there are a number of other factors that influence androgen production in PCO. The first of these is LH, which is often raised in PCOS, as are the expression levels of LH receptors [49]; the second and possibly most important mechanism driving ovarian production of androgens in PCOS is hyperinsulinemia. As discussed in more detail below, although displaying resistance in the insulin-stimulated glucose uptake pathway, theca cells in PCOS retain sensitivity to the stimulatory effects of insulin on

steroidogenesis [50, 51]. With loss of insulin sensitivity, the insulin drive to this latter pathway therefore increases and further stimulates the steroidogenic pathway enzymes, thus amplifying the intrinsic steroidogenic defect of PCO, as previously discussed.

Ovulatory Dysfunction

Being the most common cause of anovulatory infertility [52, 53], the mechanism of ovulatory dysfunction in women with PCOS has been a research interest for decades. Despite an over-recruitment of early follicles in PCOS, as discussed in the earlier sections of this chapter, the growth ceases once the follicles reach sizes of 5–8 mm in diameter [5]. Numerous hypotheses have been proposed as to the primary cause of ovulatory dysfunction of PCOS. The common finding of androgen overproduction by theca of PCOS ovaries led to this being suggested as a causative mechanism for anovulation; the validity of this assumption, however, is rendered questionable given that theca cells of PCO from ovulatory women also exhibit excess androgen production [4].

The raised serum LH often found in women with PCOS has also been postulated to play a mechanistic role in androgen excess of PCOS [4, 39, 54], as suggested by a series of experiments in which granulosa cells from individual follicles from normal and PCO were treated with LH. Progesterone levels were measured as an indicator of LH-mediated luteinization [55]. Whereas progesterone production was not evident in granulosa cells from normal ovaries until the follicle reached 10 mm in size, cells from follicles as small as 4 mm from PCOS follicles were producing progesterone, indicating *premature* luteinization [55]. Given that during spontaneous folliculogenesis, it is shortly after luteinization that the granulosa cells within the follicle cease division [56], the above data provide a possible mechanism for the failure of follicle growth and development that is the hallmark of PCOS (Fig. 4.3). It was later shown that LH receptor mRNA is overexpressed in granulosa cells from polycystic ovaries compared to normal ovaries [49], adding weight to this hypothesis.

The selection and maturation of the follicle from its small antral and antral phase is principally FSH-dependent [57]. Disordered FSH secretion is postulated as a possible mechanism for ovulatory dysfunction in PCOS; indeed, levels of FSH are slightly lower in women with PCOS and do not show the intercycle rise seen in the normal menstrual cycle. However, granulosa cells from women with PCOS express increased FSH binding and are readily responsive to FSH both in vitro and in vivo [6, 58, 59]. This was supported by clinical studies in which women with PCOS who underwent ovarian stimulation with exogenous FSH produced significantly greater levels of estradiol than those with normal ovaries [60]. Conversely, time-course response experiments revealed that estradiol levels in women with PCOS were not sustained, whereas the levels in women with normal ovaries persisted for 24 h after peaking [60]; these latter observations suggest the possibility of a defect in CYP19 aromatase activity.

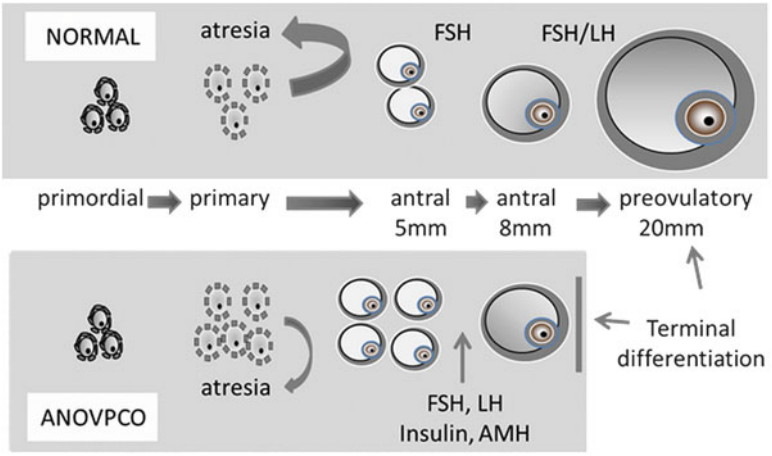

Fig. 4.3 In the normal ovary, the primordial follicle pool is laid down before birth and follicle growth is initiated after puberty. Follicles grow initially to the primary stage, at which many will die through atresia. A group of surviving antral follicles will enter the menstrual cycle, where, under stimulation by FSH, one will become selected and acquire LH receptors, a process known as differentiation. At this point, the granulosa cells have a limited remaining number of divisions and the follicle will undergo final terminal differentiation and cease growth at the LH surge. In the polycystic ovary, the primordial follicle pool appears to be of the same magnitude as in the normal ovary. Once growth commences, however, there are twice as many follicles of each phase, possibly due to a reduction in atresia. This increase in follicle number persists to the antral phases and results in the characteristic polycystic appearance of the ovary. In PCOS, a number of factors subsequently contribute to a failure to select and grow the dominant follicle. High levels of AMH production by the granulosa cells inhibit follicle growth. Insulin, which is often raised in women with PCOS, has been shown to sensitize the follicle to the effects of LH such that the granulosa cells acquire LH receptors early and, therefore, undergo early terminal differentiation and early growth cessation. Modified from Molecular and Cellular Endocrinology, 163/1–2, Franks S, Mason H, Willis D, Follicular dynamics in the polycystic ovary syndrome, 49–52, Copyright 2000, with permission from Elsevier

Appreciation of insulin resistance as a principal metabolic defect in patients with PCOS is relatively recent [61]. From the first finding of an inverse correlation between annual number of cycles and insulin sensitivity [62], there have been numerous studies showing an association between anovulation and insulin resistance [63–66]. When overweight insulin-resistant patients with PCOS lose weight and their insulin sensitivity parameters consequently improve, there is often a resumption of ovulatory cycles, further reinforcing the link between ovulatory dysfunction and insulin signalling [67–71]. The mechanisms underlying insulin resistance in adipose and muscle in PCOS is unclear, although a post-receptor binding defect has been implicated [72].

The effects of insulin on steroid production are equally important and may explain the arrest of antral follicle growth in PCOS due to premature luteinization, as discussed above [59]. Briefly, insulin was found to sensitize granulosa cells to the

effects of LH such that cells from follicles as small as 4 mm from hyperinsulinemic women with anovulatory PCO were able to respond to LH [55, 59]; in contrast, a follicle would normally have to reach approximately 9 mm in diameter in order to become responsive to LH [53, 73]. Hyperinsulinemia and insulin resistance are thus suggested to play a causative role in "premature luteinization" resulting in arrested folliculogenesis in PCOS (see Fig. 4.3) [74].

In addition to its possible role in early folliculogenesis, the fact that AMH circulates at significantly higher levels in women with anovulatory PCOS has led to a hypothesis that AMH excess may be contributory to anovulation in PCOS [75]. AMH is highest in small- to medium-sized antral follicles, and its production declines around the time of follicle selection for dominance (see Fig. 4.1). The reason for this spontaneous decline in AMH levels may be because AMH inhibits FSH- and LH-induced aromatase expression in granulosa cells and further reduces the activity of the ovary-specific aromatase promoter II, resulting in a significant reduction in estradiol production [76]. Interestingly, Pellatt et al. also demonstrated that AMH inhibits FSH-stimulated FSH receptor mRNA expression [76]. These data add to our understanding of the relevance of AMH in disturbed folliculogenesis in PCOS as AMH production was shown to be 75 times higher per granulosa cell in anovulatory PCO compared to normal ovaries [28], a level which would be sufficient to inhibit follicle growth (see Fig. 4.1). The cause of this high AMH production by PCO, however, remains to be elucidated.

Energy Balance

From the primordial resting phase through to ovulation, follicles increase in size dramatically [77]; thus, there is clearly a need for an adequate energy supply to fuel the multiplying granulosa cells and maturing oocyte within the growing follicle. The dividing granulosa cells utilize glucose primarily via the glycolytic pathway. This allows for energy production in the form of ATP and metabolites (pyruvate and lactate), which are then secreted into the follicular fluid [78–80] or passed directly to the oocyte via gap junctions [81, 82]. The concentration of glucose in the follicular fluid is comparable to plasma levels, at about 3.3 mM in humans [83], and follicular fluid glucose concentrations are positively correlated with ovarian follicle size [84].

The high capacity of the follicle to metabolize glucose is characterized by the presence of glucose transport proteins (GLUTs; gene nomenclature SLC2A), which act as shuttles to move glucose across the cell membranes by forming an aqueous pore through which glucose can move [85]. In insulin-sensitive tissues, the translocation of the insulin-regulated GLUT4 from the intracellular pool to the cell surface accounts for most of the insulin-stimulated increase in glucose uptake [85]. Given that other insulin target tissues display insulin resistance in patients with PCOS [72, 86], the possibility of the ovary also being resistant to insulin-stimulated glucose uptake in PCOS was investigated. Insulin-stimulated glucose uptake and

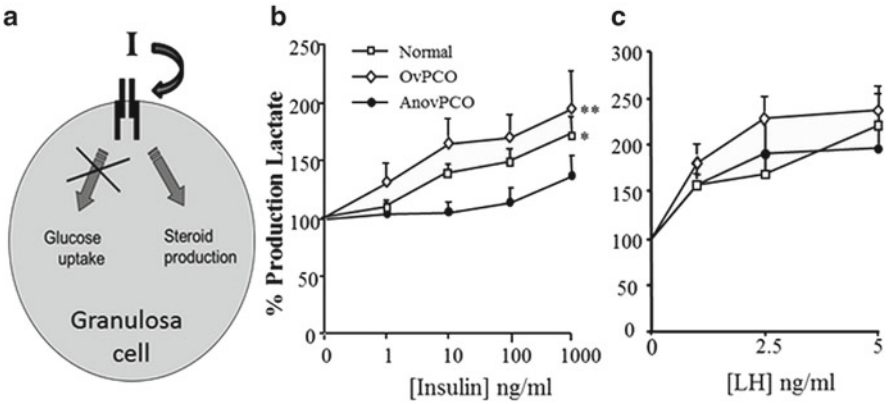

Fig. 4.4 (**a**) There is a divergence in the insulin signalling pathway in granulosa cells from women with insulin resistance. The glucose uptake pathway is resistant, as evidenced by the experiments performed in cells from women with normal ovaries, insulin-sensitive ovulatory polycystic ovaries (Ov-PCO), or insulin-resistant anovulatory polycystic ovaries (Anov-PCO). (**b**) Cells from Anov-PCO showed attenuated lactate production in response to insulin stimulation, whereas cells from insulin-sensitive women were not different to normal (data are mean ± SE of triplicate experiments. $*p < 0.005$, $**p < 0.001$ ANOVA). (**c**) In contrast, there was no defect in LH responsiveness. Modified from Rice S, Cristoforidis N, Gadd C, Nikolaou D, Seyani L, Donaldson A, et al.: Impaired insulin-dependent glucose metabolism in granulosa-lutein cells from anovulatory women with polycystic ovaries. Hum Reprod. 2005; 20(2):373–81; by permission of Oxford University Press

metabolism pathway in granulosa cells from women with PCOS was noted to be impaired as evidenced by an attenuated insulin-mediated lactate production response in cultured granulosa cells [50, 51, 87] (Fig. 4.4). The observed impairment of insulin-mediated lactate accumulation is likely to represent both attenuated glucose uptake and a reduction in glycolytic activity in cells from anovulatory women with PCOS; these phenomenon occurring in vivo may account for impaired growth and proliferation of granulosa cells due to compromised energy availability.

While impairments in glucose metabolism are well described in granulosa cells from women with PCOS, as discussed earlier, this defect appears to be confined to the insulin-mediated pathway, since there was no difference in LH-stimulated lactate production in luteinized granulosa cells collected from women with normal ovaries, ovulatory and anovulatory PCO undergoing in vitro fertilization [51]. In addition, insulin-mediated steroidogenesis remained unaltered in the insulin-resistant PCO, indicating a divergence in the pathway such that insulin-mediated glucose uptake is resistant, whereas steroidogenesis remains sensitive to insulin stimulation [50, 51]. At a molecular level, this defect of insulin action in women with PCOS appears to be secondary to a post-receptor binding defect in insulin signalling, with reduced tyrosine phosphorylation of the insulin receptor and insulin-receptor substrate proteins 1 and 2 [61, 86, 88]. Interestingly, we showed

that metformin, an insulin-sensitizing agent commonly utilized in the management of PCOS, was capable of directly activating IRS-1 and -2 in granulosa cells, leading to an increased insulin-stimulated translocation of GLUT-4 to the plasma membrane [89]. Metformin thus has the potential to increase glucose uptake and metabolism in insulin-resistant PCO and facilitate follicle growth. Indeed, a recent Cochrane review on metformin concluded that ovulation and pregnancy rates were higher in women with PCOS taking metformin [90], though whether metformin can improve the developmental competence of PCOS oocytes remains to be established.

As stated earlier, pyruvate is the preferred substrate for the human oocyte, and abnormalities in pyruvate consumption have been implicated in abnormal meiosis in oocytes from PCO. Conversely, and in keeping with earlier discussion, prior exposure to metformin significantly decreased pyruvate consumption by in vitro matured oocytes from women with PCOS [91]. A study investigating carbohydrate metabolism and meiotic status in immature oocytes donated by women with PCOS and control patients and matured in vitro showed a high pyruvate uptake in the oocytes from PCOS and that this was associated with oocyte aneuploidy [91].

Collectively, existing data indicate that the distinct phenotype of PCO is accompanied by defects in steroidogenic pathway as well as impairments in glucose uptake and metabolism that are likely to have adverse effects on both oocyte development and follicle growth.

Oocyte

Given the disordered follicular development characteristic of PCOS, and the fact that oocyte energy metabolism may be abnormal, it is pertinent to further question the health of the oocytes contained within these follicles. Evidence from in vitro fertilization suggests that despite the higher oocyte yield that is common in women with PCOS, the implantation rate per oocyte is lower in this population compared to patients without PCOS [92]. The oocyte is exposed to high intrafollicular levels of androgens and abnormal levels of other factors, including insulin, throughout its lifespan in PCO. The impact of endocrine and metabolic influences on gene expression in donated oocytes from PCO was addressed by a comparison with oocytes from normal ovaries using microarray analysis [93]. These donated oocytes by women with PCOS were morphologically indistinguishable from those obtained from controls but displayed consistently different gene expression profiles. Of interest was the fact that the majority of differentially expressed genes were up-regulated in oocytes from women with PCOS. Genes involved in the mitotic cell cycle and maternal effect genes (i.e., genes expressing proteins that are required around activation of the zygotic genome at the time of resumption of mitosis) were amongst those altered. Many of the proximal promoters of the altered genes contained putative nuclear receptor binding sites including for AR. While difficulty in access to oocytes from clinically well-characterized patient populations for similar studies remains a limitation, future endeavors are clearly needed to fully assess the

implications of an abnormal follicular environment on the developing oocyte in order to gain not only a better understanding of the reproductive capacity in PCOS but also to appreciate implications therein for the progeny of this population.

Summary

Collectively, existing data suggest that a genetic signal, thus far elusive, lays a platform for abnormalities in the development of preantral follicles; these phenomena are initiated in the prenatal ovary and persist into adult life. Abnormalities in theca cell steroidogenesis of PCO are well characterized and result in an overproduction of androgens. Androgen excess in turn is responsible for the many manifestations of PCOS and that may impact on the oocyte. The cause of the failure of follicle selection for dominance in the majority of women with PCOS manifesting ovulatory disturbances appears to be multifactorial. An interplay between insulin and LH signalling pathways contributes to premature arrest of follicle growth; an abnormal milieu of paracrine factors, particularly AMH, is strongly implicated in disturbed folliculogenesis of PCOS. Finally, the insulin-stimulated glucose uptake pathway is resistant to insulin action in the follicular somatic cells of PCOS, and the resulting impaired energy availability is an additional pathophysiologic mechanism contributing to the ovarian dysfunction of PCOS. Despite the strides in our understanding, the polycystic ovary remains an enigma, and many aspects of the ovarian dysfunction of PCOS will provide fertile research material for many years to come.

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Chapter 5
The Role of the Adrenal Glands in the Hyperandrogenism Associated with Polycystic Ovarian Syndrome

Ruth Freeman

Key Points

- As many as 30–50 % of PCOS patients have excessive androgens originating from the adrenal glands.
- Most also have androgen excess of ovarian origin.
- Early onset of adrenal androgen increase, particularly DHEA, and DHEA-S seen in children with premature adrenarche or precocious puberty often precedes the development of PCOS in late adolescence.

Adrenal Androgens and PCOS

Polycystic ovarian syndrome (PCOS) is a multietiologic disorder of young premenopausal women [1]. It is a syndrome of oligomenorrhea and hyperandrogenism associated with either clinical findings (hirsutism and/or acne) or elevated blood androgen levels. The source of the androgens in PCOS has been controversial and to this day has not been clearly defined [2].

There is no true consensus on the role of the adrenal in the pathophysiology of PCOS. An extensive review by Futterweit was published in a monograph by Springer Verlag in 1984 [2]. Some studies have shown that the androgens in this disorder may be entirely of ovarian origin, whereas others provide evidence that it is largely from the adrenal, and still others provide evidence that both the ovaries and the adrenals contribute to the hyperandrogenism of PCOS.

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The steroid-producing tissues of both the adrenals and the ovaries have a common embryonic origin, basically from the early mesonephric region. In some individuals, adrenal rest tissue can be identified within the ovary, further obscuring the source of elevated androgens. This has been described in patients diagnosed with congenital adrenal hyperplasia (CAH). Adrenal tissue responsive to ACTH is present in their gonads. This is more commonly recognized in the testis than in the ovary [3–5], making it uncertain whether the ovarian output of a steroid is due to adrenal rest or native ovarian tissue. Androgen production by the ovary may in fact cause alterations in steroidogenesis in the adrenal [6, 7]. The reverse may also be true, as elevated exogenous androgens during or prior to puberty may cause increased ovarian steroidogenesis via a positive feedback at the hypothalamo-pituitary level with consequent LH elevation [8]. In the discussion that follows, the available data will be reviewed with a systematic focus on adrenal function during childhood and adolescence in subjects who develop PCOS, then in the adult with this syndrome, and finally in the peri- and postmenopausal person who has PCOS.

The Role of the Adrenal in Childhood and Subsequent Development of PCOS

Some children who have classical CAH with excessive androgen production by the adrenals during fetal life develop PCOS after adolescence. On long-term follow up of CAH patients, Mnif et al. [9] found that 6 of 15 women also had PCOS.

Does exposure to androgens in the fetus result in an alteration in the post pubertal ovarian hormone production? In animal studies, administration of testosterone to the newborn mouse or rat results in persistent estrus and polycystic ovaries [10]. Female monkeys exposed to androgens just prior and during puberty developed increased LH pulsatility with circulating levels similar to those observed in PCOS [8]. Similar findings were seen in adolescent girls given testosterone acutely [11]. Thus, exposure of the fetus or the pubertal girl to androgens, regardless of the source, may result in altered functioning of the hypothalamo-pituitary-ovarian axis consistent with PCOS [12].

Premature adrenarche, which is known to be associated with elevated dehydroepiandrosterone (DHEA) levels, is frequently seen in children who will subsequently develop PCOS [13]. The onset of puberty in normal girls starts with breast development (thelarche) followed by the growth of pubic hair (pubarche). It has long been recognized that girls experiencing premature adrenarche (or pubarche) manifested by early onset of pubic and axillary hair are likely to go on to develop PCOS; a detailed review of the effects of premature adrenarche was published by Idkowiak et al. in 2011 [14]. Elevation in serum DHEA occurs at the time of the onset of pubic hair growth [15]. It may be due to an alteration in the function of CYP17A1 (P450c17α), an enzyme that has dual actions of (1) 17 hydroxylation of pregnenolone and progesterone (Fig. 5.1), and (2) removal of C20 and C21 from the resultant intermediary products17-OH pregnenolone and 17-OH progesterone to

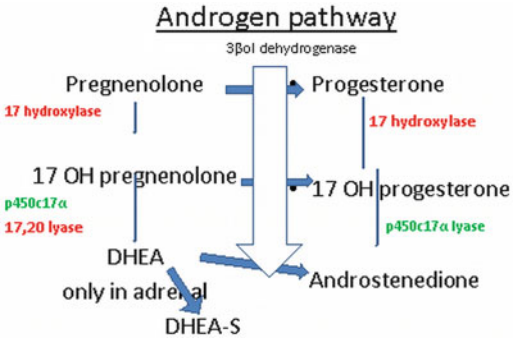

Fig. 5.1 Androgen production pathways in the adrenal and ovary. Sulfation DHEA (dehydroandrostenedione) resulting in production of DHEA-S is limited to the adrenal gland

yield DHEA and androstenedione (lyase activity). Activation of CYP17A1 in the zona reticularis of the adrenal glands is a physiological event resulting in a rise in DHEA during normal puberty [16]. Activity of CYP17A1 may be enhanced in girls experiencing premature adrenarche, resulting in excess DHEA and androstenedione. An extensive review of this paradigm in adults who started out with early pubarche (adrenarche) has been reported by Ibanez et al. [15]. Prior to puberty, lyase activity is very low. (Note: This is true of adrenal and ovary.)

Oberfield et al. reviewed the pubertal increase in the size of the zona reticularis of the adrenal and increasing function of the P450c17α activity producing increased DHEA in normal puberty, as well as the progression from premature adrenarche to PCOS in one child [17]. Conversely, girls who have precocious puberty commonly develop PCOS with early abnormal hair growth and irregular menses [18]. Alteration of the lyase pathway may be part of the pathophysiology of increasing DHEA production in the adrenal and/or increasing conversion of 17OH progesterone to androstenedione in the ovary of women with PCOS. The same enzyme is active in the adrenals and ovaries. Its control is likely due to other proteins, some of which decrease 11 hydroxylation in the adrenal, thereby increasing the amount of androstenedione due to the increased levels of the precursor 17-hydroxy progesterone (see Fig. 5.1). A study of the daughters of women who had PCOS found higher levels of DHEA-S and greater increase in ACTH-stimulated DHEA-S in 30 % of the PCOS daughters than in a control group of normal girls [20]. Thus, an alteration in the production of adrenal androgens in children may, by a diverse array of mechanisms, result in the disordered menstrual cyclicity that is characteristic of PCOS.

While abnormalities of adrenal steroidogenesis may contribute to the reproductive axis dysfunction that is characteristic of PCOS, of interest is the observation that excess lyase activity of P450c17α function may be associated with insulin resistance [19] or with an increase in IGF1. These latter data provide a mechanism that links functional differences in adrenal steroidogenic pathway with the metabolic picture of PCOS. The frequent occurrence of insulin resistance in adults who started out with early pubarche (adrenarche) has been detailed by Ibanez et al. [15].

In summary, existing data suggest that abnormal production of androgens by the adrenals early in life (in the fetus or prepubertal child) may be a precursor of the PCOS phenotype.

Adults with PCOS and the Role of Adrenal Androgens

PCOS in the adult is a complicated disorder whose origin is difficult to determine. Overall it seems that between 30 and 50 % of women meeting criteria for PCOS exhibit excess androgens of both adrenal and ovarian origin. Determining the source of androgen excess is important from the perspective of ruling out differential diagnoses (such as an adrenal or ovarian tumor); it may also allow for more specific treatment strategies.

Clinical presentation of PCOS may be somewhat insidious early in its presentation; the condition is usually manifest early in the reproductive years, usually within 5–10 years following menarche. For some, menstrual cycles may be erratic from the time of menarche, whereas for most others, menses may be predictable and cycles ovulatory in the early years, with progressive deterioration in menstrual cyclicity, signs of androgen excess, body odor related to apocrine gland activity, acne, and excess facial and body hair growth. By the time the young woman seeks medical attention, the source of the androgen/s may be difficult to ascertain.

Adrenal androgens, after conversion to estrogens in the peripheral body fat and/or liver, increase the pituitary secretion of LH, which in turn causes increased ovarian androgen production. Therefore, there are likely some women with PCOS in whom the process started with an increase in adrenal androgens that secondarily affected ovarian steroid production. Conversely, the reverse may also be true, as ovarian androgens have been shown, in vitro, to alter the adrenal production of androgens by blocking the 21 hydroxylation of 17 hydroxyprogesterone and 11 deoxycortisol [21], thus channeling the steroidogenic precursors towards androgen synthesis. Effect of estrogen in altering the production of adrenal androgens has been well summarized by Gonzalez et al. [6].

Catheterization of the adrenal and ovarian veins has been used to identify the source of excess androgen production. The studies by Kirschner et al. [22] and Wajchenberg [23] suggest that the androgens are largely from the ovary, whereas the studies by Abraham et al. provide evidence for excessive adrenal production of androgens [2]. Elevated DHEA and particularly DHEA-S has been suggested as evidence that the adrenals are the source of the abnormally high androgens in women with PCOS. Kumar et al. examined 182 patients who had PCOS and found that 30 % of black and 20 % of white subjects had excessive amounts of DHEA-S, suggesting an adrenal source of androgen excess [24]. The age-associated decline in DHEA-S was seen both in normal women as well as in those who had PCOS. Whereas in normal women most of the DHEA is produced by the adrenal, the ovary is capable of secreting DHEA as well. DHEA-S, however, is thought to be produced only by the zona reticularis of the adrenals, suggesting an adrenal source of androgen excess for those PCOS patients who have elevated DHEA-S levels. A concomitant ovarian contribution to androgen excess may exist in this population as well.

Stimulation and suppression tests are commonly used to differentiate the source of excess androgens; the ACTH and GNRH stimulation tests evaluate for adrenal and ovarian source of steroid hormone excess, respectively; similarly,

suppression of androgen levels following a trial of exogenous glucocorticoid (usually dexamethasone) reflects an adrenal source, whereas successful suppression with GnRH antagonist or following long-term use of a GnRH agonist favor an ovarian source of androgen excess.

Trial of a GNRH agonist, reducing gonadotropins to very low levels, resulted in reduced production of ovarian hormones and has clearly shown that at least some PCOS subjects only have ovarian hyperandrogenism with negligible adrenal contribution. Chang et al. in 1983 suppressed the ovarian function with long-term GnRH agonist use, reducing ovarian estradiol, estrone, and androstenedione production to levels that were comparable to castrated controls [25]. In their subjects, DHEA but not cortisol was elevated and remained unaltered by the GNRH agonist treatment, suggesting that the DHEA was essentially of adrenal origin. These authors concluded that in their PCOS patients, both adrenal and ovarian sources contributed to androgen excess. It is important to appreciate that in the short term, administration of GnRH agonist results in a flare effect, with an acute rise in pituitary release of both LH and FSH, with a resultant transient increase in ovarian androgens. When administered over a longer period of time (at least 2–3 weeks), however, suppression of pituitary gonadotropins is achieved with use of GnRH agonist secondary to a down-regulation of GnRH receptors on the gonadotrophs, with consequent ovarian suppression. A complicated study by White et al. using GnRH agonists first and then followed by ACTH stimulation testing could not show increased adrenal androgen production when ovarian function was blocked by GnRH administration [26], thus adding to the confusion of the source of androgens in this disorder.

Although adrenal suppression with potent glucocorticoids (usually dexamethasone) can show reduction of adrenal androgens, they may also alter the ovarian production of hormones and may, in fact, reduce LH secretion, which in turn would lower ovarian androgen production. In a complicated study of 60 PCOS patients and 39 normal control patients, Rosenfield et al. reported that lack of suppression of testosterone by dexamethasone provided evidence of an ovarian source of the androgens in the PCOS group [27]. These authors further proposed that greater response of DHEA after ACTH administration, which was found in roughly 30 % of the PCOS subjects, additionally suggested an adrenal source of excess androgens. ACTH stimulation tests have shown increased response with higher 17 hydroxyprogestrone levels in about 20–30 % of PCOS patients. Azziz et al. separated women with PCOS into those with elevated DHEA-S and a second group with normal or minimally elevated DHEA-S levels and found that the high DHEA-S group had an excessive response to ACTH [28]. These authors also measured ACTH response to CRH (corticotrophin-releasing hormone) stimulation and found that to be similar in both groups. In patients with demonstrable suppression of androgen levels following exogenous corticosteroid exposure, ACTH stimulation test can help identify women with PCOS from those with a diagnosis of late-onset of CAH. It is important to appreciate an overlapping phenotype between these two diagnostic entities, and some of these women may, in fact, have both PCOS and CAH, similar to the classical CAH patients who develop PCOS during adolescence. Azziz et al. [28] studied the ACTH response in PCOS subjects using a complicated system of increasing low

doses of ACTH followed by the usual 250 μg dose and demonstrated an increased output of androstenedione, which was greater than the cortisol production, which in turn was greater than the output of DHEA. The response was higher in PCOS patients than in normal controls, suggesting an increased adrenal source of androstenedione in these subjects; androstenedione is normally produced equally by the adrenal and the ovary.

In a recent study, Rosenfield et al. showed that about 27 % of the PCOS patients had excessively high response of DHEA to ACTH stimulation [27]. This was found in a group of PCOS patients who when given GnRH agonist (GnRHag) acutely to stimulate the ovary did not have the expected rise in 17 hydroxyprogesterone or androstenedione. The authors proposed heterogeneity within PCOS with *typical* and *atypical* PCOS phenotypes. Some women in PCOS in both the groups had less suppression of testosterone with dexamethasone, suggesting an ovarian (often as well as an adrenal) source of hyperandrogenism. In fact, they identified a group of women with BMI >40 who had a PCOS-like picture but responded normally to dexamethasone suppression, ACTH stimulation, and GnRHag However, not all of the very obese subjects had this finding; some of the typical PCOS group also had >40 BMI. Sahin et al., in contrast, found that the initial increase in 17 hydroxyprogesterone was greater in PCOS patients than in normal controls; after pituitary suppression with GnRHag, the ACTH response in PCOS patients was the same as in controls, suggesting an ovarian rather than adrenal source for androgens [29]. Carmina et al. have suggested that ovarian disturbance could affect the adrenal response to stimulation; these investigators provided evidence that only those subjects who at baseline have elevated 11 hydroxy androstenedione (11 hydroxylation occurs exclusively in the adrenal tissue and not at all in the ovaries) had increased adrenal androgen production in the first place [7]. These authors suppressed the ovaries with 3 and 6 months of GnRH followed by ACTH stimulation and found that in some subjects this altered the DHEA response to ACTH stimulation.

Futterweit's monograph *Polycystic Ovarian Syndrome* [2] offers an extensive review of the classic stimulation and suppression studies up through 1984 utilized for evaluating adrenal gland function in PCOS. More recently, studies have suggested that there may be many different alterations in the enzymes affecting steroidogenesis pathways in both the adrenal and the ovary. Pathways leading to the production of androstenedione and testosterone are shared, and both tissues require the cytochrome P450c17α activity for hydroxylation of the steroid molecule at the 17 position; the same enzyme is required for cleavage (lyase) of the 20 and 21 carbon side chains (see Fig. 5.1). Thus, excessive lyase activity of P450c17α in both the ovary and the adrenal can contribute to excessive production of both adrenal and ovarian androgens. Nestler et al. in 1997 reported that correction of high insulin levels in lean PCOS subjects reduced the abnormality of ovarian P450c17α; the authors utilized GnRH stimulation to assess the ovarian component [19]. Vassiliadi et al. demonstrated increased 5α reductase activity in both obese and nonobese PCOS patients as compared to normal women of comparable BMI [30]. In fact, cortisol production in these PCOS patients was shown to be higher than the controls; however, circulating cortisol levels were in the low normal range. This

suggested an exaggerated corticosteroid degradation in patients with PCOS; suggesting that the increased adrenal androgens were a side product of the compensatory increase in cortisol production. A cause–effect relationship to the observed phenomenon, however, could not be elucidated given the cross-sectional study design. Gambineri et al. suggested that increased clearance of cortisol due to 5β reductase activity resulted in increased adrenal androgen production due to elevated ACTH levels in 90 subjects with PCOS by demonstrating that these women had higher responses to ACTH than did the 45 women without the enzyme abnormality [31]. They concluded that increased inactivation of cortisol resulted in increased ACTH secretion (not proven) and a more robust response in adrenal 17 hydroxyprogesterone to ACTH stimulation. Direct measurement of ACTH by Chang et al. [32] in PCOS, however, failed to show any increase in circulating ACTH levels in PCOS patients. The ratio of cortisol:ACTH was the same as in normal women, but DHEA-S: ACTH was much higher in PCOS women than the normal control; this latter observation implies that in women with PCOS, for every unit of cortisol production there was excessive output of androgens.

Glintborg et al. reviewed data on 650 PCOS patients and found that those who smoked (260 subjects) had exaggerated 17 hydroxyprogesterone responses to ACTH stimulation [33]. This suggested increased adrenal responsiveness with increased androgen production in smokers who had PCOS. Smoking has been shown to increase the degradation of estrogens, possibly resulting in higher production of androgens as a side-product of increased or decreased estradiol production (no evidence of increased estrogen production has been demonstrated) [34].

Adrenal Androgens in Postmenopausal PCOS Women

There is little information regarding the PCOS patient's adrenal androgen production in the postmenopausal period. Whereas adrenal androgen production decreases in aging normal control perimenopausal women, in response to ACTH stimulation, PCOS women had continued high response of 17 hydroxyprogesterone into perimenopausal ages [35]. In fact, this was also true of six postmenopausal women in the same study. Markopoulos et al. reported that 20 postmenopausal women who had a history of PCOS continued to have higher DHEA-S and androstenedione levels and had a greater response to ACTH stimulation than in the normal women [36]. In these women, the response of ACTH to CRH was the same as in normal women, suggesting increased sensitivity of the adrenals to normal levels of ACTH. Schmidt et al. examined 25 women well past menopause who had previously undergone ovarian wedge resection as a management strategy for PCOS. Increased degree of hirsutism and higher free testosterone levels were observed in this population when compared to control women [37], although DHEA-S levels were comparable to the controls. No stimulation studies were conducted, however, thus limiting interpretability of these findings.

Summary

Patients with PCOS vary in the respective adrenal and ovarian contributions to androgen excess, and in as many as 30–50 %, a dual source of androgen excess may be seen. Androgen exposure early in life, such as with premature adrenarche and precocious puberty, can identify girls who may progress towards a full-blown PCOS phenotype. The endocrine milieu of PCOS may persist well into menopause. Stimulation and suppression tests may offer insightful information regarding mechanisms contributing to androgen excess, but may be limited in their interpretability due to lack of specificity. Studies of PCOS subjects with these tests also suffer from the nonspecificity of the PCOS phenotype. Further genetic and more specific enzyme testing will be necessary to clarify the pathophysiology of this common disorder.

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Chapter 6
The Hypothalamic–Pituitary Axis in PCOS

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Key Points

- Menstrual cycle dysfunction in women with PCOS is accompanied by specific neuroendocrine changes.
- Increased GnRH pulse frequency and an augmented LH response to GnRH result in an increase in the LH/FSH ratio and abnormal regulation of ovarian steroidogenesis and folliculogenesis.
- Both hyperandrogenemia and hyperinsulinemia may play a role in decreasing sensitivity of the progesterone-induced slowing of GnRH pulse frequency in PCOS.
- Obesity results in attenuation of the increased LH response to GnRH and the increased LH/FSH ratio in PCOS through direct pituitary actions.

Introduction

Polycystic ovary syndrome (PCOS) is a syndrome of ovarian dysfunction along with the cardinal features of hyperandrogenism and polycystic ovary morphology in the absence of other explanatory endocrinopathies [1]. The etiology of PCOS is multifactorial and complex, with abnormal ovarian steroidogenesis, hyperinsulinemia, and neuroendocrine abnormalities playing significant interactive roles. The vast majority of patients have menstrual irregularities, and studies have indicated

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that those with menstrual cycle dysfunction also tend to be more hyperandrogenic and hyperinsulinemic [2, 3]. Both genetic and environmental factors impact the presentation and complications associated with PCOS [4–6].

This chapter will review the hypothalamic and pituitary dynamics in normal women with ovulatory cycles and in women with PCOS, will discuss the variable patterns of neuroendocrine dysfunction in patients with PCOS, and will review factors that impact neuroendocrine dysfunction in PCOS.

Neuroendocrine Function in Normal Reproduction

Neuroendocrine regulation of the menstrual cycle involves a complex integrated network of feedback mechanisms between the hypothalamus, pituitary, and target organs. The hypothalamic–pituitary–gonadal (HPG) system is comprised of the gonadotropin-releasing hormone (GnRH) producing neurons of the hypothalamus, the pituitary gonadotropes, which secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and the ovary which responds to gonadotropins with follicular development and ovulation, and with secretion of estradiol, progesterone, and the gonadal peptides, inhibin A and inhibin B. The ovarian steroid and nonsteroidal hormones, in turn, modulate the hypothalamic and pituitary components of the reproductive axis; for review, see [7].

Frequent blood sampling studies with measurement of LH as a marker of pulsatile GnRH secretion and the use of pharmacological probes, such as GnRH antagonists, have been utilized to evaluate the physiology of GnRH secretion in studies in women with normal menstrual cycles [8] and in women with PCOS [9]. In the normal women, the frequency of pulsatile GnRH secretion is dynamically regulated across the menstrual cycle and, importantly, this dynamic regulation of GnRH pulse frequency is key to maintaining the repetitive cycles of follicle development that are required for normal reproductive function in women. The transition from the end of one cycle to the beginning of the next is marked by an increase in pulsatile LH-GnRH secretion from the luteal phase frequency of one pulse every 4 h to a pulse of every 90 min in the early follicular phase. During the mid-follicular phase, LH pulse frequency increases further to one pulse per hour, and this frequency is maintained through the mid-cycle gonadotropin surge. After the mid-cycle surge and ovulation, the GnRH pulse generator slows down to one pulse every 90 min, followed by a further decline to one pulse every 4 h during the late luteal phase. The luteal phase decrease in GnRH pulse frequency is secondary to rising progesterone levels in the presence of estrogen [8]. Although gonadal steroid levels fluctuate less radically because of prolongation of their half-life due to binding to the circulating sex hormone binding globulin (SHBG), progesterone concentrations can vary dramatically in the mid- and late luteal phases (from 2.3 to 40.1 ng/mL) in response to the relatively infrequent pulses of LH [8].

GnRH pulse frequency plays a critical role in the differential regulation of pituitary LH and FSH synthesis and secretion and thus, in the dynamic control of FSH.

At slow GnRH pulse frequencies, as seen in the late luteal phase of the menstrual cycle, GnRH receptor (GnRHR) concentrations on the gonadotrope cell surfaces are relatively low, with activation of a single signal transduction pathway stimulating the expression of the common gonadotropiñ α-subunit, and the LH β- and FSH β-subunits. Faster GnRH pulse frequencies, as seen with progression into the follicular phase of the menstrual cycle in regularly menstruating women, increased GnRHR concentrations on the gonadotrope cell membranes result in greater activation of the signal transduction pathway and stimulation of a second signal transduction pathway that specifically inhibits FSH gene expression [10]. Thus, the slow luteal phase frequencies of pulsatile GnRH stimulation of the gonadotrope result in increased synthesis of FSH, while faster GnRH pulse frequencies favor the synthesis and secretion of LH. With release from the negative-feedback effects of estradiol and inhibin A on FSH secretion during the luteal follicular transition, and inhibitory effects of luteal progesterone on GnRH pulse frequency, the FSH levels rise late in the luteal phase, thus initiating a new cycle of follicle development, setting the stage for the next ovarian cycle.

Gonadal Feedback on the Neuroendocrine System

FSH levels rise threefold in the early follicular phase in response to the decline in estradiol and inhibin A late in the luteal phase of the preceding cycle and thus release of the hypothalamic–pituitary components of the axis from negative-feedback effects of estradiol and probably inhibin A [11]. FSH release early in the follicular phase is further facilitated by the increase in GnRH pulse frequency that occurs with the late luteal phase decline in progesterone levels of the preceding cycle [7]. This luteal-follicular rise in FSH is critical for initiation of folliculogenesis and the beginning of a new cycle of follicle development. With recruitment and early development of a new cohort of ovarian follicles, estradiol and inhibin B increase and concomitantly exert a negative feedback at the hypothalamic–pituitary level, inhibiting FSH release. This mid-follicular phase decrease in FSH is important for ensuring that only a single follicle emerges as dominant and reaches maturity. While the initial increase in estradiol inhibits GnRH, LH, and FSH secretion, the exponential rise in estradiol concomitant with growth of the dominant follicle paradoxically exerts a positive feedback effect on gonadotropin secretion, triggering the mid-cycle LH surge when LH levels can rise tenfold. Ovulation occurs within 36 h after the mid-cycle LH surge; the LH levels subsequently decrease and reach a nadir by the late luteal phase. Progesterone secretion begins with the luteinization of the theca-granulosa cells, which is induced by the LH surge, and reaches peak circulating concentrations in the mid-luteal phase. In addition to progesterone, the corpus luteum also secretes estradiol and inhibin A, and their patterns of secretion follow a similar pattern to that of progesterone.

Neuroendocrine Abnormalities in PCOS

The neuroendocrine abnormalities in PCOS play a significant role in the etiology of abnormal follicular development in PCOS. There has been considerable controversy regarding the primary versus secondary origin of the neuroendocrine abnormalities identified in PCOS. Are the neuroendocrine aberrations the principal instigators of the disorder or is the neuroendocrine dysfunction in PCOS consequent to other factors, such as abnormalities in gonadal steroidogenesis? In PCOS, LH levels are disproportionately elevated in comparison to FSH, resulting in 94 % of women with irregular cycles having an elevated LH/FSH ratio [9]. The LH amplitude response to exogenous GnRH is exaggerated in women with PCOS (Fig. 6.1). Furthermore, the GnRH pulse frequency is increased in PCOS to approximately one pulse per 50–60 min [12], similar to the menopausal pulse frequency, and there is an increase in the overall amount of GnRH secreted that is similar in magnitude to the increase in pulse frequency [12]. This pattern of GnRH secretion favors the synthesis and secretion of LH over FSH. Taken together, the increase in GnRH pulse frequency and the increased pituitary responsiveness to GnRH suggest that neuroendocrine dysfunction may be a primary event in the pathogenesis of PCOS.

It is of interest that, in women with isolated polycystic ovary (PCO) morphology and with regular ovulatory cycles, gonadotropin dynamics are identical to those in normal ovulatory women [13]. Thus, in setting PCO morphology, an abnormal gonadotropin environment is required for development of menstrual dysfunction.

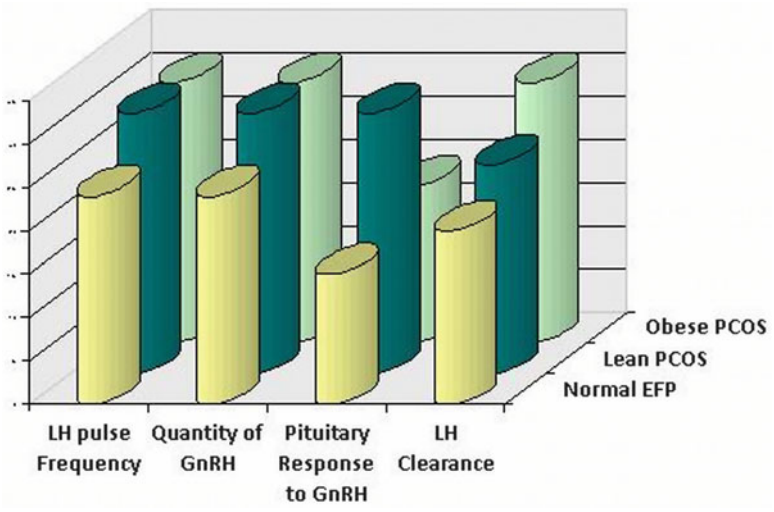

Fig. 6.1 Neuroendocrine features of lean and obese PCOS women relative to normal women in the early follicular phase (EFP). Hypothalamic function, indicated by LH (GnRH) pulse frequency and overall quantity of GnRH, is increased in PCOS but not affected by obesity, while the pituitary response to GnRH is increased in PCOS but attenuated by obesity. The effect of obesity on pituitary responsiveness and the increased clearance of LH that occurs in obese PCOS both contribute to the relative normalization of both LH levels and the LH/FSH ratio in obese PCOS

What Causes the Neuroendocrine Abnormalities in PCOS?

The Role of Androgens

In the presence of PCO morphology and an increase in the ratio LH to FSH in women with PCOS, androgen substrate production by the ovarian theca cells [13] is increased under the control of LH. The relatively lower FSH levels contribute to deficient aromatization of the androgenic substrates, by follicular granulosa cells; the net result is an increase in intra-ovarian androgens, and a milieu that is disruptive to ovarian follicle maturation and ovulation [14]. Although increased ovarian androgen production is a hallmark of PCOS, the role of androgens in the genesis of the abnormal gonadotropin dynamics of the disorder has long puzzled investigators in the field.

Restoration of normal ovulatory cycles has been observed with the removal of an androgen-producing tumor [15] and with wedge resection or ovarian drilling [16]. Despite these observations, investigators have been unable to reproduce the characteristic gonadotropin dynamics of PCOS by androgen administration. In men, testosterone decreases gonadotropin secretion through actions at both the hypothalamic and pituitary levels, only some of which are effected through aromatization to estradiol [17]. In addition, while the pituitary response to *pharmacologic* doses of GnRH is exaggerated by androgens, the opposite effect is apparent with use of GnRH in *physiological* doses wherein the LH response is reduced [17]. In normal women, as in men, acute increases in androgens decrease LH pulse frequency and increase the LH response to *pharmacologic* doses GnRH [18, 19]. In longer-term studies in female-to-male transsexuals, high-dose androgen treatment resulted in a decrease rather than an increase in LH secretion and a decrease in FSH [20]. Taken together these data suggest that androgen excess may not play a direct role in the increased pituitary response to GnRH in women with PCOS.

The Potential Role of Altered Progesterone Negative Feedback

Spontaneous ovulation or the use of a progestin transiently slows GnRH/LH pulse frequency and improves the abnormal LH/FSH ratio in women with PCOS [9]. This effect is temporary but can persist for as long as 10 days after progestin exposure is terminated. This negative feedback is mediated by progesterone binding through the PgRMC1 receptor expressed in GnRH neurons, resulting in the reduction of intracellular calcium, an inhibitory effect that is independent of other factors such as GABAergic and glutamatergic input [21].

The finding that the sensitivity to progesterone-induced slowing of GnRH pulse frequency in women with PCOS is less than in normal women [22, 23] has provided a novel way to conceptualize the effect of androgens on gonadotropin dynamics in PCOS (Fig. 6.2). In animal models, progesterone with estradiol inhibits GnRH

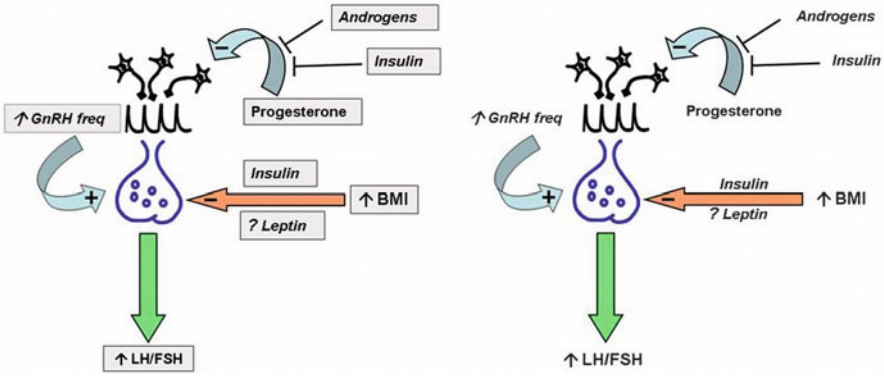

Fig. 6.2 The neuroendocrine abnormalities in PCOS are characterized by an increased frequency of pulsatile GnRH secretion and an increase in the LH response to GnRH, both of which lead to an increased LH/FSH ratio. There is evidence that women with PCOS are relatively insensitive to progesterone slowing of GnRH pulse frequency. A faster pulse frequency favors increased secretion of LH over that of FSH. Both androgens (ovarian or adrenal) and insulin inhibit the negative-feedback effect of progesterone on GnRH pulse frequency in women with PCOS, suggesting an etiologic LH and the LH/FSH ratio are inversely related to BMI in women with PCOS, an effect that is mediated at the pituitary, possibly through the effects of insulin and/or leptin

neuronal activity, and this inhibition is impaired by dihydrotestosterone [24]. Similarly, in women with PCOS, treatment with antiandrogen therapy (flutamide) for 4 weeks restored normal GnRH pulse generator sensitivity to progesterone [25]. Taken together, these findings suggest that in PCOS, increased androgens decrease sensitivity to the progesterone-induced slowing of GnRH pulse frequency that is critical for dynamic modulation of FSH release.

Both ovarian steroids and insulin alter potassium channel dynamics of hypothalamic GnRH neurons [26, 27], and there is evidence that resistance to the negative feedback of progesterone on LH pulse frequency is directly related to hyperinsulinemia in some but not all studies [28, 29]. However, unlike anti-androgens, decreasing insulin with insulin-sensitizing agents decreased gonadotropin levels in some studies while others revealed no significant impact on the sensitivity of the GnRH pulse generator to gonadal steroid feedback [30–32].

In anovulatory women with PCOS, correction of abnormal FSH dynamics by reducing the negative-feedback effect of estrogen using estrogen receptor blockers or aromatase inhibitors, or, more directly, through administration of a normal program of pulsatile GnRH, corrects the ovulatory defect [33–35] in some, but not all PCOS women. With all ovulation induction treatment modalities including use of exogenous gonadotropins, the ovulatory response is negatively affected by the severity of hyperandrogenemia, high body mass index (BMI), and insulin resistance [36, 37].

The timing of exposure to androgens during the course of development may be important in conferring risk for PCOS. Exposure to inappropriate levels of

androgens prior to puberty and perhaps even during prenatal life may impact gonadotropin secretion by decreasing the expression of hypothalamic progesterone receptors. Animal studies demonstrate that prenatal androgen exposure increases mean LH and LH pulsatility, mechanisms identified as contributory to amenorrhea, hyperandrogenemia, and possibly PCO morphology [38–40]. In rhesus monkeys, administration of moderate supraphysiologic doses of testosterone during pubertal development (1–5 years of age) results in LH hypersecretion and increased sensitivity to GnRH, a picture reminiscent of PCOS [41].

In adolescent girls, the neuroendocrine transition in early puberty is initially promoted by the increase in nocturnal LH pulse amplitude and mean FSH. In the daytime, GnRH release during puberty is impacted by negative feedback from the diurnal rise in progesterone in the early morning [42, 43]. This diurnal pattern is followed by an increase in GnRH pulse frequency throughout the 24-h day in the later stages of puberty [44, 45]. Adrenarche with increased adrenal androgen production is thought to be the initial source of androgen exposure impairing sensitivity to gonadal steroid feedback during normal pubertal maturation in girls [28]. Girls with premature adrenarche develop an accelerated LH pulse frequency similar to adult women with PCOS with a loss of the diurnal variation, resulting in an increased risk of ovarian hyperandrogenemia and PCOS [46, 47]. Early pubertal obese girls with hyperandrogenemia have increase LH pulse frequency compared to the nonhyperandrogenic girls across different pubertal Tanner stages, suggesting that abnormalities in the GnRH pulse generator occur prior to menarche [43]. Furthermore, increases in androgens, particularly testosterone, in early adolescence, correlate with increased LH pulse frequency, again consistent with the hypothesis that hyperandrogenemia may decrease sensitivity to the progesterone-mediated inhibition of GnRH [28].

Modifiers of the Neuroendocrine Axis in PCOS

The Role of Obesity

Studies in normal women and adolescent girls have now demonstrated a significant inhibitory effect of obesity on gonadotropin secretion [9, 43]. Obesity is prevalent in PCOS, occurring in approximately 30–60 % of patients and is associated with ovulatory dysfunction. Both BMI and percent body fat are negatively correlated with LH; thus, while a high LH/FSH ratio is common in PCOS, in very obese women with PCOS, this ratio may be relatively normal [9].

Given the known effects of leptin on GnRH secretion, it was expected that the effect of obesity on gonadotropin dynamics in PCOS would be mediated at a hypothalamic level. However, although there is an increase in both GnRH pulse frequency and overall amount in PCOS, there is no effect of BMI on either of these markers of hypothalamic activity [12] (see Fig. 6.1), indicating that obesity does not

exert its effect on LH secretion at the hypothalamic level and provides evidence that the effect of obesity on gonadotropin secretion is exerted at the pituitary.

The decrease in LH responsiveness to GnRH as a function of obesity supports a direct pituitary effect of factors relating to BMI at the pituitary level [9] (see Fig. 6.1), as does the increase in clearance of LH [12], which is associated with changes in the isoforms of LH secreted [48]. Both insulin and leptin have been considered as pituitary mediators of the effect of obesity on gonadotropin secretion (see Fig. 6.2).

Impact of Hyperinsulinemia

Insulin resistance to glucose uptake is observed in approximately 50–75 % of lean and obese women with PCOS [49], and hyperinsulinemia is positively associated with anovulation and hyperandrogenemia [49]. Significant attention has been paid to the potential role of hyperinsulinemia and/or insulin resistance at the level of the ovary, with studies indicating that prolonged insulin infusion increases androgen secretion in the setting of GnRH agonist-induced down regulation of gonadotropin secretion, providing evidence for a direct stimulatory effect of insulin on ovarian steroid synthesis [50].

There is also evidence that insulin acts directly at the pituitary. In the gonadotropin-derived LβT2 cell line, the insulin receptor is localized in the pituitary plasma membrane [51]. Deletions in the insulin receptor substrate (IRS-2) and the insulin receptor (IR) in rodents induce abnormalities in LH secretion, anovulation, and obesity [52, 53]. There is an increase in GnRH-induced pituitary stimulation and maintenance of fertility in pituitary insulin receptor knockout mice with diet-induced obesity [53–55]. Moreover, insulin decreases pituitary sensitivity to GnRH in euglycemic hyperinsulinemic clamp studies in women [56]. Taken together these data suggest that direct insulin-mediated attenuation of gonadotrope responsiveness to GnRH stimulation may be a key mechanism through which obesity modifies the abnormal gonadotropin dynamics in PCOS.

Role of Leptin

Leptin, secreted by the adipocytes, is a regulator of appetite and energy homeostasis and permissive in the activation of GnRH release at puberty. Leptin levels are higher in women with PCOS and directly related to BMI as in normal women. In addition, leptin levels are inversely related to LH levels [57]. In animal studies, leptin has been shown to modulate the expression of leptin receptors present in the pituitary in addition to the hypothalamus and ovary [58]. These data raise the possibility that leptin may mediate the effect of BMI on LH secretion, but further studies are required to confirm this hypothesis.

Genetic Insights into the Neuroendocrinopathy of PCOS

There are data from twin studies and population genome studies to support a strong genetic causality for PCOS with over 70 candidate genes identified. A recent genome-wide association study (GWAS) identified three PCOS susceptibility loci (2p16.3, 2p21, and 9q33.3) in the Han Chinese population which contain the susceptibility genes LHcGR, THADA, and DENND1A respectively [59]. Similar defects have been confirmed in Icelandic and European women [60]. The DENNDIA gene located on chromosome 9q33.3 encodes for DENN, which is responsible for regulating Rab GTPases. Rab proteins play a role in calcium-mediated exocytosis in pituitary cells and GnRH induced gonadotropin release [61, 62]. The genes encoding for LH/hCG and FSH receptors map to the chromosome 2p16.3 region which contains the PCOS susceptibility loci identified in Chinese and European-derived populations, suggesting that defects in these receptors may possibly play a role in the pathophysiology of PCOS [63]. The thyroid adenoma-associated gene (THADA) maps to 2p21 and was noted as a type 2 diabetes-associated gene affecting beta-cell function in the pancreas [64]. Genotype–phenotype correlation studies suggest that the variability in PCOS phenotypes may be due to genetic differences and in the GWAS in the Han Chinese population, the DENND1A gene was also associated with insulin resistance and the THADA gene was linked to dyslipidemia and hyperandrogenemia [65]. Furthermore, gene mutations in the LH receptor and LH β-subunit may impact LH bioactivity and insulin sensitivity in women with PCOS [66]. Chapter 3 in this text provides a more detailed overview of the role of genetics in PCOS.

Summary

Neuroendocrine dysfunction is an essential component of the pathophysiology of PCOS, resulting in disordered folliculogenesis and anovulation. Both genetic factors and hyperandrogenemia impact the hypothalamic–pituitary regulation of reproduction in PCOS and can determine the timing and variability of the clinical presentation of this common disorder. Hyperinsulinemia and obesity further modify the neuroendocrine axis, acting primarily at a pituitary level. Thus, PCOS is attributed to multiple factors: neuroendocrine abnormalities, ovarian dysregulation of steroidogenesis, and insulin resistance, each contributing at various levels to impact folliculogenesis and ovulation. Interventions at various levels have been shown to improve and promote appropriate ovarian follicle development and restore fertility.

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Chapter 7
Insulin Resistance and Lipotoxicity in PCOS: Causes and Consequences

Sylvain Bellanger, Marie-Claude Battista, and Jean-Patrice Baillargeon

Key Points

- Most of PCOS women are insulin-resistant and hyperinsulinemic.
- Insulin resistance seems to be an aggravating factor for hyperandrogenism.
- Abnormalities in fatty acid metabolism are common in women with PCOS.
- Lipotoxicity is known to cause insulin resistance, but may also directly induce hyperandrogenism.
- Lipotoxicity treatment improves insulin sensitivity and lowers androgen level.
- Lipotoxicity is an appealing candidate for PCOS pathogenesis since it could explain both the insulin resistance and hyperandrogenism of PCOS.

Introduction

Polycystic ovary syndrome (PCOS), a common endocrinopathy mainly characterized by androgen excess, is a major cause of anovulatory infertility in women. Therapeutic approaches often succeed in normalizing androgen production and/or restoring ovulatory cycles in PCOS women. However, the exact etiology of the syndrome remains unknown. Several studies have established links between PCOS and various metabolic anomalies that are more prevalent in the PCOS population. Indeed, metabolic syndrome (46 %), dyslipidemia (33 %), glucose intolerance

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(30 %), and type 2 diabetes risks (32 %) are all increased in PCOS women compared to the healthy population [1–3]. Other known consequences of the insulin resistance syndrome are also commonly encountered in PCOS women, including pro-coagulant [4–6] and pro-inflammatory [7–9] states and endothelial dysfunction [10–12]. Importantly, all of these conditions, which are associated with severe health consequences, share a common underlying feature: insulin resistance [13]. Accordingly, recognizing these associated metabolic anomalies will help manage not only the clinical manifestations of the syndrome, but also the long-term cardiometabolic risks of these women.

The diagnostic criteria established by the NIH conference in 1990, the Rotterdam conference in 2003, and the Androgen Excess and PCOS Society in 2006 use two criteria for the diagnosis of PCOS: (1) evidence of hyperandrogenism and (2) existence of ovarian dysfunction, manifested by oligo-anovulation or polycystic ovaries [14, 15]. However, in the clinic, the wide phenotypic heterogeneity that is observed among PCOS women makes it difficult to target and characterize the underlying causes. For example, most PCOS women present with elevated body mass index (BMI), and approximately 70 % of them display insulin resistance and compensatory insulinemia [16, 17]. Paradoxically, because a minority of women with typical PCOS display no sign of insulin disturbance [18, 19], insulin resistance does not seem to be necessary to develop the syndrome, and should, therefore, not be part of the diagnostic criteria per se. However, it is currently unknown whether insulin resistance is a causal factor or a mere contributor to the disorder, or rather just an innocent bystander. Consequently, determining the role of insulin resistance in the development of PCOS is an important challenge that needs further investigation.

Reminders Concerning Insulin Resistance

Definitions of Insulin Resistance

Physiologically, insulin exerts several effects on insulin-sensitive tissues such as the liver, skeletal muscles, and adipose tissue, promoting glucose intake, glycogen synthesis, and inhibiting lipolysis, all of which are known as its “metabolic” effects. However, insulin also exerts important “mitogenic” effects on cell proliferation and differentiation.

The glucose control system relies on a delicate balance between insulin sensitivity and secretion. Insulin resistance occurs when insulin sensitivity is impaired in such a way that insulin can no longer exert its metabolic effects on insulin-sensitive tissues. When insulin insensitivity hampers glucose uptake in target tissues, insulin secretion is usually increased, resulting in compensatory hyperinsulinemia for as long as the pancreatic beta cells can compensate. Several techniques are used to assess insulin sensitivity, the euglycemic-hyperinsulinemic clamp being the gold standard method [20]. However, it is important to distinguish the metabolic effects

of insulin from its other effects, such as mitogenic effects, which will be discussed later. Typically, in insulin resistance, the metabolic effects of insulin are preferentially disturbed, whereas the non-metabolic effects of insulin, such as its mitogenic effects, may remain intact.

Evolution of Insulin Resistance Knowledge in PCOS

The very first association between insulin resistance and pathophysiology of hyperandrogenism was actually reported in 1920 by Emile Charles Achard and Joseph Thiers; these investigators described the Achard-Thiers syndrome, in which highly virilized women developed type 2 diabetes. The characterization of PCOS by Stein and Levental occurred in 1935 [21]. Almost a century later, a large body of data affirms a relationship between endocrine and metabolic aberrations of PCOS with insulin resistance [1–3]; despite the consistency across studies, however, a cause-and-effect relationship to the observed associations remains unclear and the existence of intrinsic anomalies leading to insulin resistance in the PCOS population is still open for debate.

In 1980, Burghen and colleagues observed higher levels of insulin in obese PCOS patients compared to healthy control women of similar BMI. They also observed significant positive correlations between circulating androgen and insulin levels [22]. Others also revealed new and significant positive correlations between testosterone or androstenedione with insulin levels, either under fasting state or during an oral glucose tolerance test (OGTT) [18]. However, Dunaif and colleagues were the first to assess insulin sensitivity in a small group of PCOS women, both obese and nonobese, using the euglycemic-hyperinsulinemic clamp technique [23]. They showed lower insulin sensitivity in PCOS women ($n=29$) compared to BMI comparable healthy women ($n=18$), suggesting that lower insulin sensitivity in PCOS could be independent of obesity and, thus, intrinsic to the syndrome. Results were confirmed later by the same group [24] and others [25, 26] using different techniques to assess insulin sensitivity.

Slightly different results have also been obtained. In a study [27] based on a very heterogenous PCOS group including 49 PCOS women and 42 control women with similar BMI ranging from approximately 18 to 38 kg/m^2, lower insulin sensitivity was observed in women presenting with elevated BMI (>28 kg/m^2) but not in lean PCOS subjects. In addition, the lower insulin sensitivity observed in some lean PCOS women disappeared upon correction for truncal-abdominal subcutaneous fat distribution. Therefore, not all lean PCOS women are insulin-resistant, and those who are usually display increased abdominal fat distribution, as already described in the general population [28]. This suggests that weight and adipose tissue distribution are more important for the development of insulin resistance in PCOS women than the syndrome per se. Interestingly, a mean loss of 12.4 kg, at the end of a 14-month diet, increased insulin sensitivity in 13 obese PCOS women as compared to those seen in control women, thus suggesting once again a key role for weight in

PCOS insulin resistance [29]. In 2000, Morin-Papunen and colleagues observed hyperinsulinemia and insulin resistance in PCOS women compared to healthy women, but only in those who were obese. No significant difference was found between lean PCOS and control women [30]. This study adds to the evidence that some lean PCOS women are not insulin-resistant.

The apparent discrepancies observed among studies regarding insulin sensitivity in PCOS versus non-PCOS women are probably explained by the heterogeneity of PCOS phenotypes across studied populations. Taken together, data from the literature nevertheless suggest that insulin resistance and hyperinsulinemia may be contributing factors that potentially aggravate or even uncover PCOS in predisposed women. This may explain why, on average, PCOS women are more obese and/or insulin-resistant than controls. However, despite the robust associations, a causal role for insulin resistance and hyperinsulinemia for the development of PCOS is unclear.

Insulin and Hyperandrogenemia in PCOS

The reduced action of insulin on glycemic regulation that is observed in the setting of insulin resistance is the result of a series of alterations affecting insulin signaling in the insulin-sensitive tissues such as the liver, skeletal muscles, and adipose tissue. The compensatory hyperinsulinemia and/or alterations in the insulin signaling pathway leading to metabolic insulin resistance are suggested to directly affect the androgen biosynthesis pathway, thus contributing to the PCOS phenotype (Fig. 7.1). The next section describes how insulin influences androgen production as well as the possible mechanisms involved in PCOS hyperandrogenemia.

In Vivo Observations

In vivo, several studies have attempted to dissect the role of insulin in androgen production, either in PCOS or normal women. Different approaches aimed at reducing insulin levels in hyperinsulinemic PCOS women were first used to study the direct implication of insulin on androgen hypersecretion in PCOS. In 1989, Nestler and colleagues observed that in obese hyperinsulinemic PCOS women, a short-term decrease in fasting and glucose-stimulated insulin levels, using diazoxide for 10 days, significantly reduced both free and total testosterone levels [31]. Similarly, a randomized, controlled trial showed that a 6-month treatment with acarbose, which slows intestinal glucose absorption and thus postprandial insulinemia, significantly decreased the free androgen index in 30 obese and hyperinsulinemic PCOS women, compared to placebo [32].

These studies were conducted in obese PCOS subjects with evidence of hyperinsulinemia. However, similar benefits have also been observed in lean normo-insulinemic women with PCOS [19, 33]. In one study assessing the effect of

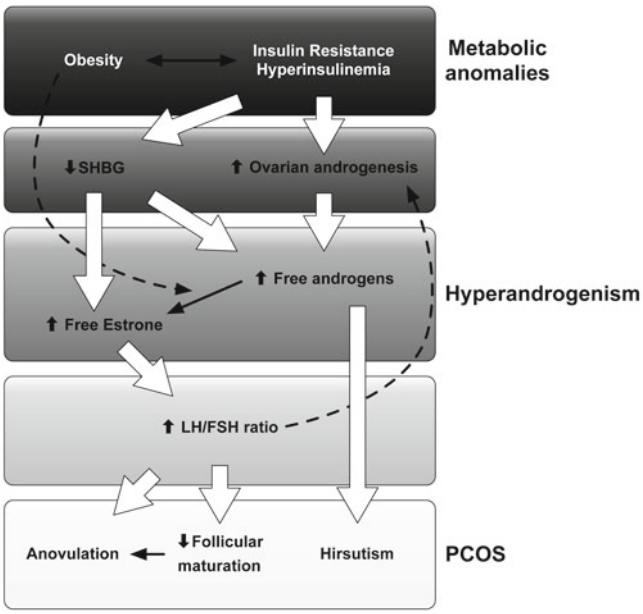

Fig. 7.1 Effects of insulin resistance and compensatory hyperinsulinemia on the development of hyperandrogenism and resulting PCOS phenotype. Insulin resistance and hyperinsulinemia are responsible for the increase in free androgen plasmatic levels, both directly, through the stimulation of ovarian androgen synthesis, and indirectly, by decreasing hepatic production of SHBG. Hyperandrogenism and obesity will promote the conversion of androgens into the estrogen *estrone*, the free form of which will be increased due to the decrease in SHBG levels. High free-estrone levels contribute to alterations in the pituitary gonadotropin release, and to a relative excess of LH which will in turn further stimulate ovarian androgenesis; the net result is impaired follicular maturation and the clinical picture of PCOS with symptoms of oligo/anovulation and hyperandrogenism (hirsutism and acne)

diazoxide-lowering insulin secretion in lean normo-insulinemic PCOS women [33], results demonstrated normal insulin sensitivity, as measured by euglycemic-hyperinsulinemic clamp followed diaxozide treatment. These women responded to insulin lowering by a significant drop in free testosterone and androstenedione levels. Importantly, these results support the contribution of insulin to hyperandrogenemia, even in PCOS women with normal insulin sensitivity. In a randomized, controlled trial, the effects of metformin or rosiglitazone treatments (two insulin sensitizers), alone or in combination, were tested against placebo in lean PCOS subjects with normal insulin levels both during fasting and glucose stimulation states; both insulin-sensitizing drugs significantly improved free-testosterone levels as compared to placebo. Interestingly, metformin lowered insulin levels in these PCOS women, an effect not seen with rosiglitazone [34, 35]. Thus lowering of androgens with metformin could be attributed to a decline in circulating insulin, similar to that seen with diazoxide, whereas lowered hyperandrogenemia with rosiglitazone may be due to restoration of the androgenic response to insulin. In

addition, a recent study conducted in nine young PCOS women, lean and obese, reported that GnRH-agonist stimulation during insulin infusion and after adrenal steroidogenesis suppression with dexamethasone, significantly increased circulating progesterone and androstenedione levels compared to when administered during a saline infusion. Moreover, testosterone levels were also higher 24 h post GnRH-agonist stimulation under hyperinsulinemic conditions [36]. Since gonadotropic hormone levels (LH/FSH) did not differ between infusion conditions, these results suggest that the hyperinsulinemic condition potentiated ovarian androgenic response to gonadotropic hormones stimulation.

Studies in normal non-PCOS women, have found that lowering insulin does not decrease basal androgen production [37] and that the induction of transitory hyperinsulinemia during a euglycemic-hyperinsulinemic clamp procedure does not increase androgen production [38]. Taken together, these observations support the above-mentioned suggestion that hyperinsulinemia in itself does not induce PCOS in a normal woman, but does contribute to hyperandrogenemia in both insulin-resistant and insulin-sensitive women with PCOS.

Insulin also indirectly contributes to hyperandrogenemia in PCOS women by lowering sex hormone-binding globulin (SHBG) levels. This carrier protein binds testosterone in the plasma, reducing levels of its free circulating bioactive form. Plasmatic SHBG levels have been shown to be lower in PCOS women [39, 40], provoking an increase in the bioavailable testosterone. In obese hyperinsulinemic PCOS women, the direct reduction in insulin levels with diazoxide for 10 days [39] or with acarbose for 6 months [32] increases SHBG levels. These results suggest that the low SHBG levels found in PCOS women are due to compensatory hyperinsulinemia associated with insulin resistance. However, lower plasmatic SHBG levels due to insulin resistance and hyperinsulinemia are not limited to PCOS, but are also commonly observed in prediabetic states or in established type 2 diabetes [41]. Moreover, associations between decreased plasmatic SHBG levels and increased risks of developing type 2 diabetes or metabolic syndrome have also been observed both in men and women [42–44].

Another important characteristic of PCOS is increased ovarian and adrenal responsiveness to LH or ACTH, respectively [45–47]. Moreover, it has been shown that suppression of LH secretion with a GnRH long-acting analog in PCOS women does not correct ovarian hypersensitivity to HCG stimulation [48]. However, treatments improving insulin resistance in lean and obese women with PCOS have been shown to curb the exaggerated androgenic response to ACTH [49, 50] and LH [51, 52], suggesting that such hyperresponsiveness is probably due to factor(s) related to insulin sensitization rather than to LH, ACTH, or ovarian steroids per se.

In Vitro Observations

Several defects in insulin action, which are mostly located at the level of the insulin signaling pathway, have been observed in vitro in different PCOS cell types or tissues.

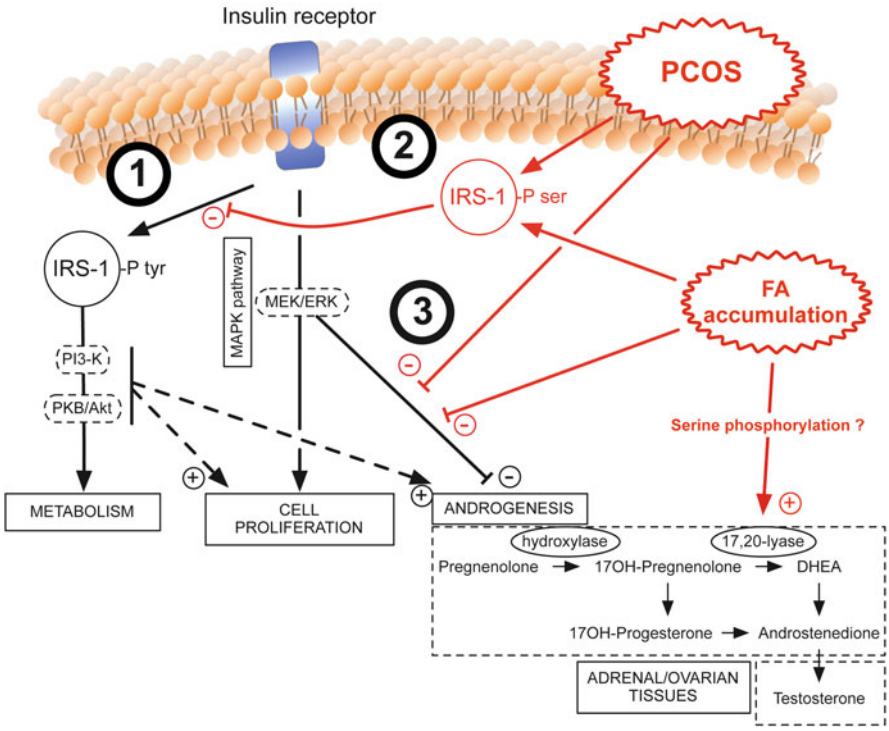

Fig. 7.2 Consequences of lipotoxicity in PCOS. A summary of theoretical mechanisms leading to the establishment of insulin resistance and androgen overproduction in insulin-sensitive tissues and androgen-secreting cells in PCOS women. (*1*) Insulin binding to its receptor activates IRS-1 protein by tyrosine phosphorylation, which in turn activates the PI3-kinase and PKB/Akt proteins that mainly mediate metabolic effects of insulin and could also affect cell proliferation and androgenesis. (*2*) Serine phosphorylation of IRS-1 observed in PCOS and insulin resistance states impairs metabolic effects of insulin by preventing its tyrosine phosphorylation. This phenomenon could potentially be caused by fatty acid accumulation. (*3*) The constitutive inhibitory effect of the mitogenic pathway of insulin (MEK/ERK) exerted on androgenesis could be removed by fatty acid accumulation; this removal phenomenon is observed in PCOS and could lead to increased androgenesis. In addition, fatty acids could directly increase the 17,20-lyase activity of the P450c17 through the serine phosphorylation mechanism by unknown serine/threonine kinase(s). Adapted with permission from Baillargeon JP. Insulin action in polycystic ovary syndrome: in vivo and in vitro. In Azziz R, ed. The polycystic ovary syndrome: current concepts on pathogenesis and clinical care. New York: Springer; 2007: 43–68. (Endocrine Updates; vol 27)

Under physiological conditions, the interaction of insulin with its receptor results in receptor dimerization and autophosphorylation by its own tyrosine kinase activity (Fig. 7.2). This enables recruitment of the first proteins involved in signal transduction, namely, the insulin receptor substrate protein family, or IRS. Thereafter, the numerous actions of insulin are mediated via two main pathways. The first is responsible for the "metabolic" effects of insulin and regulate energy balance. The "mitogenic" effects of insulin on cell proliferation and survival are mediated via the second pathway, which is aptly called the *mitogenic pathway*. When investigating

the mechanisms leading to insulin resistance and associated metabolic conditions, such as type 2 diabetes, most studies in literature have focused on defects in the metabolic pathway.

Metabolic Pathway Anomalies

Insulin metabolic pathway alterations have been studied in classically defined insulin-sensitive tissues (i.e., tissues that are sensitive to its main metabolic actions, namely myocytes, adipocytes, and hepatocytes). In PCOS, insulin sensitivity seems to decrease partly because both muscles and adipocytes have difficulty utilizing glucose under insulin-stimulated conditions, as has been well demonstrated in experimental in vitro studies [24, 53, 54].

Different mechanisms could explain this decrease in insulin-dependent glucose uptake efficiency. Studies suggest that metabolic pathway defects may involve the insulin receptor itself. For example, one study from Dunaif et al. [55] found that insulin-stimulated autophosphorylation of the insulin receptor β-subunit was decreased in fibroblast and muscle cells from women with PCOS. In these tissues, an increase in insulin-independent serine phosphorylation of the insulin receptor was associated with a decrease in insulin-induced tyrosine residue phosphorylation, which is the normal active state of the receptor. Another study, in PCOS-derived fibroblasts [56], reported that insulin receptor tyrosine autophosphorylation dropped by 40 % after insulin stimulation. In addition, treatment with a nonspecific serine kinase inhibitor or a protein kinase A specific inhibitor, respectively, totally or partially restored insulin-stimulated tyrosine phosphorylation of the receptor. Treatment of the cells with Gö6983, a protein kinase C (α, β, γ, δ, and ζ isoforms) inhibitor, had no effect. Thus, a drop in signal transduction after insulin stimulation in PCOS cells could be the result of altered basal serine phosphorylation of the insulin receptor caused by one or several yet unidentified factors.

The mechanisms of serine phosphorylation of insulin receptor have been abundantly studied in research related to type 2 diabetes [57, 58]. Amongst the main proteins recruited by the activated insulin receptor are the IRS. Following recruitment, these proteins are usually phosphorylated on tyrosine residues by the insulin receptor itself. Once again, serine phosphorylation of IRS-1 and 2, instead of tyrosine phosphorylation, impairs transmission of the insulin signal and may thus be responsible for insulin resistance in PCOS. This phenomenon has been observed in muscle fibers from PCOS women [59] displaying an increase in IRS-1 serine phosphorylation compared to muscle fibers from normal women. These results support the notion that excessive serine phosphorylation of the insulin receptor and/or IRS by a serine kinase may be involved in the metabolic insulin resistance of PCOS.

Furthermore, post-receptor defects impairing insulin signaling have also been suggested in the setting of PCOS. One of the terminal effects of metabolic insulin action is the translocation of GLUT4 glucose transporters to the cell membrane in order to allow glucose uptake in insulin-sensitive tissues. Reduced GLUT4 expression has been directly detected in adipocytes from PCOS women in several studies [60]. Post-receptor defects in the insulin signaling cascade have also been suggested

to lead to reduced GLUT4 translocation and/or expression, and, thus, deficient glucose uptake, which is typical of metabolic insulin resistance. More recently, Ciaraldi et al. [54] detected a drop of maximal insulin effect under a sub-maximal dose of insulin (0.17 nM), reflecting a loss of insulin sensitivity compared to control adipocytes. However, no difference in the absolute rate of glucose transport at a supra-physiological dose of insulin (8.5 nM) was detected between PCOS and control adipocytes [54]. This could be indicative of the presence of adipocyte insulin resistance under low doses of insulin in these women. In addition, no difference in the expression of GLUT4, or other proteins involved in the metabolic signaling pathway such as IRS-1, Akt1/2, PKCζ, CAP, and cbl, was observed between healthy and PCOS adipocytes, muscle tissue, or cultured muscle fibers in the same study. As expected, loss of insulin-dependent signal transduction could result in lower protein phosphorylation/activation rather than a drop in signaling protein expression. Surprisingly, no difference in Akt serine or threonine phosphorylation was detected between controls and PCOS-derived cells/tissues under basal or insulin-stimulated conditions. Indeed, despite increased phosphorylation of Akt by insulin stimulation in all three cell/tissues types, the degree of phosphorylation reached after stimulation was nevertheless similar in PCOS and control cells/tissue [54]. These results suggest that the signaling defects responsible for the loss of metabolic effects of insulin in adipocytes or muscle cells of PCOS women may not be located at the level of Akt activation. However, this study assessed the expression of several other insulin metabolic components rather than their phosphorylation states. Thus, the absence of protein expression differences observed in this study between PCOS and control-derived cells does not exclude the presence of phosphorylation differences not assessed here.

Mitogenic Pathway Anomalies

Besides its actions on glucose regulation, insulin also regulates cell proliferation and differentiation via the insulin mitogenic pathway. Following insulin stimulation, a cascade of mediator phosphorylations leads to the activation of several transcription factors, which in turn regulate the expression of genes involved in cell proliferation and differentiation [61]. Interestingly, defects in the metabolic pathway do not affect the mitogenic pathway and vice versa [62], since each possesses a respective set of exclusive signaling actors. Differential alterations between metabolic and mitogenic pathways have been characterized experimentally in different cell models [57, 63, 64]. For example, Books et al. [65] found that PCOS skin fibroblasts displayed metabolic insulin resistance, as demonstrated by lower insulin-stimulated glucose incorporation and glycogen formation, compared to cells from healthy controls. Mitogenic functions per se, however, seem unaltered, as evidenced by the absence of significant difference in thymidine incorporation, a marker of proliferation, between PCOS and control fibroblasts under insulin or IGF-1 stimulation.

Perturbations of insulin signaling, which occur in PCOS women, seem to affect the metabolic and mitogenic pathways differently, depending on the tissue or cell type observed. Thus, in skeletal muscle of PCOS women, while an increase in IRS-1 serine phosphorylation [59] has been associated with decreased activation of the

metabolic pathway, the mitogenic pathway was only constitutively activated (as in healthy controls) and, consequently, mitogenic effects were less stimulated in presence of insulin [66].

Existence of potential cross-talk and interactions between both insulin signaling pathways has been observed in vitro in muscle cells from PCOS women [67]. Increased MEK/ERK phosphorylation levels under basal conditions or under insulin stimulation were found that seemed to result from higher activation of the initial steps of the MEK/ERK cascade (Fig. 7.3) (i.e., the interactions between the small GTP-binding protein [Ras] and the first component of the MEK/ERK cascade the MAPKK kinase [Raf]) [68]. More importantly, inhibition of MEK1/2 activation resulted in a decrease of IRS-1 serine phosphorylation and an increase of its interaction with Akt. These results highlighted the fact that, in PCOS women, the presence of defects affecting insulin's mitogenic pathway also affect metabolic signaling, at least in specific tissues such as skeletal muscle.

Insulin-signaling defects have also been reported in steroidogenic organs in PCOS. The bulk of in vitro studies have been performed in bovine or porcine ovarian thecal cells as well as in ovarian tissue from PCOS women. It is worth noting that different insulin-signaling defects have been reported for the ovarian tissue; some are even the opposite of those observed in muscle or adipose tissue. Moreover, in the ovarian tissue, insulin-signaling defects directly impact androgen biosynthesis. Indeed, as opposed to what has been reported for muscle cells [66], cultured PCOS ovarian thecal cells displayed a significant decrease in MEK/ERK activation compared to the same type of cells from healthy women [69]. In vitro, loss of MEK/ERK activity could explain, at least in part, increased androgen production in PCOS thecal cell. In vitro, hyperactivation of MEK/ERK pathway inhibited androgen production and conversely, inhibition of the pathway significantly stimulated androgen synthesis. These results suggest that, under physiological conditions, in the ovary, the MEK/ERK pathway could exert constitutive inhibition of androgen production and that lifting of this inhibition due to insulin-signaling defects associated with PCOS could contribute to the development of ovarian hyperandrogenism.

The apparent contradictions concerning insulin-signaling defects in different tissues from PCOS women strongly suggest that a combination of genetic and environmental factors contribute to tissue-specific defects and effects. Existing data are supportive of metabolic signals, specifically impaired insulin signaling in insulin-sensitive tissues as a mechanism for androgen excess of PCOS.

Lipotoxicity in PCOS

Evidences of Lipid Metabolism Dysfunction in PCOS

Adipocyte dysfunction and abnormal fatty acid metabolism are important defects widely investigated for their implication in muscle and liver insulin resistance as well as beta-cell failure [70]. Indeed under pathological conditions, adipocyte

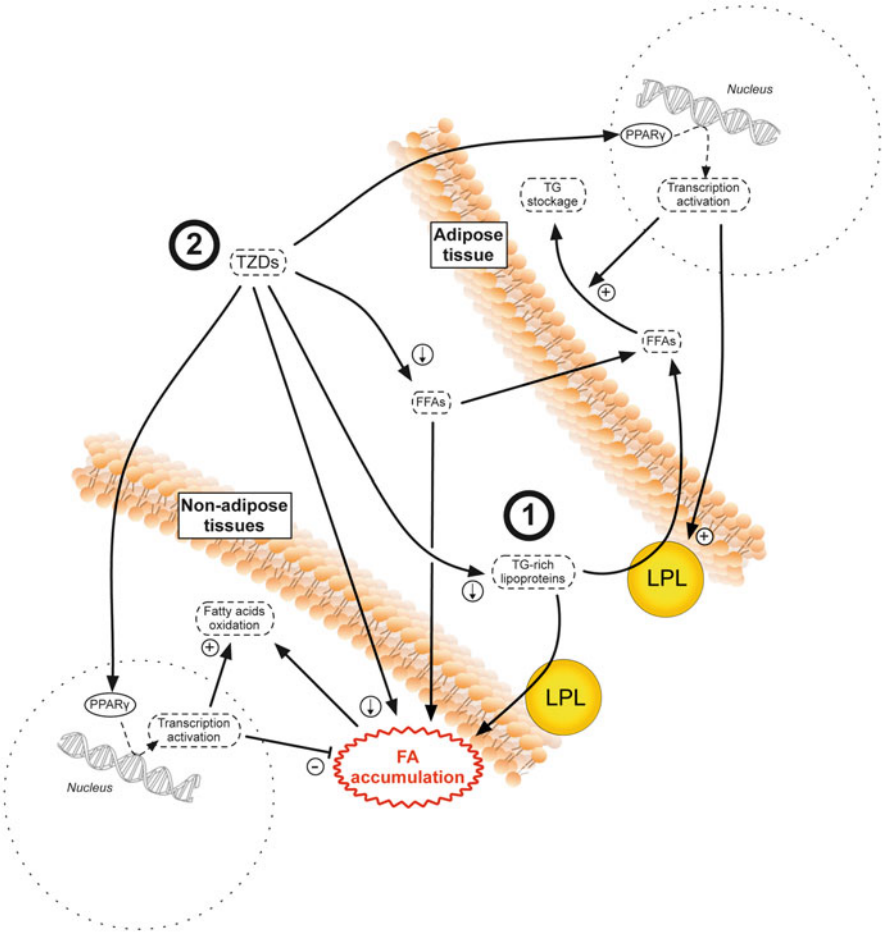

Fig. 7.3 General effects of thiazolidinediones on lipid metabolism in adipose and non-adipose tissues. (*1*) TG-rich lipoproteins release FFAs under the action of the LPL; this release is followed by the entry of fatty acids that will be stored as TG in adipose tissue or will be accumulated and oxidized in non-adipose tissues. (*2*) TZDs bind PPAR**γ**, which will in turn activate the transcription of several genes. In adipose tissue, it stimulates the LPL activity and enhances the entry and the storage of FFAs. In non-adipose tissues, it enhances fatty acid oxidation and inhibits their accumulation. Through these mechanisms, TZDs decrease lipid accumulation in non-adipose tissues and promote fat redistribution in adipose tissue. *TZDs* thiazolidinediones, *FFAs* free fatty acids, *LPL* lipoprotein lipase, *TG* triglyceride, *PPARγ* peroxisome proliferator-activated receptor gamma

dysfunction can lead to free fatty acid spillover in the circulation and overflow in non-adipose tissues. The deleterious effects of fatty acids in overexposed tissues are gathered under the term of "lipotoxicity". Thus, the lipotoxicity theory is important to characterize the perturbations that could ultimately lead to the development of type 2 diabetes [70].

In addition to lipotoxicity, adipocyte dysfunction is also associated with adipose tissue inflammation, low-grade systemic inflammation, increased secretion of adipokines implicated in insulin-resistance development (leptin, resistin, visfatin, etc.) and reduced production of adiponectin, an adipokine known to improve insulin sensitivity [70–73]. The role of inflammation in PCOS is discussed elsewhere in this book and will not be the focus of this chapter.

Lipotoxicity has not yet been widely studied in PCOS research, despite the well established link between PCOS and a risk for several metabolic complications, including type 2 diabetes [1–3]. Increased non-esterified fatty acid (NEFA) levels, during fasting state and following oral glucose, are described in both lean and obese women with PCOS as compared to a healthy population of comparable weight [74]. This suggests that PCOS women could suffer from abnormal NEFA metabolism independent of obesity. More recently, morphological and functional adipose tissue anomalies were described in 74 PCOS women with BMI ranging from 18 to 47 kg/m^2 compared to 31 control women with similar BMI [75]. The authors found an increase in mean adipocyte size along with a decrease in lipoprotein lipase activity and plasmatic adiponectin levels in PCOS women. These features provide evidence of adipose tissue dysfunction in PCOS women that could trigger lipotoxic effects in their insulin sensitive tissues and, thus, contribute to their insulin resistance.

Impact of Lipotoxicity on Androgenesis

Lipotoxicity could contribute to the insulin resistance of PCOS, as has been demonstrated in obesity [70] and other conditions characterized by insulin resistance, such as type 2 diabetes, Cushing's syndrome, and inherited or acquired states of lipodystrophy [70, 76–78]. However, a more interesting question is whether lipotoxicity could contribute directly to PCOS hyperandrogenism; if latter was indeed true, this would suggest that lipotoxicity may be a key factor in PCOS pathogenesis. To begin to answer this question, the effects of overexposure to NEFA on androgenesis have been investigated in vivo [79, 80]. Mai and colleagues first studied the effects of an intralipid/heparin (IH) infusion on androgen production in eight healthy young men [79]. The infusion of IH induced a transitory increase in whole-body circulating NEFA levels because heparin stimulates endothelial LPL activity that will hydrolyze the triglycerides of the intralipid emulsion in all tissues. The authors observed an increase in the levels of the adrenal androgens DHEA and androstenedione during a 6-h IH infusion as compared to saline infusion. In addition, they detected this increase before any increase in circulating insulin levels was apparent, suggesting that hyperandrogenic effect was independent of NEFA-induced insulin resistance and/or compensatory hyperinsulinemia [79]. Moreover, neither ACTH, LH, nor FSH levels significantly changed during the infusion, suggesting an increase in the androgen responsiveness to physiological stimuli. Two years later, the same team performed a similar experimentation on 12 healthy young women and confirmed their earlier findings. In addition, they observed an increase in the production in

both adrenal and gonadal androgens, as well as estrogens in women following IH infusion [80]. These results are the first to suggest that overexposure to circulating NEFA directly increases androgen production in healthy women.

Using bovine adrenal fasciculata/reticularis cells as an in vitro androgen-producing cell model, we recently observed similar results in vitro [81]. We reported significant effects of a 48-h exposure of these cells to palmitate, a saturated fatty acid, on ACTH- or forskolin-induced androgen production. Indeed, bovine adrenal fasciculata/reticularis cells treated with palmitate (100 μM) produced 37 and 68 % more DHEA, the main adrenal androgen, under ACTH or forskolin stimulation, respectively, when compared to untreated cells. Moreover, while we were not able to detect any changes in the expression of the key enzyme of androgenesis, the P450c17 protein, we observed significant decrease of the ERK1/2 activation under forskolin stimulation after palmitate exposure. This result suggests that palmitate could impair MEK/ERK pathway under forskolin-stimulated condition in adrenal androgen-producing cells and, therefore, induce androgen production. This hypothesis is consistent with the loss of MEK/ERK phosphorylation reported by Nelson-Degrave and colleagues [69] in ovarian thecal cells from PCOS women and the stimulation of androgen production that they observed in vitro after experimental MEK/ERK inhibition [69]. However, complementary results are necessary, and specific inhibitors of the MEK/ERK pathway should be used to confirm whether fatty acids could induce such a defect in vitro.

In PCOS, several anomalies directly affecting the key enzymes involved in steroidogenesis and more specifically in androgen production have been described. Expression of P450c17, the key enzyme responsible for DHEA and androstenedione synthesis, and the stability of its transcripts were increased in ovarian thecal cells from PCOS women [82, 83]. In addition, it has been shown recently that upstream enzymes in the steroidogenic pathway, such as P450 cholesterol side-chain-cleavage, could probably be also affected in a similar way [84]. The P450c17, which possesses two distinct activities (17α-hydroxylase and 17,20-lyase), may be regulated through serine phosphorylation mechanisms [85–87], although this has not been clearly characterized yet. Indeed, a serine phosphorylation of this enzyme can increase its 17,20-lyase activity over its 17α-hydroxylase activity, thus increasing androgen synthesis relatively to glucocorticoids. We previously mentioned the existence of abnormal serine phosphorylation at different steps of the insulin-signaling pathway that was associated with insulin resistance in PCOS women. All these findings thus suggest dysregulated serine kinase activity in PCOS that could affect both the insulin-signaling pathway and the P450c17/17,20-lyase activity. Moreover, a potential mechanism for lipotoxicity is the activation of serine kinase cascades after intracellular accumulation of reactive lipids, such as diacylglycerol (DAG), long-chain acyl-coenzyme A, and ceramides [70]. These lipotoxic mediators accumulates in NEFA-exposed cells when their ability to esterify or beta-oxidize NEFA is exceeded [70]. In muscle or liver [88], it has been shown that the insulin resistance induced by increased serine phosphorylation of IRS-1/2 was likely due to activation of a serine kinase, such as novel PKC isoforms, after intracellular accumulation of DAG, acyl-coA and ceramides [70, 89–91]. Therefore,

both PCOS insulin resistance and hyperandrogenesis may be due to an increase in the serine phosphorylation state of insulin-sensitive and androgenic cells following accumulation of these reactive lipids.

Directs and Indirect Effects of Thiazolidinediones on Androgenesis

Many studies have demonstrated that peroxisome proliferator-activated receptor gamma (PPARγ) agonists, the thiazolidinediones (TZDs), can normalize insulinemia and androgen levels in PCOS women [50, 92]; these data underscore the implication of NEFAs in the development of PCOS. TZDs, namely, rosiglitazone or pioglitazone, activate PPARγ receptors that will increase the expression of key genes for fatty acid storage in the adipose tissue and beta-oxidation in non-adipose tissues (see Fig. 7.3). Therefore, TZDs can improve pan-corporal fatty acid metabolism and improve insulin resistance in insulin-sensitive tissues [93, 94], presumably by reducing lipotoxicity. Interestingly, TZDs have been shown to significantly improve androgen levels in PCOS women [95, 96]. However, it is unclear whether these effects on androgens are due to insulin lowering following improvement of insulin resistance, or directly to improved beta-oxidation of NEFAs in androgen-secreting cells and, thus, reduction in lipotoxic effects. Our finding that 6-month treatment with a TZD significantly reduced androgen levels in lean normoinsulinemic PCOS women, without affecting insulin levels, greatly supports a direct mechanism independent of insulin stimulation [19]. Furthermore, an in vitro study suggested that pioglitazone could directly correct the increased expression of P450c17 that occurs in NCI-H295R cells after inhibition of the MEK/ERK pathway [97]. Once again, these data corroborate the results observed by Nelson-Degrave and colleagues [69] regarding a potential implication of constitutively decreased activation of the MEK/ERK pathway in PCOS hyperandrogenemia.

Summary

It is difficult to determine whether insulin resistance is the main cause, the consequence, or a bystander of hyperandrogenism in PCOS. Since insulin resistance and hyperinsulinemia do not seem to be necessary for the development of the syndrome, they are more likely contributing factors rather than being the pan essential trigger. However, depending on individual characteristics (phenotype and genotype), the contributions of insulin resistance to the development of PCOS may strongly vary amongst individual women. On the other hand, current evidence in the literature suggests that lipotoxicity in insulin-sensitive tissues is a key mechanism in the establishment of insulin resistance. Moreover, recent studies have shown that overexposure of tissues to NEFA could also increase androgen production. Together,

these data support the hypothesis that impaired NEFA metabolism in PCOS women could explain both the insulin resistance and the hyperandrogenism that characterize the syndrome. An increased susceptibility of androgen secreting cells to altered NEFA metabolism may exist in women destined to develop PCOS. This underpinning may also predispose most, but not all, women with PCOS to the same susceptibility in their insulin-sensitive tissues. The lipotoxicity paradigm can thus explain the metabolic and endocrine aberrations of PCOS. The degree of tissue susceptibility to lipotoxicity however could greatly differ among PCOS women and explain the large variety of phenotypes in PCOS, including why insulin resistance may be absent in some women with otherwise typical features of PCOS.

Future Directions

As mentioned throughout this chapter, while a spectrum of metabolic and endocrine alterations have been identified in PCOS, the exact role of each in the causation of the syndrome remains unclear. However, it will be important to clarify the causes as well as implications of these alterations. Since PCOS is associated with increased familial predisposition [98–100], it will be important to distinguish whether a specific defect is caused by genetic or epigenetic alteration, or induced by environmental factors such as lifestyle habits. Among the different alterations in steroidogenic enzyme activity described in literature, it will be important to determine whether determinants of increased activity, such as enzyme over expression, increased mRNA stability, or phosphorylation state, could be related to insulin-signaling defects or lipotoxicity. Should an association be uncovered, it will be necessary to fully characterize the mechanisms involved. The serine phosphorylation defect observed at different levels of the insulin-signaling pathway in PCOS women needs clarification and the responsible factors need to be better characterized, in order to assess whether these defects could be linked to lipotoxic mechanisms already described in literature. In addition, to validate, or refute the serine phosphorylation hypothesis of PCOS, it will be important to determine whether specific serine kinases may regulate the 17,20-lyase activity of P450c17, and then to test these hypothetic mechanisms in PCOS-derived androgenic cells. These characterizations would be important to better understand the mechanisms by which insulin sensitizers improve PCOS hyperandrogenism and ovulation, but also to develop new therapeutic strategies that target more specifically the underlying causes of PCOS development.

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Chapter 8
Obesity, Metabolic Dysfunction, and Inflammation in Polycystic Ovary Syndrome

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Key Points

- Beyond its energy-storing capacity, adipose tissue acts as an endocrine and immunological organ.
- Accumulation of excess adiposity causes dysfunction of the adipose tissue compartment that leads to chronic low-grade inflammation, metabolic derangement, and endocrine imbalance.
- Obesity exacerbates the signs and symptoms of polycystic ovary syndrome (PCOS) by worsening preexisting chronic low-grade inflammation and insulin resistance.
- Obesity worsens the risk of developing type 2 diabetes mellitus, dyslipidemia, and hypertension in PCOS.
- Diet-induced inflammation that is independent of excess adiposity can induce molecular alterations in PCOS that may be the underpinning of insulin resistance, atherogenesis, and ovarian dysfunction.

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Introduction

Obesity has grown in pandemic proportions in industrialized countries throughout the world, in part because of modern dietary habits and sedentary lifestyle [1]. More recent evidence suggest that epigenetic phenomenon related to the abnormal metabolic fetal environment of maternal obesity has significantly contributed to the pandemic [2]. Beyond its energy-storing capacity, adipose tissue acts as an endocrine and immunological organ and contains several cell types that contribute to its various functions. Obesity is a significant contributor to metabolic derangement and increases the risk of developing type 2 diabetes mellitus (DM), dyslipidemia, and hypertension. Obesity exacerbates the signs and symptoms of polycystic ovary syndrome (PCOS) by worsening metabolic abnormalities present in PCOS, such as chronic low-grade inflammation and insulin resistance. Lessons learned from the proinflammatory state of obesity have proven to be useful in understanding key inflammatory mechanisms in PCOS that are independent of excess adiposity.

Definition of Obesity and Body-Fat Distribution

Obesity is defined as an excess of adiposity in the body. In the clinical setting, obesity is defined as an increased BMI [weight in pounds/(height in inches)$^2 \times 703$ or weight in kilograms/(height in meters)^2] [3, 4]. Overweight and obese BMI ranges have been established (Table 8.1) [4]. However, when correlated with more accurate measures of body fat, lower BMI thresholds have been suggested to designate these ranges [5]. It has also been suggested that separate ethnic-based BMI designations should be established since with the current thresholds, individuals of certain ethnicities have more body fat than others with the same BMI [6].

A BMI $\geq$30 kg/m^2 is generally associated with increased body fat. However, the BMI alone cannot differentiate between lean and fat mass, the proportions of which define body composition, which in turn is affected by age, gender, and athleticism [3, 4]. The measurement of height may be less reliable in the setting of vertebral

Table 8.1 Classification of body mass index[a]

Classification		Body mass index (kg/m^2)
Underweight		<18.5
Normal weight		18.5–24.9
Class 1	Overweight	25–29.9
Class 2	Obesity	30–34.9
Class 3		35–39.9
Class 4		≥40

[a]Modified from [4]

collapse or fracture, and self-report of height may lead to subsequently inaccurate BMI determination [3]. However, obesity defined by BMI tends to be more accurate in women than in men. This is because women typically have less muscle mass than men [7]. Thus, greater body weight in the average sedentary woman tends to be more reflective of increased adiposity.

The BMI should be considered a starting point for the assessment of obesity. Indirect measures of body fat include waist, thigh, and hip circumferences, calculated waist-to-hip (WHR) and waist-to-thigh (WTR) ratios [3]. Circumference of the waist (WC) is measured at the level of the iliac crest after a normal expiration, hip (HC) at the widest point around the buttocks, and mid-thigh (TC) with slightly flexed knees and both feet flat on the floor [8].

In humans, adipose tissue is present in subcutaneous and visceral locations throughout the body. WC reflects both subcutaneous and intraabdominal/visceral adiposity and correlates most strongly with BMI; conversely, HC and TC reflect mostly subcutaneous fat and muscle [9]. Although elevated WHR and WTR suggest increased visceral adipose, these may alternatively indicate decreased muscle or subcutaneous fat [9, 10]. With aging, adiposity becomes more centrally localized within the abdomen independently of weight [3]. Since central adiposity is associated with higher morbidity, it has been recommended to measure both BMI and WC in the clinical setting [9]. Direct measures of body composition use techniques such as bioelectrical impedance, dual energy X-ray absorptiometry, computerized tomography, and magnetic resonance imaging. However, there currently is no universally recommended approach to assess body composition using either clinical or direct methods, and is typically based on the patient population and specific characteristics desired for interpretation [11]. Gender-specific thresholds for classifying obesity based on percent body fat suggest ≥25 % body fat for men and ≥30 % for women [4].

Prevalence of Obesity

The most recent data from the National Health and Nutrition Examination Survey 2009–2010 found that >78 million, or 35.7 %, of adults in the United States were obese, a prevalence that has not improved from prior years. Men and women had similar prevalence of obesity, but rates of obesity increased with age only in women, reaching 42.7 % at age ≥60 [12].

Adipose Tissue Anatomy

The adipocyte is the primary cell type of adipose tissue. Adipocytes can be brown or white and comprise 50 % of the adipose contents [10]. Aggregates of adipocytes are held in a framework by tissue collagen (Fig. 8.1) [13]. The non-adipocyte

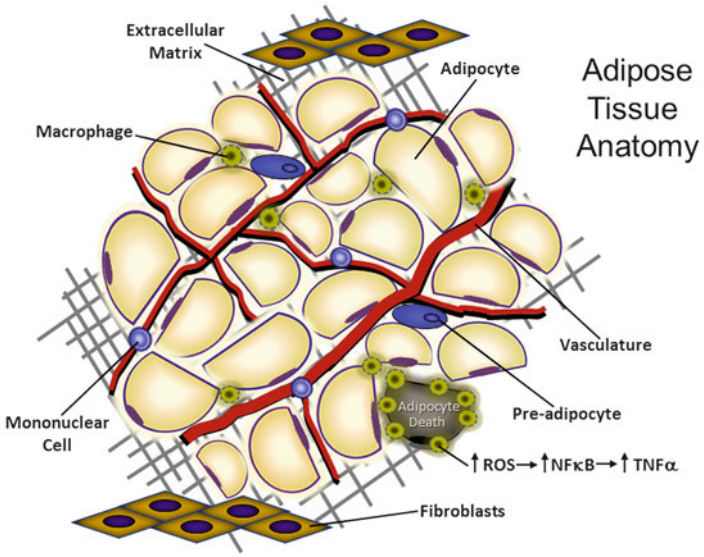

Fig. 8.1 Adipose tissue holds aggregates of adipocytes in an extracellular collagen matrix [10]. The non-adipocyte components are contained in the stromal–vascular compartment and consist of mononuclear cells (MNC), MNC-derived macrophages, pre-adipocytes, fibroblasts, and blood vessels [11]. Adipocyte death induced by hypoxia in the expanded adipose tissue of the obese leads to migration of circulating MNC into the adipose stromal-vascular compartment [81, 82]. Phagocytic activity by MNC-derived macrophages induces oxidative stress and inflammation [84, 85]

components are known as the *stromal–vascular fraction* and consist of mononuclear cells (MNC), MNC-derived-macrophages, pre-adipocytes, fibroblasts, mesenchymal stem cells, and blood vessels [14]. The brown adipocytes are more richly perfused, but each white adipocyte is still in close proximity to a capillary [13, 15]. The blood supply facilitates delivery of metabolic substrates to and removal of end products of adipocyte metabolism [16].

White adipocytes are more prevalent and appear as spherical unilocular vacuoles. The cytoplasm of white adipocytes is predominantly composed of lipid and contains small mitochondria that are pushed to the periphery. They produce leptin, which influences food intake and promotes release of stored energy during fasting in the form of free fatty acids (FFA) [10]. White adipocytes can enlarge in size (hypertrophy) by increasing lipid within their cytoplasm or undergo cell division to increase in quantity (hyperplasia). Hypertrophy increases with overall fat mass. However, adipocyte hyperplasia ensues once the cytoplasm reaches a critical size [10, 13] and is commonly seen with aging and chronic overfeeding. Brown adipocytes, in contrast, contain numerous vacuoles and large mitochondria, and are innervated by adrenergic nerves that are activated by low temperatures to generate heat [10].

Adipose Tissue Metabolism

Dietary Fat Absorption

Dietary fat absorption (Fig. 8.2) involves three steps as follows: (1) enterocyte uptake; (2) intracellular processing; (3) delivery to the mesenteric lymph. Ingested dietary fats consist mostly of triglycerides. Pancreatic triacylglycerol lipase hydrolyzes triglycerides into FFA and glycerol in the lumen of the small intestine [17]. FFA and glycerol cross into the enterocyte by simple diffusion and are shuttled into the endoplasmic reticulum with the aid of fatty acid binding proteins. The translocated FFA and glycerol are reformed into triglycerides and packaged with cholesterol into prechylomicrons. Final processing of prechylomicrons occurs in the Golgi to form chylomycrons [18]. Transport vesicles carry mature chylomicrons to the basolateral membrane for exocytosis into the mesenteric lymph, and the chylomicrons eventually enter the circulation to reach muscle and adipose tissue [17, 18].

Energy Storage and Utilization

Chylomicrons and very low density lipoproteins (VLDLs) are too large to pass through the adipose tissue capillaries. The triglycerides they contain are released into the capillary lumen and hydrolyzed by adipocyte-derived lipoprotein lipase into non-esterified free fatty acids (NEFA) (see Fig. 8.2). NEFA are subsequently transported into the adipocytes, where they are esterified to reform triglycerides [19].

Use of fatty acids as a source of energy is dependent on glucose availability. NEFA are important sources of energy during times of energy deprivation, particularly during fasting or physical exercise. Adipose tissue metabolism has opposing regulation by insulin and catecholamines. Insulin promotes fat storage in a process

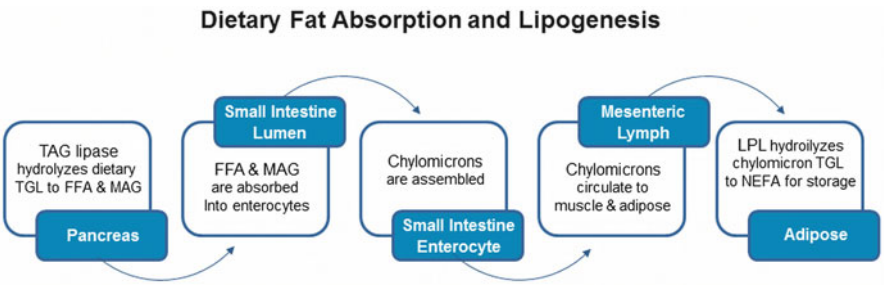

Fig. 8.2 The process of dietary fat absorption and lipogenesis occurs with the aid of enzymes in the gut and circulation that break down or reform fat molecules into various sizes for transport and eventual storage into adipose tissue. *TAG* triacylglycerol, *FFA* free fatty acids, *MAG* monoacylglycerides, *LPL* lipoprotein lipase, *NEFA* nonesterified fatty acids

called lipogenesis, whereas catecholamines promote fat breakdown in a process called lipolysis [19, 20]. Lipogenesis consists of both the de novo formation of FFA from carbohydrate stores in the liver as well as the formation of triglycerides from FFA in adipose tissue [21]. Insulin stimulates uptake of glucose into adipocytes, where it is converted to glycerol and combines with FFA to form triglycerides, which then accumulate in the adipose tissue [19].

Lipolysis consists of the breakdown of triglycerides into NEFA and glycerol and is regulated by catecholamine-induced hormone-sensitive lipase and adipose triglyceride lipase/desnutrin [19]. Other proteins, such as perilipin, either cover or uncover the core of triglycerides, controlling their exposure to these lipases [19, 20].

Adipose Tissue as an Endocrine and Immunological Organ

Steroid Hormone Peripheral Conversion: Role of Adipose Tissue

Although the adipose tissue contains enzymes that metabolize steroids, it is not a site for de novo synthesis of steroid hormones [22, 23]. Adipose tissue expresses 17β-hydroxysteroid dehydrogenase, which converts androstenedione to testosterone, a more potent androgen, and estrone to estradiol, a more potent estrogen (Fig. 8.3). Cytochrome-p450-dependent aromatase is also present in the adipose tissue and is responsible for aromatization of androgens to estrogens within fat

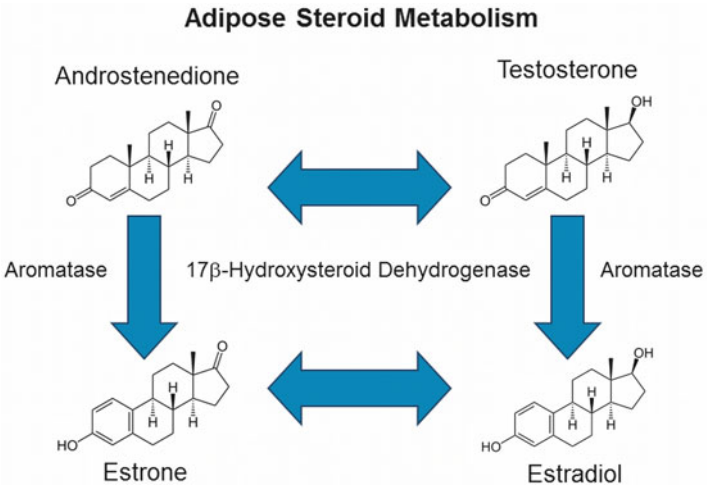

Fig. 8.3 Adipose tissue is an endocrine organ. Androstenedione and testosterone are interconverted by 17β-hydroxysteroid dehydrogenase activity and are respectively converted to estrone and estradiol by aromatase activity

stores (see Fig. 8.3) [21, 24]. Thus, the presence of excess adipose tissue in obesity increases peripheral conversion of circulating androgens to estrogens, thereby promoting a hyperestrogenic state.

Adipose tissue expresses the enzyme 11β-hydroxysteroid dehydrogenase type 1, which converts less potent circulating glucocorticoids such as cortisone and 11-dehydrocorticosterone to more potent cortisol and corticosterone, respectively [25]. Local glucocorticoid activity within adipose tissue is involved in adipocyte differentiation [25]. However, over-expression of 11β(beta)-hydroxysteroid dehydrogenase type 1 in obesity leads to glucocorticoid excess within adipose tissue. The subsequent increase in local glucocorticoid activity promotes abdominal fat accumulation, particularly in the visceral compartment similar to what is observed in Cushing syndrome [25].

Adipokine Function

Adipose tissue produces many proteins called adipokines that aid in adipose tissue metabolism and immunomodulation for host defense [14]. The substantial listing of adipokines includes proinflammatory cytokines such as tumor necrosis factor (TNFα) and interleukin (IL-6), also known as chemokines, as well as leptin and adiponectin [14]. Resident macrophages in adipose tissue can generate reactive oxygen species (ROS) and produce TNFα and IL-6. TNFα, in particular, exerts a paracrine effect on adipocytes by enhancing lipolysis that causes the release of FFA. Both macrophages and adipocytes express toll-like receptor 4 (TLR4) that can bind either FFA or lipopolysaccharide (LPS) [26]. This stimulates chemokine production from either of these cells even further to perpetuate a local and systemic inflammatory response to the offending stimuli. Interestingly enough, pre-adipocytes can transform into macrophage-like cells that are also involved in innate immunity [26].

Leptin is primarily secreted by subcutaneous adipose tissue and acts as a hormone to enhance satiety at the level of the hypothalamus for regulation of body weight [29]. Leptin also acts locally in white adipose tissue to regulate energy expenditure by stimulating triglyceride depletion without increasing FFA release. This favors the use of FFA instead of glucose as a fuel source [27–29]. Although leptin-deficient states are associated with hyperphagia and insulin resistance, obese patients are leptin-resistant despite high circulating leptin [14]. These leptin elevations promote inflammation characterized by increased chemokine production from circulating mononuclear cells (MNC) and resident macrophages of adipose tissue. In contrast, adiponectin inhibits inflammation by preventing macrophage differentiation into foam cells and decreases LPS-mediated TNFα release by macrophages. Adiponectin also improves insulin resistance by improving skeletal muscle glucose uptake and decreasing hepatic gluconeogenesis [14].

Development of Obesity

The development of obesity is a multifactorial process. However, a major component is positive energy balance. Excess macronutrient intake exceeding expenditure causes weight gain and is exacerbated by reduced satiety [1, 30]. Sedentary lifestyle reduces energy expenditure [31]. With technological advances, physical activity has decreased [31]. Fewer occupations require physical labor; transportation is readily available so fewer people walk; and entertainment such as television and computers decrease leisure activities that require physical activity [1, 31–33].

Specific dietary components play a role in the maintenance of a healthy weight and body composition versus the promotion of weight gain and adipose accumulation. Protein intake is the most satiating, while carbohydrate intake tends to suppress later food intake. Fat contains the most energy per gram, and its intake is less satiating [30]. The body can easily oxidize excess protein and carbohydrates, whereas it tends to store excess fats [31, 34, 36]. Just the same, excessive intake of carbohydrates high on the glycemic index scale leads to excessive insulin secretion. Since insulin is anabolic, these type of carbohydrates are more easily stored as fat [28]. Eating a high-fat diet has also been associated with overeating and weight gain [30, 32, 35].

Ghrelin is a hormone secreted by gastric mucosal cells that increases appetite and stimulates food intake [34, 35]. In fact, ghrelin administration has been shown to increase appetite scores and caloric intake compared with placebo [28, 36]. Leptin inhibits ghrelin secretion beyond its actions in the brain and adipose tissue [26, 27, 37]. Fasting and weight loss suppress leptin secretion [38]. A high-fat diet has a variable effect on leptin secretion but can clearly suppress the secretion of ghrelin. This physiologic ghrelin suppression in response to a meal is attenuated in obese individuals and results in a continued hunger sensation [35]. Profound weight loss has been demonstrated in leptin-deficient and severely leptin-insufficient individuals following leptin administration [39]. However, this approach has shown only a modest benefit in the more common forms of obesity [39].

Genetic factors also play a role in the development of obesity, since there is 40–70 % heritability [40]. Many genetic mutations have been reported in association with obesity, including those affecting leptin, its receptor, and post-receptor signaling [37, 38]. These genetic factors as well as other imprinted genetic syndromes, copy number variants, and susceptible genetic loci increase the susceptibility to gain weight by promoting a positive energy balance [1, 38–40]. Despite the many associations however, these findings highlight the fact that there is no single genetic abnormality to account for every instance of obesity.

Epigenetic phenomena are being recognized as significant contributors to the development of obesity in the modern era. Maternal diet high in fat content results in a lipid-rich fetal environment [41]. In animal studies, inflammation triggered by lipid excess causes DNA methylation and histone deacetylation to silence genes that are normally expressed during fetal development. This alters appetite regulation within the fetal hypothalamus and may also reprogram metabolic gene targets

located in the fetal liver, skeletal muscle, adipose tissue, and pancreas [2]. Several such genes have been shown to be regulated in this fashion, including genes for leptin, suppressor of cytokine signaling-3, and glucose transporter [42, 43]. As a consequence, obese mothers give birth to offspring who become even more obese [2, 30, 44, 45], a paradigm that can explain the growing pandemic of obesity.

Medical Disorders Associated with Obesity

Obesity is associated with a higher risk of developing impaired glucose tolerance (IGT), type 2 diabetes mellitus (DM), dyslipidemia, hypertension, cardiovascular disease, and obstructive sleep apnea [46]. In fact, the risk of cardiovascular disease increases in direct proportion to body weight [46]. The prevalence of obstructive sleep apnea increases as the percentage of total body and visceral fat increases [47].

Weight gain and physical inactivity lead to insulin resistance in the skeletal muscle, liver, and adipose tissue [48]. This in turn causes a compensatory increase in pancreatic insulin secretion and eventual pancreatic β(beta)-cell failure in the predisposed individuals. In fact, a family history of type 2 DM is a genetic marker for the development of glucose intolerance and type 2 DM [49].

The incidence of dyslipidemia is increased in obesity. VLDL transports triglycerides to peripheral tissues and is formed by a lipoprotein synthesis cascade that proceeds from apolipoprotein B (apoB) to intermediate density lipoprotein (IDL) to low-density lipoprotein (LDL) to VLDL [50]. All of these lipoproteins along with triglycerides tend to be elevated in obesity owing to delayed hepatic clearance as a result of increased hepatic secretion of VLDL-apoB [51]. High-density lipoprotein-cholesterol (HDL-C) transports cholesterol from peripheral tissues back to the liver, either directly or through reverse cholesterol transport [50]; HDL-C tends to be low in obesity as a result of increased hepatic uptake [51].

Obesity contributes to hypertension through several mechanisms. Increases in circulating leptin, insulin, and FFA activate both the sympathetic nervous system and the renin-angiotensin-aldosterone system [52]. In the latter instance, increases in plasma aldosterone levels cause abnormal sodium retention, which raises the arterial pressure [52]. Obesity is also associated with vascular inflammatory changes that lead to endothelial dysfunction, decreased nitric oxide responsiveness, increased endothelin-1, and renal functional abnormalities [52]. These vascular abnormalities cause vasoconstriction that can result in hypertension [52].

Obesity is one of the criteria for metabolic syndrome, a condition associated with a significantly higher risk of coronary artery disease and all-cause mortality even in the absence of cardiovascular symptomatology. The National Cholesterol Education Program/Adult Treatment Panel III defines the metabolic syndrome as the presence of any three of the following five clinical criteria: elevated waist circumference >88 cm (central obesity), elevated triglycerides >150 mg/dL, low HDL cholesterol <50 mg/dL, elevated blood pressure ≥130 mmHg systolic/>85 mmHg diastolic, and elevated fasting glucose >100 mg/dL [53].

Metabolic Dysfunction in PCOS: Prevalence and Risk

Women with PCOS exhibit metabolic abnormalities that are independent of obesity. There are much higher rates of glucose intolerance, type 2 DM, dyslipidemia, hypertension, and subclinical atherosclerosis in PCOS compared to the general female population [47]. In a study by Legro et al., the prevalence of IGT and type 2 DM among rural women with PCOS was 31 % and 8 % respectively, and 10 and 1.5 % in the absence of obesity. In fact, nonobese women with PCOS had higher LDL-C and higher total cholesterol compared with nonobese women without the disorder after adjusting for alcohol intake, smoking, and exercise [54]. Hypertension related to underlying endothelial dysfunction is also more likely to occur in women with PCOS even after accounting for age, BMI category, diabetes, and dyslipidemia [55, 56]. Thus, it is not surprising that the prevalence of the metabolic syndrome in PCOS is twice as high compared with women in the general population (43–47 % versus 23 %) when matched for age and BMI [57, 58].

Anovulatory infertility is associated with PCOS, in part due to hyperinsulinemia. Some studies suggest that hyperinsulinemia affects ovulation by altering the synthesis and pulsatile pattern of LH and FSH secretion, and potentiating androgen secretion and bioavailability [59, 60]. However, it remains unclear whether hyperinsulinemia can elicit these endocrine abnormalities in PCOS in the complete absence of obesity.

Impact of Obesity on PCOS

Perpetuation of Signs and Symptoms of the Disorder

PCOS with Superimposed Obesity

The prevalence of obesity in PCOS (42–74 %) is much higher than in the general population (~25 %) [61–63]. Obese individuals, regardless of whether they have PCOS, are more insulin-resistant than normal-weight women with PCOS. While obesity-related compensatory hyperinsulinemia is evident in both the fasting and postprandial states, in contrast, normal-weight women with PCOS exhibit a milder form of insulin resistance that only results in postprandial hyperinsulinemia [64].

When obesity is superimposed on PCOS, the more profound hyperinsulinemia promotes androgen production from ovarian theca cells, inhibits hepatic sex hormone-binding globulin (SHBG) synthesis, enhances pituitary LH secretion, and increases the ovarian androgenic response to LH. The superimposed obesity is also associated with hyperactivity of the hypothalamic–pituitary–adrenal axis, leading to increased adrenal androgens and worsening of the hyperandrogenic state [65].

Obese women with PCOS have lower SHBG levels than normal-weight women with PCOS. This translates into greater elevations in circulating free testosterone,

and as a consequence, there is a greater degree of hirsutism and menstrual disturbance in obese women with PCOS [66]. In fact, the treatment of anovulatory infertility in obese women with PCOS often requires higher doses of fertility drugs, and subsequent ovulation and birth rates are lower compared with those of normal-weight women with PCOS [67].

It has been proposed that PCOS itself may predispose to weight gain. Although resting energy expenditure is normal in PCOS, studies conflict as to whether thermogenesis is decreased in the postprandial state [68, 69]. Disordered hunger and satiety signals have also been reported in PCOS and may be due to ghrelin dysregulation [70, 71]. More importantly, the superimposed obesity, once entrenched, promotes further weight gain. This is because the more profound hyperinsulinemia of obesity exerts insulin's anabolic action to a greater extent, thereby leading to a more rapid adipose deposition [28].

Obesity-Induced PCOS

It is possible that obesity promotes the development of PCOS in predisposed individuals. The signs and symptoms of PCOS typically become evident around menarche in normal-weight women with the disorder [72]. In contrast, some obese women with PCOS encountered in clinical practice develop these signs and symptoms later in life following progressive weight gain to the obese range. This observation is supported by the restoration of menstrual cyclicity, normalization of hyperandrogenemia, and resolution of acne in these individuals once sufficient weight loss has been achieved with lifestyle modification or bariatric surgery [73, 74]. However, hirsutism is a more permanent malady and usually persists after weight loss, even though the progression is halted [75].

It is likely that a predisposing factor is needed for isolated obesity to induce PCOS as the syndrome is not uniformly observed in all obese women. Although the predisposing susceptibility remains unclear, the presence of polycystic ovarian morphology is a prime candidate. Polycystic ovaries (PCO) are quite common in premenopausal women, especially at an early age, with a prevalence of 24–32 % [76, 77]. In contrast, PCO are evident in the vast majority of women with PCOS [78]. Asymptomatic women with PCO do not develop the syndrome in the absence of weight excess. The isolated PCO morphology typically resolves with age even if modest weight gain below the obese range occurs in the interum [79]. Clinically relevant endocrine and metabolic abnormalities are lacking in this population [77]. Nevertheless, ovarian and metabolic dynamic testing has uncovered subclinical abnormalities of androgen secretion and insulin sensitivity that are intermediate between women with PCOS and normal ovulatory women [80, 81]. It is possible that the insulin resistance of obesity has an adverse impact on ovarian function should PCO be present at the time the critical weight limit is superceded and that PCOS develops in the process. For confirmation, long-term longitudinal studies are needed that prospectively evaluate the effects of weight gain on asymptomatic normal weight women with polycystic ovaries.

Perpetuation of Medical Risks of Metabolic Dysfunction

The risk of developing chronic medical disorders attributable to metabolic dysfunction increases in PCOS in the presence of obesity. There is a preponderance of android body habitus in PCOS, even in normal-weight women with this syndrome [82]. Consequently, most obese women with PCOS exhibit fat accumulation in the abdomen, particularly within the visceral compartment [83]. Abdominal obesity is strongly correlated with the development of insulin resistance [84]. Visceral adipose tissue in particular is highly sensitive to lipolytic stimulation by androgens that facilitates increased portal FFA availability [85]. This, in turn, leads to fat accumulation in the liver, reduced hepatic insulin clearance, and the induction of hepatic insulin resistance [86].

The prevalence of IGT and type 2 DM is further increased in PCOS as a result of the greater degree of insulin resistance related to obesity [54, 87]. Furthermore, obesity worsens the fasting lipid profile in PCOS characterized by increased triglycerides and decreased HDL-C that may also reflect the influence of the more profound insulin resistance of obesity [65, 88, 89]. Increases in circulating endothelin-1 and activation of both the sympathetic nervous system and the renin-angiotensin-aldosterone system as a result of obesity also contribute to worsening hypertension in PCOS [45]. The increased severity of all of these metabolic abnormalities when obesity is present in PCOS collectively increases the risk of incurring a cardiovascular event. Not surprisingly, the majority of women with PCOS who meet the criteria for metabolic syndrome are obese. In fact, abdominal obesity is the most common feature of metabolic syndrome in the disorder [90, 91].

Obstructive sleep apnea (OSA) occurs more often in obese women with PCOS compared to obese women who lack the syndrome [47]. In fact, the prevalence of OSA in the former approaches the higher prevalence known to occur in men [47]. OSA is particularly associated with insulin resistance, and appears to be an obesity-related phenomenon because this problem could not be demonstrated in the non-obese women with PCOS [92].

Metabolic Inflammation in PCOS

Obesity and Inflammation

It is well established that obesity is a prooxidant, proinflammatory state. Adipocyte death induced by hypoxia in the expanded adipose tissue of the obese leads to migration of circulating MNC into the adipose stromal–vascular compartment (see Fig. 8.1) [93, 94]. These MNC subsequently undergo morphological alterations to become the resident macrophages. Phagocytic activity by MNC-derived macrophages induces membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [95]. Oxidation of NADPH by NADPH oxidase

generates superoxide, a ROS that induces oxidative stress [96]. This phenomenon activates the transcription factor known as nuclear factor κB (NFκB), the cardinal signal of inflammation, by promoting its dissociation from the inhibitory protein, inhibitory κB (IκB) [97]. NFκB is thus freed to undergo nuclear translocation and subsequent DNA binding to promote transcription of a variety of inflammatory mediators that up-regulate the aforementioned molecular events and promote insulin resistance and atherogenesis.

TNFα and IL-6 are proinflammatory cytokines produced in the excess *inflamed* adipose tissue following upregulation of the NFκB inflammation pathway. These cytokines primarily originate from the MNC-derived macrophages, but also perpetuate their own production in the adipocytes through paracrine interactions [98]. While TNFα is a known mediator of insulin resistance, the impact of IL-6 on insulin resistance is variable [99–101]. IL-6, however, is clearly involved in the promotion of atherogenesis [102].

TNFα mediates insulin resistance in obesity-related diabetic syndromes by causing increased serine phosphorylation of insulin receptor substrate-1 (IRS-1) in insulin-sensitive tissues [99]. This leads to decreased expression of GLUT 4, the insulin-sensitive glucose transport protein, and a subsequent decrease in glucose transport [103]. On the other hand, IL-6 mediates atherogenesis in consort with other proatherogenic inflammatory mediators produced in response to NFκB activation such as soluble vascular cell adhesion molecule-1 (sVCAM-1) and monocyte chemotactic protein-1 (MCP-1). IL-6 stimulates synthesis of the acute phase reactant C-reactive protein (CRP) to some extent in adipose tissue, but primarily in the liver through its unique endocrine effects [104]. Whereas sVCAM-1 causes attachment of MNC to the vascular endothelium, MCP-1 facilitates migration of MNC into the vascular interstitium. CRP subsequently promotes the uptake of lipids into MNC-derived foamy macrophages within atherosclerotic plaques [105, 106]. Thus, the proinflammatory environment of the expanded adipose tissue compartment in obesity is a major driver of metabolic aberration and is often superimposed on metabolic dysfunction observed in PCOS per se.

Chronic Low-Grade Inflammation in PCOS

A genetic basis for the chronic low-grade inflammation has been observed in PCOS. Several proinflammatory genes, including those that encode TNFα, and the type 2 TNF receptor as well as IL-6 and its signal transducer are associated with PCOS [107–110].

The majority of studies addressing the status of chronic low-grade inflammation in PCOS have focused on the measurement of circulating CRP using high-sensitivity assays. In fact, a recent meta-analysis revealed that CRP is the most reliable circulating marker of chronic low-grade inflammation in PCOS [111]. CRP levels >3 mg/L are equally predictive of a cardiovascular event compared to the ATP III criteria for metabolic syndrome [112]. However, the CRP elevation in

normal-weight women with PCOS (<3.0 mg/L) is still much lower in magnitude compared to what is observed in obese individuals (>3.0 mg/L), regardless of whether PCOS is present or absent [113, 114]. Thus, CRP elevations attributable to PCOS are obscured in the presence of obesity, but are nonetheless indicative of intermediate atherosclerotic cardiovascular risk [115]. It remains to be determined whether a single static circulating marker such as CRP is reflective of inflammation at the molecular level.

Diet-Induced Inflammation in PCOS

The circulating MNC has been the object of intense investigation in efforts to increase the understanding of the mechanisms responsible for metabolic abnormalities in PCOS. Moreover, the discovery that dietary components such as glucose and lipids can trigger an inflammatory response in MNC has shed light on the molecular events that may promote insulin resistance and cardiovascular risk in PCOS.

Insulin resistance is a common feature of PCOS affecting 50–70 % of women with the disorder. The degree of insulin resistance in normal-weight women with PCOS is similar to that of obese individuals without PCOS [63, 116]. The compensatory hyperinsulinemia is considered to be a promoter of the hyperandrogenism and chronic oligo- or anovulation in the disorder, particularly when there is concomitant obesity. The insulin receptor in PCOS is genetically and functionally normal [117]. Insulin resistance in PCOS is suggested to be caused by a post-receptor defect in insulin signaling, with increased serine phosphorylation implicated as the cause of decreased insulin-stimulated IRS-1 activation and decreased GLUT 4 expression [118, 119]. The discovery that circulating levels of TNFα are elevated in PCOS independent of obesity not only served as the initial clue that PCOS is a pro-inflammatory state, but also raises the possibility that TNFα is a mediator of insulin resistance in the disorder. Moreover, the ability of TNFα to stimulate increased serine phosphorylation makes it an ideal candidate for initiating the molecular events that truncate insulin signaling in PCOS.

Ingested glucose and lipids are utilized by MNC for mitochondrial respiration during glycolysis and β-oxidation respectively [120]. Some glucose is diverted to the hexose monophosphate shunt to generate NADPH while some lipid is converted to diacylglycerol [121, 122]. Both of these processes promote phosphorylation of $p47^{phox}$, the key cytosol component of NADPH oxidase, the ROS-producing enzyme [123]. Translocation of $p47^{phox}$ to the cell membrane leads to ROS-induced oxidative stress and NFκB activation [95, 124]. This in turn promotes TNFα(alpha) and IL-6 gene transcription in a fashion similar to what occurs within excess adipose tissue of the obese [124].

In PCOS, ingestion of glucose and lipids are indeed capable of inducing a pro-oxidant inflammatory response, as evidenced by increases in MNC-derived ROS generation, $p47^{phox}$ protein content and NFκB activation that are independent of obesity [125–128]. The release of TNFα and IL-6 from circulating MNC following glucose ingestion in vivo, and glucose exposure in vitro, is also altered in PCOS

[129, 130]. In addition, these markers of oxidative stress and inflammation are associated with glucose-stimulated measures of insulin sensitivity and/or fasting measures of insulin resistance [125–128, 130]. Thus, diet-induced inflammation in PCOS can be theorized to culminate in proinflammatory signaling known to be involved in the development of insulin resistance and atherogenesis.

Glucose ingestion also activates activator protein-1 (AP-1) and early growth response-1 (EGR-1), two additional proinflammatory transcription factors involved in the development of atherothrombosis [131, 132]. AP-1 regulates the transcription of a family of matrix matalloproteinases (MMP) [133]. MMP-2 and MMP-9, in particular, are involved in extracellular matrix remodeling within the blood vessel wall [134, 135]. EGR-1 regulates the transcription of TF, the receptor for coagulation factor VII that induces thrombin generation to promote fibrin formation and platelet activation [136]. MMP-2, MMP-9, and TF are produced by MNC-derived foamy macrophages and activated vascular smooth muscle cells within the atherosclerotic plaque [137]. Excessive extracellular matrix breakdown by MMP-2 and MMP-9 culminates in atherosclerotic plaque rupture, thereby exposing TF to the circulating blood. This, in turn, triggers thrombosis, and culminates in blood vessel occlusion [138, 139].

In PCOS, circulating levels of MMP-2, MMP-9, and TF are elevated and glucose ingestion activates AP-1 and increases the protein content of MMP-2 independent of obesity [140, 141]. In contrast, glucose ingestion increases the protein content of EGR-1 and TF only in the obese, regardless of whether or not they have PCOS [142]. These atherothrombotic inflammation markers are associated with abdominal adiposity and circulating androgens [141, 142]. These findings are striking because they show that both PCOS and obesity separately contribute to an atherothrombotic state at an early age [141–143]; and that excess abdominal adiposity and hyperandrogenism may be specific risk factors for atherosclerotic plaque rupture and vascular thrombosis.

The Relative Impact of Excess Abdominal Adiposity in PCOS

The proinflammatory state of obesity contributes to the promotion of insulin resistance and atherogenesis. Even in the absence of frank obesity, excess abdominal adiposity is present in PCOS across all weight classes, with a prevalence of 30 % in normal-weight women with the disorder [144]. However, molecular inflammation marker expression in the adipose tissue is in proportion to the degree of adiposity in PCOS, and is not uniquely greater when compared to individuals without PCOS [145, 146].

Although a relative excess of abdominal adiposity, is more common in women with PCOS, it is not the cause of the proinflammatory state of the normal-weight women with this disorder. Ingestion of glucose induces a prooxidant inflammatory response, as evidenced by increases in MNC-derived ROS generation, $p47^{phox}$ protein content and NFκB activation, in normal-weight women with PCOS who lack excess abdominal adiposity (Fig. 8.4) [147, 148]. These individuals are

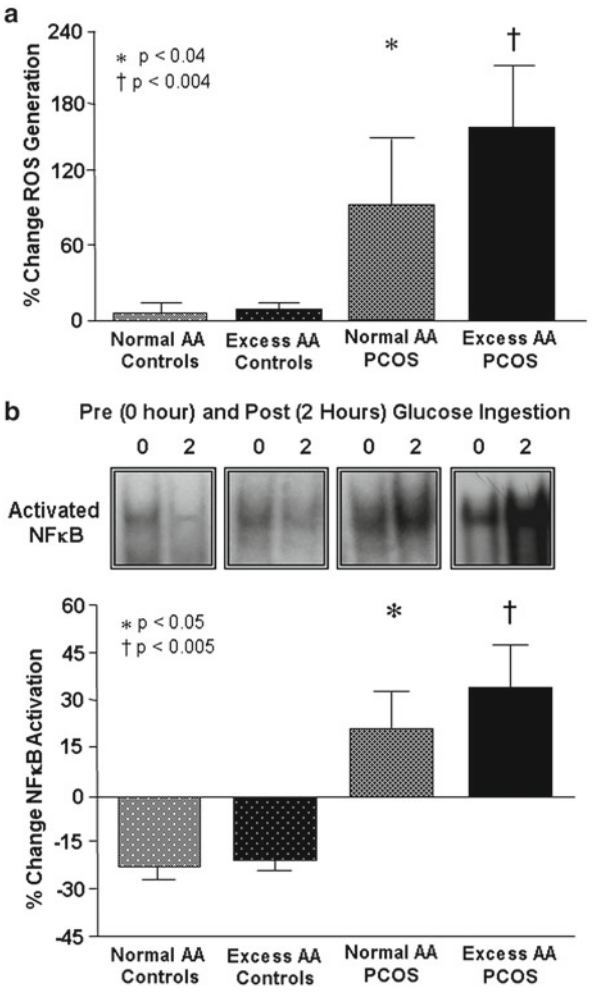

Fig. 8.4 (**a**) Change from baseline (%) in reactive oxygen species (ROS) generation from mononuclear cells (MNC) when fasting samples (pre) were compared with the samples collected 2 h after glucose ingestion (post). *Response in women with polycystic ovary syndrome (PCOS) with normal abdominal adiposity (AA) was significantly greater compared with that of either control group, $P < 0.04$. †Response in women with PCOS with excess AA was significantly greater compared with that of either control group, $P < 0.004$. Adapted from [130]. (**b**) Representative electrophoretic mobility shift assay (EMSA) bands from the four study groups showing the quantity of nuclear factor κB (NFκB) in nuclear extracts from MNC in samples collected pre- and post-glucose ingestion. Densitometric quantitative analysis comparing the change from baseline (%) in MNC-derived activated NFκB between fasting and 2-h post-glucose ingestion samples. *Response in women with PCOS with normal abdominal adiposity (AA) was significantly greater compared with that of either control group, $P < 0.05$. †Response in women with PCOS with excess AA was significantly greater compared with that of either control group, $P < 0.005$. Adapted from [131]

insulin-resistant in the face of normal TNFα and CRP levels. In contrast, markers of oxidative stress and inflammation trend higher, and TNFα and CRP levels are elevated in normal-weight women with PCOS who have excess abdominal adiposity [148]. Thus, glucose-stimulated oxidative stress and inflammation from MNC is independent of increased abdominal adiposity in normal weight women with PCOS. While further studies are needed for confirmation, the TNFα and CRP elevations evident in normal-weight women with PCOS may be a manifestation of the inflammatory load contributed by excess abdominal adiposity. Most importantly, circulating MNC have a separate and discrete role compared with inflamed adipose tissue in the development of chronic low-grade inflammation in PCOS.

In PCOS, the impact of adiposity in the development of insulin resistance and hyperandrogenism may depend on the amount of fat accumulated in a given individual. Excess abdominal adiposity is a promoter of insulin resistance in obesity, and is inversely related to measures of insulin sensitivity in studies that include obese individuals, whether or not they have PCOS [122–130, 149]. However, abdominal adiposity is not related to insulin resistance in normal-weight women with PCOS [147, 148]. This suggests that in PCOS, the amount of inflamed excess adipose tissue present in the abdomen in the absence of increased body weight may be insufficient to promote systemic insulin resistance. In studies that include obese women with PCOS, excess abdominal adiposity directly correlates with circulating androgens [150]. This is consistent with the common concept that in PCOS insulin resistance promotes hyperandrogenism [151]. In contrast, circulating testosterone is inversely related to abdominal adiposity in normal-weight women with PCOS, and is elevated to a greater extent in normal-weight women with PCOS with normal abdominal adiposity compared with those with excess abdominal adiposity [147, 148]. As a known inducer of catecholamine-induced hormone-sensitive lipase activity within the visceral adipose compartment, testosterone at these higher levels may limit the development of abdominal adiposity in normal-weight women with PCOS [85, 152]. Thus, excess abdominal adiposity contributes to the inflammatory load without necessarily playing a significant role in the promotion of insulin resistance in normal-weight women with PCOS; and the degree of hyperandrogenism in PCOS before the onset of weight gain may control the development of excess abdominal adiposity.

The Relationship Between Inflammation and Hyperandrogenism in PCOS

There is a strong association between circulating and molecular markers of oxidative stress and inflammation and circulating androgens [127–130, 143, 147, 148, 153, 154]. These observations suggest that in PCOS, hyperandrogenemia can preactivate MNC to account for the inflammation that is induced by hyperglycemia, or, conversely, that glucose-stimulated inflammation can promote excess ovarian androgen production. Data exist that are in keeping with both mechanisms [155–157].

Induction of Inflammation by Hyperandrogenism

In PCOS, there is increased ROS generation and NFκB activation in the fasting state to indicate that MNC are pre-activated [153, 154]. This can explain the increased MNC sensitivity to glucose ingestion in the disorder. In contrast, MNC of normal-weight ovulatory women are not sensitive to hyperglycemia and do not exhibit an inflammatory response to glucose ingestion [125–128]. Acute oral androgen administration to increase circulating androgen levels in normal-weight ovulatory women to the range observed in PCOS increases ROS generation, $p47^{phox}$ protein content, activated NFκB and TNFα RNA content from MNC in the fasting state, and in response to glucose ingestion [155, 156]. This prooxidant, proinflammatory response occurs in MNC in an androgen receptor-dependent fashion [157]. Thus, hyperandrogenemia to the level present in PCOS can activate MNC and increase MNC sensitivity to glucose ingestion. Hence, it is possible that hyperandrogenism, the hallmark feature of PCOS, is the progenitor of diet-induced inflammation in the disorder.

Suppression of Inflammation by Hyperandrogenism in PCOS Combined with Obesity

Circulating CRP is a useful measurement of inflammatory load [158–160]. In obese women with PCOS, CRP levels increase along with an increase in IL-6 levels and body weight and a decrease in FFA levels in response to chronic gonadotropin releasing hormone (GnRH) agonist-induced androgen suppression [161]. The ability of elevated circulating androgens to promote lipolysis may be responsible for these alterations. Once again, testosterone is known to stimulate catecholamine-induced hormone-sensitive lipase activity, which, in turn, limits adipose tissue expansion [85, 162]. Conversely, decreased lypolysis following androgen suppression can lead to adipose tissue expansion to account for the observed weight gain in obese women with PCOS during GnRH agonist treatment, and is corroborated by the fall in FFA levels. Indeed, a progressive increase in subcutaneous adipocyte size that is associated with worsening insulin resistance has been documented in PCOS when hormone-sensitive lipase activity is low [163]. Furthermore, visceral fat accumulation has clearly been demonstrated in obese women with PCOS in response to chronic GnRH agonist-induced androgen suppression [164]. The rise in IL-6 in this group during GnRH agonist treatment is most likely the result of increased IL-6 production from the excess inflamed adipose tissue, which, in turn, can stimulate hepatic CRP synthesis to account for the rise in CRP. Thus, hyperandrogenism exerts an anti-inflammatory effect in obese women with PCOS.

No Effect on Inflammation by Hyperandrogenism in PCOS in the Absence of Obesity

Normal-weight women with PCOS respond quite differently to chronic GnRH agonist-induced androgen suppression. In this group, CRP levels, along with IL-6

and FFA levels, are unaltered, and body weight does not change significantly during GnRH agonist treatment [161]. This is most likely because circulating androgens have a limited effect on subcutaneous lipolysis in normal-weight women with PCOS. Moreover, the subcutaneous adipose tissue of these individuals is catecholamine-resistant, thereby precluding adequate androgen induction of hormone-sensitive lipase activity [165]. These findings are important because they show that once the chronic state is established, hyperandrogenism does not promote inflammation in PCOS.

Direct Induction of Hyperandrogenism by Inflammation

Inflammation may be the promoter of hyperandrogenism in PCOS. It is known that the ovary is infiltrated by MNC-derived macrophages [166]. CYP17, the steroidogenic enzyme responsible for androgen production, is up-regulated in theca cells by proinflammatory stimuli and inhibited by anti-inflammatory agent such as resveratrol and statins [167, 168]. Furthermore, proliferation of theca cells from rat and human PCO is stimulated by TNFα(alpha) and suppressed by statins [169, 170]. It is possible that MNC recruited into the PCO may cause a local inflammatory response that stimulates ovarian androgen production in women with PCOS.

Summary

Adipose tissue serves multiple functions involved in normal metabolism and maintenance of normal endocrine and immune status. Accumulation of excess adiposity that culminates in obesity causes dysfunction of the adipose tissue compartment. This ultimately leads to an immune alteration characterized by inflammation, which causes metabolic derangements and endocrine imbalance. PCOS is an endocrinopathy characterized by ovarian dysfunction that is driven by metabolic abberration. Thus, it is not surprising that the signs and symptoms of this endocrinopathy manifested by hyperandrogenism and chronic anovulation, much less the risk of medical illness, are made worse when obesity is superimposed on PCOS.

The metabolic pathophysiology of obesity and PCOS in many ways runs in parallel, except that the proinflammatory effects that promote metabolic dysfunction are more pronounced in the setting of obesity compared with what is observed in PCOS alone. Inflammation triggered by diet has emerged as a key contributor to the pathogenesis of PCOS. Diet-induced oxidative stress and inflammation can induce molecular alterations that may be the underpinning of insulin resistance, atherogenesis, and the ovarian dysfunction in the disorder. It is now clear that most of these alterations are independent of excess adiposity. While it is possible that hyperandrogenism is the progenitor of the proinflammatory milieu in PCOS, androgen excess alone does not contribute to inflammation once the chronic state is established. In contrast, inflammation is capable of directly stimulating excess ovarian androgen production, aside from promoting the cascade of molecular events that characterize metabolic aberration in PCOS.

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Part III
Managing PCOS

Chapter 9
Role of Lifestyle and Diet in the Management of Polycystic Ovarian Syndrome

Amy Krystock

Key Points

- Diet therapy is without question one of the most important and effective management strategy for polycystic ovarian syndrome (PCOS).
- The health benefit of weight loss in overweight and obese women with PCOS is well documented.
- While there is much debate about the optimal balance of macronutrients that would be uniformly beneficial to women with PCOS, evidence supports that the type and amount of dietary carbohydrates are important for a woman with PCOS.
- A diet moderate in low glycemic index carbohydrates, low in saturated fat and sodium, and high in fiber from whole grain, fruits, and vegetables can improve short- and long-term symptoms of PCOS, as well as decrease the risk of chronic diseases associated with insulin resistance.
- Regular exercise must be encouraged and incorporated in the management paradigm of PCOS.

Introduction

With insulin resistance and compensatory hyperinsulinemia central to the pathogenesis of polycystic ovary syndrome (PCOS) and obesity and dyslipidemia being common accompaniments to the clinical picture, lifestyle interventions aimed at reducing insulin levels and improving insulin sensitivity are critical to the overall

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management of this disorder. While there is strong agreement that a hypocaloric diet is beneficial for overweight and obese women with PCOS, there is much debate about the balance of dietary macronutrients that could be construed as optimal for this population. Inclusion of regular exercise has also demonstrated positive impact on metabolic and clinical parameters in women with PCOS. Management strategies must prioritize body weight optimization and improving insulin sensitivity. This chapter provides an overview of lifestyle interventions that can successfully address the spectrum of metabolic derangements that are commonly encountered in PCOS and are modifiable through dietary modification and regular physical activity.

Why Diet Makes a Difference in Women with PCOS

Abnormalities in insulin-induced glucose utilization are well recognized in PCOS [1, 2]. Inability of the secreted insulin to optimally drive the cellular machinery through glucose transportation and utilization results in a reactionary increase in pancreatic output of insulin; higher levels of insulin are thus needed to facilitate insulin actions, a phenomenon called *insulin resistance*. In the presence of hyperinsulinemia and impaired insulin signaling, energy efficiency is impaired, and glucose, instead of being utilized as fuel, gets diverted towards lipogenesis and, hence, fat storage [3]. Hyperinsulinemia, in turn, encourages fatty acids to be deposited as body fat while also inhibiting its release from fat stores. The metabolic processes are subtle and yet translate into progressive weight accrual despite reportedly regular physical activity and even caloric restriction. Over time, these processes can exhaust the pancreatic beta cells; ability to adequately maintain euglycemia is thus impaired, setting a stage for development of type 2 diabetes, a disorder commonly seen in women with PCOS; the prevalence of type 2 diabetes is ten times higher among young women with PCOS than healthy controls [3]. Adiposity worsens the innately disturbed insulin signaling. Body mass excess is evident in almost two-thirds of women with PCOS and is recognized to worsen several features of PCOS, such as hyperandrogenism, hirsutism, infertility, and pregnancy-related complications. Insulin resistance is associated with an increased risk for several other disorders, including hypertension, dyslipidemia, elevated markers of inflammation, endothelial dysfunction, and heart disease. Concerningly, impaired glucose tolerance or type 2 diabetes develops by the age of 30 in 30–50 % of obese patients with PCOS [3].

Progressive weight gain, cravings for carbohydrates (CHO) and sweets, and symptoms of hypoglycemia (such as dizziness, fatigue, shakiness, irritability, headaches, postprandial exhaustion, and somnolence) are common in women with PCOS. Episodes of hypoglycemia are typically encountered when an individual goes long periods of time without eating. More typically, however, patients manifest features of *reactive hypoglycemia* following ingestion of refined CHO (e.g., candy or a large serving of sweets). Outpouring of pancreatic insulin in response to an abrupt rise in glucose, with lingering hyperinsulinemia and delayed onset of insulin action, is contributory to a precipitous drop in circulating glucose levels a few hours after ingestion of CHO load, instigating cravings for sweets as the body kicks in

defense mechanisms to counteract the hypoglycemic event. Indeed, many women with PCOS report strong, almost compulsive, uncontrollable urges for "sweets" and CHO-rich foods.

Reassuringly, insulin resistance of PCOS responds remarkably well to weight reduction through dietary change and improved lifestyle. An optimal diet for a woman with PCOS must encompass several facets. It must be calorie-appropriate to support the specific needs of the particular woman (weight maintenance, weight loss, or weight gain). Proportioning meal size and ensuring a timely intake of nutritionally balanced snacks and meals are strategies that can successfully abort the vicious cycle of insulin resistance-driven carbohydrate cravings. Lowering of dietary sugar load and choice of complex dietary CHO reduces the postprandial excursion in serum glucose and, hence, lessens the magnitude of insulin secretion in response to dietary load. Reducing postprandial insulin levels has demonstrated an increase in fat oxidation for several hours following a meal, and reduced hunger and, hence, predisposition to overeating and weight gain [4]. A balance in respective amounts of dietary carbohydrate, protein, and fat can further ensure homeostasis while mitigating insulin resistance. Eating patterns that support lower insulin levels should be central to the diet of a woman with PCOS.

Dietary Carbohydrates

The primary role of dietary CHO is to serve as fuel. Carbohydrates in diet are almost exclusively of plant origin. Milk is the only animal-derived food that contains a significant amount of CHO. Fruits, vegetables, whole grains, and legumes (beans, lentils, peas) are major sources of healthy CHO. Fruit drinks, soft drinks, cookies, ice cream, and candy bars are also examples of dietary CHO, although they represent relatively unhealthy versions of carbohydrates. Although foods containing protein and fat affect insulin secretion, ingested CHO content has a more pronounced effect on pancreatic insulin release and circulating insulin levels. The rapidity of CHO breakdown and gastrointestinal absorption following ingestion depends on the *type* of ingested CHO. The more refined and processed the CHO content of a meal, the less work the body has to exert to digest and absorb the nutrient. The glycemic effect (also called the *glycemic response*) refers to the effect a particular food has on a person's blood glucose and insulin response. The more refined varieties of dietary CHO evoke a higher glycemic response, and this phenomenon represents the basis for how many CHO-containing foods are classified.

Glycemic Index of Foods

The glycemic index (GI) of foods is a numerical system of measuring how much of a rise in circulating blood sugar the food triggers—the higher the GI number, the greater is the expected excursion in blood sugar response following ingestion of that

Table 9.1 Glycemic indices of common dietary items

High glycemic index foods
White bread
Certain cereals (cheerios, corn flakes, rice krispies)
Mashed potatoes
Honey
Soft drinks
Moderate glycemic index foods
Watermelon
Sourdough or rye breads
Cream of wheat and instant oatmeal
Orange juice
Pineapple
Low glycemic index foods
Bran cereal
Old-fashioned oatmeal
Peaches
Lentils
Milk
Sweet potatoes

particular food. Foods containing CHO that are broken down easily and raise the blood sugar quickly are referred to as *high glycemic* foods; glucose is currently considered the reference food with a GI of 100. A GI index number between 70 and 100 is considered *high*, whereas values between 50 and 70 and <50 are deemed of medium and low GI index value [5]. The fiber content of various edibles influences their respective GI indices; higher dietary fiber content slows the rapidity of glucose absorption and, hence, offers a more controlled rise in blood sugar levels. Food items containing a high fiber content are classified as *low glycemic* foods, which also promote an increased level of satiety, thus helping to control appetite and decrease hunger, and are ultimately helpful for weight management. Table 9.1 presents GI values for common food items.

There are many proponents of a low-GI diet as a means to improve insulin sensitivity and possibly improve the androgen profile of women with PCOS [6, 7]. Because the majority of women with PCOS demonstrate a marked compensatory hyperinsulinemia after CHO ingestion, there may be specific metabolic and cardiovascular benefits of consuming a low-GI value diet. However, while numerous studies have assessed the impact of a low-GI diet in the general population (non-PCOS women), few studies have specifically examined the role of a low-GI diet on women with PCOS.

In a 2010 study performed by Marsh et al., researchers compared the changes in insulin sensitivity and clinical outcomes after similar weight losses after the consumption of a low-GI diet compared with a conventional healthy diet in women with PCOS [8]. Both diets were designed as reduced-energy, moderate-to-high fiber with similar macronutrient distribution. Only the quality of carbohydrate (i.e., GI) varied between the two diets. Overweight and obese women with PCOS were assigned to

one of two groups: (1) an ad libitum low-GI diet or (2) a macronutrient-matched healthy diet. Both groups were followed for 12 months or until they reached a 7 % weight loss. With only a modest weight loss of 4–5 % of body weight, the group of women following the low-GI diet experienced a threefold greater improvement in whole body insulin sensitivity. In addition, women following the low-GI diet demonstrated improved menstrual cycle regularity (95 %) versus those women following a conventional healthy diet (65 %). While not a long-term study, this research nonetheless supports the capacity of dietary carbohydrates to increase postprandial glycemia, which ultimately, when manipulated, may be an important consideration for managing the symptoms associated with PCOS.

Another study performed by Mehrabani et al. examined the impact of the glycemic load on overweight and obese women with PCOS [9]. A total of 60 overweight and obese women with PCOS were recruited and randomly assigned to one or two hypocaloric diet groups for a single-blind clinical trial. Group 1 included a conventional hypocaloric diet (CHCH), with 15 % of dietary calories coming from protein. Group 2 included a modified hypocaloric diet (MHC), with a high-protein, low glycemic load (30 % of daily energy from protein plus low glycemic load foods selected from a list). Both diets were prescribed via 12 weekly counseling visits. While weight loss and androgen reduction were similar among both diet groups, the group adhering to the MHCD presented with a significant reduction in insulin level and overall decrease in high-sensitivity C-reactive protein when compared with the conventional diet. While research in this area does appear promising, more research regarding the long-term efficacy of low-GI diets in this population is clearly needed.

Limitations of Using the Glycemic Index for PCOS Patients

Despite the aforementioned potential for metabolic benefit in chronically hyperinsulinemic and insulin-resistant populations, an overenthusiastic focus on dietary GI has several disadvantages worth noting. First, in assigning a numerical value to a food item, GI assumes the food is eaten alone, which is often not the case. When foods are eaten in combination, indices of consumed items may influence the overall GI of the meal. Dietary fat and fiber slow the process of meal digestion and, therefore, can lower the GI of an individual food item. For example, an apple eaten alone produces a rapid rise in blood glucose. However, the rise is not as substantial if you add a tablespoon of natural peanut butter; the apple is digested at a slower rate.

The GI of the food is affected by its ripeness, processing, the length of storage, cooking methods, and its variety. For example, a yellow banana has a lower GI than a brown banana. Fully cooked pasta has a lower GI than al dente pasta, and boiled potatoes have a lower GI than a baked potato. Even beverages such as soy milk, depending upon the brand, can dramatically vary in the CHO content and, hence, GI.

Lastly, the GI does not tell us anything about the nutritional content of the food. *A low-GI food does not equate to a healthy food*. Also, just because a particular food

has a low or moderate GI does not mean that food can be consumed in abundance. The GI simply tells us how quickly a particular CHO affects blood glucose levels and, thus, pancreatic insulin response to the consumed food. Therefore, by strictly avoiding certain foods with high GI, individuals may deprive themselves of some very healthy foods. For example, watermelon has a GI of 72 and is considered a high-GI food. A chocolate bar has a GI of 68, and potato chips have a GI of 58, which classifies both as moderate-GI foods. It is evident that watermelon is a nutritionally superior choice to a chocolate bar or potato chips. Therefore, while the dietary GI deserves some consideration, it should not be the only criterion when selecting appropriate meal choices. The total amount of dietary CHO, the amount and type of fat, dietary fiber, and salt contents are additional dietary considerations that merit attention.

Guidelines for Dietary Carbohydrate

As discussed, diets high in CHO and refined and processed forms of CHO, are particularly detrimental for the insulin-resistant PCOS population. However, evidence does not support the benefits of drastically reducing or eliminating carbohydrates in the diet either [10, 11]. Given that the majority of women with PCOS have underlying issues with insulin resistance, the increased likelihood of obesity, and risk factors for developing type 2 diabetes, a diet that is moderate in CHO with a focus on low-GI foods is best suited for this population. Women with PCOS should aim for around 40 % of their total calories coming from CHO (Table 9.2). This amount is appropriate to optimize blood glucose levels while still providing many of the benefits of a low-GI diet. For a woman consuming 1,500 kcal per day, this would equate to a maximum value of 150 g of CHO spread consistently throughout the day (e.g., 20–30 g of low-GI CHO with breakfast, 35–40 g of low GI CHO with lunch, and 30–35 g of low-GI CHO with dinner). The remainder should be consumed in 2–3 low-GI CHO-containing snacks spaced equally between the three meals. A specific meal plan that fits these criteria is provided later in this chapter.

Table 9.2 Recommended daily carbohydrate consumption for women with PCOS based on daily caloric intake

Daily caloric level	Daily maximum carbohydrate level (g/day)
1,400	140
1,500	150
1,600	160
1,700	170
1,800	180
1,900	190
2,000	200

Fruit and PCOS

All fruit in its natural form is nutritious, and regular consumption should be encouraged by women with PCOS. Despite the "healthy" connotations, all fruit predominantly contain CHO Typically, one serving of fruit contains about 15 g of CHO, no protein or fat, and 60 cal. Therefore, while fruit (quantified as one small whole fruit such as apple, orange, or banana or three-quarters cup cubed fruit such as melon, strawberries, or grapes) contains many positive benefits, such as fiber, vitamins, minerals, and antioxidants, an individual with PCOS needs to be mindful of the quantity consumed; women should aim for consuming two servings of fruit per day.

Given that fruit items are poor in fat or protein, they may be best paired with a lean protein choice or modest amount of fat, such as low-fat cheese, yogurt, or nuts, to stabilize blood sugar and promote increased satiety. Women with PCOS should particularly avoid fruits in canned heavy syrup; when purchasing canned fruit, the words "extra light syrup" or "packed in juice" are optimal.

All fruit juices represent a concentrated source of sugars and low amount of fiber and are best avoided, as even small amounts can spike insulin, making weight loss difficult. It is always preferable to choose whole fruit over fruit juice, even if the juice label states "100 percent juice"!

Vegetables and PCOS

Vegetables are high in fiber and abundant in vitamins, minerals, and antioxidants. There are two main categories of vegetables: nonstarchy and starchy vegetables. Nonstarchy vegetables include spinach, lettuce, broccoli, green beans, onions, mushrooms, broccoli, zucchini, and peppers, and promote satiety. They are low in calories and in CHO, and contain fiber, so an individual can consume them without worrying about gaining weight or worsening insulin resistance. Women with PCOS can typically consume nonstarchy vegetables in unlimited amounts, and should aim for *at least* three servings of nonstarchy vegetables (each serving defined as one-half cup of cooked vegetables, one cup of raw of vegetables or one 8-oz cup of vegetable juice) daily.

Starchy vegetables have a higher CHO content and, therefore, a bigger impact on insulin levels than the nonstarchy ones, and include corn, peas, winter squashes, plantains, and all types of potatoes. Although excursions in insulin levels are much higher following ingestion of starchy vegetables, the goal is not to eliminate starchy vegetables from the diet. However, caution is advised against excessive consumption of starchy vegetables by women with PCOSs. One serving of starchy vegetables contains 15 g of CHO, 0–3 g of protein, 0–1 g of fat, and 80 cal in contrast to approximately 5 g of CHO, 2 g of protein, and 25 cal per nonstarchy vegetable serving.

Dietary Fiber and PCOS

Dietary fiber is the indigestible part of plant-based foods and passes through the gastrointestinal tract in its original form. Fruits, vegetables, nuts, seeds, beans, and whole grains all contain fiber. In the stomach, fiber content of consumed food conveys a feeling of fullness, delays gastric emptying and promotes a feeling of satiety. Additionally, in appropriate amounts, dietary fiber has been demonstrated to have a cholesterol-lowering effect (REF). The products of bacterial digestion of fiber in the colon are absorbed into the body and may inhibit the body's production of cholesterol as well as enhance the clearance of cholesterol from the blood. Lastly, fiber has been demonstrated to improve the way the body handles glucose by slowing the digestion and absorption rate of CHO. This promotes a slower, more stable rise of blood glucose and helps prevent the symptoms of reactive hypoglycemia and reactionary sugar cravings that are commonly experienced by women with PCOS. In addition, the more gradual the release of blood glucose, the longer it will take for the body to feel hungry again.

Recommendations for Fiber and PCOS

Women with PCOS should aim to consume 25–30 g of fiber per day. Products containing 3 g or more per serving are considered a good source of fiber whereas diets contains 5 or more grams of fiber per serving are considered as *high* fiber source. *Just because a food label may state "100 % wheat" or "multigrain" does not mean it is a good source of fiber*. Products that have the words "whole wheat," "whole oats," "whole rye," "bulgur," "graham flour," "oatmeal," or "wild rice" listed as the first ingredient on the food's label represent a high-quality source of fiber. Box 9.1 offers tips for including more fiber in the diet.

Box 9.1 Strategies for Optimizing Dietary Fiber Intake

- For breakfast choose a high-fiber breakfast cereal. Look for cereals with at least 5 or more grams of fiber per one-cup serving.
- Add a few tablespoons of unprocessed wheat bran or flaxseed to your favorite cereal.

Consume breads that list the words "whole wheat," "whole-wheat flour," or other whole grain as the first ingredient on the label.

- Purchase brands of bread with at least 3 g of dietary fiber per slice.
- Add beans such as lentils, black-eyed peas, lima beans, great northern beans, and chickpeas to your diet.
- Sprinkle crushed walnuts or slivered almond into your hearty salads.

Protein

Protein has many vital roles in the body. About 20 % of our total body weight is protein. The body uses protein for building and maintaining tissues, to make important enzymes, hormones, neurotransmitters, tendons, ligaments, antibodies, and other body chemicals. Women with PCOS should do their best to select lean rather than high-fat sources of animal protein. Women with PCOS are advised to substitute a portion of their dietary CHO for protein as a strategy to reduce overall dietary CHO content. Protein takes longer than CHO to digest, and dietary protein content confers a sense of satiety; thus, inclusion of lean protein in meals and snacks can improve the overall insulin response in women with PCOS. An optimal intake for protein for women with PCOS would be between 25 and 30 % of total calories consumed. For a woman following a 1,500-cal diet, this would come out to around 100–110 g of protein per day, spread equally among meals and snacks. A helpful hint is that every 1 oz of lean protein is equal to 7 g of protein. Therefore, a 4-oz chicken breast contains 28 g of protein. By consuming regular amounts of protein throughout the day, women with PCOS can help stabilize blood sugar and prevent hypoglycemia and subsequent cravings. Box 9.2 presents considerations for choosing dietary proteins.

Box 9.2 Considerations When Choosing Dietary Protein Sources

- 3 oz of protein is equal to the size of the palm of your hand or a deck of cards.
- Fish and shellfish, fresh, frozen, or canned in water, are excellent protein choices. Experts recommend heart-healthy benefits of consuming at least two servings of fish per week.
- Meat, chicken, and fish are all excellent sources of protein, as well as iron, zinc, and vitamin B_{12}. Women with PCOS should focus on the low-fat varieties as well as the leanest cuts of meat, such as the following:
  - Flank steak, round steak, sirloin, tenderloin or extra-lean ground beef (90 % lean or greater).
  - Lean ham, Canadian bacon, pork tenderloin, and center-cut loin pork chops.
  - Chicken, turkey, or game hens without the skin; extra-lean ground turkey or chicken breast.
  - At the deli counter, select low-fat, low-sodium options such as lean ham, turkey, chicken, or lean roast beef.
  - Eggs are also an excellent protein choice. Choose organic, cage-free eggs whenever possible. They are higher in omega-3 fatty acids. Egg substitutions, although they do not supply any of the healthy fats or many of the beneficial nutrients found in whole eggs, can also be consumed. One-quarter cup of egg substitute is equal to one egg.
  - Beans are a good source of fiber as well as good source of protein. All beans do contain carbohydrates, so it is important for women with PCOS to be mindful of the quantity. One-quarter cup of any of the following is equivalent to 1 oz of meat: kidney beans, lentils, chickpeas, white beans (cannellini), black beans, or pinto beans.

Lipid Abnormalities in PCOS and Relevance of Dietary Fat

Abnormalities in lipid profile are encountered in almost two-thirds of women diagnosed with PCOS [12]. Hyperandrogenemia, central obesity, insulin resistance, and hyperinsulinemia acting in conjunction are recognized as mechanisms for the observed spectrum of dyslipidemia in women with PCOS. Lipid abnormalities commonly encountered in PCOS patients are similar to those observed in diabetics and include elevated levels of low-density lipoprotein cholesterol (LDL) and triglycerides, and lower levels of high-density lipoprotein cholesterol (HDL) [13]. Hyperinsulinemia and hyperandrogenemia have been thought to cause adipocytes to undergo increased catecholamine-induced lipolysis and release of free fatty acids (FFA) into the circulation [13]. Increased FFA in the liver stimulates secretion of triglyceride-rich very low-density lipoprotein (VLDL), ultimately contributing to the commonly encountered hypertriglyceridemia in this population [14].

Dietary Fat and PCOS

The type and amount of dietary fat consumption has implications for weight management and insulin resistance. By consuming the appropriate qualities and quantities of fat in their diet, women with PCOS can actually help enhance the absorption of healthful fat-soluble vitamins, decrease their overall risk of cardiovascular disease, and even gain reproductive benefit (as the majority of the sex hormones are controlled by dietary fat). Fat also takes far longer to metabolize than dietary CHO, ultimately promoting a sense of fullness and satisfaction.

Fats and oils are made up of basic units called fatty acids. Each type of fat or oil is a mixture of different fatty acids, and independent of the type of fat consumed, all types of dietary serve as equitable sources of energy, yielding 9 cal/g.

Monounsaturated fatty acids (MUFAs) are found mainly in vegetable oils (vegetable, olive, rapeseed, and peanut), nuts and nut butters, seeds, olives, and avocadoes. Studies show that eating a diet rich in MUFAs can improve cholesterol levels, optimize insulin levels, and aid in blood glucose control [15].

Polyunsaturated fatty acids (PUFAs) are found mainly in vegetable oils, almonds, cashews, pecans, avocados, fish, and seafood. Omega-3 and omega-6 fatty acids are types of PUFAs and are considered essential fatty acids because humans are dependent on dietary sources, as these cannot be synthesized in vivo.

Omega-3 fats are particularly relevant for women with PCOS given that a balanced intake of these fatty acids confer anti-inflammatory benefit in a condition that is associated with low-grade systemic inflammation, as evidenced by elevation of multiple clinical markers such as C-reactive protein, interleukin-18, as well as endothelial dysfunction [16]. Adequate omega-3 consumption supports healthy cardiovascular, brain, mood, skin, and immune function as well as decreases cholesterol

and triglycerides and improves insulin sensitivity [17–19]. The three major types of omega-3 fatty acids are EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), and ALA (alpha-linolenic acid). EPA and DHA are found in fatty cold-water fish (like salmon, mackerel, blue fish, albacore tuna, sardines, lake trout, and herring) as well as fish-oil supplements. ALA is found in seeds, nuts, beans, green leafy vegetables, flaxseed, and canola and soybean oil.

Although *omega-6* fatty acids also play an integral role in health, their extreme prevalence in the Western diet—in vegetable oils, animal fats, bakery goods (donuts, cookies)—is contributory to systemic inflammation. *The optimal ratio of omega-6 to omega-3 in healthy diet is 4:1*, whereas the typical American diet presents with a ratio of 20:1. This imbalance triggers a genetically programmed inflammatory response in the body. Therefore, most women with PCOS could greatly benefit from decreasing their intake of omega-6 fatty acids and increasing their intake of omega-3 fats. Experts recommend a minimum of 200 mg of DHA per day from either fatty fish or fish oil pills [15]. This can also be obtained by consuming at least 12 oz of fatty fish per week, whereas vegetarians or vegans can take flaxseed oil, a rich source of ALA. Strategies for maximizing dietary intake of omega 3 fatty acids are summarized in Box 9.3.

Saturated fatty acids are found mainly in foods from animal sources like high-fat cuts of meat, dairy products made from whole milk (butter, ice cream, cheese. and sour cream), and chicken skin. Some vegetable oils, such as coconut, palm kernel, and palm oil, also contain saturated fat. Saturated fats are reported to raise both total and LDL cholesterol. These foods should be eaten sparingly. National dietary guidelines recommend that saturated fat intake be less than 10 % of total calories consumed. For example, if a person consumes 1,500 cal per day, she should consume less than 17 g of saturated fat.

Trans fatty acids are liquid vegetable oils that have been chemically processed to become semisolid at room temperature through the addition of hydrogen atoms. Trans fatty acids, also called "partially hydrogenated" oils, are used in some margarines, fried foods, cakes, cookies, and processed snack foods to improve the flavor, texture, and shelf-life. Surprisingly, many commercial peanut butters also contain

Box 9.3 Tips for Increasing the Omega-3 Content of the Diet

- Add salmon or tuna to a salad with chopped walnuts.
- Select oils that provide omega-3 fatty acids such as canola or walnut oil.
- Sprinkle ground flaxseed on your yogurt, cereal, or salad.
- Cook spinach or kale and blend it into a fruit smoothie.
- Spread margarine or peanut butter with added omega-3 fatty acids on whole-wheat toast.
- Grill, bake, or broil your favorite seafood with a bit of olive oil (bluefish, salmon, tuna, etc.).

trans fats. Trans fats are easily oxidized to form free-radical chain reactions that, in turn, can damage cell membranes and body tissues, and compromise immune function. Research confirms that consumption of trans fat may promote inflammation, premature aging, and various cancers. Dietary trans fat content can adversely affect the risk of coronary disease by raising LDL cholesterol levels, lowering HDL cholesterol levels, and raising triglyceride levels [20]. Indeed, in a large, prospective study conducted on more than 800,000 women enrolled in the Nurse's Healthy Study, researchers found that a higher dietary intake of saturated fat and trans fat was associated with an increased risk of coronary disease [20]. By replacing the saturated and trans fat content of the diet with unsaturated fats, there appears to be a clear benefit on blood lipids [21, 22]. Dietary restriction of trans fats should be a priority in women with PCOS, who are inherently deemed at an enhanced risk for premature atherosclerosis. Women with PCOS should aim for 25–30 % of their total calories coming from fat. For a woman following a 1,500-cal diet, this would equate to 40–50-g fat intake per day, with poly- and monounsaturated forms of fat constituting the bulk of total daily intake. Fish, nuts and nut butters, avocados, seeds, and olives offer healthy sources of dietary fat.

Dietary Calcium

Dairy foods are excellent sources of protein, calcium, and other important nutrients. Beyond its benefit for skeletal health, adequate dietary calcium is suggested to hold implications for blood-pressure lowering and have relevance for insulin signaling; it is also suggested to have a role in fat metabolism [23, 24]. Table 9.3 presents calcium-rich foods and their relative calcium content. Women with PCOS should aim for 1,200–1,500 mg per day, which equates to about three servings per day, with dietary sources being preferable to supplementation strategies.

Mozzarella, farmer's cheese, feta, and low-fat string cheese are all excellent low-fat dietary options; these choices are lower in saturated fat and calories than their full-fat counterparts (less than 5 g of fat per ounce).

Table 9.3 Foods rich in calcium

Food	Amount	Calcium content (mg)
Milk (1 % or nonfat)	8 oz	300
Yogurt (low-fat)	8 oz	350–400
Cheese	1 oz	200
Canned sardines (with bones)	3 oz	375
Canned salmon (with bones)	3 oz	170
Leafy greens	1/2 cup	100–150

Dietary Sodium and PCOS

Because women with PCOS have a predisposition to premature atherosclerosis and propensity for endothelial dysfunction, attention to dietary sodium may be relevant. While sodium occurs naturally in most foods such as milk, beets, and celery, it is a common additive to various food products in forms such as monosodium glutamate, sodium nitrite, sodium saccharin, baking soda (sodium bicarbonate), and sodium benzoate. Sodium is also found in condiments such as Worcestershire sauce, soy sauce, onion salt, garlic salt, and bouillon cubes. Processed meats, such as bacon, sausage, and deli meats, and canned soups and vegetables are all examples of foods that contain added sodium. Fast foods are generally very high in sodium. Sodium intake should be consistent with the dietary guidelines for healthy Americans and be no more than 2,300 mg (1 teaspoon table salt) per day. Adults with high blood pressure should have no more than 1,500 mg per day, and are encouraged to choose foods labeled as "reduced-sodium," "no-salt-added," or "unsalted" products.

Caloric Needs and PCOS

An individual's calorie needs are dependent on a variety of factors, including activity level, age, sex, weight, body composition, and an individual's own metabolism. While there are many different ways for determining daily caloric needs, one of the most established methods is referred to as the Harris Benedict equation [25], which first establishes an individual's metabolic rate (BMR) and then determines the total allowable calories based on the individual's activity level. BMR represents how many calories an individual burns at rest and includes calories expended for sustaining cardiac activity, breathing, digesting food, body temperature regulation, as well as maintaining functioning of muscles and various other organs. A woman's BMR can be calculated as follows:

Step 1: $\text{BMR} = 655 + (4.35 \times \text{weight in pounds}) + (4.7 \times \text{height in inches}) - (4.7 \times \text{age in years})$

Step 2: Once BMR is established, an individual's activity level must be accounted for and designated a numerical value termed "activity factor." Daily caloric estimate needed to maintain an individual's current weight is achieved by multiplying BMR by the activity factor, as follows:

Sedentary (little or no exercise): $\text{BMR} \times 1.2$
Lightly active (light exercise/sports 1–3 days/week): $\text{BMR} \times 1.375$
Moderately active (moderate exercise/sports 3–5 days/week): $\text{BMR} \times 1.55$

Very active (hard exercise/sports 6–7 days a week): BMR × 1.725
Extra active (very hard exercise/sports and physical job or 2× per day training): BMR × 1.9

As an example, let's apply the Harris Benedict equation to a lightly active, 5 ft, 3 in. 35-year-old woman who weighs 150 lb.

Step 1: Calculate BMR
$655 + (4.35 \times 150) + (4.7 \times 63\ in.) - (4.7 \times 35) = 1{,}439$ cal

Step 2: Multiple BMR × activity factor
1,439 cal × 1.375 = 1,980 cal

For this woman to maintain her current weight of 150 lb, she could consume around 2,000 cal per day. Typically, if someone is looking to lose weight, they would subtract 500 cal per day from their maintenance caloric needs. This is based on the assumption that 1 lb of body fat is equal to about 3,500 cal. Therefore, by deducting 500 cal per day for 7 days theoretically should result in loss of around 1 lb of body fat per week.

It is important to appreciate that the preceding calculations are only estimates and may not suit all populations. Many overweight, insulin-resistant women have a challenging time losing weight and may benefit from seeking guidance of a registered dietitian to establish a more personalized dietary regimen that takes into account the individual and unique metabolic needs and profiles. Registered dietitians within different regions of the United States can be identified through visiting www.eatright.org and clicking on the link "Find a Registered Dietitian."

Behavior Modification in PCOS Management

Snacking and Optimal Meal Patterns

Eating small, frequent meals and snacks not only decreases food cravings and prevents binges, but it also provides a steady flux of energy and helps prevent low blood sugar. Given an inherent tendency towards reactive hypoglycemia and CHO cravings, women with PCOS are an ideal population to benefit from consumption of small and frequent snacks spaced every 2–4 h. The optimal meal patterns would be to consume breakfast within 1 h of waking, a snack 2–3 h later, eat lunch 2 h later, consume another snack 2–3 h later, and then eat dinner after another 2 h If hungry after dinner, a third snack can be consumed, if needed. Box 9.4 lists items to be considered as snack substitutes.

The guidelines for snacks allow for flexibility. A snack should roughly be around 60–100 cal, 0–20 g of CHO, 0–7 g of fat, and a modest amount of protein (2–8 g), if possible.

Box 9.4 List of Recommended Snack Substitutes

- 6 oz plain nonfat Greek yogurt (90 cal)
- 1 small piece fresh fruit with 1/4 cup of low-fat cottage cheese (100 cal)
- 1 small apple sprinkled with cinnamon and 1 tablespoon sugar-free syrup, baked (80 cal)
- 2 graham crackers squares with 1 teaspoon peanut butter (90 cal)
- 12 almonds or 20 peanuts (83 cal)
- 1/2 cup whole-grain cereal and ½ cup low-fat milk (100 cal)
- 1/2 cup cooked edamame beans (100 cal)
- 2 tablespoons hummus with 12 grape tomatoes, 2 stalks celery (71 cal)
- 6 medium shrimp with cocktail sauce (60 cal)
- 1 hardboiled egg (75 cal)
- 1 part-skim mozzarella string-cheese stick (70 cal)
- 1 medium tomato stuffed with 1/3 cup reduced-fat cottage cheese (100 cal)

Importance of Exercise for PCOS Management

Regular exercise has long been accepted as an effective part of weight management and overall maintenance of physical and mental health. Physical activity can reduce insulin levels, improve insulin sensitivity, and optimize lipid profile; it may also improve self-esteem, depression, and anxiety in women with PCOS.

Exercise-associated improved glucose homeostasis relates to an up-regulation of the expression and/or activity of proteins involved in insulin signal transduction in the skeletal muscle [26]. Numerous studies have examined the impact of exercise specific to women with PCOS. In a study performed by Vigorito et al., 90 young women with PCOS were randomly subdivided into two groups, each composed of 45 subjects. The PCOS-T (trained) group underwent a 3-month structured exercise training program, whereas the PCOS-UnT (untrained) group maintained an unaltered lifestyle. This study demonstrated that a 3-month structured exercise training program induced a significant improvement in cardiopulmonary functional capacity and insulin sensitivity and also led to a significant reduction in BMI and CRP levels in a wide overweight PCOS population [27]. In yet another study by Brown et al., researchers demonstrated that moderate-intensity exercise without significant weight loss improved several components of the lipoprotein profiles of women with PCOS. There were significant reductions in the concentration of large VLDL/chylomicrons and medium/small HDL, and increased large HDL and average HDL size in the exercise group compared to controls. Further, there were reductions in calculated triglycerides and VLDL-triglycerides in the exercise group compared to controls [28]. These findings support the recommendation to increase physical activity in women with PCOS to obtain improvements in the overall metabolic picture and to reduce overall risk for cardiovascular morbidity in this population.

Putting It All Together

Sample Menu Plans for PCOS

1,200–1,400-cal Sample Menu

Breakfast

1¼ cup of strawberries
1 plain nonfat Greek yogurt
1/2 cup high-fiber cereal
6 walnuts

Lunch

3 oz of grilled chicken
2 cups of Romaine lettuce with 1 cup of assorted nonstarchy vegetables
1 tablespoon of olive oil
1 tablespoon of balsamic vinegar
1 small orange

Dinner

3 oz of grilled salmon
1 cup of green beans with 2 teaspoons butter
1 cup of cooked wild rice
1 cup of 1 % milk

Snacks

1 oz of low-fat cheese (<3 g of fat)
1 whole grain granola bar

Totals: 1,350 cal, 120 g of carbohydrates (35 % total calories), 25 g of fiber (100 % of needs), 52 g of fat (36 % of total calories), and 108 g of protein (32 % of total calories).

Exercise Recommendations

The Physical Activity Guidelines for Americans supports the recommendation that physical activity should be no less than 150 min total per week of moderate-intensity exercise. Activities such as brisk walking, water aerobics, ballroom and line dancing, general gardening, tennis (doubles), or sports in which one catches and throws (baseball, softball, volleyball) are all considered to reflect moderately intense physical activity. When exercising moderately, an individual should be able to talk but not sing. If the individual chooses to increase the intensity of exercise (while decreasing

the duration), recommendations are for 75 min total per week. Vigorous-intensity activities include race walking, aerobic dance, biking faster than 10 miles an hour, hiking uphill, heavy gardening, jumping rope, martial arts (such as karate), jogging, running, swimming fast laps, tennis (singles), and any sport that involves a significant amount of running (basketball, soccer, field hockey). During vigorous activity, an individual should not be able to say more than a few words before breathing deeply. Activities that strengthen the muscles, improve balance, and preserve bone, such as weight training, using resistance bands, weight-bearing aerobics, and heavy gardening, are recommended to be included 2 or more days per week.

Summary

The majority of women with PCOS are overweight and therefore likely to benefit from weight-reduction strategies. Successful weight loss can be achieved through a combination of dietary modifications and restrictions. Minimizing intake of simple CHO, saturated fats, and omega-6 fatty acids, optimizing dietary fiber and omega-3 fatty acid content, ensuring against spells of starvation and encouraging intake of frequent and small meals are strategies that will facilitate improvements in metabolic as well as phenotypic burden of PCOS. Regular physical activity of moderate intensity in conjunction with the specified dietary modification is sure to further enhance the overall health benefit.

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Chapter 10
Role of Insulin-Sensitizing Drugs in PCOS Management

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Key Points

- Insulin resistance is recognized as contributory to the clinical picture of PCOS.
- Metformin is the insulin-sensitizing drug of potential benefit for women with PCOS who are planning to conceive.
- In the management of PCOS-related subfertility, ovulation-induction therapy citrate remains the first line treatment.
- Metformin-alone therapy may benefit the nonobese, while metformin combination therapy with clomiphene citrate may benefit obese women with PCOS who are attempting to conceive.
- Metformin co-treatment during IVF/ISCI cycles does not improve any of the reproductive endpoints in women with PCOS, except for a significant reduction in the risk of ovarian hyperstimulation syndrome.
- The use of metformin during pregnancy to prevent spontaneous miscarriages in PCOS is not supported by existing data.
- Insulin-sensitizing drugs offer no advantage over established anti-androgenic drugs for ameliorating the symptoms of androgen excess.
- There is no established role for insulin-sensitizing drugs in the long-term prevention of type 2 diabetes mellitus and coronary artery disease in PCO.

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Polycystic Ovary Syndrome and Insulin Resistance

Nearly eight decades following its original description, polycystic ovary syndrome (PCOS) remains a poorly understood entity. Various pathophysiologic mechanisms have been proposed, including intrinsic ovarian, adrenal, and hypothalamic-pituitary pathways, but none was shown to provide a complete explanation for the development of this condition. Much interest has accrued lately over the metabolic aspects of the syndrome, rendering insulin resistance with compensatory hyperinsulinemia the mechanism most widely studied today [1–3].

Increased insulin resistance is observed in 20–30 % of nonobese and 70–80 % of obese women with the syndrome [4–6]. Affected women tend to be more insulin-resistant than weight-matched women in the general population [7]. Although obesity is a recognized risk factor for decreased insulin sensitivity [8], at least one aspect of the insulin resistance hypothesis in the context of PCOS is thought to be largely obesity-independent [7]. Hyperinsulinemia is believed to stimulate ovarian androgen production by direct action on the theca cells and by potentiating LH effects [9]. The local build-up in follicular androgenic signal has been shown to promote premature follicular atresia [10], primary and pre-antral follicle arrest, and impaired follicle dominance [11], leading to anovulation [12]. High insulin state further potentiates adrenal androgen secretion by enhancing adrenal response to ACTH [13]. Insulin has further been implicated in impairing progesterone inhibition of the gonadotropin-releasing hormone pulse generator [14–16], and suppression of hepatic synthesis of sex hormone-binding globulins (SHBG).

More than 50 % of women with PCOS are obese [17]. Since abdominal obesity, expressed by increased waist-to-hip ratio, is considered an independent factor for reduced fecundity [18–20] and increased menstrual disorders [4, 21], behavioral therapy aimed at weight reduction has been proposed as a first-line management strategy for improving fertility in the overweight and obese women with PCOS [22]. The effectiveness of this approach, however, is highly dependent on personal drive in the context of well-structured lifestyle modification programs [23, 24]. The main limitation to such programs, however, lies in the difficulty of safeguarding benefits over time. For this reason, much attention has been diverted lately towards finding an alternative pharmacologic approach to the management of PCOS.

Insulin-Sensitizing Drugs: Background

The management of PCOS has traditionally focused on the presenting complaint/s; i.e. infertility treatment through ovulation-induction strategies, hormonal manipulation for the management of menstrual irregularities, and anti-androgenic therapy for dealing with features of hyperandrogenism (hirsutism and acne). An interest in the role of insulin-sensitizing drugs (ISDs) as a means of reducing compensatory hyperinsulinemia in the hope of improving metabolic and reproductive functions in women with PCOS has grown measurably over the past decade [25, 26].

Metformin

Metformin is an FDA-approved biguanide for the management of type 2 diabetes mellitus (T2DM). Although its mechanism of action remains obscure, metformin was shown to activate adenosine monophosphate-activated protein kinase (AMPK) pathway, inhibiting hepatic production of glucose, reducing oxidation of fatty acids, and increasing peripheral tissue uptake of glucose [27–29]. Metformin is believed to lower fasting serum insulin levels in insulin-resistant states without inducing hypoglycemia [30–32], and helps reduce insulin requirements in insulin-dependent and non-insulin-dependent diabetes. These effects, however, are not universal particularly in the PCOS population. While inducing a significant drop in fasting insulin levels in nonobese women (BMI <30 kg/m^2) with PCOS (mean difference (MD) −5.65 mIU/L, 95 % CI −10.25 to −1.06; 4 trials; 85 women), metformin treatment [26] failed to show a similar effect in the obese PCOS population (BMI ≥30 kg/m^2) (MD −2.72 mIU/L, 95 % CI −6.50 to 1.05; 12 trials; 488 women).

Bothersome side effects of metformin may be experienced in about 30 % of patients, and not uncommonly can be a reason for treatment discontinuation. These are predominantly gastrointestinal in nature, consisting of bloating, abdominal discomfort, nausea, vomiting, and diarrhea [33]. A Cochrane systematic review [26] revealed a fourfold increase in the incidence of gastrointestinal disturbances when metformin was used compared with placebo (OR 4.27, 95 % CI 2.4–7.59; 5 trials; 318 women). Symptoms are usually dose-dependent, and can be mitigated by a gradual increment in the daily drug dose from 500 to 2,000 mg over a period of 4 weeks.

A rare but serious complication of metformin therapy is lactic acidosis, which has been reported in high-risk conditions such as renal insufficiency, liver disease, and congestive heart failure, with fatality rates approaching 50 % [29, 30]. A clinically insignificant decrease in vitamin B_{12} absorption has also been reported in association with metformin use [29].

Metformin remains nonetheless the preferred ISD for utilization in the management of PCOS due to the vast experience reported in the literature on its use and potential for metabolic benefit [30]. It is a Class B drug with no proven teratogenic risks in animals, and no reported untoward fetotoxic effects in humans [12, 30, 33].

Thiazolidinediones

Insulin sensitizing effects of thiazolidinediones (TZDs) are mediated through nuclear gamma-peroxisome proliferator activator receptor (PPAR) binding, inducing gene transcription and activation [30]. TZDs improve insulin signaling, enhance glucose uptake in adipose tissue and muscle, and confer multiple effects on lipid metabolism and inflammatory pathways [33]. Randomized clinical trials nevertheless failed to demonstrate any measurable reduction in fasting serum insulin levels in women with PCOS following either rosiglitazone [34, 35] or poiglitazone therapy [36]. While TZDs exhibit a greater potency at increasing peripheral glucose uptake, metformin was found to be more effective in decreasing hepatic glucose production [33].

Enthusiasm for TZD use in PCOS management is dampened not just by a predisposition towards weight gain seen with this class of ISDs [33, 36–39], but also by safety concerns as TZD use has been associated with increased risk for cardiovascular events, namely, coronary artery disease, myocardial infarction, and nonfatal heart failure [40, 41]. More recently, an increased risk of bladder cancer has raised additional concern [42], further constraining their use in healthy individuals. Fluid retention leading to edema and dilutional anemia has also been reported in association with TZD use [30, 33, 41]. Concern over the incidence of hepatoxicity as a result of troglitazone use led to its discontinuation and removal from the market [29, 30]. Moreover, rosiglitazone and poiglitazone are classified category C drugs with proven teratogenicity in animals, further limiting their use in reproductive age women [41].

Clinical Use of ISDs in PCOS Management

The scope of metformin use in the treatment of PCOS has changed critically over the last decade. Earlier studies conveyed a highly favorable and promising profile expressed by marked improvement of hyperinsulinemia, reduction of hyperandrogenemia [9], restoration of ovulatory function [43], increase in pregnancy rates [44, 45], and decrease in first-trimester spontaneous abortions in women. Most of these studies, however, were either observational or randomized, involving very small numbers of participants [12]. The largest randomized controlled trials were published only in 2006 and 2007 by Moll et al. [46] and Legro et al. [47], and included 225 and 626 women, respectively.

Effects of ISDs on Reproductive Endpoints in Women with PCOS-Related Subfertility

The role of clomiphene citrate (CC) as a first-line treatment for women with anovulatory PCOS is long established [48, 49]. There is high-quality evidence demonstrating that CC is associated with significantly higher ovulation and pregnancy rates than placebo [22, 50, 51]. It is estimated that about 80 % of women with PCOS will ovulate on CC therapy and that 50–60 % will achieve a pregnancy after six cycles of treatment [52]. However, much debate exists about the role of metformin for improving fertility outcomes in women with PCOS [47, 50, 51, 53–56].

Metformin use in the context of PCOS was found by several studies to be associated with improved menstrual cyclicity [31, 57, 58], as more regular menstrual patterns were observed compared with placebo (OR 1.72; 95 % CI 1.14–2.61; 7 trials; 427 women) [26]. Compared to OCPs, however, metformin therapy was demonstrated to be less beneficial by two RCTs (OR 0.08, 95 % CI 0.01–0.45; 2 trials; 35 women) [59, 60].

Legro et al. evaluated the rate of ovulation as a secondary outcome in a randomized controlled design in 626 infertile women with PCOS over a period of 6 months [47]. Women receiving metformin alone had significantly lower ovulation rates compared with combination CC and metformin therapy (absolute difference 31.4 %, 95 % CI 24.7–38.0). Increased ovulation rates over placebo were also reported with metformin therapy (OR 1.81, 95 % CI 1.13–2.93; 16 trials; 1,208 cycles) [26]. For one woman to achieve ovulation, the number needed to treat using metformin was estimated to be 4.0 [55]. When compared to CC therapy, metformin was found to be similar in inducing ovulation in the nonobese women (BMI <30 kg/m^2) with PCOS (OR 0.87, 95 % CI 0.60–1.26; 2 trials; 497 cycles), but much inferior in the obese PCOS population (OR 0.43, 95 % CI 0.36–0.51; 2 trials; 2,044 cycles) [26]. Combining metformin with CC therapy offered an added benefit to ovulation rates compared to CC alone treatment (OR 1.74, 95 % CI 1.50–2.001; 8 trials; 3,265 cycles) [26, 61].

While improvements in ovarian and reproductive function were also demonstrated when rosiglitzone was used in women affected with PCOS, yielding a significant increase in menstrual regularization and ovulation rates compared to placebo [36, 38, 62, 63], the earlier specified concerns relating to TZD's limit their use in the reproductive age women seeking fertility.

When BMI status was used as a discriminatory factor in a Cochrane systematic review of 44 randomized controlled clinical trials (RCTs) investigating reproductive parameters in women with PCOS on metformin treatment [26], statistical heterogeneity was significantly improved. Compared to placebo, the use of metformin alone was associated with significantly increased clinical pregnancy rates in nonobese women (BMI <30 kg/m^2) with PCOS (OR 2.35, 95 % CI 1.44–3.82; 4 trials; 413 women), but not in obese population (OR 2.21, 95 % CI 0.98–4.98; 4 trials; 294 women). Compared to CC therapy, metformin alone achieved better clinical pregnancy outcomes in nonobese women (OR 1.94, 95 % CI 1.19–3.16; 3 trials; 349 women), and detrimental outcomes in the obese (OR 0.34, 95 % CI 0.21–0.55; 2 trials; 500 women). When metformin was combined with CC treatment, the improvement in clinical pregnancy rates seemed to be confined solely to the obese women (OR 1.76, 95 % CI 1.26–2.47; 7 trials; 695 women).

It thus appears that metformin-alone therapy may be relatively more effective in modifying insulin signaling in nonobese women leading to improved reproductive endpoints. In the obese however, insulin resistance may appear to express a more severe phenotype and, therefore, may be less amenable to ISD monotherapy. Any measure of modification to the IR state in this population with metformin use may be expected to facilitate response to CC therapy [26].

Looking at live birth rates as a reproductive endpoint, the use of metformin alone (OR 1.80, 95 % CI 0.52–6.16; 3 trials; 115 women) or in combination with CC (OR 1.16, 95 % CI 0.85–1.56; 7 trials; 907 women) failed to show any benefits compared with CC alone therapy [26]. Two systematic reviews of RCTs further found insufficient evidence to establish a difference between metformin and CC in terms of live births in women with PCOS [50, 51, 61].

Metformin did not confer any protective effect against spontaneous miscarriages for pregnancies achieved during the course of treatment when compared with those conceived on no treatment (OR 0.36, 95 % CI 0.09–1.47; 3 trials; 279 women) or following CC therapy (OR 1.24, 95 % CI 0.60–2.58; 4 trials; 173 women) [26]. These findings were supported by other systematic reviews [50, 51, 61]. Contrary to previous belief, however, the findings of several clinical trials have lately demonstrated a miscarriage prevalence rate in infertile women with PCOS comparable to the general population [46, 47, 64–66]. This may explain the lack of measurable improvement in this reproductive endpoint found following metformin therapy. Previous studies indicating a two- to fivefold increased risk [53] and 58.3 % baseline prevalence [67] are now placed under critical review, because they suffered serious shortcomings, such as small sample sizes and biased PCOS phenotypes.

None of the available clinical trials reported on cost-effectiveness analyses of available drug therapies for the management of PCOS related clinical concerns.

Priming with metformin before initiating CC treatment may be of benefit although the optimal duration of pre-treatment with metformin remains poorly understood. Although several study designs reported on 4- to 12-week periods of pre-treatment with metformin [44], two studies demonstrated that ultra-short 2-week metformin pretreatment is also associated with improved ovulation and pregnancy outcomes [68, 69]. Unfortunately, there are no well-designed RCTs to determine the most appropriate duration of ISD pre-treatment for the management of subfertile women with PCOS [70].

Effects of ISDs on Reproductive Endpoints in Women with CC-Resistant PCOS

About 20 % of women with PCOS are found to be resistant to CC effect on follicular growth and development, requiring consideration for alternate means of intervention [71]. Several studies have been conducted to assess the effectiveness of using ISDs in women with CC resistance [9, 12, 71–74]. A meta-analysis of RCTs [26] recently demonstrated a significant improvement in ovulation rates in these women when metformin was used as a co-treatment (OR 4.86, 95 % CI 2.43–9.74; 5 trials; 179 cycles). Pioglitazone was also found to improve pregnancy rates in women with CC-resistant PCOS [75]. Short-term treatment with rosiglitazone was shown to be effective in inducing ovulation in CC-resistant women with the obese phenotype of the syndrome [76]. These findings seem biologically reasonable because they imply that the amelioration in the metabolic milieu by ISDs improves the likelihood of CC-resistant women to respond to ovulation-induction agents.

Another method of inducing ovulation in women with PCOS is “laparoscopic ovarian drilling” (LOD), which has shown to be effective in CC-resistant women in meeting reproductive endpoints; a particular advantage of LOD lies in reducing multiple pregnancy risk that is well recognized with the use of medical strategies for achieving ovulation induction [77–79]. LOD nevertheless is an invasive procedure

that has been associated with post-surgical pelvic adhesions and reduced ovarian reserve [77], generating serious concerns regarding its use as a primary ovulation-induction tool in women with PCOS. Results on menstrual cycle regularity, ovulation, and pregnancy rates were comparable when metformin/CC combination treatment was used compared with LOD [78, 79]. The findings of a recent Cochrane systematic review [80] in which 26 RCTs involving women with CC-resistant PCOS were analyzed demonstrated a significant improvement in live births when the combination treatment was used compared to LOD (OR 0.44, 95 % CI 0.24–0.82; 2 trials; 159 women). There were no differences in miscarriages (OR 1.43, 95 % CI 0.70–2.91; 3 trials; 441 women) and multiple pregnancies (OR 0.1, 95 % CI 0.01–1.94; 3 trials; 441 women) between both groups. A cost analysis further showed that metformin/CC combination treatment was significantly less expensive than LOD when used over a 6-month period (50 EUR versus 1,050 EUR; $P < 0.05$) [77].

Although LOD was shown to be successful in improving fertility outcomes in women with CC-resistant PCOS, metformin/CC combination treatment appears to offer an equally efficacious approach with the added advantages of being less invasive and less costly.

An entire chapter in this text addresses surgical approach to achieving ovulation in further depth.

Effects of ISDs on Reproductive Endpoints in Women with PCOS Undergoing In Vitro Fertilization and Intracytoplasmic Sperm Injection Cycles

Experience with in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles identifies that women with PCOS may experience longer treatment durations, yield larger number of oocytes, express higher serum estradiol levels, and are at a particularly increased risk for ovarian hyperstimulation syndrome (OHSS) compared to those with other etiologies for infertility; lower conception rates following embryo transfer have been suggested for women with PCOS undergoing IVF [81].

In women with PCOS undergoing assisted reproductive techniques (ART), the use of ISDs, such as metformin has been evaluated by several studies [5]. The plausible working hypothesis was that suppression of hyperinsulinemia and hyperandrogenism may prove beneficial in improving ovarian response, enhance pregnancy endpoints, and reduce adverse effects [5]. A Cochrane systematic review [82] analyzing data from six RCTs found no evidence that metformin co-treatment with IVF/ICSI cycles reduced either the mean gonadotropin dose (MD 193.20, 95 % CI −33.49 to 419.88; 6 trials; 465 cycles) or the number of stimulation days (MD 0.03, 95 % CI −0.32 to 0.37; 6 trials; 465 cycles), compared to placebo. In addition, metformin use failed to reduce significantly the number of oocytes retrieved (MD −0.13, 95 % CI −1.43 to 1.17; 6 trials; 457 cycles) and the peak serum estradiol levels (MD 3.51, 95 % CI −0.18 to 7.19; 6 trials; 346 cycles). When reproductive endpoints were evaluated, metformin treatment did not improve clinical

pregnancies (OR 0.71, 95 % CI 0.39–1.28; 5 trials; 426 cycles), miscarriages (OR 0.84, 95 % CI 0.40–1.75; 4 trials; 589 cycles), or live births (OR 0.77, 95 % CI 0.27–2.18; 3 trials; 272 cycles).

While metformin use during IVF/ICSI treatments did not impact on fertility related end points including cycle outcome, a significant reduction in the risk of developing OHSS however was demonstrated with metformin therapy (OR 0.27, 95 % CI 0.16–0.47; 5 trials; 449 cycles) [82]. Reasons for this protective effect are unclear but are seemingly independent of the total number of oocytes retrieved and/or peak estradiol serum levels achieved.

Effects of ISDs on Endocrine and Clinical Endpoints in Women with Symptomatic PCOS-Related Androgen Excess

The androgen excess state appears to play a central role in determining the PCOS phenotype, as affected women often present with the manifestation spectrum of acne and hirsutism [83]. The oral contraceptive pill (OCP) has traditionally constituted the main pharmacologic management approach to symptoms of hyperandrogenism in women with PCOS. Since about 15–20 % of women on OCPs suffer significant side effects [84], alternative pharmacologic therapies have been sought. Given the established role of hyperinsulinemia in the development of hyperandrogenism, it seemed plausible to explore the role of ISDs in improving the clinical symptoms of androgen excess in the affected women [83, 85].

Several clinical trials evaluated the effects of metformin on the endocrine milieu of PCOS. Compared with placebo, the use of metformin was found to be significantly more effective in reducing total serum testosterone levels (MD −0.60 nmol/L, 95 % CI −0.73 to −0.48; 14 RCTs; 610 women) [26], albeit without improving serum sex hormone binding globulin (SHBG) levels (MD −0.05, 95 % CI −2.33 to 2.24; 15 trials; 626 women). Compared with OCP use however, metformin alone was significantly less effective in reducing total serum testosterone levels (MD 0.54 nmol/L, 95 % CI 0.22–0.86; 3 trials; 69 women) and the free androgen index (MD 3.69 %, 95 % CI 2.56–4.83; 3 trials; 69 women) [86]. The addition of metformin to OCPs did not yield any additional benefit for total serum testosterone levels (MD 0.05 nmol/L, 95 % CI −0.80 to 0.89; 2 trials; 68 women) [87, 88].

High-quality research aimed at investigating the effects of ISDs on improving hirsutism and acne in women with PCOS is scarce. A metaanalysis demonstrated no difference in effect between metformin and OCPs in improving severity of hirsutism as assessed by Ferriman-Gallway (FG) and/or visual analogue scores in the affected women (MD −0.18, 95 % CI −0.67 to 0.32; 3 trials; 69 women) [86]. These findings concur with the results of a more recent systematic review (MD −0.5, 95 % CI −5.0 to 3.9; 5 trials) [89], which also found no difference when metformin was used in combination with the anti-androgen flutamide versus flutamide alone (MD 0.9; 95 % CI −0.4 to 2.2; 2 trials) [89]. One study, however, showed a significant reduction in FG hirsutism scores when metformin was combined with OCPs compared to OCP alone therapy (MD −2.82, 95 % CI −5.47 to −0.17) [88].

A single randomized controlled study has evaluated the effect of ISDs on acne in women with PCOS [83] and demonstrated no difference in subjective acne scores between metformin and OCP use (WMD 0.90, 95 % CI −0.40 to 2.20; 34 women).

Effects of ISDs on Metabolic Endpoints in Women with PCOS

Insulin resistance is now recognized as a key player in the pathogenesis of PCOS, and the affected women are deemed at risk for developing T2DM [30, 33]. Women with impaired glucose tolerance (IGT) at baseline are particularly at risk, as 59 % of them are expected to develop T2DM [90] over a period of 6 years, a conversion rate of 8.7 % per annum [90]. Obesity confers an estimated tenfold increase in the risk of developing this metabolic disorder. Metformin treatment was shown to reduce significantly fasting insulin levels in nonobese women with the syndrome (BMI <30 kg/m^2) (MD −5.65 mIU/L, 95 % CI −10.25 to −1.06; 4 trials; 85 women), but not in the obese PCOS population (BMI ≥30 kg/m^2) (MD −2.72 mIU/L, 95 % CI −6.50 to 1.05; 12 trials; 488 women) [26]. Compared with placebo, while the effect of metformin on fasting glucose levels was also statistically significant, the difference was deemed as of limited clinical relevance (MD −0.15 mmol/L, 95 % CI −0.25 to −0.06; 14 trials; 596 women). The Diabetes Prevention Program Research group reported on a lower incidence of T2DM in persons of both genders following metformin (7.8 cases per 100 person years) and lifestyle intervention therapy (4.8 cases per 100 person years) compared with placebo [91]. However, lifestyle intervention was associated with significantly better and more sustainable weight reduction compared to metformin [91]. One RCT evaluated the development of T2DM in women with PCOS and found no differences when metformin was used compared with OCPs (OR 0.17, 95 % CI 0.00–8.54; 18 women) [59]. Based on this limited body of data, the place for long-term prophylactic treatment with metformin in preventing progression to T2DM in women with PCOS is unclear.

Hypertension has also been described to be associated with PCOS [92]. Metformin was shown to reduce systolic blood pressure (MD −3.59 mmHg, 95 % CI −5.13 to −2.04; 7 trials; 379 women) but did not affect diastolic blood pressure (MD −0.14 mmHg, 95 % CI −1.35 to 1.07; 6 trials; 292 women) in treated women [26]. Metformin use was shown to have no effect on cholesterol (MD −0.14 mmol/L, 95 % CI −0.31 to 0.02; 10 trials; 562 women) and triglyceride levels (MD 0.14 mmol/L, 95 % CI −0.05 to 0.32; 7 trials; 309 women) [26].

Numerous markers of cardiovascular disease (CVD) risk are identified to be elevated in women with PCOS, such as serum levels of C-reactive protein (CRP) and homocysteine [93], in addition to increased carotid intima-media thickness [94]. The prevalence of metabolic syndrome among women with PCOS is estimated to be about 43 % [95], projecting a cluster of additional risk factors for CVD in this population [96]. Epidemiologic studies, although limited, have however failed to demonstrate evidence for an increased incidence of cardiac events in reproductive age women with PCOS [97]. Although metformin use is suggested to reduce the risk of microangiopathy and macroangiopathy among patients with T2DM [98] and

to reverse impaired endothelial function among women with PCOS following 6 months treatment [99], no RCTs are available to evaluate impact of metformin use on the outcome measures of stroke and myocardial infarction in women with PCOS.

Chronic annovulation, and hence lack of progesterone exposure, as well as hyperinsulinemia are suggested mechanisms for the increased risk for endometrial cancer in women with PCOS [100]. While in vitro data suggest that metformin therapy may offer protection against the development of endometrial cancer [101], unfortunately, data on the risks of endometrial cancer in women with PCOS who have been placed on metformin therapy are currently nonexistent.

There is little doubt that the management of PCOS in women should extend well beyond the immediate reproductive needs to address the metabolic concerns that hold far-reaching adverse health implications for these women [25, 33]. A systematic review, however, found insufficient data to make any conclusions on the relative efficacy of ISDs for long-term prevention of diabetes mellitus, cardiovascular disease, and endometrial cancer in women with PCOS [86]. Long term data supporting far reaching benefits of lifestyle interventions however do exist, and underscore a need for sustaining efforts at achieving and maintaining healthier life style in women with PCOS given their risk profile for myriad health hazards.

Summary and Implications for Practice

- For women with PCOS in general, data supporting a role of ISD's for prevention of T2DM, cardiovascular disease, hypertension, or endometrial cancer are sparse to nonexistent.
- For the management of ovulatory infertility in the nonobese PCOS population (BMI <30 kg/m^2), there is moderate-quality evidence demonstrating that metformin monotherapy improves the odds of ovulation and chance of achieving clinical pregnancy [26].
- There is moderate-quality evidence demonstrating the absence of reproductive benefit when metformin is combined with CC therapy in the nonobese population of women with PCOS-related subfertility. CC-alone therapy remains the mainstay pharmacological therapy for this group.
- For obese women (BMI ≥30 kg/m^2) with PCOS-associated subfertility, there is low-quality evidence showing the failure of metformin monotherapy to improve reproductive endpoints [26]. In view of the considerable side effect profile, metformin monotherapy may not be recommended for fertility management in this group of women. There is moderate-quality evidence to support a beneficial effect of metformin in combination with CC therapy in increasing the likelihood of ovulation and clinical pregnancies [26].
- For women with CC-resistant subfertility, there is moderate-quality evidence to support that metformin co-treatment increases ovulation rates [26]. There is also low-quality evidence demonstrating that metformin/CC combination therapy may be associated with higher live births than laparoscopic ovarian drilling [80]. Women with CC-resistant PCOS may be given the benefit of a trial of medical

ovulation induction using combination therapy prior to committing to the more invasive and expensive alternative of LOD.

- For women with PCOS undergoing IVF/ICSI treatments, there is moderate evidence to support the failure of metformin co-administration to improve the clinical outcomes of live births, clinical pregnancies, or miscarriages. There is moderate evidence demonstrating a significant reduction in the risk of OHSS with metformin co-treatment, when hCG is used to trigger final oocyte maturation [82].
- For women with symptomatic PCOS-related androgen excess, there is limited evidence available to support that metformin monotherapy improves hirsutism and acne, compared with more established anti-androgenic drugs. There is insufficient evidence demonstrating that the addition of metformin to the OCP is more effective than OCP alone in improving hirsutism or acne [86].

It should be emphasized that the strengths of the conclusions in this chapter remain limited by severe weaknesses related to the quality of existing evidence. Despite the availability of numerous meta-analyses, most suffered from major heterogeneity within studies and inclusion of poorly powered clinical trials. Differences in ethnic backgrounds, geographic locations, diagnostic criteria for PCOS, presence of infertility confounders, and influence of fertility co-treatments, inconsistencies in methodology of ISD administration, outcome endpoints, study design, and varying follow-up periods are some of the factors identified as source of heterogeneity within existing data. The suggested recommendations were based on the current state of evidence and readers are encouraged to stay attuned to accruing data on the subject.

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Chapter 11
Role of Statins in PCOS Management

Israel Ortega and Antoni J. Duleba

Key Points

- Ovaries of women with PCOS are characterized by hyperplasia of theca cells.
- In PCOS, theca cells produce excessive amounts of androgens.
- PCOS is associated with increased oxidative stress and systemic inflammation.
- Statins are inhibitors of the mevalonate pathway, and possess antioxidant and anti-inflammatory properties.
- Statins reduce proliferation and increase apoptosis of ovarian theca cells, and inhibit androgen production by theca cells by reducing expression of the CYP17A1 gene.
- In women with PCOS, statins reduce androgen levels, decrease ovarian size and improve menstrual cyclicity.

Introduction

The use of statins as therapy for polycystic ovary syndrome (PCOS) is still experimental. In this chapter we will present the rationale as well as in vitro and clinical evidence in support for considering statins in the management of PCOS. Furthermore, we will discuss limitations and risks of using statins and, finally, we will speculate on future directions of research on the use of statins in PCOS.

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In order to justify the concept that statins may be useful in the management of PCOS, we will first review the key features of this syndrome and the mechanisms of action of statins. PCOS is the most common endocrine disorder, affecting 5–10 % of women of reproductive age [1–3], although the prevalence of PCOS in overweight and obese women is considerably higher, reaching almost 30 % [4]. Women with PCOS present a variety of clinical signs and symptoms, including menstrual dysfunction, features of androgen excess, and infertility. Insulin resistance with resulting hyperinsulinemia is also common among women with PCOS, along with increased risk for dyslipidemia, hypertension, diabetes, and related cardiovascular consequences [5]. In addition, prolonged anovulation in this population represents a risk factor for the development of endometrial pathologies including endometrial cancer [6].

Since the description of PCOS by Stein and Leventhal in 1935, several different sets of diagnostic criteria for PCOS have been considered, including those proposed by National Institutes of Health 1990 conference [7], the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine [8], and the Androgen Excess/PCOS Society [9]. All these definitions include at least two of the following three criteria: hyperandrogenism, oligo or anovulation, and/or polycystic ovarian morphology with exclusion of other diagnoses, such as congenital adrenal hyperplasia, nonclassic adrenal hyperplasia, androgen-secreting tumor, Cushing syndrome, hyperprolactinemia, and thyroid disorders.

Androgen Excess and Polycystic Ovarian Morphology

Androgen excess, represented by biochemical and/or clinical hyperandrogenism, is the central defect of PCOS and is present in up to 90 % of women with PCOS [10]. Clinical manifestations of hyperandrogenism include hirsutism, male-pattern balding, and acne. Although the ovary represents the main source of androgens, adrenal hyperandrogenism is also recognized in PCOS patients [11]; however, neither the mechanisms nor magnitude of adrenal contribution to the androgen excess of PCOS are clearly understood [12].

While the etiology of ovarian hyperandrogenism is still disputed, inherent steroidogenic defects in PCOS theca cells, abnormal stimulation of theca cells (e.g., by insulin, growth factors, oxidative stress), and/or excessive number of theca cells are suggested mechanisms. In vitro studies indicate that the enhanced steroidogenic potential of PCOS theca cells is associated with increased mRNA expression and activity of the main enzymes involved in androgen biosynthesis, such as side-chain cleavage enzyme (P450scc, encoded by *CYP11A1*), 3-beta-hydroxysteroid dehydrogenase type 2 (3-β-HSD, encoded by *HSD3B2*) and 17α (alpha)-hydroxylase/17,20-lyase (P450c17, encoded by *CYP17A1*) [13–15]. In particular, enhanced P450c17 enzyme activity and expression may account for hyperandrogenism in PCOS [16]. In addition, dysregulation of several signal-transduction pathways has been shown to play a role in androgen excess in PCOS women. For example, inhibition of mitogen-activated protein kinase kinase 1 (MAPK1) signaling has been demonstrated in cultures of PCOS theca cells, and seems to mediate the action on theca androgen production [16, 17].

The etiology of androgen excess in PCOS women is likely multifactorial and may involve both extraovarian and intraovarian factors. First, impaired gonadotropin dynamics may play a role in excessive androgen production in PCOS. Increased luteinizing hormone (LH) pulse frequency and amplitude may directly increase androgen synthesis, whereas relatively reduced follicle-stimulating hormone (FSH) levels, by virtue of lesser stimulation of aromatase, may contribute to decreased conversion of androgen to estrogen, and hence worsen ovarian androgen excess [18]. Furthermore, increased levels of insulin and bioavailable insulin-like growth factor I (IGF-1) may stimulate theca steroidogenesis, by inducing expression of key enzymes involved in androgen synthesis as well as by promoting proliferation and reducing apoptosis of theca-interstitial cells [19]. In addition, insulin further contributes to hyperandrogenemia in PCOS by stimulating adrenal androgen production and by inhibiting hepatic production of sex hormone-binding globulin (SHBG), which in turn leads to an increase in bioavailable androgens [20, 21]. Several intraovarian paracrine factors may also play a role in ovarian androgen excess. For example, Anti-Müllerian hormone (AMH) has an inhibitory role during folliculogenesis and thus may indirectly increase androgen production by inhibiting FSH action and/or by blocking aromatase activity [22]. Other intraovarian factors, such as activin and inhibin, may also affect theca steroidogenesis and proliferation [23, 24].

Polycystic ovarian (PCO) morphology is a prominent feature of the disorder and has been defined as the presence of 12 or more follicles of 2–9 mm and/or an ovarian volume of more than 10 mL [8]. However, this finding is not unique to PCOS as PCO morphology is observed in 20 % of regularly cycling women who do not meet other criteria of PCOS. Conversely, not all PCOS women show PCO morphology [25]. The polycystic appearance of the ovaries is reported to hold prognostic relevance in relation to risk of endometrial carcinoma, and in selection of an appropriate dose of medications during ovulation induction for infertility management. Pillay et al. demonstrated an association between PCO morphology and endometrial cancer in premenopausal women [26]. Additionally, PCO are closely related to increased incidence of both multiple gestation and ovarian hyperstimulation syndrome (OHSS) in women undergoing ovulation induction therapy. The rate of multiple pregnancy in women with PCO undergoing ovulation induction with gonadotropins may be as high as 36 % [27]. A meta-analysis published by Tummon et al. reported an almost sevenfold increased risk for the development of OHSS among women with PCO compared to controls with normal ovarian morphology [28].

The above findings underscore the need to develop therapy of hyperandrogenism in PCOS that may address excessive androgen production by theca cells as well as excessive growth of ovaries (especially the thecal compartment).

Metabolic Consequences of PCOS

Another important aspect of PCOS involves alterations in metabolic status and associated increase in risk for type 2 diabetes (T2DM) and cardiovascular detriment. Women with PCOS are more likely to develop metabolic syndrome, including disturbances of carbohydrate metabolism, obesity, dyslipidemia, and

hypertension. According to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP-III) guidelines, metabolic syndrome in women is defined as the presence of at least three of the following criteria: (1) serum fasting glucose >100 mg/dL (5.6 mmol/L); (2) waist circumference >88 cm; (3) serum triglycerides >150 mg/dL (1.7 mmol/L); (4) serum HDL cholesterol <50 mg/dL (1.3 mmol/L); (5) blood pressure >130/85 mmHg [29]. The prevalence of the metabolic syndrome has been reported in 43 % of women with PCOS, which is twofold higher than that for age-matched controls [30].

Insulin resistance is a common feature of PCOS observed in the majority of obese and in many non-obese women with PCOS [31]. The molecular basis for insulin resistance, though not yet fully understood, may be related to a post-receptor defect involving reactive oxygen species (ROS)-mediated phosphorylation of the insulin receptor substrates 1 and 2 (IRS-1, IRS-2), which leads to an abrogation of insulin signaling via its receptor [32, 33]. Among women with PCOS, 30 % have impaired glucose tolerance (IGT) and an additional 7.5 % meet criteria for type 2 diabetes [34]. In view of these observations, the Androgen Excess Society recently issued a position statement urging providers to screen all PCOS patients for impaired glucose tolerance using a 2-h oral glucose tolerance test at least once every 2 years [35]. Insulin resistance is worsened by the coexistence of obesity, which is also increased in the PCOS population and exacerbates several other metabolic abnormalities in these women. Obese women with PCOS have greater insulin resistance than weight-matched controls or lean PCOS subjects [36, 37].

In the long term, PCOS women are at high risk of dyslipidemia, leading to increased cardiovascular risk and possibly cardiovascular disease and even mortality [38]. The pattern of dyslipidemia in women with PCOS includes elevated total cholesterol levels, low-density lipoproteins (LDLs), very LDLs (VLDLs), and triglycerides with concomitantly reduced levels of high-density lipoproteins (HDLs) [39]. At least one lipid abnormality is seen in up to 70 % of women with PCOS [40]. These derangements in the lipid profile correlate with the hyperinsulinemia and hyperandrogenemia of PCOS, and treatment of these conditions may improve the lipid abnormalities and thus mitigate the associated clinical implications [41].

Endothelial structure and function is impaired even among young patients with PCOS without additional risk factors for cardiovascular disease [42]. A recent meta-analysis showed that carotid intima-media thickness (CIMT), a marker of subclinical atherosclerosis, is higher in women with PCOS compared with control subjects [43]. In addition, homocysteine levels are also higher in women with PCOS, representing another independent risk factor for cardiovascular disease by inducing cytotoxic effects on vascular endothelium via mechanisms involving increased inflammatory cytokine expression, altered nitric oxide bioavailability, and induction of oxidative stress [44, 45]. Other markers of endothelial function, such as endothelin-1, are also altered in PCOS women [42]. This impairment of vascular endothelium and increased markers of atherosclerosis may have relevant clinical implications. Indeed, women with PCOS have increased day-time blood pressure after adjusting for BMI, body fat distribution, and insulin resistance, which may indicate a pre-hypertensive state and thus a further risk factor for cardiovascular disease in these women [46].

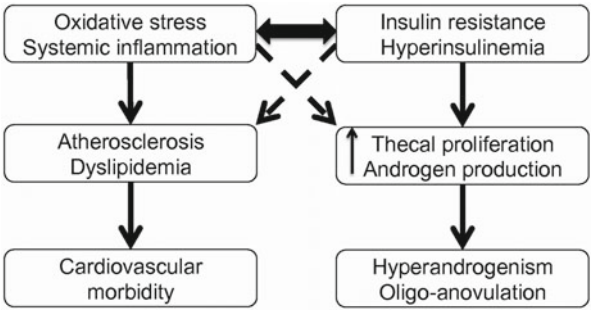

Fig. 11.1 Proposed pathophysiology and sequelae of polycystic ovary syndrome. Oxidative stress, systemic inflammation, and insulin resistance are interrelated processes involved in stimulation of theca-interstitial cell compartment and endothelial dysfunction. Cardiovascular disorders, hyperandrogenism, and anovulation are final consequences

In view of the above findings, it is apparent that comprehensive management of PCOS should include reduction of long-term cardiovascular risks.

Oxidative Stress and Systemic Inflammation in PCOS

Oxidative stress leads to molecular damage to cellular and tissue structures and is increased in patients with diabetes, cancer, and cardiovascular disease [47–49]. Women with PCOS exhibit elevation in several biomarkers of oxidative stress, including malondialdehyde (MDA; a marker of lipid peroxidation), superoxide dismutase (SOD) activity, and protein carbonyl content [49, 50]. Furthermore, reduced glutathione and decreased level of haptoglobin, a protein with antioxidant properties are described in women with PCOS suggesting a decreased antioxidant reserve in this population [49, 51, 52].

Oxidative stress may aggravate androgen excess in PCOS via at least two mechanisms (Fig. 11.1). First, modest oxidative stress induced by hypoxanthine and xanthine oxidase stimulates proliferation of theca-interstitial cells [39] and may thus contribute to hyperplasia of ovarian theca and stroma. Second, oxidative stress impairs insulin signaling resulting in compensatory hyperinsulinemia, which, in turn, further stimulates theca-interstitial cell steroidogenesis [53].

Increased oxidative stress and decreased antioxidant capacity may also contribute to the increased risk of cardiovascular disease in women with PCOS [49]. For example, advanced glycation end products (AGEs), the products of non-enzymatic glycation and oxidation of proteins and lipids, activate the production of endothelin-1 (ET-1), a peptide that causes endothelial dysfunction and cardiovascular sequelae, in women with PCOS [54]. Furthermore, oxidative stress and chronic inflammation are closely interrelated; indeed, growing evidence supports the concept of a vicious circle, whereby inflammation generates ROS, while oxidative stress promotes inflammation [55]. Such a vicious circle has been reported in the endothelium and in adipose tissues [56]. Notably, elevated C-reactive protein (CRP), a marker of

systemic inflammation, is commonly encountered in women with PCOS; CRP is a risk factor for cardiovascular events and correlates with increasing plaque vulnerability and propensity to thrombosis [57]. Additionally, other markers of low-grade chronic inflammation, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), have been associated with the pathophysiology of atherosclerosis, have been related to PCOS [58].

Current Treatments and Their Limitations

Ideally, therapy of PCOS should reduce hyperandrogenism, normalize ovarian physiology and morphology as well as improve metabolic profile and reduce cardiovascular risks. However, despite a growing number of studies evaluating pharmacological interventions in PCOS, currently available treatments are not fully satisfactory.

Metformin is a biguanide that reduces hepatic gluconeogenesis, improves glucose uptake by the peripheral tissues, and increases fatty acid oxidation [59]. Despite its overall favorable effect on hyperandrogenism [60], systemic inflammation, and on the endocrine profile [61], recent clinical trials failed to demonstrate a significant improvement of metabolic function [62, 63]. Thiazolidinediones improve insulin sensitivity in the peripheral tissues and exert beneficial effects on blood pressure, endothelial function, and systemic inflammation [63]. However, thiazolidinediones are associated with weight gain, and their effects on dyslipidemia are disappointing [64]; furthermore, recent evidence reveals an adverse long-term risk profile of these medications. Acarbose is a complex oligosaccharide inhibiting alpha-glucosidase and, hence, decreasing digestion of polysaccharides, reducing glucose absorption, and lowering postprandial insulin levels [64]. Clinical data about the effect of acarbose on metabolic profile in women with PCOS are scarce and controversial [65, 66]. Naltrexone, a competitive nonselective antagonist of opioid receptors, reduces appetite and exerts profound effects on insulin release [67]. Other adjuvant treatments, such as orlistat and vitamin D, improve insulin sensitivity, but their effects on lipid profiles still remain controversial [68, 69].

In conclusion, current treatments available for the management of PCOS have significant limitations, and there is an urgent need for more effective therapies aimed at the broad spectrum of endocrine and metabolic aspects of PCOS. The following sections will discuss how statins may fulfill these requirements. To this end, we shall review the mechanism of action of statins and subsequently will discuss how these actions may ultimately benefit women with PCOS.

Mechanisms of Action of Statins

Statins are competitive and reversible inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the mevalonate pathway (Fig. 11.2) [70]. Statins are effective in improving lipid profile and

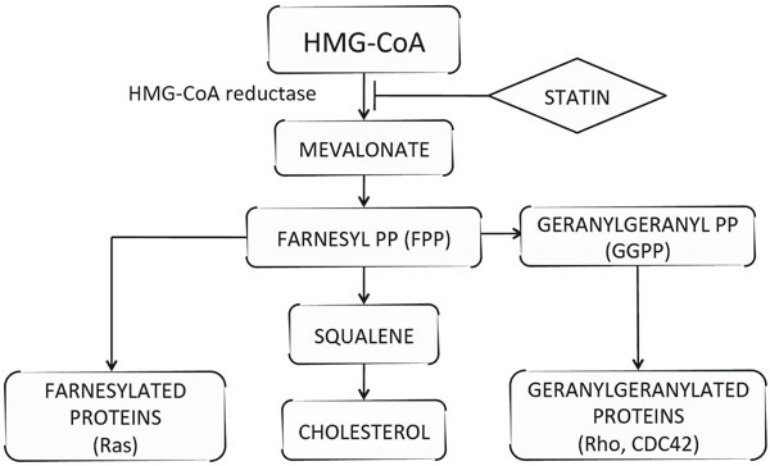

Fig. 11.2 Outline of the mevalonate pathway and its major products. Statins competitively inhibit HMG-CoA reductase, the rate-limiting step of cholesterol synthesis. FPP and GGPP are the intermediate downstream products of mevalonic acid, involved in farnesylation and geranylgeranylation of proteins, respectively. Adapted from [102]

reducing risk of cardiovascular diseases, being the first-line treatment of choice in patients with elevated cholesterol and those diagnosed with coronary heart disease [71]. In addition to their cholesterol-lowering effect, statins also exert many other potentially beneficial effects, including improvement of nitric oxide-mediated endothelial function as well as exhibit anti-inflammatory and anti-proliferative actions [72, 73].

The mevalonate pathway is responsible for the synthesis of cholesterol and other important biomolecules such as coenzyme Q, dolichols, and isoprenoids; these biomolecules are involved in several cell functions, such as membrane integrity, protein isoprenylation, and energy homeostasis [74]. Inhibition of this pathway leads to decreased production of several biologically active downstream products, including cholesterol and substrates of isoprenylation (farnesyl pyrophosphate [FPP] and geranylgeranyl pyrophosphate [GGPP]) [75].

Isoprenylation consists of the covalent addition of two types of isoprenoids: FPP (farnesylation) or GGPP (geranylgeranylation) to cysteine residues at or near the carboxyl terminus of many proteins, especially small G proteins, facilitating their membrane attachment and function [76]. These proteins are monomeric G proteins with molecular masses of 20–40 kDa [77], and are referred to as "small GTPases." Particularly relevant small GTPases are members of the Ras superfamily, such as Ras, Rho, Rac, and Cdc42; their function depends on their association with the cytoplasmic leaflet of cellular membranes, a process that requires farnesylation of Ras and geranylgeranylation of Rho, Rac, and Cdc42. These proteins are involved in many key cellular functions, such as cell growth, differentiation, cytoskeletal function, and vesicle trafficking [78–80]. Hence, statins, by reducing the synthesis of isoprenoids, may alter a wide range of cellular functions.

Interestingly, crosstalk between the insulin signaling pathway and the mevalonate pathway has been reported. Insulin stimulates the phosphorylation and activation of farnesyl transferase and geranylgeranyltransferases I and II [81, 82], increasing the isoprenylation of Ras and other small GTPases. This elicits phosphorylation of the MAPK cascade through activation of the Ras-Raf-Erk1/2 signaling pathway, leading to mitogenic responses [83]. In addition, oxidative stress shares the same signal transduction pathways with insulin and IGF-1, specifically the Erk1/2 and mTOR/p70s6k pathways [84, 85]. A convergence of the actions of insulin/IGF-1 and ROS at the Erk1/2 and mTOR/p70s6K pathways may explain the similar effects of these compounds on cell growth [19].

Isoprenylation also plays a role in oxidative stress, as isoprenylated proteins affect generation of ROS by NADPH oxidase. This multicomponent electron transport system consists of two known membrane-bound components, gp91-phox and p22-phox, and two other well-characterized oxidase components, p47-phox and p67-phox, both found in the cytosol fraction. Previously, isoprenylated Rac1 and Rac2, members of the Rho family, have been implicated in the regulation and assembly of the components of NADPH oxidase [86]. Therefore, disruption of isoprenylation may lead to cellular disturbances, owing to a decreased generation of intracellular ROS.

In Vitro Studies of Statins

In vitro, statins exert anti proliferative actions on different tissues and cell types, such as vascular smooth muscle cells [87], thyroid cells [88], and cardiomyocytes [89], as well as on a broad range of cancer cell types, including melanoma [90], meningioma cells [91], lung and breast cancer cells [92, 93]. In the ovary, statins inhibit the proliferation of rat theca-interstitial cells. Initial studies on rat cells have shown that the statin mevastatin induces a concentration-dependent inhibition of theca-interstitial cell DNA synthesis as assessed by radiolabeled thymidine incorporation assay [94, 95]. This inhibition was observed in the presence and in the absence of serum, suggesting that decreased supply of cholesterol is unlikely to be the primary mechanism of the mevastatin-induced block of proliferation. Furthermore, inhibitory effects of mevastatin were partly abrogated by mevalonic acid, indicating that mevastatin-induced inhibition of theca-interstitial cell proliferation may be due to decreased isoprenylation [95]. Similarly, another statin, simvastatin, induced an inhibition of theca-interstitial cell DNA synthesis, and this effect was abrogated by FPP and GGPP but not by squalene or cholesterol. In addition, direct inhibitors of farnesyl transferase and geranylgeranyl transferase reduced cell proliferation [96]. Thus, these findings indicate that statins inhibit theca-interstitial cell proliferation, at least in part, by reduction of isoprenylation.

The above inhibitory effects of statins on cell proliferation were verified in human theca-interstitial cells [97]. Both mevastatin and simvastatin induced an inhibition of DNA synthesis in cells obtained from women with and without PCOS; these effects

were observed both in the absence and in the presence of 22-hydroxycholesterol, indicating that statins inhibit proliferation of human theca-interstitial cells irrespective of the availability of cholesterol in normal and PCOS ovaries.

In addition to the inhibition of cell proliferation, statins also reduce steroidogenesis. Several reports have demonstrated the statin-induced inhibition of steroid production in steroidogenic cells, such as Leydig cells [98] and adrenocortical cells [99]. Mevastatin has been shown to inhibit the production of progesterone and testosterone in cultures of rat theca-interstitial cells [94]. In a recent study, simvastatin decreased androgen levels in a time- and concentration-dependent fashion, and this inhibitory effect correlated with a decrease in *Cyp17a1* mRNA levels, the gene encoding the key enzyme regulating androgen biosynthesis [100]. The addition of 22-hydroxycholesterol increased androgen levels as well as *Cyp17a1* mRNA expression and abrogated the inhibitory effects of simvastatin. Furthermore, the addition of substrates of isoprenylation (GGPP and FPP) significantly reduced simvastatin-induced suppression of *Cyp17a1* mRNA expression and androgen levels. Thus, the inhibitory actions of simvastatin on androgen production may be mediated by several mechanisms related to the inhibition of HMG-CoA reductase and, hence, a decreased production of several biologically active downstream products of the mevalonate pathway, including cholesterol and substrates of isoprenylation.

The total amount of steroids secreted by the ovary depends on two factors: the total number of steroid producing cells in the ovary and the steroidogenic capacity of individual cells. Statins inhibit both proliferation and steroidogenesis of theca-interstitial cells and consequently may be relevant to treatment of conditions associated with thecal hyperplasia and hyperandrogenism, such as seen in PCOS.

Clinical Studies

Over the past decade, several studies evaluated the effects of statins on clinical, endocrine and metabolic profiles of women with PCOS (Fig. 11.3). The first prospective, randomized clinical trial demonstrating the inhibitory effect of a statin on androgens in women with PCOS was published in 2006 [101]. In this study, patients were randomly allocated to a Statin Group (simvastatin, 20 mg daily plus oral contraceptive pill [OCP]; $n=24$) or an OCP Group (OCP alone; $n=24$). After 12 weeks of treatment, serum testosterone levels decreased by 41 % in the Statin Group and by 14 % in the OCP Group. Furthermore, there was a greater decline of LH in the Statin Group compared to Control Group (43 % vs. 9 %), resulting in a greater decrease of LH/FSH ratio (44 % vs. 12 %). In several studies, simvastatin also improved clinical hyperandrogenism, characterized by both hirsutism and acne [62, 101, 102].

This inhibitory effect of statins on androgen production is in agreement with previous clinical trials reporting decreased testosterone levels in men using statins [103, 104], and has been confirmed by recent studies on PCOS patients using other statins [105]. For example, Kaya et al. carried out a randomized clinical trial in PCOS patients comparing effects of two statins, simvastatin and atorvastatin, on

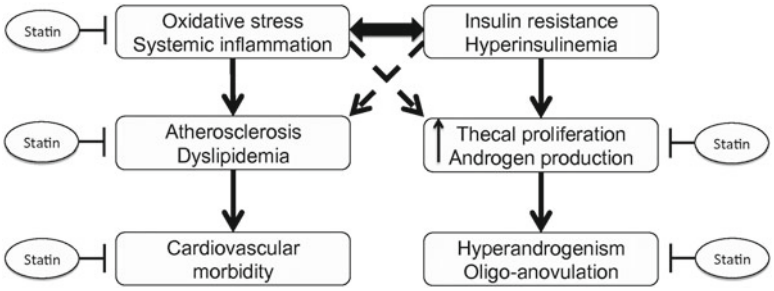

Fig. 11.3 Rationale for the use of HMG-CoA reductase inhibitors (statins) for the treatment of polycystic ovary syndrome. *Upper arrow* indicates increased; *solid line* indicates established cause and effect; *dashed line* indicates proposed pathway

hyperandrogenism and other clinical/metabolic aspects; although both statins were effective in reducing hyperandrogenemia, simvastatin had a greater effect on decreasing the level of testosterone in PCOS women [106]. Furthermore, metformin-induced inhibition of ovarian steroidogenesis has been shown to be potentiated by a combined therapy using atorvastatin in conjunction with metformin [107]. In another study, comparable and significant decreases of total testosterone and free-testosterone levels were observed following treatment with simvastatin or treatment with metformin; however, a significant decrease of DHEAS was observed only following treatment with simvastatin [62].

It should be noted that the statin-induced improvement of ovarian function appears not to be mediated by improved insulin sensitivity as determined by fasting and post-glucose challenge levels of insulin and glucose. Indeed, simvastatin had no significant effect on fasting insulin and glucose levels as well as the fasting measure of insulin sensitivity (QUICKI) [62, 101]. Nonetheless, these observations should be interpreted with caution since another statin, atorvastatin at a dose of 20 mg daily for 12 weeks improved insulin resistance measured by homeostasis model assessment for insulin resistance (HOMA-IR) by 20 % compared to a Control Group, and this effect correlated with the reduction in free-androgen index (FAI) [105]. Further long-term studies comparing different statins are needed to resolve this issue.

The effects of statins on lipid profile are consistent and highly significant. In the first report evaluating simvastatin plus OCP (Statin Group) in comparison to OCP alone (OCP Group) in women with PCOS, total cholesterol declined by 10 % in the Statin Group, whereas in the OCP Group it increased by 8 % [101]. Low-density lipoprotein cholesterol levels decreased in the Statin Group by 24 % but not in the OCP Group, whereas high-density lipoprotein cholesterol levels increased by 9 % in the Statin Group and by 13 % in the OCP Group. Finally, triglyceride levels did not change in the Statin Group but were significantly increased in the OCP Group [101]. These findings are consistent with subsequent studies, whereby simvastatin and atorvastatin markedly improved lipid profile in PCOS women [62, 105].

In addition to improvement of lipid profile, statins also reduce several other cardiovascular risk factors in women with PCOS, including improvement of markers

of endothelial dysfunction and indices of low-grade chronic inflammation. Banaszewska et al. carried out a prospective crossover trial demonstrating that in women using OCPs, simvastatin decreased high-sensitivity C-reactive protein (hs-CRP), a marker of systematic inflammation, and levels of soluble vascular cell adhesion molecule 1 (sVCAM), a marker of endothelial dysfunction [102]. These findings are consistent with subsequent studies, whereby a similar statin-induced improvement of systemic inflammation in PCOS women has been shown after simvastatin as well as atorvastatin therapies [7, 62, 105, 106]. In addition, atorvastatin decreased malondialdehyde (MDA) concentrations, a marker of oxidative stress in patients with PCOS [108]. Taken together, these findings provide convincing evidence that statin therapy significantly reduces cardiovascular risk factors even in this young, but at-risk, population.

Despite the promise, little is known about long-term clinical and biochemical effects of statins on PCOS. To date, the longest study was carried out by Banaszewska et al., who randomized 139 patients with PCOS to simvastatin alone, metformin alone, or simvastatin plus metformin; clinical, endocrine, and metabolic parameters were evaluated after 3 and 6 months of treatment [62]. Subjects in all groups experienced a significant improvement of menstrual regularity, with the greatest increase in the number of spontaneous menses observed in women receiving simvastatin. Similarly, ovarian volume decreased significantly in groups treated with simvastatin, whereas the decline of ovarian volume in women treated with metformin alone was of borderline statistical significance. This simvastatin-induced decline in ovarian volume occurred in parallel with improvement of hyperadrogenism (Table 11.1). Overall, this study indicated that in the long-term, simvastatin may be superior to metformin in actions on clinical and biochemical aspects of PCOS.

Limitations

Interesting and promising studies on the effects of statins on PCOS should be viewed with caution and with recognition of potential risks and limitations. While statins are usually considered to be safe, their use, especially in the long-term, is not risk-free. Despite an overall favorable risk profile, statins, especially at high doses, may exert adverse effects on liver as reflected by elevation in transaminases, myopathy, and even rhabdomyolysis [109]. Furthermore, serious adverse effects have been reported with concomitant use of statins with other drugs, including fibrates, macrolide antibiotics, antiarrhythmics, cyclosporin, and protease inhibitors [110]. In view of these concerns, the FDA recently reduced the recommended simvastatin doses.

One of the greatest concerns regarding the use of statins in reproductive age women is the potential for teratogenicity, although these concerns have not been conclusively substantiated to date in humans. Based on animal data, several studies failed to demonstrate teratogenicity of statins [111], whereas others found that at high doses, statins may be associated with impairment of placental and skeletal formation [112, 113]. In humans, several studies evaluated effects of statin exposure

Table 11.1 Change of parameters after 6 months of treatment in comparison to baseline values[a]

Variable	Simvastatin (S) vs. baseline ($N=28$)	P-value	Metformin (M) vs. baseline ($N=33$)	P-value	Effect of S+M (SM) vs. baseline ($N=36$)	P-value	Between groups P-value (pair-wise comparisons)
Number of spontaneous menses per 6 months	+1.6±0.2 (71.3 %)	<0.001	+1.1±0.2 (33.1 %)	<0.001	+1.7±0.2 (73.3 %)	<0.001	**0.02** (S vs. M: $P=0.03$ SM vs. M: $P=0.02$ S vs. SM: $P=0.86$)
Volume of both ovaries (mL)	−2.99±0.67 (−14.1 %)	<0.001	−1.24±1.31 (−5.4 %)	0.06	−1.49±0.80 (−7.3 %)	0.04	0.42
BMI (kg/m^2)	−0.35±0.15 (−1.4 %)	0.03	−0.93±0.14 (−4.0 %)	<0.001	−1.35±0.34 (−5.3 %)	<0.001	**0.02** (S vs. M: $P=0.09$ SM vs. M: $P=0.21$ S vs. SM: $P=0.005$)
Hirsutism (Ferriman/ Gallwey score)	−1.1±0.1 (−12 %)	<0.001	−0.84±0.35 (−8.9 %)	<0.001	−1.0±0.15 (−11.7 %)	<0.001	0.52
Acne (Score)	−0.93±0.13 (−76 %)	<0.001	−0.75±0.12 (−62 %)	<0.001	−1.06±0.14 (−67 %)	<0.001	0.23
Total testosterone (ng/mL)	−0.22±0.03 (−25.6 %)	<0.001	−0.15±0.04 (−25.6 %)	<0.001	−0.16±0.03 (−20.1 %)	<0.001	0.42
Free testosterone (ng/dL)	−0.28±0.06 (−20.3 %)	<0.001	−0.30±0.08 (−23.2 %)	0.001	−0.27±0.08 (−17.5 %)	0.003	0.97
DHEAS (μmol/mL)	−1.64±0.43 (−17.1 %)	0.001	0.54±0.37 (6.1 %)	0.15	0.59±0.31 (6.7 %)	0.07	**P<0.001** (S vs. M: $P<0.001$ SM vs. M: $P=0.92$ S vs. SM: $P<0.001$)
SHBG (nmol/L)	−5.19±2.20 (−10.7 %)	0.03	2.27±1.96 (5.2 %)	0.25	0.43±1.78 (1.1 %)	0.81	**0.03** (S vs. M: $P=0.01$ SM vs. M: $P=0.50$ S vs. SM: $P=0.05$)
LH (IU/L)	0.15±1.16 (1.4 %)	0.90	−1.9±0.77 (−19.6 %)	0.03	−0.09±1.1 (−0.9 %)	0.93	0.35

FSH (IU/L)	−0.56±0.26 (−9.2 %)	0.04	−0.67±0.29 (−10.7 %)	0.03	0.12±0.31 (2.3 %)	0.71	0.12
Prolactin (ng/mL)	−1.60±1.80 (−9.1 %)	0.38	−2.24±0.94 (−12.9 %)	0.02	−5.00±0.97 (−26.7 %)	<0.001	0.12
Total cholesterol (mg/dL)	−35.4±6.1 (−18.9 %)	<0.001	2.81±4.63 (1.6 %)	0.55	−34.5±5.6 (−18.9 %)	<0.001	P<**0.001** (S vs. M: *P*<1.001 SM vs. M: *P*<0.001 S vs. SM: *P*=0.91)
LDL cholesterol (mg/dL)	−32.6±5.0 (−31.6 %)	<0.001	2.40±4.20 (2.5 %)	0.57	−31.8±4.4 (−31.9 %)	<0.001	**0.001** (S vs. M: *P*<0.001 SM vs. M: *P*<0.001 S vs. SM: *P*=0.91)
HDL cholesterol (mg/dL)	−2.62±2.74 (−3.7 %)	0.35	0.55±2.13 (0.9 %)	0.80	−0.79±1.8 (−1.2 %)	0.66	0.61
Triglycerides (mg/dL)	−3.38±4.20 (−5.0 %)	0.42	12.8±7.5 (17.5 %)	0.09	−13.4±7.3 (−15.0 %)	0.07	**0.02** (S vs. M: *P*=0.11 SM vs. M: *P*=0.006 S vs. SM: *P*=0.31)
hs-CRP (mg/L)	−0.55±0.29 (−33.5 %)	0.03	−1.32±0.66 (−55.7 %)	0.01	−0.83±0.78 (−33.5 %)	0.10	0.78
sVCAM (μg/L)	−85.7±25.6 (−12.7 %)	0.003	−62.2±23.6 (−8.2 %)	0.01	−68.5±23.0 (−10.1 %)	0.005	0.79
Fasting glucose (mg/dL)	−2.85±2.37 (−3.4 %)	0.23	−3.13±2.05 (−3.8 %)	0.13	−3.36±2.12 (4.0 %)	0.12	0.99
Fasting insulin (μU/mL)	−0.29±0.57 (−4.1 %)	0.62	0.72±1.00 (11.1 %)	0.47	−1.73±0.76 (−20.9 %)	0.02	0.08
Insulin Sensitivity Index	0.31±0.55 (5.1 %)	0.58	0.34±0.47 (6.0 %)	0.47	0.31±0.54 (5.1 %)	0.56	0.99

Each value represents mean± SEM; percent change in brackets

[a]Reproduced with permission from Banaszewska B, Pawelczyk L, Spaczynski RZ, Duleba AJ. Effects of simvastatin and metformin on polycystic ovary syndrome after six months of treatment. J Clin Endocrinol Metab 2011;96(11):3493–501, Copyright 2011, The Endocrine Society

during the first trimester of pregnancy and did not demonstrate any significant increase in the risk for malformations [114, 115]. However, since statins are currently listed by the FDA as pregnancy category X medications, their use is contraindicated in pregnancy, and sexually active women of reproductive age should use reliable contraception when considering statin therapy.

Another important area of concern is the potential for statins to affect glucose metabolism, particularly for a population that is innately at risk for developing diabetes, such as women with PCOS. To date, the evidence regarding the role of statins in the development of T2DM is not yet clear [116–118]. In the first study evaluating the statin-diabetes association, Freeman et al. reported an inverse association between pravastatin use and diabetes incidence in the West of Scotland Coronary Prevention Study (WOSCOPS) published in 2001; notably, the investigators relied on nonstandard criteria for diagnosis of diabetes [119]. In another observational study, statin use has been linked to an increased risk of T2DM, and this association remained significant after adjusting for other potential confounders, such as age, race/ethnicity, and physical activity [120]. A recent meta-analysis of six randomized clinical trials concluded that the relationship of statin therapy to incident diabetes remains uncertain [121].

Future Directions

There is a growing interest in the development of new adjuvant therapies that could successfully address the myriad endocrine and metabolic concerns in PCOS and in identifying strategies that may potentiate beneficial effects of statins at lower doses, and, hence, improve tolerance while mitigating possible adverse effects such as on glucose metabolism. One candidate for such adjuvant therapy is resveratrol (*trans-3*,5,4′-trihydroxystilbene), a natural polyphenol found in grapes, nuts, berries, and red wine. This phytoestrogen appears to have a potential for a broad range of beneficial actions, including anticarcinogenic, cardioprotective, neuroprotective, anti-inflammatory, and antioxidant activities [122–127]. Resveratrol affects multiple signaling pathways and early clinical data indicate that resveratrol may reduce insulin resistance and thus decrease the risk of development of T2DM [128]. Resveratrol has been shown to be the most potent natural activator of sirtuins, a family of nicotinamide adenine dinucleotide^{+}-dependent deacetylases, involved in crucial cellular processes, including DNA repair, transcriptional silencing, as well as glucose and lipid metabolism [129, 130]. This property may account for resveratrol-induced beneficial effects on lifespan extension, cell survival, neuroprotection, and improvement of obesity and insulin resistance in some biological models [131]. Resveratrol also downregulates phosphoinositide kinase-3 (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathways in several cell types [132, 133] including cancer cell lines [134–136]. In addition, in vivo studies have also demonstrated that resveratrol may interfere with the mevalonate pathway, reducing serum cholesterol by down-regulating hepatic HMGR mRNA expression in hamsters fed a high-fat-diet [137].

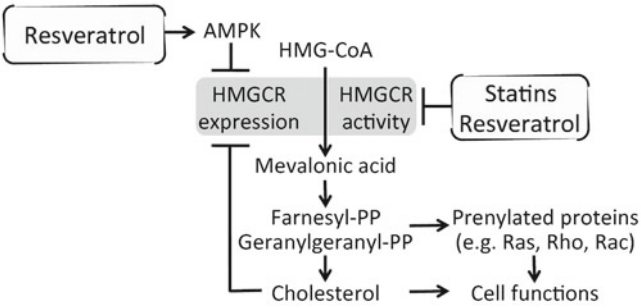

Fig. 11.4 Proposed diagram for the inhibition of HMG-CoA reductase by resveratrol (at the level of HMG-CoA reductase expression and activity) and by statins (at the level of competitive inhibition of HMG-CoA reductase activity). Products of the mevalonate pathway, such as cholesterol, inhibit HMGCR expression

In the ovary, resveratrol has been shown to promote apoptosis and reduce theca-interstitial cell growth as well as inhibit insulin-induced theca-interstitial cell growth using a rat in vitro model [138]. Furthermore, a recent study has demonstrated that resveratrol reduces theca-interstitial androgen production primarily by inhibiting *Cyp17a1* mRNA expression, and this inhibition may be mediated, at least in part, by blocking the activity of the serine-threonine kinase/protein kinase B pathway [139]. Thus, resveratrol may decrease both the number of steroid-producing cells as well as the steroidogenic capacity of each individual theca-interstitial cell by exerting its inhibitory effects on proliferation and steroidogenesis, resulting in a decrease of the total amount of androgens secreted by the ovary. Recently, resveratrol has been demonstrated to potentiate simvastatin-induced inhibition on theca-interstitial cell proliferation in a dose-dependent manner by blocking the mevalonate pathway via distinctly different mechanisms than statins; simvastatin reduces HMGCR activity while indirectly inducing HMGCR mRNA and protein expression, whereas resveratrol inhibits HMGCR mRNA and protein expression as well as HMGCR activity (Fig. 11.4) [80].

In view of these considerations, there is an emerging interest in evaluating the potential beneficial effect of combined therapy using statin in conjunction with resveratrol in anticipation that such therapy may improve endocrine metabolic and cardiovascular aspects of PCOS. These findings are consistent with a previous in vivo study, whereby simvastatin in combination with resveratrol was shown to be more cardioprotective than simvastatin alone using an ischemic rat heart model [140].

Summary

Hallmarks of PCOS include menstrual cycle irregularities, androgen excess, enlarged polycystic ovaries, and a broad range of metabolic and cardiovascular risks. Statins emerge as promising therapeutic agents targeting most of the endocrine and metabolic underpinnings of PCOS. Despite the promise however, routine

clinical use of statins in reproductive age women with PCOS cannot be yet recommended, and longitudinal studies that comprehensively evaluate benefits and safety of this class of agents in diverse populations of women with PCOS are urgently needed to assess and ensure long-term safety of statins. Furthermore, in view of a potential for teratogenicity, reproductive age women must ensure concomitant use of reliable contraception when considering use of statins for clinical indications.

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Chapter 12
Managing PCOS-Related Infertility: Ovulation Induction, In Vitro Fertilization, and In Vitro Maturation

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Key Points

- PCOS accounts for approximately 80 % of women with ovulatory infertility.
- Lifestyle modification with caloric restriction and physical exercise should be considered as first-line intervention when managing infertile women with an overweight/obese PCOS phenotype.
- Clomiphene citrate (CC) remains the first-line treatment for ovulation induction in anovulatory women with PCOS, although aromatase inhibitors have shown promise as an alternative method for ovulation induction.
- Gonadotropin use is an option for CC-resistant and CC-failure patients, although treatment-related risks of ovarian hyperstimulation and multiple pregnancies may be exaggerated in the PCOS population. Gonadotropin therapy should therefore be reserved as a second-line strategy for women with PCOS and approached with caution.
- In vitro fertilization (IVF) is a reasonable option for women with PCOS who have additional factors for infertility (tubal occlusion, male factor) or for those who have failed prior treatment with CC or gonadotropins.
- In theory, in vitro maturation (IVM) is a potentially useful strategy for the management of PCOS-related infertility; in reality however, the reported pregnancy rates with IVF and IVM are significantly lower than seen with conventional IVF. More research is needed before IVM can be offered as a reliable treatment option for women with PCOS.

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Introduction

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting up to 15 % of all reproductive-age women [1]. Among women with anovulatory infertility, PCOS accounts for nearly 80 % of the cases and affects approximately 20 % of couples trying to conceive [2]. A diagnosis of PCOS is based on features of oligo-ovulation and/or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic-appearing ovaries on ultrasound [3]. Endocrine abnormalities of the reproductive axis include hypersecretion of pituitary luteinizing hormone (LH) that is attributed to an accelerated gonadotropin-releasing hormone (GnRH) pulsatile activity, ovarian theca-stromal cell hyperactivity, and hypofunction of the follicle-stimulating hormone (FSH)—ovarian granulosa cell network [2]. These abnormalities result in higher ovarian androgen secretion, arrest of follicular growth, and intermittent or lack of ovarian cyclicity. Given these endocrine abnormalities, infertility is a common feature that often prompts medical evaluation. This chapter aims to address the different management options available for treating ovulatory infertility in women affected by PCOS.

Infertility Evaluation

Although ovulatory dysfunction/anovulation is the likely culprit for infertility in women with PCOS, a thorough evaluation must be undertaken to rule out any additional contributing factor(s). Evaluation should include assessment of tubal patency and a semen analysis, in addition to testing the endocrine and metabolic milieus. Disproportionately elevated serum levels of pituitary gonadotropin LH (luteinizing hormone) are seen in up to 40 % of women with PCOS [4]. The elevated serum LH levels are attributed to an increase in the amplitude and frequency of LH pulses, which is suggested to be detrimental not only to the success of the ovulation induction cycle, but have also been inconsistently associated with an increased incidence of miscarriage [4]. Serum levels of anti-Müllerian hormone (AMH), an established marker of ovarian reserve, are higher in women with PCOS compared to healthy controls, and attributed to the larger cohort of primary, secondary, and pre-antral follicles typical of PCOS [5, 6]. Thyroid-stimulating hormone (TSH) and prolactin levels should also be obtained to rule out any underlying thyroid or pituitary disorder contributing to ovulatory infertility.

Endocrine and metabolic evaluations of PCOS have been addressed in distinct chapters within this text; suffice it to state that beyond attainment of a successful pregnancy, overall well-being of the woman and the future conceptus should be the primary goal. Optimization of the metabolic picture as well as normalization of glycemic and dyslipidemic profiles through lifestyle changes and indicated pharmaceutical interventions should be considered prior to initiating targeted fertility medications. Additionally, obese and insulin resistant women with chronic

anovulation are deemed at risk for endometrial pathology such as endometrial hyperplasia or occult endometrial cancer; an endometrial biopsy should be considered in at risk women to rule out underlying endometrial pathology before proceeding with infertility treatment. Preconception counseling is additionally encouraged for those deemed at an enhanced risk for pregnancy-related complications. A multidisciplinary approach is thus an optimal paradigm for managing PCOS-related infertility.

Lifestyle Modifications and Weight Loss

In women, body mass index (BMI) correlates with increased rate of cycle disturbances and infertility [4, 7–9]. Central obesity manifested as an increased waist:hip ratio (WHR) appears to have a negative association with the probability of conception per cycle (hazard ratio 0.706, 95 % CI, 0.562–0.887) than overall body mass excess or age [10]. Observational studies have shown that weight loss is associated with improved spontaneous ovulation rates in women with PCOS, and pregnancies have been reported after losing as little as 5 % of the initial body weight [11–13].

In addition to influencing the time to conception, which is often delayed in obese women, excess body mass is recognized to adversely affect also the response to fertility treatments [14]. Indeed, higher doses of ovulatory medications are needed to achieve an ovulatory response in the setting of obesity [15, 16]. Given that almost two-thirds of women with PCOS are overweight to obese, optimization of body mass and improving metabolic consequences thereof through lifestyle modifications (caloric restriction, portion size, food choices, and regular physical activity) should be considered as first-line intervention in women with the overweight/obese PCOS phenotype. Although the ideal amount of weight loss for an individual woman prior to attempting infertility treatment remains unclear, strong consideration should be made for obese women to lose 5–10 % of body weight prior to starting any medical therapy.

Medical Strategies for Achieving Ovulation

Clomiphene Citrate (Clomid, Serophene)

Clomiphene citrate (CC), a selective estrogen receptor modulator (SERM), remains the first-line treatment for ovulation induction in anovulatory women with PCOS [2]. The mechanism for the ovulatory effect of SERMs lies in the blockade of estrogen-mediated negative feedback on the hypothalamo-pituitary axis. Abrogation of the estrogen-mediated suppression results in an increased secretion of FSH from the anterior pituitary, which in turn is responsible for initiating the cascade of events that culminate in follicular growth, development, and selection to dominance [17].

An alternative SERM, tamoxifen, has also been used with similar effects, although it is not generally considered as a first-line therapy [2].

CC is a racemic mixture composed of two stereoisomers, en- and zu-clomiphene, both of which are thought to contribute to the estrogen receptor antagonism [18]. En-clomiphene is the more potent isomer and the one responsible for its ovulation-inducing action. The half-life of en-clomiphene is relatively short; therefore serum concentrations rise and fall quickly after treatment [19, 20]. Zu-clomiphene has a long plasma and tissue retention, with measurable concentrations in plasma for at least 1 month after a single 50-mg dose of CC [21]. These properties are reflected in the significant accumulation of zu-clomiphene from cycle to cycle across multiple cycles of CC administration [21]. The cost of CC is low, the oral route of administration is patient-friendly, and its safety is well established when properly utilized. The goal of treatment is to mimic a physiological ovulatory cycle with monofollicular development and ovulation from a single follicle, while minimizing the risks of ovarian hyperstimulation syndrome (OHSS) and multiple gestations [22].

Given the prolonged half-life of its isomers, a short course of CC is recognized to suffice in achieving folliculogenesis. The standard practice is to administer CC for 5 days starting early in the menstrual cycle after onset of a spontaneous or progestin-induced menstrual bleeding; commonly utilized treatment protocols in clinical practice consist of daily CC from cycle day 3–7 or from cycle day 5–9. Ovulation, conception rates, and pregnancy outcomes are similar with either treatment start protocols [23]. The starting dose of CC is 50 mg/day and may be incrementally increased to 150 mg/day (recommended maximum dose by the American College of Obstetricians and Gynecologists (ACOG) and ESHRE/ASRM Committee) until ovulation occurs [2, 14, 24]. The US Food and Drug Administration recommends not exceeding 100 mg/day of CC. Some consider higher doses (200–250 mg), particularly in obese patients, however the benefit of these high doses is not proven [2, 25]. Most conceptions occur within the first six ovulatory cycles, and over 75 % of patients ovulate at a dose of less than 150 mg/day [26].

Approximately 20–25 % of women are CC-resistant (i.e., remain anovulatory despite using maximum CC dosages), requiring an alternative approach to their management [27]. In CC-resistant patients, some advocate extending the treatment period to greater than 5 days or using a step-up approach in which a second administration of CC at a higher dose is provided without interrupting the stimulation plan [28, 29]. The addition of metformin, a biguanide anti-hyperglycemic agent, has been suggested as an adjunct strategy for women resistant to CC, although the data are equivocal, and current recommendations are that use of metformin be for metabolic indications as there is no direct benefit of using metformin for induction of ovulation or to achieve live birth [30–32].

Although up to 85 % of women ovulate with CC, only 30–40 % of women will conceive following CC therapy after six cycles [33–35]. Degree of obesity, magnitude of hyperandrogenemia, hirsutism, and the patient's age have all been related to likelihood for treatment success [26]. Persistent local anti-estrogenic effect of CC on the endometrium (secondary to the accumulation of the isomer zu-clomiphene) and the prolonged depletion of estrogen receptors may create an adverse milieu for

implantation, thus explaining reduced live-birth rates with CC despite successful ovulation; alternatively, anti-estrogenic effects of CC at the level of the endocervical glands may contribute to a hostile cervical milieu which hinders sperm penetration [33, 36]. It is estimated that 15–50 % of patients using CC may be affected by poor cervical mucus and suboptimal endometrial development as assessed by transvaginal ultrasound [36].

Success of CC cycles has not shown to be impacted by the stringency of cycle monitoring nor by the ovulatory stimulus (exogenous hCG administration versus endogenous LH surge). While ultrasound surveillance of ovarian response is not necessary to predict cycle success, evidence of a mono-follicular response and information on endometrial thickness may be particularly meaningful for individual patients. The addition of an ovulatory trigger dose of human chorionic gonadotropin (hCG) has not been shown to improve CC-related ovulation, pregnancy, or miscarriage rates [37]. However, for individual patients, an ovulatory trigger allows for timely scheduling of adjunct procedures such as intrauterine insemination, and the timeliness and predictability of subsequent events may mitigate treatment-related stress for others.

The risk of multiple pregnancies with CC is between 7 and 10 % with the majority being twin gestations; the risk of triplets is 0.3–0.5 %, for quadruples 0.3 %, and for quintuplets 0.1 % [38]. The increased risk of multi-fetal gestation seen with ovulation induction medications is yet another reason to treat at the lowest effective dose. The incidence of OHSS in CC cycles is difficult to determine confidently because definitions in the literature differ among studies. Mild OHSS symptoms (transient abdominal discomfort, mild nausea, slight distention) with use of CC is not uncommon; however, expectant management is typically only required. The risk of severe OHSS resulting in ovarian enlargement, ascites, oliguria, and intractable nausea and emesis is rare (<0.1 %) [17]. Other adverse side effects associated with CC include hot flushes, abdominal distention, and, rarely, visual disturbances (blurred vision, scotomata, or light sensitivity) [39, 40].

There has been considerable concern on whether CC use increases the incidence of ovarian and breast cancer. The incidence of ovarian cancer is increased in nulliparous women and decreased in parous women and in those who use oral contraceptive pills, suggesting that *incessant ovulation* may predispose to development of ovarian cancer [41]. Therefore, apprehension on whether treatment that induces ovulation may cause an increase in the risk of ovarian cancer became of heightened concern. Initial case–control studies in the 1990s raised credence to this notion however, there were methodological flaws in the studies [42, 43]. More recently, numerous studies have confirmed that while infertility per se is associated with an increased risk of ovarian cancer, however there is no evidence that ovulation-inducing drugs, such as CC, enhance that risk any further [44–49]. The risk of developing breast cancer with CC use has been conflicting, with some studies showing no association with CC or other ovulation-inducing medications, whereas others show a possible increased risk in certain subgroups [46, 49, 50]. A meta-analysis of 23 published case–control ($n=8$) and cohort ($n=15$) studies did not show an increased risk of breast cancer associated with CC or other ovulation induction medications (RR 1.08, 95 % CI 0.98–1.19) [51].

The existing data are thus reassuring regarding the risk of ovarian or breast cancer in relation to use of ovulation induction strategy; prolonged treatment (>6 cycles), however, should be avoided, principally because it offers little benefit for success. Patients who are CC-resistant should be offered an alternative treatment modality, whereas those unable to achieve a pregnancy despite multiple ovulatory cycles (3–6) should be encouraged to consider more definitive fertility treatment options (discussed as under).

Aromatase Inhibitors

Aromatase inhibitors (AI) have been proposed as an alternative ovulation induction strategy to CC therapy. Aromatase is part of the cytochrome P450 enzyme family and is found in highest concentrations within the ovarian granulosa cells of premenopausal women, in addition to other sites such as adipose tissue, brain and skin fibroblasts [52]. Inhibition of the aromatase enzyme decreases the aromatization of androgens to estrogen; thus, AIs are a therapeutic option for estrogen-dependent conditions such as breast cancer and endometriosis [52]. Following AI exposure in premenopausal women, the profound decline in circulating and tissue levels of estrogens releases the hypothalamo-pituitary axis from estrogenic negative feedback and subsequently increases the pituitary release of FSH [53]. The increased FSH stimulates follicular development and is the basis for the use of AIs for ovulation induction. Similar to CC, the AIs are efficacious following oral administration, and a relatively low cost makes them an attractive alternative to CC.

Letrozole, a type 2a third-generation aromatase inhibitor, is the most commonly used AI for ovulation induction. Dosing paradigm of AIs is similar to that of CC [54, 55]. Similar to CC, the dose of letrozole is incrementally increased from a starting dose of 2.5 mg daily to a maximum of 7.5 mg/day for 5 days until ovulation is achieved. Two recent meta-analyses of six randomized control trials compared letrozole with CC in women with PCOS who were therapy-naïve, deemed CC resistant, or CC failures. Per cycle ovulation rates and per patient pregnancy rates were comparable between patients treated with AI compared to CC, even though letrozole was associated with significantly fewer mature follicles and significantly lower estrogen concentrations per cycle [27, 56, 57].

Early in their use as ovulation induction agents, an initial report had suggested concerns for AI-related teratogenicity; an abstract presented in 2005 described an increase in fetal cardiac and bone abnormalities with letrozole use in premenopausal women [58]. However, subsequent larger investigations have failed to show an association between AI exposure and congenital malformation risk [59, 60].

The role of AIs in the algorithm for ovulation induction is still unclear. Due to the relatively short half-life (2 days), AIs induce ovulation without any anti-estrogenic effects on the endometrium, as seen with CC and hence theorized to offer a more physiological approach for treatment success [61]. Consequently, AIs may be particularly beneficial for women with CC resistance or CC failure. Results from a large multicenter randomized control trial conducted in North America that evaluated

efficacy of letrozole versus CC for ovulation induction are keenly awaited [62]. This study will hopefully elucidate some of the uncertainties relating to the use of AIs as an ovulation induction strategy in the management of PCOS-related infertility.

Gonadotropins

The first gonadotropins for ovarian stimulation were purified from the urine of postmenopausal women in the 1960s and labeled as human menopausal gonadotropin (hMG) [63]. Although human menopausal gonadotropins, which contain equal amounts of FSH and LH activity, are still widely used today, an increasing trend towards using recombinant human FSH (rFSH) is evident.

Failure to respond to common ovulation induction strategies such as CC or AIs is an indication for a trial of gonadotropin therapy in anovulatory patients, including women with PCOS. Anovulatory PCOS patients who have failed to ovulate, failed to become pregnant after 4–6 ovulatory cycles of CC, or had other issues (i.e., thin endometrial lining) are candidates for gonadotropin therapy [64]. Gonadotropin therapy, whether with administration of rFSH, urinary purified FSH (uFSH) or hMG, is given for a transient period to initiate and maintain follicular growth of few follicles. Recognizing that the ovarian response with the latter strategy is "less controlled' than resulting from an endogenous gonadotropin release as is achieved with CC and AIs, it is important to closely monitor the ovulatory response with gonadotropin use. Therefore, the use of gonadotropins comes at the expense of an escalation in risks for multifollicular ovarian response, multiple pregnancy, and OHSS.

A cautious approach to ovarian stimulation with gonadotropin use in PCOS patients is reflected in the two commonly employed *step-up* and *step-down* treatment protocols. The *step-up* protocol is based on the principle of small increments in gonadotropin dosing until follicular development ensues. Typically, a low starting dose (25–75 IU) is chosen, which is then adjusted by small increments after 5–7 days, only if there is no evidence of follicular response (as evidenced by rise in circulating estradiol (E2) level and/or growth of ovarian follicle/s on serial transvaginal ultrasound monitoring). Once follicular response is observed, the same gonadotropin dose is maintained until attainment of a single dominant follicle [2]. In contrast to the cautious *step-up* approach, the *step-down* regimen starts with a higher gonadotropin dose (100–150 IU), with rapid decrease in dosing once follicular development is observed (typically with attainment of a leading follicle of about 12–13 mm diameter size). The rationale is to deprive the nonselected smaller follicles in the cohort by decreasing the gonadotropin dose and thus causing arrest of their growth [2]. Both protocols have similar rates of mono-follicular development [2].

During ovulation induction with gonadotropins, serial transvaginal ultrasound and serum E2 level assessments are commonly utilized to assess the magnitude of ovarian response. Patients must be counseled regarding the risk of multiple pregnancies and for the development of OHSS; cycle cancellation should be considered for patients deemed at a disproportionate risk for either.

While absolute guidelines are lacking, the risk for high-order multiple pregnancy must be individualized in relationship to the number of developing follicles and the patient's age. The rate of mono-follicular ovulation using the low-dose gonadotropin regimens is approximately 70 %, with a pregnancy rate of 20 % per cycle [65]; patients must be counseled regarding risks of multiple pregnancy (6–36 %) and severe OHSS (<1–5 %) [2]. Although specific normative cut-offs vary, in 2006 the ASRM Practice Committee suggested that caution was indicated when there was a rapidly rising serum E2 level or if the E2 concentration reached in excess of 2,500 pg/mL [66]. Once the follicle(s) size approaches 16–18 mm, ovulation is triggered with exogenous hCG followed by intrauterine insemination or timed intercourse. In previous studies, cycle cancellation was considered if three or more follicles 16 mm or larger were observed, whereas other more stringent studies recommend no more than two follicles greater than 14 mm in order to prevent the risk of multiple pregnancies and OHSS [67–70]. An alternative means to save a cycle with too many follicles is to switch the patient to an IVF cycle in order to better control the risk of multiple pregnancies (i.e., transferring a single embryo).

In Vitro Fertilization

In vitro fertilization (IVF) is the most efficacious of existing fertility treatment strategies, albeit is also the most costly and invasive approach to infertility management. While IVF is clearly indicated for women with PCOS with concomitant tubal disease or severe male factor, this strategy should be considered for women failing to achieve successful pregnancy following multiple attempts at ovulation induction.

Women with PCOS undergoing IVF treatment have similar pregnancy, miscarriage, and live-birth rates compared to those of non-PCOS patients, as evidenced by a large systematic review and meta-analysis of nine observational studies comparing 793 cycles of women with PCOS with 1116 cycles of matched controls [66, 70]. IVF cycles undertaken in PCOS population are characterized by slightly longer stimulation (1.2 days longer), higher number of developing follicles, higher E2 levels, and retrieval of higher number of cumulus-oocyte complexes (COC) (2.9 more COC) than in women without PCOS [71]. Cycle cancellation rates are higher in patients with PCOS undergoing IVF compared to those with other infertility etiologies (12.8 % vs. 4.1 %) and are due to either absent or limited ovarian response to exogenous gonadotropins, or to an exaggerated response with escalated risk of developing severe OHSS [2].

Treatment strategies employed during IVF aim at achieving "controlled" growth and development of a cohort of ovarian follicles that is available and responsive to exogenous gonadotropin stimulus, while concomitantly suppressing the hypothalamo-pituitary axis. In the absence of a suppressive strategy, escalating serum E2 levels concomitant with development of ovarian follicles can initiate an endogenous LH surge with subsequent ovulation, thus defeating the purpose of the management strategy. The long luteal *GnRH agonist* protocol has been tried and

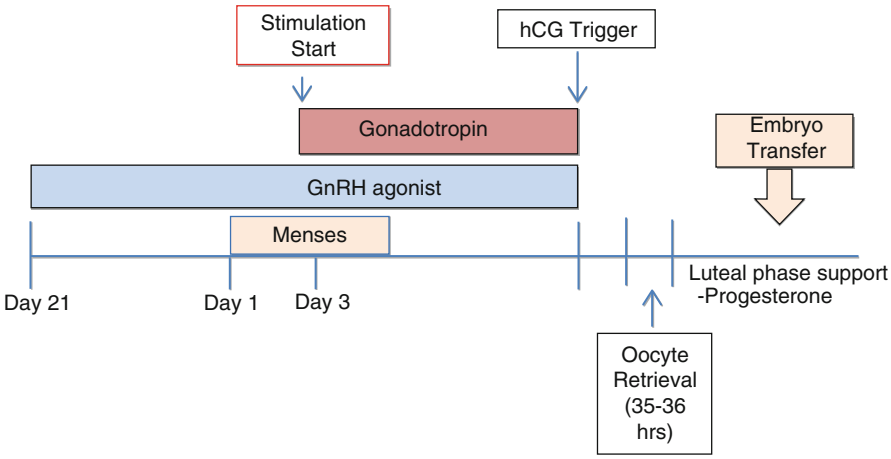

Fig. 12.1 Luteal (long) GnRH agonist protocol wherein ovarian suppression is initiated in the late luteal phase of the preceding menstrual cycle

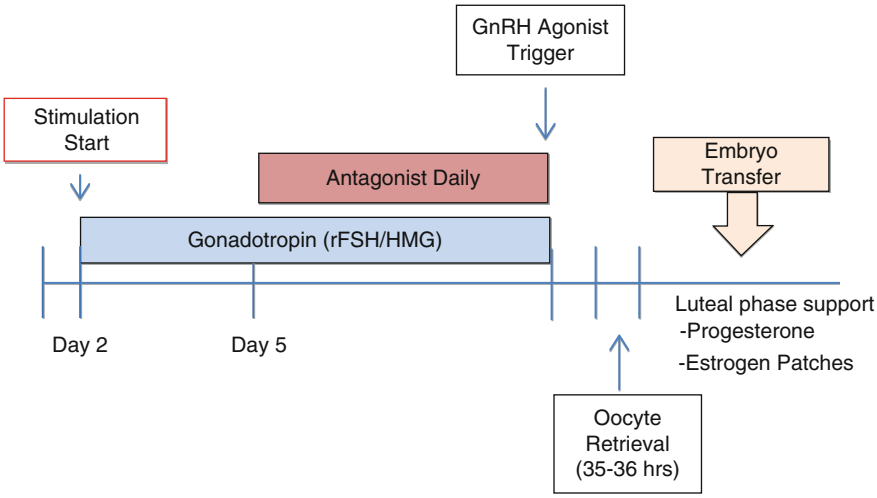

Fig. 12.2 GnRH antagonist protocol with GnRH agonist ovulatory trigger

tested for the longest time wherein suppression of the hypothalamo-pituitary axis is initiated during the late luteal phase of the menstrual cycle followed by initiation of controlled ovarian hyperstimulation with use of gonadotropins initiated after the onset of menses (Fig. 12.1). Recent years have seen an upsurge in the utilization of *GnRH antagonist* protocol particularly in women with PCOS (Fig. 12.2) wherein hypothalamo-pituitary suppression with a GnRH antagonist is initiated a few days after stating gonadotropin therapy. In the fixed protocol, GnRH antagonist is started either around day 5–6 of gonadotropin stimulation, regardless of the follicular size

or E2 level; in the flexible protocol however, GnRH antagonist is introduced when a leading follicle of about 13 mm is visualized on ultrasound and estradiol levels of about 200–400 pg/mL. Four randomized controlled trials have compared the fixed (on day 6) versus a flexible (by a follicle diameter of 13 mm) protocol of GnRH antagonist administration, and found no significant difference in live-birth outcomes [72]. A meta-analysis of eight randomized control trials comparing the GnRH antagonist protocol to the long GnRH agonist protocol in PCOS women undergoing IVF with intracytoplasmic sperm injection (ICSI) found no difference in ongoing pregnancy rate (OR = 0.91, 95 % CI, 0.67–1.22) or clinical pregnancy rate (OR = 0.87, 95 % CI, 0.64–1.19) [72].

The risk of OHSS is particularly exaggerated in women with PCOS [73]. Attention to dosing regimen and to stimulation protocols are strategies that may reduce the risk of severe OHSS in women with PCOS undergoing IVF. The GnRH antagonist protocols are associated with a lesser risk for OHSS compared to the long GnRH agonist regimen in high-risk patients [72]. Ragni et al. showed that antagonist cycles have a shorter stimulation period with fewer numbers of follicles and a lower E2 level on the day of HCG administration [74]. These findings are consistent with several other studies comparing mid-luteal GnRH agonist and antagonist cycles [75–77]. In addition to decreasing the risk of OHSS, the number of cycles that were cancelled in high responders was significantly less when the antagonist protocol was utilized [74].

The antagonist protocol provides the option to use a GnRH agonist trigger to induce oocyte maturation, instead of hCG, which has been shown to decrease the rates of OHSS in these high-risk patients (see Fig. 12.2) [78, 79]. Exogenous hCG has routinely been used for final oocyte maturation due to its LH homology. Unlike the natural mid-cycle LH surge, the hCG-mediated LH activity has a long half-life and spans several days (8–10 days) into the luteal phase [80]. The supra-physiological LH activity of hCG stimulates the multiple corpora lutea which leads to high progesterone and E2 levels and up-regulation of vascular growth factor (VEGF), fibroblast growth factor (FGF2), and numerous cytokines [80]. VEGF is a key substance that induces vascular hyperpermeability, making capillaries "leaky" which results in a fluid shift from the intravascular system to the abdominal and pleural cavities [81]. Supraphysiologic production of VEGF due to the prolonged effect of exogenous hCG is thought to be the main process underlying OHSS [81]. In contrast, when GnRHa are used during an antagonist protocol, the GnRHa displaces the GnRH antagonist in the pituitary, activating the GnRH receptor which leads to a flare of gonadotropins release. This surge in gonadotropin release is similar to what is evident in natural cycle. The main difference between a GnRHa-induced surge and a natural cycle is that the GnRHa surge is of shorter duration lasting 24–36 h compared to the 48 h seen in a natural cycle [82–84]. GnRHa trigger nearly eliminates the risk of OHSS because the luteolysis induced by the short half-life of endogenous LH is significantly shorter than the prolonged half-life of hCG [85].

Undertaking of IVF in women with PCOS thus involves particular attention to the choice of treatment protocol (GnRH agonist versus antagonist), dose of gonadotropin, choice of regimen to achieve final oocyte maturation (hCG or GnRH

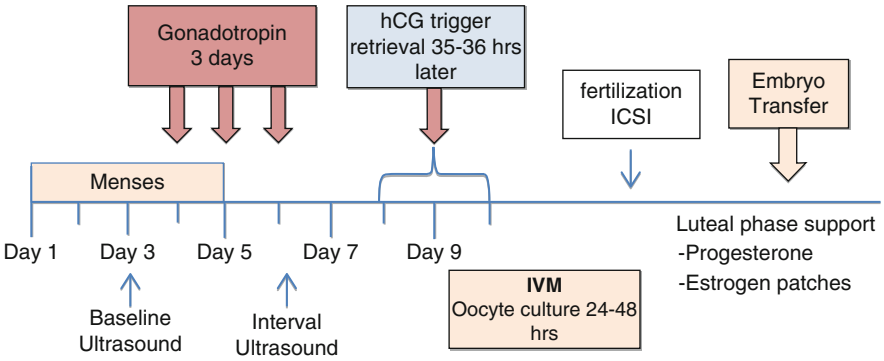

Fig. 12.3 In vitro maturation (IVM)—IVF protocol utilizing low gonadotropin dose priming, shortened follicular phase with harvesting of immature eggs followed by IVM of oocytes

agonist trigger), close monitoring of ovarian response, and preempting risk for severe OHSS. The success rates of IVF for women with PCOS are reassuring and limiting the number of embryos transferred can mitigate the risk of multiple pregnancies. Thus, young women with PCOS are ideal candidates for consideration of elective single embryo transfer.

In Vitro Maturation

In vitro maturation (IVM) entails a process somewhat similar to a traditional IVF cycle except that immature oocytes are retrieved and allowed to attain maturity in vitro over a 24- to 48-h period. Access to a laboratory with expertise in gamete biology and to special culture media is requisites for this undertaking. Since the first reported human pregnancy with IVM in 1991 by Cha et al., there have been many successful pregnancies reported, and over 2,000 babies have been born with this alternative approach [86, 87]. Compared to conventional IVF cycles, the treatment protocol for IVM is more convenient, requiring less medications and minimal monitoring; given that exposure to exogenous gonadotropins is limited, IVM has fewer side effects and a reduced risk for OHSS when compared with conventional IVF protocols.

An IVM treatment cycle typically begins after a naturally occurring or induced menstruation (Fig. 12.3). A baseline ultrasound is recommended on cycle day 1–3 to assess ovarian morphology and evidence of early follicular recruitment; baseline hormone parameters (FSH, E2) are also obtained. A second ultrasound is routinely done on cycle day 6–8 in order to monitor early selection of the dominant follicle and to assess the endometrial thickness. Transvaginal oocyte retrieval, of both mature and small immature follicles, is usually performed on days 8–10 depending on the size of the spontaneously achieved leading dominant follicle, which should not exceed 12–14 mm [88, 89]. Once the dominant follicle has reached an appropriate size, hCG is typically administered for oocyte maturation; hCG in IVM cycles has been shown

to improve the oocyte retrieval rate during aspiration as well as improve IVM cycle outcomes in women with PCOS [90]. Exogenous endometrial priming is essential in IVM protocols; typically, E2 supplementation is initiated at the time of egg retrieval and luteal progesterone support is then added 1–2 days later. Both E2 and progesterone supplementation are continued until 8–10 weeks of gestation, a point when luteo-placental shift in production of these steroid hormones is established.

Pretreatment with gonadotropins prior to oocyte retrieval in an IVM cycle remains a matter of debate [91–96]. There are conflicting data on IVM from natural cycles compared with gonadotropin-primed cycles; however, it appears that FSH priming may be of some benefit. Gonadotropin-primed cycles consist of a short course (3 days) of low-dose gonadotropin administration (75 IU hMG) initiated on day 3–4 of the cycle (see Fig. 12.3) [91]. Cha and Chian have shown more rapid progression of meiosis I and increased first polar body extrusion after FSH-primed IVM cycles when compared to natural cycles [91]. Furthermore, several other studies have demonstrated improved meiotic maturation after 48 h in culture with gonadotropin priming in IVM cycles compared to non-primed cycles [92–95]. Mikkelsen et al. found that the percentage of oocytes reaching metaphase II was significantly higher in the FSH-primed group compared to the non-primed group, however there was no difference in rate of oocyte fertilization or cleavage between the two groups [96]. In contrast, a study of 60 women randomized to either FSH priming or no priming found that there was no significant difference in the maturation rate (76.5 % vs. 71.9 %), fertilization rate (75.8 % vs. 69.5 %), or pregnancy rate (31.4 % vs. 36.4 %) between the two groups [97].

There is no randomized controlled trial that compares IVM and conventional IVF treatment cycles. The few case–control studies that compared IVM to IVF show that although IVM may be safer and simpler than conventional IVF, the success rates are significantly lower in IVM. Gremeau et al. compared 97 women undergoing IVM with 97 women undergoing conventional IVF in women with PCOS and found a significantly lower clinical pregnancy rate (50.5 % vs. 19.6 %) and live-birth rate (44.3 % vs. 16.5 %), respectively, in the IVM group [98]. These findings are consistent with other case–control studies, which show pregnancy rates of 22–27 % with IVM [99, 100]. Thus, while IVM may be an appealing strategy for younger women with PCOS because it nearly eliminates the risk for developing OHSS, enthusiasm for this option is somewhat dampened by the lesser success rates when compared with conventional IVF.

Summary

Management of ovulatory infertility of PCOS requires a detailed assessment and risk quantification and choice of therapy needs to be individualized to the clinical profile of each patient. Patients should be reassured and counseled regarding the spectrum of available options. Serious consideration must be given to achieve optimization of health parameters and risk reduction prior to initiating therapy;

importance of lifestyle modification for the overall well-being of patient cannot be minimized. For patients with ovulatory dysfunction as the primary mechanism for infertility, a stepwise approach is recommended with ovulation induction with use of CC or AIs being the first-line approach. The goal of infertility management is to achieve a singleton pregnancy while minimizing the risks of OHSS and multiple pregnancies. Consideration for the long-term health of the patient and for the safety of the pregnancy should be the primary goal of all health-care providers.

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Chapter 13
Managing the PCOS-Related Symptoms of Hirsutism, Acne, and Hair Loss

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Key Points

- Polycystic ovary syndrome is the most common endocrine disorder in reproductive-aged women.
- Dermatologic manifestations commonly experienced by women with PCOS include hirsutism, acne vulgaris, and androgenic alopecia or female hair loss, and are attributed to androgen excess.
- Combined hormonal contraceptive pills and anti-androgen agents are commonly utilized as treatment options for women with features of androgen excess.
- Cosmetic therapies may also be helpful in women who either cannot tolerate or whose symptoms are not adequately responsive to hormonal treatments.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women, with an estimated prevalence of 6–10 % based on US National Institutes of Health (NIH) report [1]. According to the European Society of Human Reproduction and American Society for Reproductive Medicine consensus workshop [2], a diagnosis of PCOS can be made based on the presence of at least two out of the

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following criteria: (1) androgen excess manifested by either clinical features of hyperandrogenism (such as hirsutism, acne, or androgenic alopecia), or evidence of hyperandrogenemia, (2) ovulatory dysfunction (oligo-ovulation or anovulation), and (3) polycystic appearance (defined as ultrasound evidence of 12 or more small follicles measuring 2–9 mm in diameter and/or increased ovarian volume of >10 mL) of at least one ovary on ultrasound examination. Although most women with PCOS have both clinical and biochemical evidence of androgen excess, discordance between clinical phenotype and hormonal milieu may be evident in some [3]. Hirsutism and acne are commonly recognized hallmarks of androgen excess; the relationship between androgenic alopecia and hyperandrogenemia, however, is less clear. Obvious manifestations of more excessive androgen elevation resulting in clinical virilization, such as deepening of the voice or clitoromegaly, warrant a thorough investigation for other etiologies, such as androgen-secreting tumors of the ovary or adrenal gland and genetic disorders, including late-onset congenital adrenal hyperplasia (CAH). As such, PCOS is a diagnosis of exclusion, an aspect that has been addressed elsewhere in this text.

Hirsutism

Hirsutism, defined as the increase in *terminal hair* growth in the androgen-dependent areas of the body (the upper lip, chin, mid-chest, abdomen, and back), can be identified in approximately 5–10 % of reproductive-age women [4]. Although idiopathic and familial variants to hirsutism are recognized, PCOS is probably the most common recognized contributor to hirsutism, with a prevalence of 57–82 % in women who are bothered by excess facial and body hair [5, 6]. Manifestations of androgen excess usually become evident around puberty, with both ovarian and adrenal androgens contributing to the clinical picture.

Although androgen excess underlies most cases of hirsutism, there is, however, only a modest correlation between the quantity of hair growth and circulating levels [3]. Androgen bioactivity is regulated in the hair follicle, primarily by the activity of enzyme 5-α reductase, which converts free testosterone to the more potent dihydrotestosterone (DHT) [7]. Two types of this enzyme have been identified; type 1 5-α reductase is found in the sebaceous glands and genital skin area, while type 2 is found primarily in the hair follicle and the scalp [7]. Type 2 5-α reductase activity is increased in disorders that have elevated circulating testosterone, and contributes to a dysregulation of hair follicle growth, prompting velus to terminal hair transition [7]. Androgen excess also increases hair follicle size, hair fiber diameter, and the proportion of time terminal hairs remain in the anagen (growth) phase [8]. Pertinent to the pathophysiology of PCOS, expression of 5-α reductase is additionally stimulated by insulin and insulin-like growth factor. It is thus apparent that hair growth excess in women with PCOS does not depend solely on circulating androgen concentrations, but also on local factors and variability in end-organ sensitivity and that insulin resistance is a likely contributor to the excess local androgen.

Evaluation of Hirsutism

A thorough history and physical exam, along with a biochemical evaluation of androgens, will help provide an idea of the degree of hyperandrogenemia and offer clues as to possible specific cause of androgen excess.

A detailed menstrual history should include information on age at menarche (early onset of menarche is relatively commonly encountered with PCOS as well as in cases of late-onset CAH), on regularity of the menstrual cycle, and on features suggestive of ovulatory cycles (such as mid-cycle cervical mucus changes, premenstrual breast tenderness, and premenstrual syndrome); information regarding history of hormone use for menstrual regulation and details regarding fertility-related concerns are also pertinent.

The age of onset and the rate of progression of the hirsutism and/or other clinical manifestations of hyperandrogenism are particularly relevant, especially when considering androgen-secreting tumors as a differential diagnosis; a rapid onset of symptoms and rapidity of progression in the severity of hirsutism makes a chronic disorder such as PCOS less likely.

Consideration must be given to potential iatrogenic contributors to the clinical picture; features of hyperandrogenism are commonly encountered as bothersome side effects of a number of commonly utilized pharmaceutical agents (e.g., danazol, phenytoin, cyclosporine, and anabolic steroids, to name a few) [9].

Any significant family history for menstrual irregularities or infertility may indicate an inherited enzymatic deficiency such as CAH, particularly in certain ethnic groups (e.g., women of Ashkenazi Jewish descent) [10].

The physical examination must quantify the distribution and severity of hirsutism with notation of hair growth along androgen-dependent versus androgen-independent areas; presence of *vellus hair* in the latter regions may be a source of bother to some women, but is considered as normal. *Hypertrichosis,* defined as increased hair growth in the androgen-independent areas, can be commonly seen in the setting of systemic disorders such as hypothyroidism, anorexia nervosa, or malnutrition [11].

Severity of hirsutism should be objectively assessed; the Ferriman–Gallwey screen was derived from a cohort of Caucasian women and represents one of a few tools that are available to the health-care providers to quantify the severity and pattern of hair excess [12]. The modified Ferriman and Gallwey score (FGS) assesses severity of hair growth along nine different androgen-dependent facial and body areas; the presence and pattern of hair growth are graded from 0 (absent) to 4 (dense). A FGS >8 is considered to reflect objective hirsutism, although one study revealed that approximately 50 % of both black and white women with FGS between 3 and 5 had evidence of an androgen excess disorder on further evaluation; these authors suggest lowering the normal FGS threshold to ≤ 3 [13].

Since the inter-observer measure of evaluating hirsutism is generally poor [14] and, often, women have gone through measures of hair removal prior to their initial evaluation by a physician, a biochemical evaluation is usually prudent, although the

Endocrine Society suggests against testing for elevated androgen levels in women with isolated and mild hirsutism, as the likelihood of identifying a medical disorder that would change management or outcome is relatively low [15]. The Androgen Excess Society guidelines, however, suggest measuring serum androgens in *all* women with hirsutism [16]. Although serum total testosterone concentration is considered a standard biochemical test in the evaluation of hirsutism, the accuracy of the currently available assays, particularly for women, remains questionable [16]. Despite evidence of hyperandrogenemia, most women with PCOS have serum total testosterone concentrations less than 150 ng/dL, and often in the normal range; in women, serum total testosterone levels above 150 ng/dL warrant consideration of an ovarian androgen-secreting tumor that merits further evaluation with a pelvic ultrasound.

Dehydroepiandrosterone (DHEA) is an adrenal androgen precursor that is elevated in a proportion of women with hirsutism; serum levels of DHEA-S (sulfate), the stable metabolite of DHEA, should thus be additionally assessed in the setting of hirsutism. Significant elevations in serum DHEA-S levels (greater than 700 μg/dL) should raise suspicion for an adrenal tumor as a source of androgen excess and merit further evaluation by abdominal imaging (e.g., by computed tomography). Late-onset CAH must be considered as a differential diagnosis when evaluating women with hirsutism, particularly in women of Ashkenazi Jewish decent [10]. A morning value of 17-hydroxyprogesterone (17-OHP) greater than 200 ng/dL in the early follicular phase of the menstrual cycle is suggestive of CAH and must be further pursued by undertaking an adrenocorticotropic hormone (ACTH) stimulation test. If there is an exaggerated response to ACTH (cosyntropin) (a 17-OHP value greater than 1,500 ng/dL), a diagnosis of CAH is made [17].

Finally, Cushing's syndrome should remain a consideration in the list of differential diagnoses when evaluating a woman with evidence of clinical hirsutism, particularly when there is evidence of centripetal obesity, hypertension, and/or the presence of purple cutaneous striae. A 24-h urine collection for estimation of urinary free cortisol (UFC) is a sensitive screening test. A UFC level more than three times the upper limit of normal should prompt further investigations to identify underlying mechanisms for cortisol excess (details of which are beyond the scope of this undertaking) or a referral to an medical endocrinologist for further evaluation and management.

Treatment of Hirsutism

The 2008 Endocrine Society guidelines recommend that the degree to which the excess hair bothers an individual patient should guide management decisions [15]. Treatment efficacy and patient expectations should be addressed at inception, and the patient should be informed that pharmacotherapy may not completely eliminate excess hair growth. Rather, with therapy, excess hair may become less coarse, grow more slowly, and require less frequent use of depilatory methods over time. In addition, the patient must realize that pharmacotherapy is not likely to eliminate

Table 13.1 Management approach to hirsutism

Evaluation	History
	Rapidity of progression in symptoms
	Menstrual
	Medical
	Medication
	Common tests
	Total (and free) testosterone
	DHEA-S
	17-OH progesterone
	24-h UFC (if suspecting cortisol excess)
	Pelvic ultrasound (if indicated)
Pharmacotherapy	OCPs
	CPA
	Anti-androgens
	Spironolactone
	Flutamide
	Finasteride
	GnRH agonist
	Glucocorticoids
	Eflornithine hydrochloride cream
	Insulin-sensitizing agents
Cosmetic	Shaving
	Waxing
	Epilation
	Depilation
	Laser and light therapies
	Electrolysis

DHEA-S dehydroepiandrosterone sulfate, *17-OH progesterone* 17-hydroxy progesterone, *UFC* urinary free cortisol, *OCP* combined oral contraceptive, *CPA* cyproterone acetate (not available in the United States), *GnRH agonist* gonadotropin-releasing hormone agonist

terminally differentiated hair and be encouraged to complement medical therapy with physical depilatory strategies to maximize overall benefit. Furthermore, it is imperative to inquire about the patient's perspective on fertility, as almost all the available therapies (Table 13.1) for targeting hirsutism are contraindicated for women seeking pregnancy.

Pharmacotherapy for Managing Hirsutism

The goal of medical treatment of hirsutism is to reduce the circulating levels of total androgens and of free testosterone, and limit the actions of androgens at the hair follicle itself. Due to the duration of the hair growth cycle, any treatment is unlikely

to offer perceptible benefit in the short term; patients should be advised to pursue treatment, provided it is well tolerated, over at least a 6-month period before deeming any tried strategy as ineffective.

Combined oral contraceptive pills (OCPs) are the first-line pharmaceutical recommendation for managing hyperandrogenemia and hyperandrogenism of PCOS. Suppression of ovarian steroidogenesis with the use of OCPs is accompanied by a decline in ovarian androgen production. An increase in the hepatic production of sex hormone-binding globulin (SHBG) occurs in response to the estrogenic component of OCPs, which in turn decreases the amount of free testosterone available. Indeed, serum free testosterone was reported to decrease by at least 50 % in hyperandrogenic women treated with an ethinyl estradiol/norethindrone acetate-containing OCP regimen [18]. There is also a suggested reduction in adrenal androgen production [19] and perhaps a slight inhibition in the binding of androgens to their innate receptors consequent to OCP exposure. Overall, beyond the contraceptive benefit, improvements in hair growth and acne are commonly seen with use of OCPs. Subjective improvement in hirsutism with OCP use has been reported in the range from 60 to 100 % [20, 21]. Others have noted a 25 % improvement in actual hair growth as an objective measurement [21], while some record longer intervals of use of depilatory measures after 8 months of treatment [22]. While choosing an OCP regimen, consideration should be given to the progestin component, given the varying androgenicity potential of the available progestin; OCPs containing low androgenicity (e.g., desogestrel and norgestimate) or anti-androgenic progestins such as drosperinone or cyproterone acetate (CPA) may be preferentially considered for women with features of hyperandrogenism [20, 23, 24]. Notably, however, an increase in both venous and arterial thromboembolic events has recently been suggested in users of drosperinone compared to levonorgestrel-containing OCP regimens [25]; thus, judicious caution must be exercised in the decision to use and choice of regimen for non-contraceptive indications.

Anti-androgens are commonly employed in conjunction with OCPs as a second-line strategy for the management of bothersome hyperandrogenism. Spironolactone, an aldosterone agonist, is structurally similar to the progestin drosperinone and competes with DHT for binding to the androgen receptor [26]. Spironolactone has also been shown to exhibit 5-α reductase inhibitory activity [27]. A 2008 Cochrane meta-analysis from the Menstrual Disorders and Subfertility Group reported that in the two trials that compared 100 mg of spironolactone with placebo, significant differences were reported for subjective improvements in hair growth (OR 7.18, 95 % CI 1.96–26.28), and spironolactone is recognized as an effective treatment for hirsutism [28].

Off-label use of flutamide, a nonsteroidal androgen receptor antagonist, for the management of hirsutism is well described, as is use of finasteride, a type 2, 5-α reductase inhibitor that impairs the production of DHT. Given their teratogenic potential (i.e., potential for adversely affecting the development of external genitalia in an inadvertently exposed male fetus), anti-androgens must not be used as single agents by reproductive-age women who are deemed at risk for unplanned pregnancy, and a concomitant contraceptive strategy must be instituted for women who

have the potential to conceive. Flutamide appears to be as effective as spironolactone in treating hirsutism, as was demonstrated in a 2008 prospective randomized study that compared the effectiveness of flutamide and spironolactone plus an OCP in the 6-month treatment of hirsutism in 29 women with PCOS. Hirsutism was graded according to the modified FGS; a significant, albeit comparable decrease in FGS was observed in the flutamide and spironolactone plus OCP groups, and neither emerged as a preferred strategy [29]. Finasteride similarly has demonstrated comparable efficacy to spirinolactone in the management of hirsutism [30]. While hepatotoxicity associated with flutamide, even at low doses [31], limits its utilization for indications such as hirsutism, finasteride, in contrast, is a relatively safe agent, barring its potential for teratogenicity (as discussed earlier).

CPA is a 17-hydroxyprogesterone derivative that competitively inhibits the action of DHT at its receptor [32]. Effectiveness of CPA in reducing hirsutism in women with PCOS is well studied. A recent double-blinded randomized controlled trial compared the effects of CPA alone to an OCP containing desogestrel alone or drospirenone alone in 171 patients; after 12 months of use, CPA significantly increased SHBG and decreased the free androgen index in addition to significantly decreasing the modified FGS [32]. CPA is commonly used either as the progestin component of OCP regimen, or in a higher dose as monotherapy for the management of hirsutism, but it is not available in the United States.

Suppression of the hypothalamic-pituitary-ovarian axis (e.g., with the use of a gonadotropin-releasing hormone [GnRH] agonists) has been shown to improve androgen levels in women with PCOS. Seventeen randomly assigned hirsute women received 6 months of treatment with leuprolide depot plus conjugated estrogen and medroxyprogesterone acetate (MPA) or just an OCP. Baseline gonadotropin and androgen levels were obtained at 0 and 28 weeks of treatment, and hirsutism was evaluated subjectively by patient self-evaluation and objectively by FGS and by determination of facial hair density, outer hair shaft diameter, and growth rate, determined both photographically and in plucked hairs [33]. Those receiving leuprolide plus estrogen and MPA demonstrated a significant decrease in the actual hair growth rate (using the photographic method) when compared to the OCP-alone group [33]. However, concurrent estrogen replacement must be considered for women who opt for long-term use of this treatment modality to mitigate bone loss that would occur secondary to resulting hypoestrogenism [34]. Because of an overall adverse profile, long-term GnRH agonist therapy should be reserved for patients who fail to achieve adequate response to first- and second-line strategies available for the management of hirsutism.

Hyperinsulinemia and insulin resistance are encountered in almost 60 % of patients with PCOS [35]. Excess insulin and elevated levels of LH are both recognized to stimulate ovarian androgen synthesis. An improvement in androgen profile is well described following use of insulin-sensitizing strategies in women with PCOS, albeit inconsistently. Randomized treatment with either troglitazone, an insulin-sensitizing agent, or placebo in 410 premenopausal women with PCOS did show a significant decrease in FGS in patients treated with troglitazone compared with placebo; a concomitant decrease in free testosterone with troglitazone use was

further noted [36]. However, a 2008 meta-analysis performed as part of the Endocrine Society's Clinical Guidelines for the Evaluation and Treatment of Hirsutism in Premenopausal Women concluded that low-quality evidence suggests that insulin sensitizers provide limited or no important benefit for hirsutism [15].

Glucocorticoids have traditionally been used to manage hirsutism and ovulatory dysfunction in women with late-onset CAH; resumption in menstrual cyclicity with this strategy is well described, whereas efficacy against hirsutism remains equivocal. Exogenous glucocorticoids suppress hypothalamic corticotropin-releasing hormone production and, therefore, the production of pituitary corticotropin and adrenal androgen production, ultimately decreasing the amount of downstream androgens. Treatment was offered to 28 women with hirsutism diagnosed with the nonclassical form of CAH due to 21-hydroxylase deficiency [37] and in women with unspecified hyperandrogenemia and hirsutism, including those with PCOS [38]. No clear benefit was seen in either of these two populations when evaluating glucocorticoids against other treatment modalities, including CPA and spironolactone. Given their relative lack of benefit for hirsutism and an adverse effect profile that is not minimal, the 2008 Endocrine Society guidelines suggest against glucocorticoid therapy for routine treatment of hirsutism, with exceptions made for women with nonclassic CAH who do not respond to or cannot tolerate OCP or antiandrogen therapies [15].

Finally, topical eflornithine hydrochloride cream 13.9 % (Vaniqa), an inhibitor of enzyme ornithine decarboxylase in the skin, has been FDA-approved for treating excess of facial hair. After 24 weeks of use in women with excessive, unwanted facial hair, eflornithine hydrochloride cream was superior to placebo (58 % as compared to 34 %, respectively) in reducing hair growth, as demonstrated by objective and subjective methods [39]. Furthermore, combined eflornithine and laser therapy demonstrated a more rapid response when compared with laser treatments alone [40]. In general, noticeable results take approximately 6–8 weeks and pretreatment levels of hair will return once the therapy is discontinued. In addition, use of Vaniqa is quite expensive and generally not covered by most insurance plans, which may limit its practical use for many patients.

Non-pharmacotherapy Strategies for Managing Hirsutism

Temporary Measures

Several non-prescription options are available for managing hair excess and are commonly utilized, particularly, in conjunction with pharmacological interventions. Temporary methods include shaving, epilation, waxing, and depilation. Shaving is the mechanical process of removing hair down to just below the surface of the skin; while promptly addressing the hair excess, efficacy is short-lasting, requiring daily use in many cases. Skin irritation, risk of folliculitis keratosis, and discoloration particularly limit utilization of this strategy for the management of excess facial hair.

Plucking or epilation is a relatively safe and inexpensive method of hair removal that allows hair to be absent for 6–8 weeks in duration, although it may be time-consuming and uncomfortable. Potential for folliculitis, however, does exist. Hair plucked during the anagen phase of growth may have lasting benefit, as there is a likelihood of destroying the dermal papilla, which would lead to permanent hair removal. Waxing is another form of epilation that involves applying a layer of sticky wax to the area where hair removal is desired; it is of particular benefit when dealing with large surface areas that merit attention.

Depilation is the use of chemicals containing thioglycolates, which dissolve the hair by disrupting the disulfide bonds present in the hair itself. The dissolved and disintegrated hair shaft can be wiped away, with resulting cosmetic benefit; hair loss through use of depilatory agents, however, is temporary, with results lasting approximately 2 weeks. In addition, contact dermatitis can occur with the use of depilatories [41].

Although not an actual method of hair removal, bleaching with the use of a product that contains hydrogen peroxide is a relatively common method for masking the presence of undesired hair; side effects include irritation and possible skin discoloration.

Permanent Measures

Photoepilation therapies such as laser may be used to reduce hair density via selective photothermolysis, a process that directs energy and sufficient heat into the target area of hair to destroy the hair bulb; the surrounding areas remain relatively untouched. This process usually results in a reduction in hair growth; new hairs in the treated area are lighter and thinner in nature, although multiple treatment sessions are usually required before any benefit may become apparent, and one is unlikely to achieve complete resolution of excess hair. A prospective study of six laser treatment sessions in 60 women with PCOS resulted in a poorer than expected reduction in hair counts and hair-free interval following treatments [42]. However, in a 2005 randomized controlled trial of five laser treatments versus five sham laser treatments in 88 PCOS women with facial hirsutism, a significantly greater decline in the severity of facial hair was perceived by women in the intervention group compared to those assigned to sham intervention; an improvement in psychological quality of life was further appreciated [43]. Laser therapy is most ideal for women who have light-colored skin, as women who have dark skin have an increased risk of long-term pigment changes and burns. A newer laser therapy for hair removal in individuals with dark skin is the long-pulsed neodymium:yttrium-aluminum-garnet (Nd-YAG) laser, which has demonstrated efficacy in dark-skinned individuals and a low rate of pigmented changes [44]. Patients typically have 4–6 treatments spaced approximately a month apart in order to achieve satisfactory cosmetic benefit. Once the primary course is complete, patients may require maintenance treatments once every 6–12 months to remove the smaller vellus hairs that may grow back [45].

Electrolysis, a therapy that is performed by inserting a needle into the hair follicle and applying an electrical current that creates heat, is technically the only permanent method of hair removal [46]. The therapeutic efficacy is based on permanent destruction of the rapidly dividing cells of the matrix and the follicle itself. Electrolysis can be effective in reducing hair counts and creating a permanent removal of hair in 75–85 % of patients [41]; however, it is a painful process and can cause inflammatory pigment changes and scarring. In comparing laser therapy versus electrolysis, a European study enrolled 25 women with facial hirsutism to split face treatments of both electrolysis and laser, over six treatment sessions. Patients were evaluated with respect to reduction in hair counts, side effects, and discomfort during treatment; hair regrowth was assessed at 3, 6, and 9 months following treatment. Ninety-six percent of the patients preferred laser to electrolysis treatment, with higher efficacy and satisfaction with former compared to the latter strategy [47].

Acne

The prevalence of acne in women with PCOS has been estimated to be 10–34 %, with as high as 50 % prevalence reported in adolescent girls [48]. In a study of 52 women ages 18–35 years of age with mild acne versus 59 age-matched controls, PCOS was significantly more common in the patients with acne than in age-matched controls [49]. Pathogenesis of acne entails the formation of comedones due to increased sebum accumulation, which allows bacterial colonization with *Propionibacterium acnes* (*P. acnes*), and subsequent inflammation. Androgens contribute to the development of acne by stimulating sebum production, growth and secretory function of the sebaceous glands, and setting the stage for bacterial colonization and ensuing inflammatory response. The relationship of androgens to the development of acne is well established; adults with an aberrant androgen receptor do not produce sebum from sebaceous glands and do not develop acne [50]. Androgen-responsive sites for acne include the face, neck, mid-chest, and upper back, areas that are commonly affected in women with PCOS.

Treatment for Acne

The goals of therapy for acne are to reduce the amount of sebum production, clear the existing comedones, and reduce the amount of bacterial colonization and inflammation (Table 13.2). For patients suffering from sequelae of severe acne (i.e., scarring), dermabrasion strategies offer a potential for reducing the severity of scarring.

Table 13.2 Management approach to acne

Evaluation	History
	Rapidity in progression of symptom
	Menstrual
	Medical
	Medication
	Common tests
	Total (and free) testosterone
	DHEA-S
	17-OH progesterone
	Additional tests as summarized in Table 13.1 (if indicated)
Systemic pharmacotherapy	OCPs
	CPA
	Oral antibiotics
	Oral retinoids
	Oral anti-androgens
	Spironolactone
	Flutamide
	Finasteride
Topical agents	Benzyl peroxide
	Retinoid cream
	Antibiotic cream

DHEA-S dehydroepiandrosterone sulfate, *17-OH progesterone* 17-hydroxy progesterone, *OCP* combined oral contraceptive, *CPA* cyproterone acetate (not available in the United States)

Systemic Treatment

In addition to slowing hair growth and improving bothersome hirsutism, OCPs are of proven benefit against acne and seborrhea. OCPs, particularly those preparations with low androgenic progestins, are indicated therapy for women with excessive ovarian androgen production such as PCOS. Hormonal therapy is thought to improve acne by inhibiting the action of androgens at the pilosebaceous units. As discussed under the section on hirsutism, the estrogen component of the OCP regimen suppresses ovarian androgen production, increases SHBG, and reduces the level of biologically available circulating free androgen. In a prospective study of 30 women with acne, treatment with a biphasic OCP containing ethinyl estradiol (EE) and desogestrel resulted in a resolution of acne in 77 % of patients after a treatment course of 9 months [51]. This beneficial effect was also seen in a study of 250 women with acne vulgaris who were randomly assigned to either an OCP containing ethinyl estradiol and norgestimate or placebo for 6 months. Significant improvements in the total number of lesions as well as in patient and investigator perspective were observed in the OCP group compared to those assigned to placebo [52]. The effectiveness of OCP use in treatment of acne was also validated in a recent 2012 Cochrane review evaluating 31 trials of OCP use in women with hyperandrogenemia [53].

Although all low-dose OCPs are estrogen-dominant, which makes these agents anti-androgenic in nature, drosperinone- and CPA-containing OCPs offer additional benefit due to the anti-androgenic nature of these progestins. A pooled analysis of two placebo-controlled randomized trials with a total of 893 females with moderate facial acne found that patients treated with a drosperinone-containing OCP (3 mg drosperinone and 20 μg EE) for six cycles were more likely to attain clear skin than those receiving placebo (odds ratio [OR] 3.41, 95 % CI 2.15–5.43), and achieved a greater mean reduction in acne lesion counts [54]. In a head-to-head comparison of another drosperinone-containing OCP regimen (35 μg EE plus 3 mg drosperinone) against a standard OCP formulation (35 μg EE and norgestimate 0.180, 0.125, 0.250 mg), patients receiving drosperinone-based regimen had a superior therapeutic efficacy for facial acne compared to the standard formulation [55]. It is important to keep in mind that progestin-only oral contraceptive and injectable MPA contraceptive strategies not only are unlikely to offer cutaneous benefit, but more importantly, can exacerbate acne [56]; these latter strategies should be avoided in women bothered by acne.

In some cases of moderate to severe acne, patients may require treatment with a combination of an OCP and an oral antibiotic simultaneously. Oral antibiotics improve inflammatory acne by inhibiting the growth of *P. acnes* within the pilosebaceous unit. The effectiveness of an oral antibiotic, like tetracycline or doxycycline, has been studied in multiple randomized trials [57]. Systemic antibiotics produce more rapid clinical improvement than topical preparations, but may induce side effects such as vaginal candidiasis or gastrointestinal distress. However, in order to achieve sufficient results, antibiotic treatment often requires several months of use to achieve an adequate response. Optimally, antibiotics are prescribed for daily use for less than 6 months with subsequent discontinuation as acne improves [58]. For patients who are on a combination OCP and oral antibiotic, the only antibiotic that may reduce contraceptive efficacy of the OCP regimen is rifampin [59].

Anti-androgenic agents discussed under the section on hirsutism have been studied for their efficacy against acne. Both spironolactone and flutamide (i.e., agents that block the androgen receptor directly at the site of the pilosebaceous unit and thereby decrease sebum production) can be used in the management of acne. However, existing data do not consistently demonstrate greater effectiveness of use of spironolactone over an OCP in acne management, and the adverse effect profile of flutamide does not warrant its use for acne treatment. A 2009 Cochrane review of randomized trials found insufficient evidence to confirm the efficacy of spironolactone in women with acne [28], although several published small studies have reported treatment benefit. A 2005 prospective study reported on treating 35 patients with acne with spironolactone; the authors suggest that the majority of patients had significant improvement in facial lesions [60]. Spironolactone therapy may be considered for women whose moderate to severe acne has not responded to conventional OCP trial and who prefer to avoid exposure to the teratogenic oral isotrentinoin (see below). It is also important to remember that since spironolactone is a potassium-sparing diuretic, and patients should be monitored for possible development of hyperkalemia, particularly when using a combination of spironolactone and

a drosperinone-containing OCP, where monitoring of serum potassium level is recommended at baseline and again at 4–6 weeks after starting therapy [61].

For patients who are unresponsive to the conventional hormonal regimens discussed above, oral isotretinoins, vitamin A derivatives that bind to retinoic acid receptors within the keratinocytes, are considered the most efficacious strategy against moderate to severe inflammatory acne. This class of drugs decreases sebum and the proliferation of *P. acnes*, inhibits development of comedones, and is anti-inflammatory in nature. Treatment duration usually lasts for a minimum of 6 months. The major disadvantage of this treatment is potential teratogenicity and a side-effect profile that includes dryness of the mucous membranes, stiffness, fatigue, headache, inflammatory bowel disease, conjunctivitis, peeling of the skin on the palms and soles, transient abnormalities in liver function tests, and elevation in serum lipids [62].

Topical Treatment Options for Acne

Topical treatment, including topical retinoids, will aid in decreasing comedones and bacterial colonization but usually do not decrease the amount of sebum production [63]. The topical retinoids include all-trans retinoic acid (t-retinoin), adapalene, and isotretinoin (tazarotene). Pooled results of two randomized controlled trials of 847 patients with acne vulgaris treated with isotretinoin for 12 weeks showed a significantly greater reduction in total lesion count compared with placebo (43 % vs. 23 %) [64]. In order to minimize the chance of irritation, it is recommended to start with the lowest-strength preparation (0.025 % cream), and then increase the potency as needed and tolerated. Most topical retinoid treatment options predispose patients to photosensitivity, and use of sun-cream protection is recommended.

Topical anti-bacterial agents such as tetracycline, clindamycin, and erythromycin, either used alone or in combination with a topical retinoid, can offer benefit. In general, these agents are better tolerated than t-retinoin or benzoyl peroxide, the original gold standard for acne treatment. Benzyl peroxide has potent anti-bacterial action but weak comedolytic activity, and exposure to higher concentrations can lead to significant skin irritation.

Female Hair Loss

Androgenic alopecia, also known as female pattern hair loss, is a hereditary condition deemed a sequel to disruption of proper androgen signaling [65]. This results in decreased proliferation of follicle epithelia and progressive miniaturization of terminal hairs on the scalp and is the most common type of hair loss that occurs in women [66]. Androgenic alopecia commonly occurs at the time of menopause, and is attributed to a relative androgen excess seen following cessation of ovarian function; androgenic alopecia is also described in conditions such as PCOS, when elevated androgens persist, is generally non-scarring, and does not permanently destroy the

hair follicle. As such, it is important to specifically ask about hair loss and not just excess hair growth in women who have PCOS or other disorders of androgen excess. Typically, hair growth is cyclical and comprised of three distinct phases—the growth phase (anagen), the involutional phase (catagen), and the resting phase (telogen). Normally, the majority of hair follicles are in the anagen phase and as hair is shed from the telogen phase, the same numbers of follicles enter the anagen phase [67]. The loss of hair that occurs in androgenic alopecia is due to a shortening of the anagen phase, resulting in a shorter, thinner hair shaft; varying degrees of partial hair thinning are commonly encountered, primarily at the vertex and frontal areas of the scalp [66].

The diagnosis of androgenic alopecia is sometimes difficult to make, as often women use varying methods to hide the hair loss. In addition, there are multiple hair and scalp disorders that may present with clinical features that resemble androgenic alopecia, making the diagnosis not always clear. Hair loss can be categorized as either scarring and non-scarring alopecia [68]. Scarring alopecias are conditions that lead to the irreversible cessation of hair cycling and permanent hair loss, while in non-scarring alopecias, the hair follicle is not permanently damaged and, often, a spontaneous or treatment-induced regrowth is a possibility. Telogen effluvium is a disorder that may be most difficult to distinguish from androgenic alopecia because patients present with acute or chronic non-inflammatory, diffuse hair loss that often follows a significant event, such as childbirth, a major illness, or severe trauma. Hair loss occurs in all areas of the scalp, although the hair loss may be most evident in the temporal area [68]. Another diffuse form of hair loss includes alopecia areata, a disorder that is characterized by a global reduction in pigmented hair density, often detected in circumscribed areas. In addition, these patients may have nail abnormalities and patchy hair loss on other body sites, findings that are not typically associated with androgenic alopecia [68]. Although a scalp biopsy is usually not needed to diagnose androgenic alopecia, it may be helpful in cases when the clinical evaluation does not provide a definitive diagnosis, as in distinguishing androgenic alopecia from telogen effluvium or diffuse alopecia areata and, furthermore, in differentiating scarring and non-scarring alopecia [69]. The Ludwig scale is one of the most commonly used grading systems that describes and classifies the distinctive features of androgenic alopecia into three grades of severity referred to as Ludwig grades I, II, and III [70]. These pictorial diagrams may be helpful in determining the extent of hair loss and assessing progress during and after treatment (Fig. 13.1).

Treatment of Female Hair Loss

Topical minoxidil (Rogaine) and oral finasteride (Propecia) are the only drugs approved by the US FDA for treatment of androgenic alopecia. Minoxidil, approved for use in women as well as men, promotes hair growth by increasing the duration of anagen and enlarging suboptimal follicles via a mechanism involving nitric oxide [71]. Two double-blind studies enrolling 550 women ages 18–45 years with androgenic alopecia demonstrated the efficacy of 2 % minoxidil, citing improvement in hair growth from 13 to 50 % compared to 6–33 % in the placebo group [72, 73].

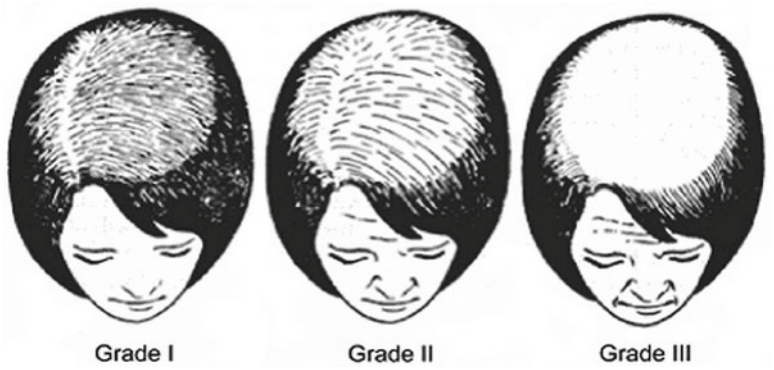

Fig. 13.1 Ludwig Scale for the classification of female pattern baldness in androgenic alopecia. Grade I (mild): Perceptible thinning of the hair on the crown, limited in the front by a line situated 1–3 cm behind the frontal hair line. Grade II (moderate): Pronounced rarefaction of the hair on the crown within the area seen in Grade I. Grade III (extensive): Full baldness (total denudation) within the area seen in Grades I and II. Reprinted with permission from Ludwig E. Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex. Br J Dermatol 1977 Sept;97(3):247–54. Copyright © 2006, John Wiley and Sons

Furthermore, in a 48-week duration randomized controlled trial undertaken in 381 women ages 18–49 years with female pattern hair loss, 5 % topical minoxidil was noted to be superior to the 2 % formulation in improving non-vellus hair count and scalp coverage [74]. Minoxidil must be used long-term, as relapse in hair miniaturization soon follows cessation of topical therapy. Contact dermatitis is a common side effect of topical minoxidil treatment.

Although finasteride (Propecia) is approved for managing hair loss, albeit only in men, off-label use of this agent is described in women suffering from androgenic alopecia. Data on efficacy of finasteride for female hair loss are, however, conflicting. Observational studies seem to suggest that finasteride may be effective for female pattern hair loss [75, 76], although this impression was not confirmed in a randomized trial [77]. Although objective evidence of efficacy is thus limited, finasteride may be considered for the management of female pattern hair loss in patients who fail topical minoxidil treatment.

Limited evidence supports some efficacy of spironolactone and CPA in managing androgenic alopecia. In an observational study in 40 women, daily use of 200 mg spironolactone was associated with stabilization of hair loss or even hair regrowth; similar results were observed with use of 50 or 100 mg of CPA in another group of 40 women [78]. As discussed in the section on hirsutism, potential for teratogenicity with use of oral anti-androgens must be kept in perspective when considering these agents for the management of androgenic alopecia, and concomitant use of a reliable contraceptive option must be stressed for women of child-bearing potential.

Potential benefit of laser therapy for hair loss seems counterintuitive given the discussion on efficacy of lasers against hair excess. However, a paradoxical sequel to laser therapy for managing unwanted hair is the development of hypertrichosis and growth of terminal hair; these sequelae may occur after several months within

Table 13.3 Management approach to female hair loss

Evaluation	History
	Onset and rapidity in progression of symptom
	Menstrual
	Medical/stress
	Medication
	Common tests
	Total (and free) testosterone
	DHEA-S
	17-OH progesterone
	FSH/LH/estradiol
	TSH
	Scalp biopsy
Systemic pharmacotherapy	Minoxidil
	Spironolactone
	Finasteride
Local/cosmetic	Laser and light therapies
	Surgery (hair transplant)

DHEA-S dehydroepiandrosterone sulfate, *17-OH progesterone* 17-hydroxy progesterone, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *TSH* thyroid-stimulating hormone

and/or proximal to areas treated with laser devices [79]. Low-level light therapy has recently increased in popularity as a stand-alone or adjunctive treatment, in treating androgenic alopecia, although a consensus written by hair-loss experts states that recommendation for use is only based on anecdotal experience [80]. The first independent blinded study of low-level light therapy for the treatment of androgenic alopecia was performed in seven patients, who noted an increase in the number of terminal hairs, and an increase in shaft diameter after treatment; however, these data were found not to be statistically significant [81]. Overall, evidence for the efficacy of light therapy in patients with female hair loss is underwhelming (Table 13.3).

A permanent improvement in female hair loss can be achieved through surgical therapy, primarily hair transplantation using follicular units [82]. Patients must have stable androgenetic alopecia and an adequate reservoir of hair for transplantation. Hair follicles are removed from a non-balding occipital scalp area, generally not affected by hyperandrogenism, and transplanted into the affected balding areas [83]. Patients can continue to lose non-transplanted hairs within susceptible areas following hair transplantation, resulting in diminishing satisfaction with the results; thus, the use of medical therapy following hair transplant is usually recommended.

Summary

In summary, hirsutism, acne, and/or female hair loss are common and bothersome accompaniments to a diagnosis of PCOS, and are significant contributors to psychological stress and a poor self-image. Hyperandrogenemia and hyperinsulinism are

recognized as contributory mechanisms to hirsutism and acne, and the mainstays of management for these common disorders include strategies that suppress androgen production (such as use of OCP), antagonize androgen action (such as use of anti-androgens), address the physical burden (e.g., laser, depilation), and slow hair growth (e.g., topical enflornithine). Mechanisms underlying female pattern hair loss are relatively less understood; genetic predisposition is recognized to play an important role. Available therapeutic options are relatively few and efficacy is far from absolute.

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Part IV
Unique Considerations in PCOS

Chapter 14
PCOS in Adolescence: Diagnostic Dilemmas and Management Considerations

Tania S. Burgert

Key Points

- Diagnosis of PCOS poses unique challenges given overlapping symptoms of puberty.
- While menstrual irregularity is common within the first 2 years of onset of menarche, menstrual disturbances may be the earliest sign of PCOS in adolescents and merit evaluation.
- Hyperandrogenemia is the key to PCOS diagnosis in adolescence.
- Similar to adults, relevance of insulin resistance to the pathophysiology of PCOS also holds true for the adolescent population.
- Oral contraceptives and metformin are safe and effective treatment strategies commonly utilized for symptom control and risk reduction in adolescents with PCOS; long-term data in this population are however lacking.
- Existing data underscore a need for long-term follow-up of adolescents with PCOS to quantify long-term risks relating to the diagnosis, and to gain a better understanding of benefits of insulin sensitizing strategies that are increasingly being employed in this population.

Introduction

Until recent years, the diagnosis of polycystic ovary syndrome (PCOS) was reserved for adult women presenting with sub/infertility tied to irregular menses/anovulation and hirsutism/hyperandrogenism [1]. With increasing awareness of multifactorial

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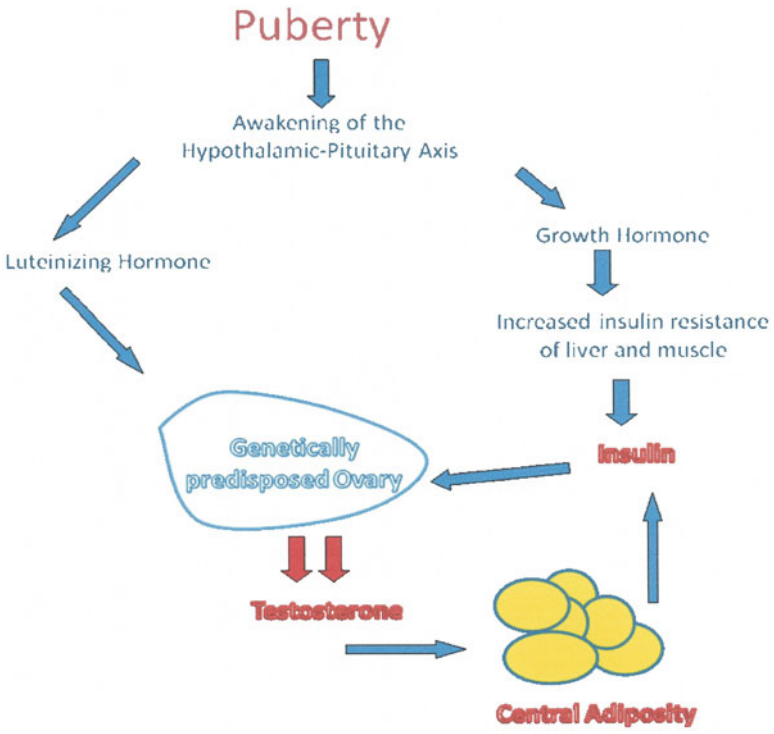

Fig. 14.1 Hormonal paradigm in pubertal PCOS

diseases in younger populations, PCOS has become a more frequent consideration among pediatric providers. However, transferring adult diagnostic criteria for PCOS to the adolescent population has proven to be most challenging, mainly due to overlapping symptoms of normal puberty. In puberty, central axis immaturity and physiologic insulin resistance are often coupled with ovarian anovulation and mild acne/hirsutism, mimicking a PCOS phenotype. In many cases, the differentiation to bona fide PCOS lies merely in the degree of clinical and biochemical expression. However, puberty is the first test of ovarian handling of insulin and LH stimuli and therefore offers an opportunity for early diagnosis of this clinical condition that may have lifelong health implications. Genetically predisposed adolescents may exhibit an exaggerated ovarian response to physiologic and non-physiologic (e.g., obesity) stimuli at puberty, with an ensuing hormonal cascade that is well recognized in the context of PCOS (Fig. 14.1).

The overlap in symptoms of physiologic puberty and PCOS had led to a diagnostic hesitation, likely leaving young women under-evaluated and under-treated until reproduction is desired. Given the potential medical implications of untreated PCOS such as metabolic syndrome/type 2 diabetes mellitus (T2DM), infertility, and endometrial cancer, early detection may have multiple health benefits. As with most conditions in the pediatric age group, very few studies have been conducted looking at the long-term benefits of early interventions for adolescent PCOS. However, data are available

for a group of non-obese adolescents who developed PCOS after being diagnosed with premature adrenarche, a common condition recognized as heralding PCOS. For this group of adolescents, Ibanez and de Zegher have extensively studied the metabolic effects of various interventions and have collected long-term and early intervention data [2–5]. It appears that early diagnosis and treatment improves menstrual symptoms, body composition, and, most importantly, cardio-metabolic profile in patients with PCOS. Unfortunately, once therapy is discontinued, many of the metabolic benefits cannot be sustained [6].

This chapter reviews the diagnostic challenges, the early metabolic manifestations, and treatment options for adolescent PCOS.

Diagnosis of PCOS in Adolescence

To date there is no unified approach to the diagnosis of PCOS in adolescence. Even among members of the North American Society for Pediatric and Adolescent Gynecology (NASPAG), there remains considerable heterogeneity in the approach to diagnosing adolescents [7].

Tackling the Definition

In general terms, PCOS is defined by a constellation of signs and symptoms, after other organic causes have been excluded. Such conditions include adrenal disorders (i.e., late-onset congenital adrenal hyperplasia), thyroid disorders, primary or secondary ovarian insufficiency, hyper-prolactinemia, androgen-producing tumors, and pregnancy. The first unifying approach to adult PCOS was proposed during a 1990 consensus meeting at the National Institutes of Health (NIH). The so-called NIH Criteria require the presence of chronic anovulation and clinical and/or biochemical hyperandrogenism. In 2003 another PCOS consensus workshop in Rotterdam, Netherlands broadened the definition to allow ultrasound evidence of polycystic ovaries (PCO) to substitute for either anovulation or hyperandrogenism. Hence, three following potential phenotypes of PCOS were created.

1. Anovulation + hyperandrogenism
2. Anovulation + PCO on pelvic ultrasound (non-hyperandrogenemic phenotype)
3. PCO + hyperandrogenism

Varying studies have tried to implement the Rotterdam diagnostic criteria to the adolescent populations. In most of these examinations, inclusion of the non-hyperandrogenemic phenotype created a diagnostic dilemma, especially in young women [8, 9]. Appreciating a need for clarification, the Androgen Excess Society (AES) convened in 2009 to define PCOS by the presence of hyperandrogenism *plus* one of two signs of disturbed ovarian physiology: either ovarian dysfunction (e.g., reflected in disturbed menstrual cycles) or ultrasound evidence of the typical PCO morphology (detailed as under).

For adolescents, an important finding has emerged over the last years since the AES definition. Beyond the clinical presentation, the presence of biochemical hyperandrogenemia (despite the absence of clinical hyperandrogenism) is the singular finding that crystallizes out PCOS from other pubertal "noise" of adolescence [10]. Still actual comparable testosterone cut-offs across laboratories remain elusive, since laboratory assays vary widely between laboratories and that normative data for testosterone levels in the adolescents is lacking [11]. Given these diagnostic dilemmas, Carmina et al. have suggested that for adolescents the diagnosis of PCOS can be securely made only when hyperandrogenemia *and* chronic anovulation *and* ovarian morphologic changes (enlarged ovaries or classic PCO morphology) are evident. In contrast, PCOS diagnosis is only *probable* when hyperandrogenemia and anovulation alone (AES criteria) manifest during adolescence [12].

Teasing Apart Diagnostic Parameters

Irregular Menstruation/Anovulation

While menarche is the first sign of an intact hypothalamic-pituitary-ovarian (HPO) feedback loop, the HPO system may take up to 5 years to mature fully [13]. Therefore, "physiological" anovulation has become the main assumption in examining adolescents with menstrual irregularity. In support of this assumption is the description of 244 unselected post-menarchal girls (mean age 15.2 years) of which 51 % had menstrual irregularity that was not consistently associated with other features of PCOS [14]. However, this study applied a narrow definition of a regular menstrual pattern (cycles were considered abnormal if they varied by >4 days from month to month). It is therefore conceivable that the authors' designation of a "normal" cycle was too narrow in its application to adolescents. Contrary to these findings, a prospective follow-up study found that 62 % of women diagnosed with menstrual disturbance during adolescence were still irregular in their mid-20s and 59 % of those fulfilled criteria of PCOS [15]. Furthermore, a large Finnish Cohort of adolescent girls with irregular menses by self-report had higher testosterone levels than their eumenorrheic counterparts, indicating that irregular menses at age 15–16 years may denote a non-physiologic state [16]. The authors suggested that a regular menstrual pattern at age 15–16 years excludes the risk of hyperandrogenemia with a specificity of 72 %. Interestingly, irregular menstrual cycle patterns at age 15 were more predictive of oligo-amenorrhea at age 18 than hormonal profiles [17].

Ultrasound Evaluation

The Rotterdam Criteria, which first introduced the application of ultrasound evidence in the diagnosis of PCOS, defined PCO as having 12 or more small follicles (2–9 mm) and/or enlarged ovarian volume greater than 10 cm^3 [18]. Unilateral manifestation

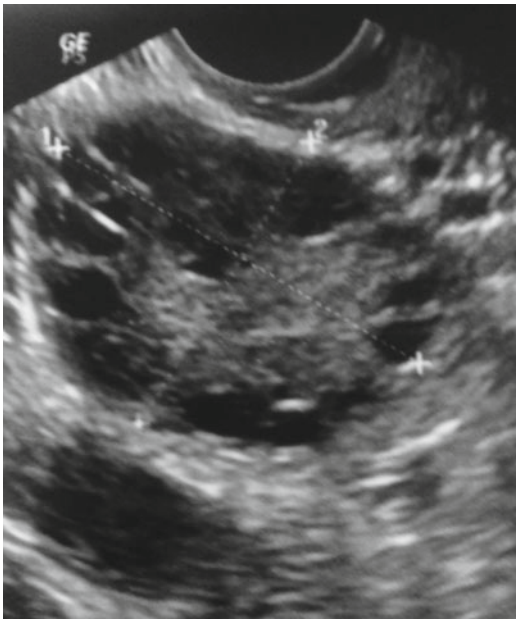

Fig. 14.2 Transvaginal ultrasound image of a polycystic ovary, demonstrating peripheral alignment of small follicles (pearl necklace alignment) with dense central stroma. Reprinted with modification from Balen AH, Laven JS, Tan SL, Dewailly D. Ultrasound assessment of the polycystic ovary: international consensus definitions. Human Reprod Update 2003 Nov–Dec;9(6):505–14, by permission of Oxford University Press

is sufficient for a PCO diagnosis. An important technical aspect of ultrasound examination is the timing within the menstrual cycle. Standardized care suggests ultrasounds to be performed in the early follicular phase (days 3–5) of a spontaneous menstrual cycle or 3–5 days after a progestin withdrawal bleed.

The application of ultrasound to the diagnosis of PCOS in adolescents is intriguing and might serve as an opportunity to differentiate between physiologic irregularity of menstruation and PCOS. However, a potential caveat in the application of ultrasound examination during adolescence is the physiological multi-follicular appearance of pubertal ovaries. Differentiation between the two nevertheless is possible. Multi-follicular ovaries characteristically have follicles of varying sizes strewn amidst normal ovarian stroma. In contrast, the PCO demonstrates pathogenic hypertrophy of central stroma leading to a peripheral displacement of the follicles; a ballooning effect is thus apparent within the ovary with central clearing and peripheral alignment of follicles (*string of pearls* appearance). Recent reports for adult women have suggested that consideration be paid not just to the total ovarian volume but also to the stromal/ovarian ratio in the evaluation of PCO (Fig. 14.2) [19, 20].

Further obscuring the diagnostic evaluation is the preferential application of the transabdominal scanning technique over the transvaginal approach to most adolescents, which may limit the assessment of ovarian morphology. However, with improved ultrasound technology, this is becoming less of a concern [21]. Nevertheless, reference data regarding normal ovarian size in adolescence have not been unified. A recent study comparing 86 Caucasian adolescents with PCOS based on NIH criteria to 48 adolescents without PCOS found that those with PCOS had a significantly larger ovarian volume (9.6 ± 4.4 cm^3) versus non-PCOS

girls (4.6 ± 1.9 cm^3). The study concluded that an ovarian volume higher than 5.6 increased the risk of PCOS by 15-fold for adolescents [21]. Trans-rectal ovarian volume examination in 69 Chinese adolescents with PCOS and 26 controls had similar findings on ovarian volume and suggested that a maximal normal ovarian volume for this population should be at 7.82 cm^3 versus the Rotterdam consensus of 10 cm^3 [22]. In a study examining the correlation between menstrual pattern and ovarian appearance on ultrasound, there was a significant correlation between ovarian morphology and menstrual symptoms [23]. The authors of this study found that 45 % of girls with oligomenorrhea had PCO-appearing ovaries, whereas only 9 % of normocyclic girls had PCO-appearing ovaries.

In an intriguing study examining ultrasound findings of PCO in biochemically and clinically asymptomatic adolescents, one-half of the "asymptomatic" subjects demonstrated androgen hyper-responsiveness to a gonadotropin-releasing hormone agonist (GnRHa) challenge [24]. This could indicate subclinical PCOS-type ovarian dysfunction that may progress to expressed PCOS over time or with environmental/lifestyle triggers [24]. In contrast, another study that performed ovarian ultrasound on young "asymptomatic" volunteers (age 18–25 years) questioned the utility of ultrasound in the diagnosis of PCOS. In this study, 33 % of asymptomatic subjects had PCO morphology [25]. However, testosterone levels were higher in those volunteers with PCO morphology, suggesting that there may have been a selection bias in those women who signed up for ovarian ultrasound examination. Furthermore, given the results of the aforementioned study, subclinical ovarian dysfunction may have been present in some of the "healthy volunteers." Similar conclusions were drawn by a study of 232 Australian adolescent volunteers that found that 30 % met PCO criteria based on ovarian volume alone [26]; this latter study, however, did not examine the relationship between PCO morphology and hyperandrogenism. Interesting work by Sir-Petermann et al. showed that daughters of women with PCOS when compared to controls (daughters of women without PCOS) had significantly larger ovarian volume through all Tanner stages [27]. Despite the increasing number of studies examining the utility of transabdominal ultrasound in the diagnosis of adolescent PCOS, more normative and PCO-specific data need to be collected before ultrasound evaluation can rise to a veritable diagnostic aid in the accurate diagnosis of adolescent PCOS in adolescents.

Hyperandrogenism

Clinical hyperandrogenism is difficult to interpret in adolescents. Acne, for example, is a very common, transitory phenomenon during adolescents and therefore not diagnostic in PCOS [28]. Hirsutism and androgenic alopecia are usually less marked in adolescents because it takes time for hyperandrogenemia to affect the hair follicles [29]. Still persistent and progressive facial hair during adolescents should prompt an evaluation for hyperandrogenemia in any female. For adult women with concern for hirsutism, the modified Ferriman–Gallwey scoring system is used and includes

examination of body areas such as the chin, chest, upper arm, upper and lower back, thigh, groin, and lower and upper abdominal area [30]. However, since adolescents may only manifest upper-lip hair [31], a high hirsutism score should not be sought as the prime manifestation of hyperandrogenism in adolescents [32].

Biochemical hyperandrogenism, on the other hand, is helpful in the diagnosis of androgen excess adolescents [10] and for adolescents, determining total testosterone and sex-hormone binding globulin (SHBG) is recommended for diagnosis. The most sensitive indicator of biochemical hyperandrogenemia is the determination of free testosterone through equilibrium dialysis [33]. However, this method is not widely available and is technically complex and expensive. Alternatively, the free androgen index (FAI) correlates well with free testosterone measured through equilibrium analysis and can be calculated from measures of total testosterone and SHBG [34]. Although there still is significant variability across laboratories, the best methods for evaluation of total testosterone are either immunoassays after extraction and chromatography or tandem mass spectrometry [35]. In general, adolescent normative data for total testosterone and FAI are lacking and so hyperandrogenism in adolescents is defined as >SD above the mean for the particular assay used. However, very high total testosterone levels in any laboratory above 200 ng/dL may suggest the presence of an ovarian androgen-secreting tumor and would warrant further evaluation [36].

For complete androgen evaluation in adolescents with possible PCOS, a measure of adrenal androgens such as 17-OH progesterone and DHEA-S are important to rule out variants of late-onset congenital adrenal hyperplasia (>200 ng/dL) [12] as well as to assess the contribution of adrenal androgen production to the clinical features [33]. An elevation of DHEA-S above 800 μg/dL should prompt further investigation into an adrenal androgen-secreting neoplasm [36]. All androgen hormones should be measured in the morning, preferably in the follicular part of the menstrual cycle [12].

Table 14.1 provides an overview of aspects to consider when diagnosing PCOS during adolescence.

Tending to What Ultimately Matters: Metabolic Complications of PCOS

Even though obesity and insulin resistance are not part of the PCOS definition, they are closely linked morbidities that tie into perpetuating the clinical phenotype and ultimately are responsible for the development of T2DM, the most serious metabolic and cardiovascular risk in PCOS. Other metabolic risk factors such as dyslipidemia, hypertension, impaired glucose metabolism, and obstructive sleep apnea (OSA) either pave the road to T2DM, or are found at the time of diagnosis as concomitant comorbidities.

Table 14.1 Evaluation for PCOS in adolescent population

Assessment	Considerations	Implications
Supporting PCOS		
Clinical	Menstrual disturbance of oligomenorrhea, primary or secondary amenorrhea. Physical examination of acanthosis nigricans, central weight distribution, facial hair/hirsutism	
	Family history of PCOS, infertility, gestational diabetes, type 2 diabetes	
Laboratory	Elevation of morning total testosterone, free testosterone (low sex-hormone binding globulin)	>200 ng/dL suggestive of ovarian androgen-secreting tumor
Imaging	Transabdominal ultrasound with findings of spherical enlarged ovary (ies) where mostly the central stroma is increased—peripheral arrangement of small follicles (string of pearls)	
Ruling out other conditions		
Laboratory	Morning 17-hydroxyprogesterone	>200 ng/dL suggestive of late-onset congenital adrenal hyperplasia
	Dehydroepiandrosterone-sulfate	Adrenal hyperandrogenism/>800 μg/dL suggestive of adrenal tumor
	Thyroid stimulating hormone	Thyroid disorder
	Luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol (E2)	Elevated FSH/LH and low E2 suggest primary ovarian insufficiency. Normal to low FSH, LH, and low E2 suggest hypothalamo-pituitary dysfunction
	Urine/serum human chorionic gonadotropin	Rule out pregnancy
	Prolactin	Prolactinoma or drug-induced cause for elevated prolactin
	Urinary cortisol assessment/ dexamethasone suppression test	Rare conditions such as Cushing's syndrome are not routinely ruled out but should be considered if clinical suspicion arises (e.g., adolescent with elevated blood pressure)

The Metabolic Syndrome

The metabolic syndrome is a constellation of metabolic disturbances that is associated with cardiovascular disease. While the diagnosis of the metabolic syndrome in adults and adolescents remains disjointed across the scientific world, the most common definitions require at least three of the following criteria: (central) obesity, dyslipidemia (related to either triglycerides or HDL), hypertension, or impaired glucose metabolism [37]. Adolescents with PCOS are 4.5 times more likely to have metabolic syndrome than age-matched adolescents from the Third National Health and Nutrition Examination Survey (NHANES III) after adjusting for BMI [38].

Interestingly, this study found that the odds of having metabolic syndrome increased with every quartile increase in testosterone after adjusting for BMI and insulin resistance. A more recent study of 244 adolescent girls with PCOS found that metabolic syndrome was present in 11.8 % of the subjects [14]. In this study, a cluster analysis further revealed that about one-third of adolescents were at high risk for developing metabolic syndrome. Another study confirmed the presence of metabolic syndrome in 10.8 % of adolescents with PCOS compared to 1.7 % of adolescents in a control group without PCOS [39].

Obstructive Sleep Apnea

Recent findings in respiratory medicine point to OSA as not only an inconvenient consequence of obesity but also an important cardio-metabolic condition that in and of itself worsens insulin resistance, glycemic control, and adiposity. Interestingly, while OSA is closely linked to obesity in the general population, it is much more prevalent in women with PCOS when compared to age and BMI obese controls without PCOS [40]. While the pathogenetic link between PCOS and OSA remains to be elucidated, there have been suggestions that insulin resistance [41] central adiposity, and testosterone levels may play a mechanistic role in the prevalence of OSA in PCOS. Hyperandrogenemia, being the differentiating factor of PCOS, has led to a hormonal explanation of OSA in PCOS. In a recent review, the effect of testosterone on sleep disorder breathing was attributed to its promotion of visceral fat as well as neck fat deposits, its affect on central drive for breathing, and its alteration of lung mechanism [42]. Despite the increasing evidence that OSA is an important health risk for women in PCOS [41, 43, 44], it remains unscreened for by gynecologist and endocrinologists alike [45]. When it comes to adolescents with PCOS, the OSA screening is mostly based on the degree of obesity and not on the diagnosis of PCOS. Therefore, there are few adolescent studies examining OSA in a purely PCOS population, some of which could not confirm a link between PCOS and OSA [46]. However, a recent controlled study found that OSA was four times more prevalent in obese adolescents with PCOS than in BMI-matched adolescents without PCOS [47].

Prediabetes and Diabetes

The most important cardiovascular risk-enhancing condition in PCOS is type 2 diabetes mellitus (T2DM). Also, prediabetes, as defined by either abnormal fasting or abnormal glucose tolerance during standard glucose tolerance testing, is not only an antecedent to T2DM but in and of itself a cardiovascular disease risk factor [48]. In adolescent medicine, obesity, a family history of T2DM, and the presence of acanthosis nigricans on skin examination are the red flags that prompted thorough testing of glucose metabolism in adolescents. However, adult studies have demonstrated that prediabetes and T2DM are present in non-obese women with

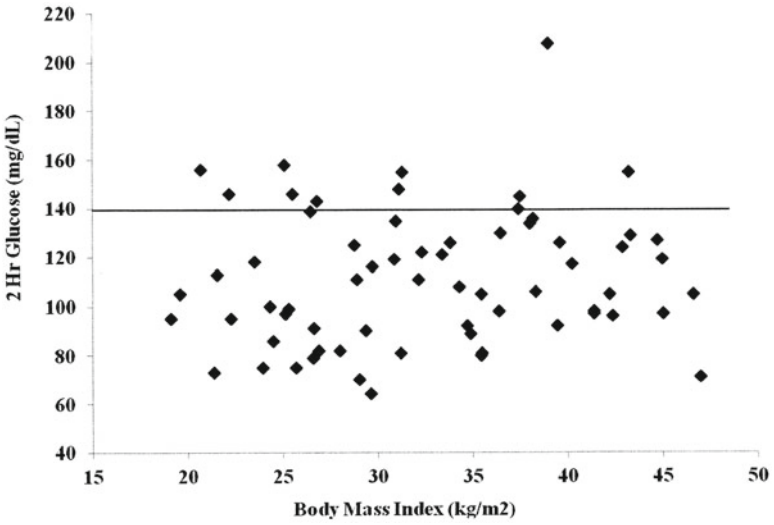

Fig. 14.3 Impaired glucose tolerance (IGT), as defined by a 2-h stimulated serum glucose ≥140 mg/dL, occurred across the spectrum of BMI in adolescents with PCOS. One obese adolescent met criteria for type 2 diabetes, based on a 2-h serum glucose >200 mg/dL

PCOS and, therefore, the diagnosis of PCOS in and of itself should prompt a metabolic evaluation [49].

In pediatric medicine, data on the prevalence and presence of prediabetes and type 2 diabetes are just emerging, and there is not much information in subgroups such as PCOS. Depending on the population examined, prediabetes has a prevalence between 4.7 % in obese Italian youth [50], 8.9 % in obese German youth [51], 20 % in obese Bangladeshi youth [52], and 21 % in a mixed ethnic obese cohort in the United States [53]. However, these examinations do not necessarily exclude the presence of PCOS in their obese cohorts. In the few adolescent studies examining only those with PCOS diagnosis, the data on disturbance in glucose metabolism vary between 6 % and 33 % and are often confounded by the overwhelming presence of obesity [54–56]. In a study in Indian adolescents with PCOS, where obesity is less confounding but there is more of a heritable risk of insulin resistance and diabetes, impaired glucose metabolism was present in 9.7 % [57].

We recently set out to examine glucose metabolism based on PCOS diagnosis only and found that impaired glucose metabolism was present in adolescent PCOS across the BMI spectrum [58] (Fig. 14.3).

Similar findings were reported in a contemporaneous study conducted in Chinese youth with PCOS. In this study, 14 % of non-obese adolescents and 25 % of obese adolescent with PCOS had IGT and none of the control subjects [59]. Further supporting a prediabetes risk in PCOS are the results of a series of studies examining metabolic and other hormonal features in pubertal daughters of women with PCOS. Compared to BMI-matched controls, daughters of women with PCOS

Table 14.2 Metabolic considerations after diagnosis of PCOS in an adolescent population

	Considerations	Implications
Laboratory	Fasting glucose, insulin, and 2-h oral glucose tolerance test with 75 g of glucose	Examine for prediabetes, diabetes, insulin resistance
	Fasting lipid profile	Examine for dyslipidemia as knowledge may impact on treatment choice, e.g., caution is advised when using oral contraceptive regimen in the setting of moderate to severe triglyceridemia
	Sleep study	Examine for obstructive sleep apnea

have significantly higher 2-h insulin levels during OGTT than matched controls at all stages of puberty [27]. These findings are intriguing and support the increased (and BMI-independent) risk for abnormalities in glucose homeostasis in adolescents on the road to PCOS.

Given the metabolic implications that go hand in hand with the diagnosis of PCOS, additional evaluation may be considered, as outlined in Table 14.2.

Tried and True (?) Treatment of Adolescent PCOS

Although several avenues of therapy have been suggested, there are no published guidelines for treating adolescents with PCOS. Similar to adult therapy, treatment in adolescence focuses on the dual approach of (1) symptomatic relief and (2) preventative strategy. The three interrelated targets of therapy are anovulation, hyperandrogenemia, and insulin resistance. To date, Ibanez et al. have contributed the majority of systematic studies examining therapeutic modalities in lean adolescents with PCOS [2, 60, 61]; these authors have moved the field forward in terms of therapeutic options for young girls diagnosed with PCOS. Given the narrow ethnic and clinical PCOS spectrum of the Ibanez and Zegher cohorts, the transferability of the data to obese adolescents of other ethnic backgrounds still needs to be examined.

Lifestyle

Intensive lifestyle modification, even with only a modest change in BMI has shown to improve menstrual cycle regularity and normalize serum androgens in adolescents with PCOS [62, 63]. In most lifestyle studies, adolescents received intense nutrition education and psychological support and participated in group exercise classes at least once a week. However, even when enrolled in an intensive lifestyle program for a year, only 26/59 obese girls with PCOS lost >0.2 SDS in BMI [45]. Maintaining lifestyle changes outside of clinical trials is even more challenging and, therefore, pharmacotherapy often becomes necessary.

Hormonal Contraceptive Approach

Combined oral contraceptives pills (OCPs) containing both an estrogen and progestin component, not only regularize the menstrual cycle but also alleviate clinical features such as acne and hirsutism [64]. Specifically, the estrogen component of the pill increases hepatic production of SHBG, which leads to a reduction in circulating free and bioavailable androgens. The progestin component of OCPs ensures endometrial decidualization and thus protects against proliferative endometrial pathologies, including endometrial hyperplasia. Therefore, the potential risk for endometrial cancer resulting from prolonged periods of anovulation is reduced. By negative-feedback effect on pituitary LH and thereby lowering LH-driven ovarian androgen production, progestins can also contribute to the improving hyperandrogenemia associated with OCP use. Fourth-generation progestins such as drospirenone have an added anti-androgenic effect and therefore have been deemed more effective in improving PCOS symptoms [65]. One study was even able to demonstrate that switching to the anti-androgenic fourth-generation progestin OCP from a third-generation progestin OCP improves adiposity in adolescent PCOS [66]. In recent years, concerns regarding somewhat higher thromboembolic risk with fourth-generation progestins compared to older third-generation formulations have been suggested and have contributed to a somewhat cautious stance regarding the use of pills containing fourth-generation progestins even in the adolescent populations [67–69].

Another consideration in prescribing OCPs in overweight adolescents with PCOS is the negative effect on lipid profiles and insulin resistance [70, 71]. The higher the estrogen dose and the more androgenic the progestin, the more unfavorable effects can be observed on lipid and glucose metabolism. A study comparing a combination product of 30 μg ethinylestradiol (EE) with either a third- or fourth-generation progestin found that both led to similar increase in HDL and triglycerides profile in lean women with PCOS. However, fourth-generation progestin combination kept the LDL levels stable, therefore increasing the HDL:LDL ratio, leading to a beneficial constellation in terms of vascular disease [72]. Another study in adult non-obese women with PCOS demonstrated that low-dose EE (30 μg EE) did not affect gluco-insulinemic homeostasis [73]. However, studies are lacking in comparing differentiated effects of OCPs in obese versus non-obese women with PCOS [74]. Also, estrogen-containing OCPs are contraindicated in adolescents with Factor V Leiden mutations, greatly enhancing thromboembolic risk. Sometimes family moral/religious or stigma concerns about oral contraceptives also prompt younger patients to seek alternative treatment to their PCOS. In such cases, uterine protection can also be obtained by inducing periodic withdrawal bleeds by a short course of progesterone every 2–3 months.

Metformin

Metformin is an insulin sensitizer with main action in the liver. Given the perpetuating effect of insulin on androgens, metformin was the first anti-diabetic drug to be examined in the context of treating the PCOS condition in adult women [75].

Since then, many studies have confirmed the benefits of metformin for restoring cycle regularity, fertility, and favorable metabolic profile for adult women. In 2001 the first studies emerged examining the effect of metformin in adolescent PCOS. For a group of 18 non-obese girls with PCOS and a history of premature adrenarchy, Ibanez et al. found that 14 (78 %) had restored menstrual cycle regularity by 6 months of metformin therapy [60]. Six months of metformin restored normal menstrual cycles in 10/11 obese subjects [76] and improved both ovarian and adrenal hyperandrogenism in another group of obese subjects [77]. A double-blind placebo-controlled study in obese girls with PCOS found that metformin/lifestyle versus placebo/lifestyle was more effective in reducing testosterone levels and menstrual cycle regularity than lifestyle alone [78]. Albeit there may be a prescribing bias of metformin to obese adolescents with PCOS, it is equally effective in lean adolescents in restoring menstrual regularity [79]. Metformin in combination with oral contraceptives and anti-androgens has been extensively studied by Ibanez and Zegher for girls of the Catalan region of Spain. Intriguing are their recent data on starting metformin at the presentation of premature adrenarche, a common antecedent to the later manifestation of PCOS [80]. In terms of body composition and metabolism, the authors show that earlier treatment appears to be more effective than the later treatment [5] and may even augment final height by delaying early menarche [81].

Metformin is a good choice for the lean and overweight young adolescents who are not in need of contraception and who don't suffer from severe acne and hirsutism that would require more immediate symptomatic therapy with OCPs. Metformin has an excellent safety profile in patients with healthy kidney and liver function and can be used in patients with concern of thromboembolic risk. Menstrual regularity in both lean and obese adolescents will likely be restored after 6 months of therapy. Should endometrial build-up from prolonged amenorrhea be a concern, then a short-course progesterone-induced withdrawal bleed may be indicated before starting metformin.

If the dose is slowly and incrementally increased to 1,500–2,000 mg daily, divided twice or thrice daily over a course of 3–4 weeks and taken with food, GI side effects such as diarrhea can be avoided in most patients [82, 83]. Long-term use, however, may decrease levels in B_{12} [84]; therefore, a supplemental daily multivitamin that contains B_{12} can prevent this long-term use effect.

Combining OCPs and Metformin

Not too many clinical researchers other than Ibanez and Zegher have examined varying drug combinations in adolescent PCOS [2]. From their data, we know that metformin has additive beneficial effects, especially in improving body composition and dyslipidemia. For obese adolescents, a recent double-blind placebo-controlled study confirmed the beneficial effect of additive therapy [63]. They first compared single-agent therapy (OCP vs. lifestyle vs. metformin) over a 6-month

time period. Subsequently, they examined combinations of lifestyle plus OCP with either metformin or placebo. In this study, lifestyle alone was highly effective in reducing FAI with an increased SHBG. OCPs improved androgen profile but had negative metabolic effects with increased C-reactive protein and LDL cholesterol. Interestingly, adding metformin to OCP and lifestyle improved total testosterone and HDL and waist circumference. It therefore appears that adding metformin to OCPs ameliorates some of the negative metabolic effects of OCPs. An important consideration when treating adolescent PCOS is that OCPs are often desired therapy for their contraceptive benefit. Monotherapy with metformin, on the other hand, can enhance fertility by way of restoring normal ovulatory cycles and therefore may increase the risk of teen pregnancy. Hence, combining both therapeutics may sometimes be the best choice, especially for sexually active overweight adolescents with cardiometabolic risk.

Anti-androgens

Hyperandrogenemia is not only closely tied to the pathogenesis of the condition but also responsible for the metabolic complications. Hyperandrogenemic obese adolescents have greater risk of metabolic syndrome and inflammatory state than non-hyperandrogenemic, similarly obese counterparts [35, 85]. Therefore, anti-androgen therapy may be indicated not only to benefit cosmetic manifestations such as hirsutism and acne but also as a metabolic management strategy.

Flutamide, a nonsteroidal drug with a potent androgen receptor blocker, has been very effective in both cosmetic and metabolic effects when added to oral contraceptives and metformin in a PCOS treatment regimen [66, 86, 87]. However, few pediatric investigators have studied flutamide due to the widespread concern about its hepatotoxic effect. It appears, though, that this effect is dose-dependent and not idiosynchratic. In low dose (62.5 mg/day), flutamide did not affect the liver transaminases, as demonstrated by an epi-analysis of 12 prospective studies, each with at least 20 patients treated for at least 12 months [88]. While Ibanez and Zegher have published most of the data about safety and efficacy of flutamide in combination therapy for adolescent PCOS, a recent observational study confirmed low-dose flutamide safety for other young women with hirsutism [89].

Spironolactone, used for decades as a potassium-sparing diuretic, was found to have anti-androgenic effect in the 1970s [90]. Its anti-androgenic effect is carried out through a dual mechanism of androgen receptor blockade, and via spironlactone's inhibition of 5-alpha reductase, thus reducing the conversion of testosterone to its biologically more potent metabolite, di-hydrotestosterone. Studies in adult women have shown higher doses of 100 mg daily to be safe and effective in improving hirsutism scores over time. Spironolactone, while more commonly used in the United States to help with hirsutism, has the unnerving side effect of irregularizing menses. Therefore, it is often used in combination with OCPs in patients with PCOS.

Table 14.3 Efficacy of commonly utilized therapeutic options for managing PCOS-related problems in adolescents

Treatment	Menstrual dysfunction	Hyperandrogenism	Metabolic benefit
Insulin sensitizer Metformin	±	±	+
Oral contraceptive pill (OCP)	+	+	−
Cyclic progesterone	+	−	−
OCP + metformin	+	+	+
Anti-androgen	±	+	±

In young women at risk for pregnancy, both anti-androgens must only be used with secure contraceptives due to the potential effect of undervirilization of a male conceptus.

Table 14.3 identifies efficacy of the commonly utilized therapeutic regimens against the common symptoms of PCOS.

Summary

While the manifestations of PCOS during adolescence may be similar to those seen in the adults, diagnostic dilemmas abound and diagnostic criteria are less studied in this population compared to the adults. A timely diagnosis of PCOS in the afflicted adolescent allows an opportunity of not only successfully addressing the burdensome symptoms but also initiating preventative strategies aimed at mitigating long-term sequelae of this chronic disorder.

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Chapter 15
Considerations and Challenges for Pregnancy in Polycystic Ovary Syndrome

Christina Shih-chi Han and Erika F. Werner

Key Points

- PCOS is associated with an increased risk of pregnancy loss, gestational diabetes, preeclampsia, perinatal mortality, and operative delivery.
- PCOS does not appear to affect rates of congenital anomalies.
- Well-designed prospective studies using standardized definitions are necessary to elucidate the true effects of PCOS on adverse outcomes in pregnancy.

Scope of the Problem

Although the relationship of polycystic ovarian syndrome (PCOS) with subfertility is well established, postconception effects of PCOS on pregnancy remain debated. Obstetrical complications associated with PCOS may include miscarriage, gestational diabetes, hypertensive diseases of pregnancy, birth-weight alterations, perinatal mortality, and increased risk of operative delivery (Table 15.1) [1]. Unfortunately, the heterogeneity of the syndrome and the paucity of large randomized controlled trials studying pregnancy in PCOS limit our understanding of causal relationships between PCOS and adverse pregnancy outcomes. This chapter reviews the current literature and the pathophysiology proposed to explain a propensity for adverse pregnancy outcomes in women with PCOS.

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Table 15.1 Possible obstetrical complications associated with PCOS

Early pregnancy loss
Gestational diabetes mellitus
Hypertensive disorders of pregnancy
Alterations in birth weight
Perinatal morbidity and mortality
Increased risk for operative (cesarean) delivery

Effects of PCOS on Pregnancy

Early Pregnancy Loss

The prevalence of early pregnancy loss, defined as miscarriage during the first trimester, has been reported in women with PCOS to be as high as 30–50 % [2]. This rate is approximately threefold higher than reported rates of 10–15 % for women without PCOS [2]. Conversely, in women who have a history of prior miscarriages, polycystic ovaries (PCO) were identified on ultrasound imaging in up to 40–82 % of the subjects, compared to 23 % of unselected women [3, 4]. However, the presence of morphologically abnormal ovaries on imaging may not translate to an increased risk of miscarriage in a subsequent pregnancy [4].

Although the association of PCOS with early pregnancy loss has been repeatedly reported in earlier studies, the relevance of PCOS for recurrent pregnancy loss remains contested. The broad variance in reported rates of ovarian findings in these studies can be attributed to the use of non-standardized diagnostic criteria, discrepant modes of imaging (transabdominal vs. transvaginal), and variable subject selection criteria [5]. Furthermore, it is important to note that these studies reported only the findings of PCO, and not the presence or the absence of other clinical and biochemical alterations that define the syndrome [6].

The pathophysiology of early pregnancy loss in PCOS has been attributed to the various metabolic and endocrinologic abnormalities commonly encountered in women with PCOS, including obesity, hyperinsulinemia, hyperandrogenemia, abnormal pituitary gonadotropin profile with elevated luteinizing hormone (LH) concentrations, and endometrial dysfunction [1, 5]. Body mass excess is commonly encountered with more than one-third of PCOS patients being obese, i.e., having body mass index (BMI) greater than 30 kg/m^2 [7]. In a retrospective study evaluating women with PCOS who conceived on ovulation induction therapy utilizing low-dose gonadotropins, overweight body habitus (BMI between 25 and 27.9 kg/m^2), compared to individuals of normal BMI, was associated with an increased risk in miscarriage at or below 8 weeks gestation (60 % vs. 27 %, $p<0.05$) [7]. A negative impact of obesity on maintenance of pregnancy in the PCOS population was confirmed in a prospective observational cohort study of 270 women from Kuwait with 36 pregnancies [8]. Live birth was noted in 97.2 % of women with BMI 18–24 kg/m^2, 63.6 % of women with BMI 30–34 kg/m^2 ($p=0.001$), and 60 % of women with BMI$\geq$35 kg/m^2 ($p=0.04$).

A large cohort study further argues that obesity may be the primary mechanism underlying early pregnancy loss in women with PCOS; the authors observed that the risk of pregnancy loss in PCOS fades after adjusting for obesity [9].

These studies support the empiric recommendation that weight reduction and lifestyle modifications should be integral to the management of PCOS patients seeking to conceive. While benefits of weight reduction for reproductive success are widely canvassed, however, it should be noted that a recent Cochrane review (2011) found insufficient well-designed literature on the effect of lifestyle interventions on reproductive outcomes [10]. Further studies are thus needed to confirm a benefit to preconceptional weight loss, particularly in the PCOS population.

Hyperinsulinemia is common to PCOS and obesity and has been proposed as an independent risk factor for miscarriage, irrespective of BMI [11]. Proposed mechanisms of hyperinsulinemia-induced early pregnancy loss include excess transplacental transport of glucose to the fetus [12], and alterations in levels of serum glycodelin [13], insulin growth factor-binding protein-1 (IGFBP-1) [13], and plasminogen activator inhibitor 1 (PAI-1) [14]. Glycodelin and IGFBP-1 are major endometrial secretory proteins that may play important roles in endometrial receptivity during the implantation period and in the maintenance of pregnancy [13]. PAI-1 activity is the major determinant of hypofibrinolysis, and overexpression may result in higher rates of venous thromboembolism and adverse pregnancy outcomes, including recurrent miscarriage, intrauterine growth restriction, placental abruption, preeclampsia, and intrauterine fetal demise. Treatment with metformin may reverse the thrombophilic state induced by PAI-1 overexpression, thereby preventing adverse pregnancy outcomes resulting from thrombosis-induced placental insufficiency [15].

Hyperandrogenemia has been described in subjects experiencing recurrent early pregnancy loss, both with and without PCOS [5]. Comparison between studies is difficult due to measurements of different androgens, including total testosterone, free testosterone, and calculated free-androgen index [6]. Abnormal endometrial development [16], reduced expression of endometrial protein PP14 [16], and detrimental effects on oocyte quality [17] are proposed as mechanisms relating androgen excess to early pregnancy loss. Endometrial protein PP14 correlates well with luteal phase endometrial dysfunction [16]. Negative correlation between plasma androgen concentrations and uterine PP14 concentrations in the women with recurrent miscarriages suggests that high androgen concentrations may result in an abnormal secretory endometrium and hence a suboptimal milieu for successful implantation.

Another common feature of PCOS that has been implicated in early pregnancy loss is the abnormally elevated circulating LH. Elevated LH is a neuroendocrine hallmark of PCOS which results from persistent rapid pulsatile secretion at an exaggerated amplitude [18]. Proposed theories regarding the pathophysiology of early pregnancy loss in PCOS include the premature maturation and aging of oocytes and dysfunctional endometrial development secondary to the abnormally elevated LH. Although early studies suggested a link between LH hypersecretion and miscarriage [19], recent data have been unable to corroborate the earlier findings [5, 20]. Variations in findings can be attributed to the difference in experimental assays and design [20]. Suppression of elevated LH levels before conception in a subset of women with a history of recurrent pregnancy loss and PCO does not appear to improve pregnancy outcomes [21].

Ovulation induction strategies are commonly utilized for the management of ovulatory infertility in women with PCOS and include clomiphene citrate, gonadotropin, laparoscopic ovarian drilling (LOD), and use of metformin [5]. Clomiphene

citrate, a mixed estrogen agonist–antagonist, is frequently used as a first-line strategy to induce ovulation in PCOS with anovulation. However, in patients who experience recurrent miscarriage after successful conception, it is uncertain whether clomiphene offers any protective advantage [5]. Gonadotropin treatment is commonly utilized to induce ovulation in anovulatory women who are clomiphene resistant. However, there have been no studies evaluating the effects of gonadotropins in the management of recurrent miscarriage. Ovarian drilling, a surgical procedure thought to decrease androgen-producing tissue in the PCOS ovary, is an accepted alternative therapy in clomiphene-resistant patients [22]. The ongoing pregnancy rate after LOD, followed by clomiphene citrate or gonadotropin therapy (if anovulation persists), seems equivalent to rates seen with the use of recombinant follicle-stimulating hormone alone, although pregnancies after ovarian drilling carry a lower risk of multiple gestation [22]. Rates of miscarriage at less than 12 weeks gestation were comparable between LOD and gonadotropin therapy arms. Lastly, metformin, an oral anti-hyperglycemic medication, initially held promise in decreasing miscarriage risk and increasing live birth rate in women with PCOS, compared to clomiphene citrate and LOD [23, 24]. However, this latter impression was not confirmed by a meta-analysis that failed to establish an efficacy of metformin in reducing miscarriage rate in women with PCOS [25, 26].

Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is defined by the American Congress of Obstetrics and Gynecologists as carbohydrate intolerance that begins or is first recognized during pregnancy [27]. Rates of GDM in pregnancies in the general US population are currently reported to be 3–9 %, although this number is expected to continue to escalate secondary to the parallel obesity epidemic, sedentary lifestyles, urbanization, and older maternal ages at conception [28]. Complications of GDM in pregnancy include fetal macrosomia, polyhydramnios, birth trauma, instrumental vaginal delivery, cesarean section, and higher perinatal mortality. Although universal screening for GDM at the beginning of the third trimester is standard protocol for most institutions within USA, early screening for GDM in the first and second trimester in high-risk populations is also widely accepted [27]. A recent large randomized clinical trial evaluating the efficacy of GDM treatment showed that dietary counseling, home blood glucose monitoring, and insulin therapy effectively reduce serious perinatal morbidity and may also improve the woman's health-related quality of life [29].

More than 50 % of nonpregnant patients with PCOS exhibit resistance to the action of insulin [30]. Up to 40 % of all women with PCOS will develop type 2 diabetes mellitus or impaired glucose tolerance during their reproductive years, or by the age of 40 [31]. During normal gestation, the inherent insulin resistance of PCOS is further compounded by physiologic changes of pregnancy designed to accommodate and prepare for the nutritional requirements of the developing fetus, especially in the third trimester. Placental hormones, such as human placental lactogen, cortisol, progesterone, and estrogen, all alter maternal glucose homeostasis by

inducing peripheral insulin resistance [32]. In patients with underlying insulin resistance, such as those with PCOS, the glycemic balance may be further tipped towards GDM. Indeed, women with PCOS demonstrated a significantly higher chance of developing GDM (OR 2.94, 95 % CI 1.70–4.08) in a meta-analysis [33]. A subgroup analysis of five higher validity studies from the same meta-analysis further reinforced the increased risk (OR 3.66, 95 % CI 1.20–11.16) [33]. Conversely, in a study comparing women with a previous history of GDM with those without such a history, a higher prevalence of PCO by ultrasound (41 % vs. 3 %, $p<0.0001$) and other clinical and endocrinologic stigmata of PCOS (hirsutism, $p<0.01$; irregular menstrual cycles, $p<0.01$; higher BMI, $p<0.001$; and higher concentrations of androstenedione, $p<0.01$; testosterone, $p<0.01$; and LH/FSH ratio, $p<0.01$) were apparent in women with a history of GDM [34].

As with early pregnancy loss, a potential confounder in studies relating GDM to PCOS is obesity [35]. In a meta-analysis of studies evaluating the effect of BMI on development of GDM, the risk of carbohydrate intolerance is significantly positively correlated with pre-pregnancy BMI [36]. Likelihood for developing GDM in the overweight, moderately obese, and morbidly obese women was observed to be linear with odds ratio for developing GDM of 1.97 (95 % CI 1.77–2.19), 3.01 (95 % CI 2.34–3.87), and 5.55 (95 % CI 4.27–7.21), respectively. Nonetheless, even after controlling for BMI, the increased risk of developing GDM remained evident in women with PCOS [33].

Metformin, an insulin sensitizer and an inhibitor of gluconeogenesis, is a commonly used pharmacotherapy in nonpregnant patients with PCOS as a strategy for improving insulin resistance and hyperandrogenemia of PCOS; addition of metformin to ovulation induction strategy is suggested to improve treatment success with clomiphene citrate [35, 37].

The frequent use of metformin in the diabetic and PCOS populations, and its favorable safety profile, therefore led to a hypothesis that metformin may decrease the risk of GDM in patients with PCOS [11]. Metformin treatment in early pregnancy, however, has not been shown to reduce incident GDM rates compared to placebo controls (17.6 % vs. 16.9 %, $p=0.87$) [38]. This lack of improvement was further confirmed in a more recent study [39]. However, efficacy of metformin as a management strategy for GDM has demonstrated reassuring safety profile with perinatal complication rates comparable to those treated with the gold standard, insulin. Additionally, women with GDM managed on metformin demonstrated lesser weight gain from enrollment to term compared to term (0.4 ± 2.9 vs. 2.0 ± 3.3, $p<0.001$) [40].

Hypertensive Disorders of Pregnancy

Hypertensive disorders during pregnancy are the second leading cause of maternal mortality in the United States (after thromboembolism). Accounting for almost 15 % of maternal deaths [41], these disorders are estimated to affect 6–8 % of all pregnancies [42]. Hypertensive disorders in pregnancy are categorized into chronic hypertension, preeclampsia/eclampsia, preeclampsia superimposed on chronic

hypertension, and gestational hypertension [42]. In patients without evidence of chronic hypertension (defined as elevated blood pressure before 20 weeks of gestation), the onset of elevated blood pressure with proteinuria (persistent 1+ urine on dipstick testing or >0.3 g protein in a 24-h urine specimen) after 20 weeks of gestation qualifies for the diagnosis of preeclampsia whereas evidence of blood pressure elevation in the absence of proteinuria qualifies as gestational hypertension [42]. Elevated blood pressure with seizures that cannot be attributed to other causes is known as eclampsia. Some known risk factors for hypertensive disorders of pregnancy include diabetes, autoimmune disease, renal disease, and obesity [43].

Women with PCOS demonstrated a significantly higher chance of developing a hypertensive disorder of pregnancy in a meta-analysis (OR 3.67, 95 % CI 1.98–6.81) [33]. A subgroup analysis of two higher validity studies also revealed a significant increased risk of any hypertensive disorder of pregnancy (OR 3.71, 95 % CI 1.72–17.49). Women with PCOS also demonstrated a significantly higher chance of developing preeclampsia (OR 3.47, 95 % CI 1.95–6.17). However, all studies in which preeclampsia was an endpoint reported a lower parity, higher BMI, or more multiple pregnancies among women with PCOS versus controls. Nulliparity, obesity, and multiple gestations are known risk factors for preeclampsia, which may contribute to the increased rates of hypertensive disorders of pregnancy [42, 43].

Alterations in Birth Weight

The increased risks of gestational diabetes and preeclampsia for women with PCOS have led to hypotheses that the offspring in this population may be at an enhanced risk for a spectrum of growth aberrations that include macrosomia at one extreme, and small-for-gestational age (SGA) at the other [1]. Existing literature however is equivocal and a lack of consistency in observed relationships can in part be attributed to differing definitions of macrosomia and SGA utilized across published studies. In a meta-analysis, pooled results from 12 studies showed statistically, albeit not clinically, significantly lower neonatal birth weight among infants of women with PCOS (mean weight difference, −38.4 g; 95 % CI −62.2 to −14.6) [33]. Subgroup analysis of four studies in which controls were matched for confounders showed no significant difference in neonatal birth weight. PCOS babies also showed no significant increase in the incidence of macrosomia or growth retardation.

Perinatal Morbidity and Mortality

In a meta-analysis of five studies with 162 pregnancies with PCOS and 725 control pregnancies, a statistically and clinically significant increase in perinatal mortality was observed in the offspring of women with PCOS compared to controls (OR 3.07; 95 % CI 1.03–9.21) [33]. Reported causes for perinatal mortality in offspring of pregnancies affected by PCOS include lethal malformations, cervical insufficiency, sepsis, and placental abruption. However, as with many other pregnancy

complications, most studies showed a higher BMI in women with PCOS compared to controls, which is a recognized independent risk factor for perinatal mortality [44].

A large Swedish study utilizing the national birth registry, published after the previous meta-analysis, reported that women with polycystic ovary syndrome are at increased risk of adverse pregnancy and birth outcomes that cannot be explained by the increased use of assisted reproductive technologies in this population. Infants born to mothers with PCOS were more prone to be large for gestational age (aOR 1.39; 95 % CI 1.19–1.62), meconium aspiration (aOR 2.02; 95 % CI 1.13–3.61), extreme prematurity (aOR 2.21; 95 % CI 1.69–2.90), and Apgar scores <7 at 5 min (aOR 1.41; 95 % CI 1.09–1.83) [45].

Mode of Delivery

In a meta-analysis of eight studies, women with PCOS were noted to have a higher incidence of delivery by cesarean section compared to controls (OR 1.56; 95 % CI 1.20–2.02) [33]. The total number of deliveries, proportion of cesarean delivery, and indications for cesarean delivery were not reported in the meta-analysis. However, when a subgroup analysis was performed on three higher validity studies, no significant increased risk of cesarean delivery was observed (OR 0.92; 95 % CI 0.54–1.58). This increased risk in surgical delivery in some studies was attributable to the differences in body habitus between PCOS and control groups. There were no differences between rates of spontaneous vaginal and forceps or vacuum-assisted vaginal deliveries (OR 1.37; 95 % CI 0.80–2.35).

Summary

PCOS is associated with an increased risk of early pregnancy loss, gestational diabetes, and preeclampsia. Other reported adverse outcomes, such as alterations in birth weight, increased risk of perinatal mortality, and operative delivery, remain debated due to conflicting data between studies. Additional well-designed prospective studies using standardized definitions are necessary to elucidate the effects of PCOS on adverse outcomes in pregnancy. Nonetheless, the reported increased risk of adverse pregnancy outcomes warrants preconceptional and antenatal counseling and stringent antenatal surveillance of pregnant women with PCOS.

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Part V
Role of Surgery in PCOS Management

Chapter 16
Role of Surgery in the Management of PCOS: Rationale and Considerations for Bariatric Surgery

Héctor F. Escobar-Morreale

Key Points

- Obesity is the major factor responsible for metabolic disorders in patients with PCOS.
- Lifestyle modification is effective for prevention of metabolic disorders in obese patients with PCOS, but it is rarely effective in the long term due to difficulty in sustaining the newly adopted lifestyle by the majority.
- Bariatric surgery in severely obese women may entirely resolve the clinical picture of PCOS.
- Fertility may be restored after weight loss in women with PCOS submitted to bariatric surgery.
- Avoidance of the pregnancy-related and lifetime risks associated with severe obesity compensates for the innate procedure-related risks in appropriately selected patients.

Introduction

Polycystic ovary syndrome (PCOS), the most common endocrine disorder in premenopausal women [1, 2], is characterized by the association of androgen excess with chronic oligoovulation and/or polycystic ovarian morphology, provided that other disorders such as hyperprolactinemia, nonclassic congenital hyperplasia, and androgen-secreting tumors have been excluded [3, 4].

Metabolic disorders and classic and nonclassic cardiovascular risk factors cluster in women with PCOS from very early in life [5]. A disproportionately higher risk of

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early-onset type 2 diabetes and evidence of premature atherosclerosis are well recognized in this population, underscoring the need for timely institution of metabolic prevention strategies in women with PCOS. This chapter focuses on the major role that obesity plays in the association of PCOS with metabolic disorders and the scarce, but promising, evidence suggesting that bariatric surgery may be a very useful tool for the management of PCOS and associated metabolic conditions.

Role of Obesity in the Pathophysiology of PCOS and in the Development of Metabolic Disturbances

McAllister and colleagues [6] demonstrated a decade ago that exaggerated androgen secretion by ovarian theca cells is a primary feature of PCOS. After several passes in primary culture, theca cells from women with PCOS secreted increased amounts of androgens, and their steroid precursors, compared with cells obtained from women without androgen excess disorders, and PCOS theca cells showed increased expression of all the enzymes involved in androgen synthesis [6]. The fact that these cells maintained an increased capacity for androgen synthesis when isolated from the endocrine and metabolic milieu characteristic of women with PCOS strongly suggests that androgen excess is a primary ovarian defect in this syndrome [6].

However, insulin resistance and compensatory hyperinsulinism also contribute to the pathogenesis of PCOS, because insulin acts as a co-gonadotropin at the ovary, facilitating androgen secretion [7].

We have recently hypothesized that women with PCOS suffer from a vicious circle whereby androgen excess favoring the abdominal deposition of fat further facilitates androgen secretion by the ovaries and adrenals in PCOS patients (Fig. 16.1) [8]. The possibility that androgens influence body fat distribution and visceral adipose tissue dysfunction is supported by recent studies. Women with PCOS present with increased thickness of intraperitoneal and mesenteric fat depots and, when considered together with non-hyperandrogenic women and with men, the thickness of visceral adipose tissue depots correlated positively with serum androgen concentrations and negatively with serum estradiol levels [9]. Moreover, both genomic and proteomic nontargeted studies of visceral adipose tissue indicated substantial differences in the gene expression profiles and in the proteomes of severely obese patients with PCOS compared with control women [10, 11]. Of note, the promoter regions of several of the genes dysregulated in visceral adipose tissue of women with PCOS contain putative androgen response elements, suggesting that androgen excess might influence adipose tissue dysfunction in these patients [12].

The aforementioned vicious circle might start very early in the life of affected women, even during fetal development [13]. Animal models of prenatal androgenization demonstrated that, in mammals, in utero exposure to excessive androgen concentrations favors the development of abdominal adiposity and endogenous

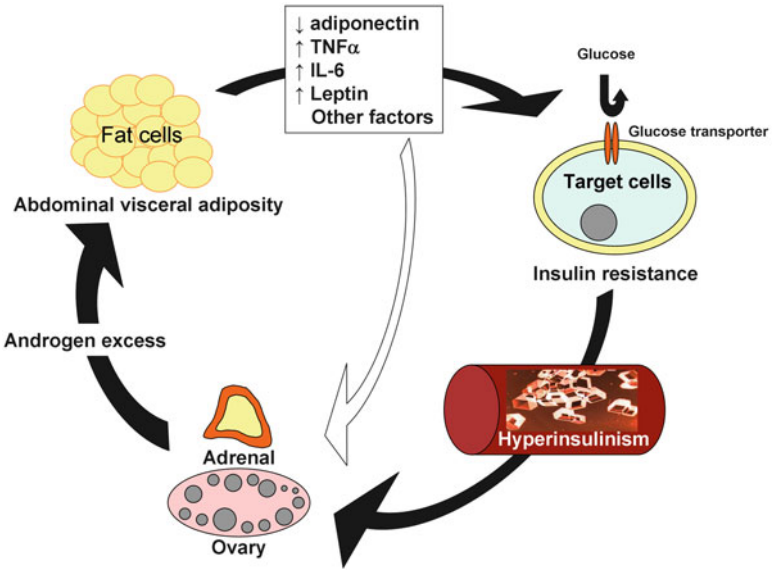

Fig. 16.1 Unifying hypothesis explaining the interplay between the polycystic ovary syndrome (PCOS) and abdominal adiposity as the result of a vicious circle represented by the *black arrows*: androgen excess favors the abdominal deposition of body fat, and visceral fat facilitates androgen excess of ovarian and/or adrenal origin by the direct effects (*white arrow*) of several autocrine, paracrine, and endocrine mediators, or indirectly by the induction of insulin resistance and hyperinsulinism. Reprinted from Escobar-Morreale HF, San Millan JL. Abdominal adiposity and the polycystic ovary syndrome Trends Endocrinol Metab. 2007;18:266–72, Copyright 2007, with permission from Elsevier

androgen excess after birth [14, 15]. The familial aggregation of PCOS suggests an inherited basis for this disorder [16]. Therefore, female fetuses from PCOS mothers may be exposed to excessive androgen levels during pregnancy if they inherit the abnormal mechanisms leading to androgen excess, and this exposure may condition the development of PCOS and abdominal adiposity after birth, as suggested by animal studies.

Girls born from mothers with PCOS may present with increased umbilical vein androgen levels at birth [17], supporting the hypothesis that androgen excess begins during fetal life in humans; yet other studies failed to reveal increased androgen levels in such girls [18, 19]. The samples used in these studies (venous or mixed cord blood obtained at delivery) may account for these discrepancies; as such samples may contain a certain amount of androgens of maternal origin from the mother. Further studies using umbilical artery blood, which comes from the fetus, or actual blood samples from the newborns are definitely needed to resolve this controversy.

Of note, the development of exogenous obesity aggravates the vicious circle of androgen excess, abdominal adiposity and adipose tissue dysfunction, insulin

resistance, and further androgen excess in women with PCOS, increasing markedly the metabolic and cardiovascular risks of affected women.

However, our very recent metabolomic data indicate that insulin resistance is not universal in PCOS [20]. Even if patients with PCOS as a group were hyperinsulinemic and insulin-resistant compared with the controls, non-obese patients with PCOS showed a metabolic profile consisting of suppression of lipolysis and increased glucose utilization in peripheral tissues. PCOS patients as a whole showed decreased 2-ketoisocaproic and alanine concentrations, suggesting utilization of branched-chain amino acids for protein synthesis and not for gluconeogenesis [20]. These metabolic processes required effective insulin signaling; hence, insulin resistance was not present in all tissues of these women, and different mechanisms, such as a decrease in insulin clearance, possibly contributed to their hyperinsulinemia. On the contrary, in obese women with PCOS the increase in plasma long-chain fatty acids, such as linoleic and oleic acid, and glycerol suggests increased lipolysis, possibly secondary to impaired insulin action at adipose tissue [20]. *Therefore, obesity appears to be the major determinant of metabolic heterogeneity in PCOS.*

The metabolic heterogeneity of PCOS may be explained by the existence of a continuum in the relative contribution of androgen excess, on the one hand, and of abdominal adiposity and insulin resistance, on the other [8]. At one extreme of this spectrum, women presenting with severe androgen excess may develop PCOS without the participation of any other pathophysiological mechanism (Fig. 16.2). At the other extreme of the spectrum, women with mild androgen excess only develop PCOS when another pathophysiological mechanism, such as abdominal adiposity, obesity, insulin resistance and/or hyperinsulinemia, is also present (see Fig. 16.2). Obviously, the most severe phenotypes are observed in patients presenting with both severe androgen excess and severe obesity. But in order to develop PCOS, a primary defect in androgen secretion, from mild to severe, is needed. In the absence of such a defect, PCOS cannot develop, explaining how approximately 40 % of morbidly obese women do not suffer the syndrome, even in the presence of massive abdominal adiposity and severe insulin resistance [21].

Although abdominal adiposity, metabolic dysfunction, and markers of subclinical atherosclerosis may also be present in non-obese women with PCOS [22–24], obesity is clearly related to the development of metabolic disorders in women with PCOS [25], explaining why not every women with PCOS is at increased risk of metabolic and cardiovascular disease [5]. Accordingly, the frequency of dyslipidemia and disorders of glucose tolerance in women with PCOS increases markedly with obesity [26–28]. In Spaniards, abdominal adiposity is an intrinsic characteristic of PCOS; yet obesity increases markedly the amount of fat in the visceral adipose tissue depots of these women [9]. In fact, it is obesity that is actually responsible for the association of PCOS with the metabolic syndrome [29], hypertension [30], hyperuricemia [31], and decreased health-related quality of life [32].

Therefore, obesity is a major player in the association of PCOS with metabolic dysfunction, and its prevention and management must be a priority when designing strategies for the long-term management of PCOS [5, 33].

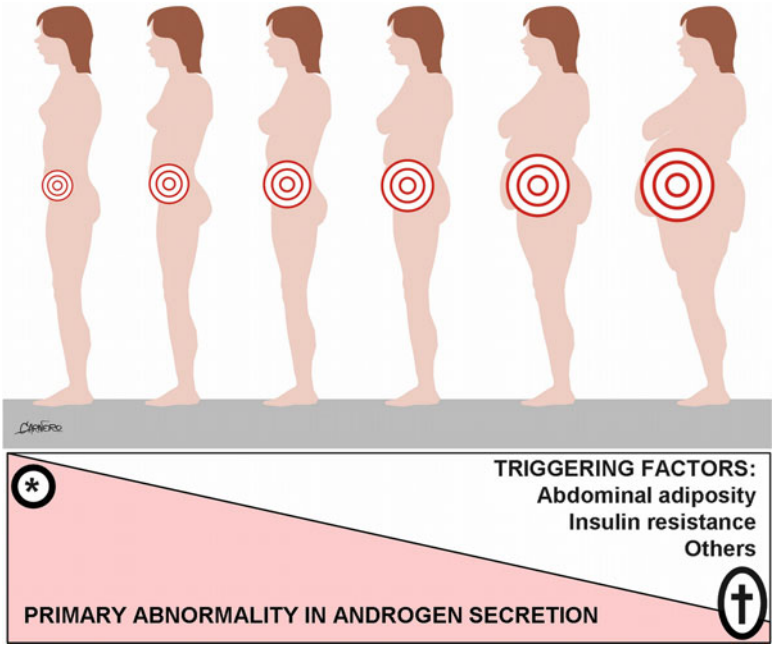

Fig. 16.2 The PCOS as the result of the interaction of a primary abnormality in androgen synthesis, manifesting as androgen excess, with environmental factors such as abdominal adiposity, obesity, and insulin resistance. At one extreme (*asterisk*), in some patients the disorder is severe enough to result in PCOS, even in the absence of triggering environmental factors. At the other extreme (*dagger*), a very mild defect in androgen secretion is amplified by the coexistence of abdominal adiposity, obesity, and/or insulin resistance. Between the two extremes, there is a spectrum in the severity of the primary defect in androgen secretion, explaining the heterogeneity of PCOS patients with regard to the presence of obesity and metabolic comorbidities. Yet all patients share a primary defect in androgen secretion. Reprinted from Escobar-Morreale HF, San Millan JL. Abdominal adiposity and the polycystic ovary syndrome Trends Endocrinol Metab. 2007;18:266–72, Copyright 2007, with permission from Elsevier

Role of Surgery in the Management of Metabolic Dysfunction of PCOS

The cornerstone for management of metabolic dysfunction in PCOS is lifestyle modification, especially diet-induced weight loss [5, 33]. Indeed, evidence supports that lifestyle intervention improves body composition, hyperandrogenism, and insulin resistance in women with PCOS [34]. However, there is not enough evidence for the effect of lifestyle intervention on improving glucose tolerance or lipid profiles and no literature assessing, in women with PCOS, clinical reproductive outcomes, quality of life, and treatment satisfaction in the relation to lifestyle modification strategies [34]. Furthermore, long-term efficacy of lifestyle intervention in patients with PCOS is still to be proven. In general, the magnitude of weight loss usually

attained after caloric restriction combined with increased physical activity is usually moderate, in the range of 5–10 % of the initial body weight, and is frequently not maintained for long periods of time [35, 36].

The evidence supporting the efficacy of pharmacological treatment for metabolic dysfunction in PCOS is unfortunately far from conclusive, with the possible exception of the use of metformin for glucose intolerance and diabetes and of lipid-lowering drugs in dyslipidemic patients [37].

In this context, surgical management of obesity has emerged as an alternative therapeutic approach for metabolic dysfunction in PCOS when lifestyle intervention and drug treatment fail. It must be highlighted that there is not enough evidence at present to universally recommend bariatric surgery for patients with PCOS and metabolic dysfunction. The available data for bariatric surgery in obese patients with PCOS however are very promising, especially considering that the prevalence of PCOS diagnosis is reportedly higher in women submitted to bariatric procedures compared with the general population [21, 38, 39]. In fact, PCOS diagnosis and menstrual abnormalities are possibly the most common accompaniments of morbid obesity when the analysis of comorbidities is restricted to the adolescents [40, 41].

Bariatric surgical procedures induce weight loss by inducing restriction of caloric intake through reduction of the reservoir capacity of the stomach, by inducing malabsorption through bypass of the small bowel absorptive surface area or diversion of the biliopancreatic secretions that facilitate absorption, or a combination of above mechanisms [42]. The most commonly used bariatric procedures nowadays include laparoscopic adjustable gastric banding, vertical banded gastroplasty, Roux-en-Y gastric bypass, and biliopancreatic diversion. Bariatric procedures usually result in effective weight loss, and a substantial majority of patients with diabetes, hyperlipidemia, hypertension, and obstructive sleep apnea experience complete resolution or improvement of these complications [42].

A few years ago, we screened for PCOS a series of 36 morbidly obese premenopausal women who elected to undergo bariatric surgery at our hospital. Seventeen of the 36 met the 1990 National Institute of Child Health and Human Development criteria for PCOS [43], 5 had idiopathic hyperandrogenism, and 14 had no evidence of androgen excess or ovarian dysfunction [21]. These groups were comparable in terms of BMI, waist circumference, waist to hip ratio, blood pressure, and pulse rate [21]. One patient with PCOS presented with diabetes and hypertension, another had diabetes and dyslipidemia, and two were hypertensive, but their glucose tolerance and lipid levels were normal. Only one of the regularly menstruating hyperandrogenic women had hypertension, whereas three of the nonhyperandrogenic women had hypertension, one had diabetes, and one had dyslipidemia.

Of the women with PCOS, 11 were submitted to biliopancreatic diversion and 4 were treated by laparoscopic gastric bypass [21]. The choice of procedure was dictated by the increasing expertise with laparoscopic gastric bypass of the surgeon performing bariatric surgery. We were able to follow-up 12 of these patients after weight-loss stabilization and a mean loss of weight of 41 ± 9 kg [21]. The diagnosis of PCOS could not be sustained in any of these women after weight loss: hirsutism improved and even normalized; androgen levels returned to the normal range in all but one woman (Fig. 16.3); all the patients regained regular menstrual cycles; and in

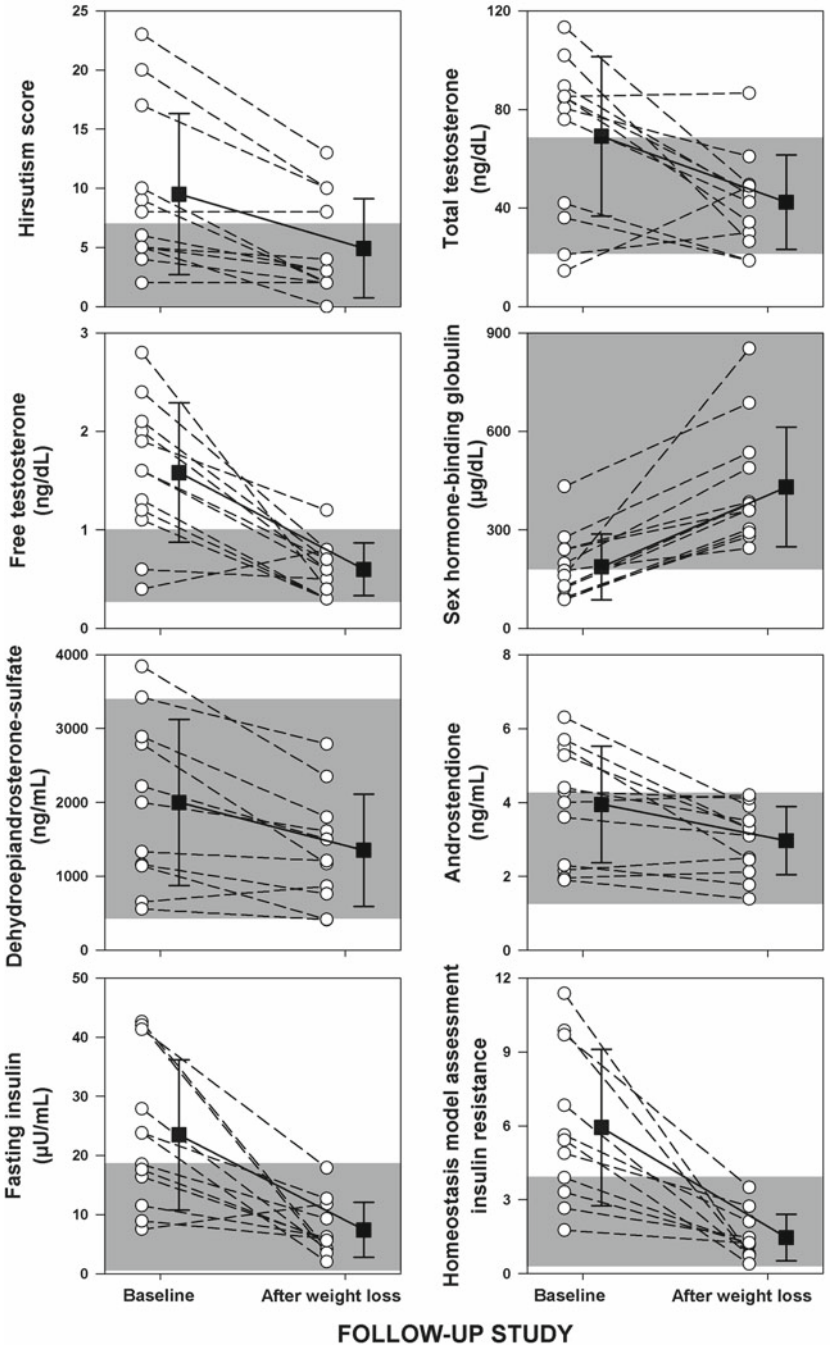

Fig. 16.3 Clinical and biochemical characteristics of the morbidly obese PCOS patients submitted to bariatric surgery, before and after surgical weight loss. *White circles* are individual values, and black squares are mean ± SD. The shaded areas represent the reference range for each variable. All paired comparisons are statistically significant ($P < 0.02$). Reprinted with permission from Escobar-Morreale HF, Botella-Carretero JI, Alvarez-Blasco F, Sancho J, San Millan JL. The polycystic ovary syndrome associated with morbid obesity may resolve after weight loss induced by bariatric surgery. J Clin Endocrinol Metab. 2005;90:6364–9. Copyright 2005, The Endocrine Society

the ten patients in whom we were able to measure serum progesterone concentrations during the luteal phase of the menstrual cycle, these levels indicated recent ovulation [21]. These favorable changes paralleled the improvement in insulin resistance, as homeostasis model assessment of insulin resistance values returned to the normal range after weight loss (see Fig. 16.3) and was accompanied with improvement or resolution of diabetes and hypertension [21]. Similar results have been reported using laparoscopic gastric bypass in the United States [44]. Retrospective evaluation of 24 oligomenorrheic women with PCOS submitted to bariatric surgery revealed that after marked weight loss hirsutism improved in 21, menstrual cycles were regular in all the patients, and five patients who were considered infertile before surgery were able to conceive spontaneously [44]. The improvement in PCOS symptoms was paralleled by improvement or resolution of diabetes, hypertension, and dyslipidemia in most of the women presenting with these conditions before surgery [44]. An extended report of this series including 31 patients with PCOS showed a 100 % postoperative conception rate in the subset of infertile PCOS subjects desiring pregnancy after 9 years follow-up [45]. In another prospective 6-year study of patients undergoing laparoscopic adjustable gastric banding, the features of PCOS improved in 48 % of women specifically with regard to menstrual cycles, fertility, and/or hirsutism [46], and this figure increased to 60 % in the adolescents [40]. A retrospective analysis of a very large series of patients with PCOS submitted to surgical treatment of obesity further confirmed the marked improvement of metabolic dysfunction in PCOS after bariatric surgery [47].

One of the most heartening effects of bariatric surgery is the potential of restoring fertility in given that PCOS is a major contributor to ovulatory infertility. Earlier studies focusing on pregnancy outcomes in women who had undergone bariatric surgical procedures suggested concerns regarding a potential increased risk of intrauterine growth restriction, possibility of complications of surgery, including internal hernia and need for strict nutritional supplementation during pregnancy; recent data however are reassuring with theoretical risk being more than counterbalanced by the avoidance of the many pregnancy associated risks that are well recognized in the setting of severe maternal obesity [48, 49]. Beyond facilitation of ovulation and attainment of pregnancy, beneficial effects of surgery-induced weight loss for a future pregnancy include reduced rates of maternal complications such as gestational diabetes and preeclampsia, and reduced incidence of premature delivery, low birth weight, and macrosomia compared with obese women; indeed, pregnancy outcomes of women who attained successful weight loss following bariatric surgery were similar to those of non-obese controls [48, 49]. Furthermore, assisted reproductive techniques, such as in vitro fertilization, are safe and effective in women with prior bariatric surgery [50]. The current recommendations include delaying pregnancy for at least 1 year after the bariatric procedure in order to avoid the initial period of rapid weight loss following surgery, nutritional supplementation tailored to the individual patient and the type of bariatric procedure performed, and close follow-up of fetal growth during pregnancy [49].

While the bulk of evidence derives from the effects of bariatric procedures in severely obese women, a recent clinical trial compared bariatric surgery with

conventional treatment in moderately obese (body mass index 30–40 kg/m^2) patients recently diagnosed with type 2 diabetes and suggested that the beneficial effects of bariatric surgery on metabolism are not restricted just to severely obese subjects [51]. These data introduce the possibility of exploring a role for bariatric surgery in moderately obese women with PCOS who manifest evidence of metabolic dysfunction, are deemed at an enhanced lifetime risk for chronic conditions such as diabetes and cardiovascular disease, who are either unable to sustain lifestyle modification strategies or in whom such interventions fail to significantly impact on the overall clinical picture.

Despite the promise of bariatric surgery, as outlined earlier, one cannot minimize the importance of appropriate patient selection for surgical consideration as morbidities and even mortality relating to bariatric procedures are well described. Of the 36 patients reported by our group a single mortality event occurred and 1 of the 36 patients submitted to bariatric surgery died as a consequence of postoperative surgical complications followed by sepsis of abdominal origin and multiorgan failure [21]. In the past, perioperative mortality has been reported in as many as 1.5–2 % of bariatric surgical cases, yet these figures have been reduced to less that 0.3 % from more recent registries involving many thousands of patients [52]. Moreover, serious perioperative complications reportedly occur in 1–4 % of patients [52].

Summary

Obesity aggravates all the manifestations, risks, and metabolic comorbidities of PCOS. While lifestyle modification and dietary interventions constitute a first-line management strategy, these approaches are unlikely to be effective in the long term in a significant proportion of this population. Given that most pharmacological approaches are at best of modest benefit, bariatric surgery offers a promising alternative therapeutic approach in obese to morbidly obese patients with PCOS and for those with evidence of metabolic comorbidities. Despite the promise, however, existing data on bariatric surgical management of PCOS are limited, and large-scale prospective studies addressing not only the amelioration of PCOS symptoms and reproductive success but also the long-term consequences are definitely needed before bariatric surgery gets considered in the routine management paradigm for PCOS.

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Chapter 17
Surgical Management of Polycystic Ovary Syndrome: A Contemporary Viewpoint on Place of Ovarian Surgery in PCOS Management

Phoebe H. Leonard, Jani R. Jensen, and Gaurang S. Daftary

Key Points

- Ovarian surgery for PCOS should be reserved as a second line for individuals who are unresponsive to conventional medical therapy (such as those exhibiting clomiphene resistance) or for those who are unable to undertake medical therapy (due to clinical or financial constraints).
- Ovarian surgery may be more cost-effective than trial of gonadotropin for addressing ovulatory dysfunction of PCOS, with less risk for multifetal gestation and ovarian hyperstimulation, although with comparable pregnancy rates.
- Surgical techniques vary, offer comparable efficacy, and may be individualized based on the expertise of the practitioner and access to surgical tools. The conventional approach of ovarian wedge resection has long been superseded by minimally invasive surgical techniques (ovarian drilling utilizing microlaparoscopy, fertiloscopy, and ultrasound-guided ovarian drilling) that confer clinical benefit while mitigating the long-term sequelae associated with wedge resection.

Introduction

Polycystic ovary syndrome (PCOS) is a complex disorder that is characterized by an array of clinical, endocrine, and metabolic manifestations. In order to accommodate the heterogeneity of this disease, the diagnostic criteria have been generalized

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and therefore are broadly inclusive, despite differences in severity of the clinical picture [1, 2]. In general, PCOS patients present with symptoms and laboratory anomalies that stem from hyperandrogenism (hirsutism, acne) as well as ovulatory dysfunction (oligoovulation/anovulation) with ensuing menstrual aberrations (oligorrhea/amenorrhea). Ovulatory dysfunction is an obvious contributor to infertility in this population. Given the high prevalence of PCOS in the general population, PCOS is, therefore, also the commonest cause of ovulatory infertility. Treatment of PCOS is aimed at arresting or reversing the progressive endocrine and metabolic anomalies associated with the disorder. Although ideally the treatment should focus on targeting the inciting endocrine event(s), the exact pathophysiology of PCOS still remains unclear and therapeutic approaches, therefore, empirically attempt to override abnormal signaling pathways.

While medical approach is the mainstay in PCOS management, therapeutic efficacy consequent to surgical elimination of ovarian tissue has long been recognized in women with PCOS (further detailed in next section). The ovarian stroma, especially theca cells, is a primary source of androgens and therefore hyperandrogenemia [3]. Excision of populations of theca cells is thus expected to diminish the synthesized and secreted androgen load, resulting in amelioration of hyperandrogenemia. Indeed, improvements in the abnormal endocrine and metabolic milieu of PCOS following partial removal of ovarian tissue are well described [4]. A decline in the circulating androgen levels, as a result, is purported to restore the sensitivity of the hypothalamic–pituitary–ovarian axis; normalization of the gonadotropic axis is suggested to underlie with the resumption of ovulation, menstrual regularity, and restoration of fertility consequent to "de-bulking" of the polycystic ovaries.

History

Reports from as early as the 1920s describe an association between obesity and menstrual irregularity as well as between hirsutism and infertility. Polycystic ovary syndrome was first described by I.F. Stein and M.L. Leventhal in Chicago in 1935 [5–7], and the authors published their findings in a landmark paper entitled "Amenorrhea Associated with Bilateral Polycystic Ovaries" in the American Journal of Obstetrics and Gynecology in that same year [8]. Practicing in an age when laparotomy had facilitated surgical approach to the ovaries, Stein and Leventhal operated on these patients only to discover that their ovaries were enlarged to 2–4 times the normal size, with numerous small fluid-filled cysts. Whereas some of the ovaries were flat and "oyster-like," in general, they all appeared dull, grayish, and polycystic. Thus, ever since the initial description of the syndrome, an association was made between the characteristic symptomatology and the presence of enlarged and polycystic ovaries. Stein and Leventhal initially performed wedge biopsies of the ovaries to more fully elucidate the underlying pathology. Although no hallmark pathological lesion was identified, they serendipitously noticed that their biopsied patients resumed regular menstruation within a few months of the procedure; many

of whom also spontaneously conceived thereafter. Of 96 patients subjected to ovarian wedge resection by Stein and Leventhal, 71 had experienced infertility, and in this subset, 63 achieved spontaneous pregnancy. Additionally, from the entire cohort, all except three patients had a resumption of regular menstrual cyclicity [6, 7, 9]. Ovarian wedge resection thereafter became a therapeutic option rather than a diagnostic modality.

However, despite the initial enthusiasm based on high success rates, ovarian wedge resection fell out of favor with time due to a high incidence of postoperative adhesions and concerns about a significant loss of endocrinologically active normal ovarian tissue [10, 11]. In its place, medical therapy, which was considered a safer and more efficacious option, became the mainstay for induction of ovulation in women with PCOS.

Contemporary Modes of Ovarian Surgery in PCOS

Given the concerns of adhesion formation and ovarian tissue destruction after wedge resection, a less invasive laparoscopic approach known as ovarian drilling or diathermy (LOD) was developed by Gjønnæss in 1984 [12]. Using minimally invasive surgical concepts, focal destruction of the ovarian stroma is achieved utilizing ablative strategies that have ranged from diathermy to laser tissue vaporization. In the initial report, diathermy was utilized to create up to ten individual punctures into the ovarian cortex; Gjønnæss treated 62 women with PCOS, of whom 92 % ovulated successfully within 3 months following the procedure; regular menstrual cyclicity was established in 86 % (51/62 patients). Of the 35 patients presenting with infertility, 24 became pregnant (69 %); interestingly, 7 of 9 patients determined to be clomiphene-resistant ovulated spontaneously, whereas the remaining two became responsive to clomiphene in the postoperative period. In a subsequent larger series of 212 patients, the ovulation and pregnancy rates following were 92 and 84 %, respectively [13]. As observed in their initial cohort, patients that remained anovulatory after surgery did, however, respond to clomiphene and eventually achieved pregnancy. Inclusion of this group increased the overall pregnancy rate to 89 %. Although lean patients had higher spontaneous ovulation rates (96–97 %) compared to the obese (70 %), the pregnancy rates were similar in both groups once ovulation had been established (92–95 %).

The reproductive outcomes of clomiphene-resistant PCOS patients who underwent LOD were evaluated in a recently updated Cochrane Database analysis comprising 25 randomized controlled trials (the original review had consisted of nine studies) [14]. The primary outcomes evaluated were live birth and multiple pregnancy rates, whereas rates for miscarriage, ovulation and pregnancy, and the incidence of ovarian hyperstimulation syndrome (OHSS), impact on quality of life, and treatment related costs were evaluated as secondary outcomes. Amongst 1,034 patients from eight comparative studies, the live birth rate following LOD was 34 % compared to 40 % with medical therapy; live birth rates following LOD

were comparable to seen with clomiphene citrate, gonadotropins, or aromatase inhibitors but inferior to seem with combination of clomiphene and metformin (159 patients; OR: 0.44 [95 % CI: 0.24–0.82]; $p < 0.01$). In contrast, there was a clear advantage in favor of LOD in terms of reduction in the multiple pregnancy rate in comparison to gonadotropin therapy (166 patients; OR: 0.13 [95 % CI: 0.03–0.52]; $p < 0.004$). Interestingly, no difference in multiple pregnancy rates was seen when LOD was compared to either clomiphene or aromatase inhibitors.

Operative Techniques

In general, in order to minimize morbidity and reduce the likelihood of pelvic adhesion formation, a minimally invasive approach is preferred for ovarian drilling unless contraindications to laparoscopic approach exist (Fig. 17.1). Traditional laparoscopy has been used in the majority of reports and trials on LOD. There does not appear to be a difference in either the postoperative hormone profile or ovulation and pregnancy rates with regard to the type of thermal technique (harmonic scalpel, monopolar cautery, bipolar cautery, or laser coagulation) employed for ovarian thecal decompression [15, 16]. Each of these minimally invasive approaches creates focal areas of damage in the ovarian cortex and the stroma; the extent of ovarian tissue damage with LOD approach is minimal compared to seen with the originally described ovarian wedge resection technique.

A significant concern with any pelvic surgery is the potential risk for excessive, unintended ovarian tissue destruction that spreads beyond the area intended for surgical ablation. Excessive ovarian cortical destruction could potentially result in

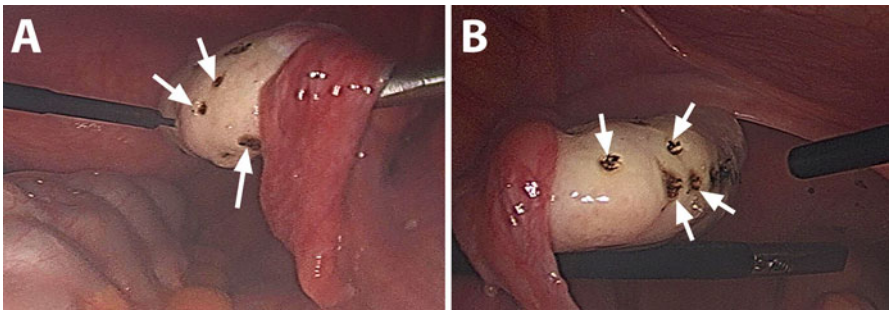

Fig. 17.1 Surgical management of PCOS-related ovulatory infertility by laparoscopic ovarian drilling. Electrocautery was delivered to the ovarian cortex laparoscopically to the right (**a**) and left (**b**) ovaries in a 23-year-old nulliparous patient. Ovarian cortexes were punctured with a monopolar needle cautery set at 30 W coagulation for approximately 3–4 s at each puncture site. The patient had been experiencing oligoovulation/anovulation with irregular menstrual cycles. Although she desired regular cycles and wanted to have the option of conceiving, she was not willing to undertake treatment by medical ovulation induction secondary to ethical and religious beliefs. Individualized treatment by laparoscopic ovarian drilling was therefore the option of choice in this carefully selected and counseled patient. *White arrows* indicate ovarian drilling sites

diminished function with consequent adverse sequelae on ovarian endocrinology and fertility. In order to determine the minimal energy requirement for effective ovarian stromal destruction and resumption of ovulation, Amer et al. treated 30 clomiphene-resistant PCOS patients with LOD [17]. Using a specially designed monopolar electrode, 1–4 punctures were made to a depth of 8 mm each into the ovarian stroma, delivering 150 J of energy per puncture site. Ovulation and pregnancy rates were highest (67 % each) in the subgroup of patients that received four punctures per ovary—an average of 600 J/ovary or 1,200 J/patient. In contrast, in patients receiving only one puncture per ovary, the corresponding rates were 33 and 0 %, respectively. The authors, therefore, recommend that an ovarian dose of 600 J is required for optimal tissue response [17]. In another study, 26 patients were treated with five punctures and 37 patients with ten punctures per ovary [18]. The clinical and laboratory outcomes of both groups were similar. The authors concluded that the efficacy of five punctures per ovary was sufficient treatment. From these studies, it appears that there is a threshold effect that peaks at 4–5 punctures per ovary.

Monopolar diathermy is probably the most commonly employed strategy for achieving ovarian stromal reduction. Caution is warranted regarding site of ovarian puncture; site of ovarian puncture should be remote from the fallopian tube as is possible in order to avoid subsequent fimbrial adhesions and thereby cause iatrogenic tubal occlusion with consequent tubal factor infertility [19].

Recently published reports have cited success of LOD using microlaparoscopy, a procedure that can be performed in the office setting under a combination of intravenous sedation and local anesthesia utilizing a malleable 2.2-mm fiber-optic laparoscope introduced through a specially designed 12 cm × 2.5 mm metal sleeve that fit over a Veress needle [20]; a high-frequency 2.2-mm electrocautery probe was used for ovarian drilling during this procedure. With technological advances and increasing prioritization of low-cost treatments, previously used diagnostic modalities such as fertiloscopy or transvaginal hydrolaparoscopy are being revived as relatively less invasive methods that can permit reliable ovarian access for focal decompression. These techniques hold promise for minimally invasive approaches for ovarian drilling. Transvaginal hydrolaparoscopy was initially used in the 1960s and 1970s but was abandoned because trans-abdominal laparoscopy provided a more panoramic view of the pelvic and abdominal cavity, and better access for surgical treatment. This technique uses a small culdotomy incision with a 4-mm endoscope that has a 30° angle [21–25]. While less invasive than conventional laparoscopy, these minimally invasive approaches are not currently in widespread use.

Transvaginal ovarian follicle aspiration been shown to be as effective as LOD for clomiphene resistance [26, 27]. In a technique that mimics that of transvaginal oocyte retrieval, small ovarian follicles are aspirated under ultrasound guidance through 3–6 transvaginal punctures made. The procedure can be safely undertaken in an office setting. Other investigators have successfully used an ultrasound-guided laser technique for ovarian drilling [28]. However, there are concerns that the cortical blood flow may be disrupted with this technique [29]; given the small numbers and preliminary nature of such novel approaches, further confirmatory studies using larger patient numbers are needed to establish both safety and efficacy of ovarian drilling utilizing transvaginal laser.

Endocrine Events After Ovarian Surgery

Dysregulated endocrinology with an overall androgen excess is the underlying driver of PCOS-associated pathophysiology. Androgen excess, either relative or absolute, is a hallmark of PCOS, and the magnitude of hormonal imbalance is often reflected in the severity of symptomatology. Although numerous theories abound about the signaling event/s responsible for the panoply of hormonal aberrations, of PCOS, the instigating mechanisms remain far from clear. Disproportionately elevated levels of luteinizing hormone (LH) [30, 31], hyperandrogenemia [32, 33], hyperinsulinemia and insulin resistance [34, 35] are commonly encountered in women with PCOS and recognized to underlie the common symptoms as well as co-morbidities of PCOS [36, 37].

In most studies, there is an immediate decrease in circulating levels of androgens and androgen precursors (testosterone, dihydrotestosterone, androstenedione, and 17-hydroxyprogesterone) of ovarian origin following ovarian drilling, notable as early as within 24 h of the procedure [38–49]. Moreover, the decrease in androgen levels following ovarian drilling is described to persist, spanning observation periods in different studies that ranged from a few days to a few years [38]. However, most of the studies that reported persisting endocrine benefit following ovarian drilling utilized electrocautery and comprised fewer than 20 patients. Given the size, heterogeneity, and differences in study design, it is thus difficult to draw definitive conclusions regarding long-term effects of this strategy.

In contrast to the consistent reduction in serum androgen levels after ovarian surgery, the overall effect on estradiol concentrations is less clear. This is partly attributable to differences in size and heterogeneity of studies, but more importantly also to the lack of consistent menstrual cycle phase dependent monitoring. In studies where measurements correlated with cycle phase, there was either no change in the early and mid-follicular estradiol levels, or some increase in the late follicular and luteal levels, the latter evident in those that responded with resumption of menstrual cyclicity. Lower estradiol levels in the early proliferative phase may be due to decreased tonic LH stimulation of theca cells resulting in a lower intra-ovarian androgen load. In turn, the lower thecal follicular androgen output results in diminished substrate for granulosa cell aromatase and therefore lower estradiol levels. Higher estradiol levels later in the menstrual cycle thus likely reflect resumption of ovarian folliculogenesis following surgical intervention. Ovarian surgery however did not have any significant impact on progesterone concentration [50].

A transient elevation in serum LH levels is commonly noted on the day after ovarian surgery, with a more consistent and sustained decline in levels thereafter. The decrease in LH levels (except for the preovulatory surge) can reportedly persist for several years following ovarian surgery [51]. The decline in LH levels following ovarian surgery is attributable to decreased LH pulse amplitude; LH pulse frequency reportedly remains unchanged [52]. The magnitude of decline in circulating LH was noted to be greater in those achieving spontaneous ovulation and/or conception following ovarian surgery compared to the nonresponders [38]. Similar to LH, FSH

levels also increased in most studies in the immediate postoperative phase and then declined over time to pretreatment values [39, 53]. Pituitary FSH secretion is driven by hypothalamic GnRH, which in turn is responsive to feedback regulation by the sex steroids. FSH is also inhibited by inhibin, which is produced by ovarian granulosa cells. Although the data on the impact of ovarian surgery on inhibin are limited, inhibin levels appear to decline in the immediate postoperative period followed by a gradual return to baseline levels within a few weeks following the procedure [53]. Destruction of the ovarian stroma and granulosa cells can thus be hypothesized to underlie the decline in inhibin levels notable immediately following the procedure, thereby explaining the rise in endogenous FSH levels and creation of a more physiological milieu that is conducive to follicular growth.

In contrast to the amelioration in hyperandrogenemia of ovarian origin following ovarian surgery, serum levels of adrenal dehydroepiandrosterone sulfate (DHEAS) are essentially unchanged [54].

Repeated LOD in PCOS

Given concerns of adhesion formation and a detriment to ovarian reserve consequent to focal destruction of ovarian tissue, is there a role for repeated LOD in the management of PCOS? To answer this question, 20 patients who had previously undergone LOD 1–6 years prior to the study (to which 12 had and 8 had not responded), were subjected to repeat intervention [55]. Following the repeat LOD procedure, the ovulation and pregnancy rates were 83 and 66 %, respectively, in the 12/20 who had achieved resumption of ovulation following the first procedure, whereas in the remaining eight (nonresponders to first intervention), the respective rates were 25 % (2 out of 8) and 29 % (2 out of 7). As suggested by this single study, although there appears to be a short-term fertility-related benefit from repeat procedure, especially in prior responders, the long-term effects of repeated LOD procedures both with respect to risk of pelvic adhesions and for ovarian detriment, remain unclear. In the absence of robust data, caution is advised regarding consideration of repeat LOD, which should be entertained only after exhaustion of the existing repertoire of medical management options.

Conclusion

Although medical management is the mainstay, focal surgical intervention such as LOD is an effective treatment option for a select group of patients with PCOS seeking fertility treatment who fail to respond to common ovulation induction agents (such as clomiphene or aromatase inhibitors) and are either deemed at an enhanced risk for gonadotropin therapy related sequelae (such as multiple pregnancy or ovarian hyperstimulation syndrome) or cannot afford the financial burden

relating to gonadotropin use. Almost 20 % of patients with PCOS demonstrate resistance to clomiphene [56]; given that this subgroup is particularly at risk for developing ovarian hyperstimulation syndrome as well as multiple pregnancy with gonadotropin exposure, these patients may represent an ideal population to benefit from LOD. Indeed, pregnancy rates with LOD are comparable to seen with ovulation induction and gonadotropin therapy, albeit with minimal iatrogenic risk of multiple pregnancy. For patients not seeking fertility who are also not candidates for hormonal therapy, LOD may be considered as a management strategy but only after detailed discussion on the risks and benefits relating to this procedure. In an appropriately selected patient, LOD can be considered as a relatively safe and financially viable option that offers a potential for long-term benefit for PCOS-related symptomatology. There are a wide variety of techniques that can be employed for LOD and with no formal recommendations by professional societies. Based on the consensus of the literature and our own clinical experience, we would recommend a monopolar needle approach, set between 20 and 30 W, with approximately 5–10 punctures depending on size of the ovary.

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Part VI
Recognized Risks and Emerging Concepts

Chapter 18
Long-Term Morbidity: Cardiovascular Disease, Cancer, and Depression in PCOS

Lauren W. Milman and Anuja Dokras

Key Points

- Women with PCOS have an increased risk of several traditional risk factors of cardiovascular disease (CVD) including obesity, dyslipidemia, hypertension, metabolic syndrome, and diabetes.
- Women with PCOS have an increased prevalence of markers of subclinical atherosclerosis including increased carotid intima–media thickness (IMT), increased coronary artery calcification, and endothelial dysfunction compared to control populations.
- Currently available CVD risk-assessment tools may underestimate the risk of CVD in the PCOS population.
- There is an increased risk of endometrial cancer in the PCOS population.
- Limited evidence suggests an increased risk of ovarian cancer in women with PCOS.
- Previously suggested risk of breast cancer has not been substantiated in women with PCOS.
- Women with PCOS are more likely to have increased depressive symptoms and depression compared to controls.

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Cardiovascular Disease

Cardiovascular disease (CVD) is the leading cause of death for women in the USA, and is evident in approximately one-third of women [1]. Several traditional risk factors of CVD are prevalent in women with PCOS including obesity, dyslipidemia, hypertension, metabolic syndrome, diabetes, and depression [2–5]. Patients with both diabetes and metabolic syndrome have been shown to have an eightfold risk of CVD-related mortality than those without these diagnoses [6]. A recent meta-analysis has shown that women PCOS have a higher prevalence of both diabetes and metabolic syndrome than weight- and age-matched controls [7–9].

Standardized tools can be applied to assess an individual's long-term risk for a cardiovascular (CV) event. The Framingham risk score uses age, gender, total and HDL cholesterol, smoking status, systolic blood pressure, and use of an antihypertensive agent to calculate a 10-year risk of developing angina, myocardial infarction, or coronary death [10]. The Reynolds risk score uses similar data, with the additional inclusion of serum high-sensitivity C-reactive protein, a newer CV risk biomarker, and parental history of myocardial infarction [11]. However, these tools are likely to underestimate the true risk in women, since up to 20 % of women have a CV event without an identifiable major risk factor. Moreover, women are more likely to present with a stroke as the first manifestation of CVD, an entity that is not considered in the above risk score calculations. A notable limitation of applying traditional risk assessment strategies for identifying CV events in the PCOS population is that women are typically diagnosed with PCOS in the second and third decades of life, accounting for a low *absolute risk* based on decreased duration of CV risk exposure. However, a young patient with a low absolute risk score, such as a woman with PCOS, may have a significantly increased *relative risk* compared to others of the same age. Therefore, there is an urgent need to develop and validate CV risk screening tools that can be used in reproductive-age women. The androgen excess-PCOS (AE-PCOS) society has published guidelines for risk assessment in women with PCOS using traditional CV risk factors [3]. All women with PCOS should have a cardiometabolic risk assessment. Timely and appropriate treatment in this young group will likely reduce future morbidity and mortality. In women who do not have significant risk factors, regular screening should be recommended [3].

Subclinical Atherosclerosis

To better determine the risk of CVD in the asymptomatic PCOS population, several surrogate markers for subclinical atherosclerosis have been studied. These include changes in carotid intima–media thickness (IMT), the presence of coronary artery calcification (CAC), as well as assessment of endothelial function of conduit and resistance vessels.

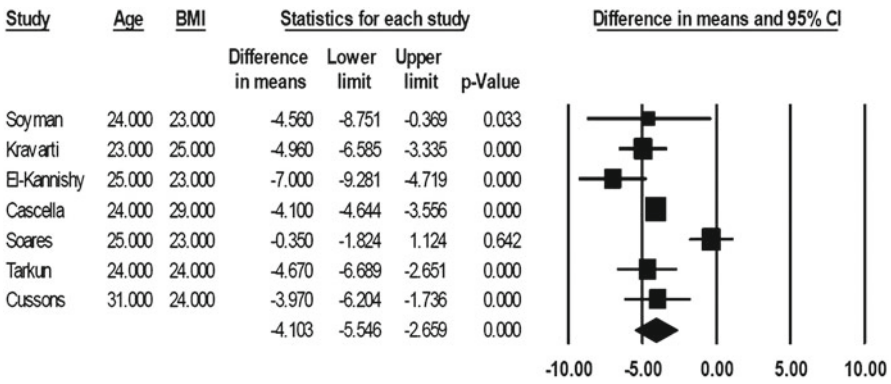

Fig. 18.1 Forest plot of individual study difference in mean FMD (%) between PCOS and control women in those studies that adjusted for the effect of age, BMI, smoking, and used only the Rotterdam diagnostic criteria [72] for PCOS [15]

Endothelial Function

Vascular dysfunction may be one of the earliest events in the process of developing atherosclerosis, and studies suggest that those subjects with vascular dysfunction are at increased risk of having a CV event [12]. Both obesity and insulin resistance, entities that are prevalent in the PCOS population, are associated with increased endothelial dysfunction [13]. Endothelial function can be measured noninvasively via flow-mediated dilation (FMD) of the brachial artery, where a response is measured to increased blood flow after transient ischemia. A decrease in this vascular response suggests underlying endothelial dysfunction. Our study showed that obese PCOS women have significantly reduced vascular dilatation in response to endothelium-dependent and -independent mechanisms compared to the lean PCOS population, although this difference was not statistically significant compared to obese and lean controls, respectively [14]. A recent meta-analysis has shown that 13 of 20 studies report a significantly lower FMD in women with PCOS compared to controls, with a pooled mean FMD 3.4 % (95 % CI 1.9–4.9) lower in the PCOS group. A sub-analysis of subjects with age- and Body mass index (BMI)-matched controls, and those with PCOS diagnosed by the Rotterdam criteria [15] showed a decrease of FMD of 4.1 % (95 % CI 2.7, 5.5) in the PCOS group (Fig. 18.1) [15]. The analysis is limited by substantial heterogeneity between the studies, but there appears to be overall evidence that women with PCOS have impaired FMD than controls, even after adjusting for age and BMI.

Carotid Intima–Media Thickness

Carotid IMT is measured by Doppler ultrasound, and its accuracy depends on the sonographer's skills. An increased carotid IMT positively correlates with the presence

Table 18.1 Prevalence of coronary artery calcification (CAC) in women with PCOS compared to controls

Author, year	*n*	Study population (study design)	Outcome measure/results
Shroff (2007) [22]	24 Cases 24 Controls	Obese, premenopausal (cross-sectional)	Prevalence of CAC (>0) OR = 5.5 (95 % CI = 1.03, 29.45) $p < 0.03$
Christian (2003) [70]	36 Cases 71 Controls	Premenopausal, age 30–45 (cross-sectional)	Prevalence of CAC (>0) OR = 1.99 (95 % CI = 0.68,5.82) $p = 0.21$ (NS)
Talbott (2004) [71]	61 Cases 85 Controls	BMI <35, age 40–61 (prospective)	Prevalence of CAC (>0) OR = 2.31 (95 % CI = 1.00, 5.33) $p = 0.049$
Talbott (2008) [24]	149 Cases 166 Controls	All BMI (cross-sectional)	Prevalence of CAC (>10) OR = 1.90 (95 % CI = 1.04, 3.48) $p = 0.037$
Chang (2011) [25]	144 Cases 170 Controls	All BMI (cross-sectional)	Prevalence of CAC (>10) 3.6 % vs. 5.7 % (controls) (matched for age, BMI) $p = 0.41$ (NS)

of coronary atherosclerosis [16], and is prognostic of risk of CV events [17]. In 2011, Meyer et al. conducted a meta-analysis that included 19 studies that compared mean carotid IMT in 1,123 women with PCOS and 923 controls. There was a statistically significant mean difference of 0.072 mm (95 % CI 0.040–0.105) between the PCOS subjects and the controls in the seven "highest quality studies," that reported a reproducibility statistic and used both the left and right carotid artery measurements [18]. Thus, women with PCOS appear to have a higher carotid IMT, consistent with evidence of premature atherosclerosis, and hence CVD and are deemed at an increased risk of CV events.

Coronary Artery Calcification

Both electron beam and multislice computed tomography (CT) are used to noninvasively measure CAC as a marker of total coronary atherosclerosis burden [19]. This marker can be used to assess the risk of having a myocardial infarction or sudden cardiac death [20] and has been shown to independently predict all-cause mortality [21]. Compared to assessment of endothelial function and carotid IMT, fewer studies have measured CAC in the asymptomatic PCOS population. Table 18.1 highlights five studies, three of which detected a significantly increased prevalence of CAC in women with PCOS. In particular, Shroff et al. studied a young, obese premenopausal population; the average age in the PCOS group was 32 years (SD ± 6.5), and BMI was 36 ± 5.4 kg/m^2. The prevalence of CAC in the PCOS group was 33 % compared to 8 % in the control group. After matching for age, BMI, and other cardiovascular risk factors, the prevalence of CAC was significantly increased in the PCOS population compared to the controls (odds ratio [OR] 5.5 (95 % CI 1.03–29.45) [22]. Interestingly, in another study, the prevalence of CAC in young women with PCOS (31 %) was comparable to that noted in age-matched men from the same geographic region (Muscatine study) [23]. In the two largest studies,

however, evidence regarding the prevalence of CAC in women with PCOS is conflicting. Talbott et al. found a statistically significantly increased prevalence of CAC in PCOS patients compared to the controls, while Chang et al. found no difference in CAC between those with and without PCOS [24, 25]. Both studies accounted for potential confounding influences of age and BMI in their design and analyses; however, the cohort in the Talbott study had a higher mean age compared to the Chang study (48 vs. 41 years). Collectively, existing data are supportive of concerns that young asymptomatic women with PCOS demonstrate evidence of subclinical atherosclerosis.

Risk of Fatal or Nonfatal Cardiovascular Events

Despite evidence of a plethora of CV risks being prevalent in this population, evidence for CVD-related morbidity and mortality in women with well-defined PCOS is, however, limited. The definition of PCOS has undergone changes over the past few decades, and longitudinal studies in large cohorts of women with PCOS are lacking. Most of the available data are derived from studies that included women with a retrospective diagnosis of PCOS and not based on currently accepted definitions [21, 26]. One study reported that, after adjusting for confounders, women with polycystic appearing ovaries were more likely to have stenotic CAD (i.e., more coronary artery segments with >50 % diameter loss) than women with normal appearing ovaries ($n=142$) [26]. Eight additional studies were included in a systematic review, five of which found an increased risk associated with cardiovascular endpoints in the PCOS population, while three studies found no difference in cardiac events compared to age-matched women [27]. A prospective study followed 31 of 35 women with PCOS (diagnosed in 1987 via histology at wedge resection, and confirmed by Rotterdam criteria in 2008) and 160 age-matched controls into late menopause. In this cohort, women with PCOS did not have a significantly increased risk of CV-related morbidity or mortality over the 21-year follow-up period, with subjects followed to a mean age of 70 years old [28]. Additional longitudinal data are available from the Nurses' Health Study, which followed 82,439 women over a period of 14 years, and assessed the risk of CVD-related events based on menstrual cycle irregularity as the only known diagnostic feature of PCOS. Compared to women reporting a history of regular menstrual cycles, those who acknowledged *usually irregular* or *very irregular* cycles had an increased risk for nonfatal or fatal coronary events with a multivariate relative risk (RR) of 1.38 (95 % CI 1.06–1.8) and 1.88 (95 % CI 1.32–2.67), respectively [29]. A recent meta-analysis by de Groot et al. included five studies assessing the risk of both nonfatal and fatal CHD and stroke in women with PCOS. Using the random effects model, the pooled RR was 2.02 (95 % CI 1.47–2.76) for CHD or stroke when evaluating women with PCOS compared to those without PCOS. When limited to the only two studies that adjusted for BMI, RR decreased to 1.55 (95 % CI of 1.27–1.89), albeit remained of statistical significance [30]. Collectively, existing evidence suggests that women with PCOS are more likely to manifest evidence of early onset CVD and are at an increased risk

for developing adverse CV-related outcomes. However, larger prospective studies with long-term follow-up of well-characterized populations of PCOS cases are needed to better clarify the magnitude of risk for CV related outcomes as well as to better identify any subpopulations within the diagnostic group of PCOS that may be particularly vulnerable to CV outcomes. In the meantime, there is consensus that women with PCOS be recognized as being at risk for CVD and that CVD prevention should be a critical part of the management of women with PCOS. Successful implementation of CVD prevention will include increasing physician awareness and adherence to available guidelines [37].

Cancer

Overall, cancer diagnoses account for an increasing percentage of morbidity and mortality worldwide. Existing data suggest that a diagnosis of PCOS may confer an enhanced risk for certain gynecological cancers, including endometrial, ovarian, and breast cancer. Understanding the associated risks for cancer in women with PCOS can be helpful in determining the best strategy to evaluate and counsel these women.

Endometrial Cancer

Several comorbidities that are prevalent in women with PCOS, including obesity [31], insulin resistance, type 2 diabetes, and hypertension, are also associated with an increased risk for endometrial cancer [32]. Chronic anovulation, a hallmark of PCOS, predisposes these women to a spectrum of proliferative endometrial pathologies including endometrial polyps, endometrial hyperplasia, and endometrial cancer. A milieu of chronic and unopposed estrogen exposure is one of the likely pathophysiological mechanism underlying the observed relationship and chronic anovulation is recognized as one of the strongest of risk factors for endometrial cancer (RR of 3.1 95 % CI 1.1–7.3) [33]. In addition, it is possible that peripheral aromatization of androgens, as well as the peripheral aromatization of estrogen associated with obesity, confers and additional endometrial cancer risk. Insulin resistance and the associated excess of Insulin-like Growth Factor 1 has also been implicated in cancer cell development and growth [34].

Despite the observed associations, an independent effect of PCOS on the risk of endometrial cancer has been difficult to establish given confounding influences of obesity and hyperinsulinemia that often coexist in this population. A recent meta-analysis on risk of endometrial cancer in PCOS included 4,056 women; 667 cases of endometrial cancer were identified. Overall, an increased likelihood of endometrial cancer was observed in those diagnosed with PCOS compared to the controls, OR 2.7 (95 % CI 1.0–7.29). Although three of the four studies found an increased

risk of endometrial cancer, this risk reached statistical significance in only one study [35]. Another meta-analysis reported a similar association after including one additional study that reported an overall OR of 4.0 (95 % CI 1.7–9.3). After adjusting for BMI, oral contraceptive use, age, parity, and history of diabetes, the strength of association was noted to be attenuated (OR 2.2; 95 % CI 0.9–5.7) [36, 37].

There are limited data on prevention and treatment outcomes of endometrial cancer specifically in women with PCOS. The mainstay of management lies in appreciating the risk of endometrial cancer in women with PCOS, instituting surveillance through judicious endometrial sampling, particularly in those experiencing protracted periods of oligomenorrhea, and offering endometrial protection thorough periodic exposure to progestins [38]. There is some data to suggest that metformin has antitumor properties and its use may confer protection against endometrial cancer [39, 40].

Ovarian Cancer

Overall there is controversial and somewhat contradictory data regarding the association of ovarian cancer with PCOS. Certain entities that are recognized as risk factors for ovarian cancer, such as nulliparity, infertility, and possibly ovulation induction treatment, are also commonly encountered in women with PCOS. Treatment of infertility resulting in live birth as well as oral contraceptive use, however, can be considered as protective [41].

One of the largest studies assessing this association included 476 cases of invasive and borderline ovarian cancer from a cohort of 4,547 women. Seven of the 476 women with ovarian cancer self-reported a diagnosis of PCOS, compared to 24 of 4,081 controls leading the authors to conclude that PCOS was associated with an increased risk of ovarian cancer (OR 2.4 95 % CI 1.0–5.9); analyses adjusted for age, infertility history, parity, and oral contraceptive use. In concordance with the literature supporting a protective effect against ovarian cancer risk with oral contraceptive use, their stratified analysis adjusting for age also found that a subgroup of women with PCOS who never used oral contraceptives had even greater likelihood of being diagnosed with ovarian cancer compared to non-PCOS controls (OR = 10.5 (95 % CI 2.5–44.2). Women with PCOS who used oral contraceptives for more than 3 months were no more likely to be diagnosed with ovarian cancer than their non-PCOS-matched controls [42]. Other studies have calculated the risk of death from ovarian cancer in this population. In particular, one longitudinal study assessed causes of mortality in 786 women with PCOS (defined as "*definite PCOS*" with both histologic evidence from ovarian biopsy and clinical evidence of ovarian dysfunction, and "*possible PCOS*" with histologic evidence with missing clinical information or clinical diagnosis by a gynecologist). The calculated standardized mortality rate for ovarian cancer was 0.39 (95 % CI 0.01–2.17) [43] in both definite and possible PCOS combined compared to national death rates, suggesting that the likelihood of death caused by ovarian cancer in patients with PCOS was lower compared to the national data.

Table 18.2 Risk of PCOS in women with a history of breast cancer

Author, year	*n* (PCOS/androgen-related disorder)	Study population	Outcome measure/results
Gammon (1991) [48]	10 Cases 9 Controls	Age 20–54 years Total population: 4,730 cases 4,688 Controls Primary exposure: history of infertility	OR = 1.13 (95 % CI = 0.46, 2.98) adjusted for age and number of pregnancies
Talamini (1997) [45]	8 Cases 8 Controls (premenopausal) 6 Cases 8 Controls (postmenopausal)	Age 20–74 years Total population: 2,569 cases 2,588 Controls	OR = 1.0 (95 % CI = 0.4, 2.8) (premenopausal) OR = 0.8 (95 % CI = 0.4, 1.7) (postmenopausal)
Baron (2001) [46]	100 Cases 78 Controls (acne) 60 Cases 52 Controls (hirsutism) 22 Cases 14 Controls (polycystic ovaries)	Age 50–75 years Total population: 5,659 cases 5,928 Controls	OR = 1.4 (95 % CI = 1.0, 1.9) (acne) OR = 1.2 (95 % CI = 0.8,1.8) (hirsutism) OR = 1.6 (95 % CI = 0.8, 3.2) (polycystic ovaries)

Breast Cancer

As with the gynecologic cancers, some features of PCOS have been described to be as possible risk factors for breast cancer. There is evidence to suggest that both obesity and diabetes may be associated with increased risk of breast cancer in women older than 35 years, or post-menopause [44, 45]. In contrast, limited data suggest that a diagnosis of diabetes prior to age 35 trends towards a protective effect [46]. In addition, a history of chronic anovulation may contribute to a decreased risk of breast cancer [47].

Three case-control studies have provided data assessing the possible relationship between PCOS and breast cancer. Table 18.2 provides information for each study where identification of breast cancer has been made by histologic confirmation. Gammon et al. used physician records to confirm the diagnosis of PCOS; Baron et al. relied on patient recollection of physician diagnosis of acne, hirsutism, and/or polycystic ovaries and Talmini et al. used patient report of a PCOS diagnosis [48]. None of these studies found a statistically significant risk of breast cancer with PCOS or androgen-related disorders. Overall there does not appear to be a strong association linking PCOS and breast cancer risk.

Depression

Women with PCOS are at an increased risk for mood disorders, anxiety disorders, decreased health-related quality of life, and emotional distress [49, 50]. Mood disorders, including major depressive disorder (MDD), dysthymic disorder,

and depression not otherwise specified [51], are prevalent in the general population, affecting more than 15 million American adults. It has been well documented that women are 70 % more likely than men to experience depression in their lifetime [52], and those diagnosed with major depression are more likely to have an earlier age of onset of this diagnosis [53]. In the USA, it has been reported that the prevalence of MDD in reproductive-age women is approximately 13 % [54].

Several studies have shown an increased prevalence in abnormal depression scores and the presence of depression in patients with PCOS compared to controls [55–59]. We conducted a study with 206 PCOS subjects and controls and screened them for mood and anxiety disorders using the PRIME-MD PHQ [60]. Subjects who had abnormal screens had a clinical interview by a psychiatrist in order to confirm the diagnosis of depression. Our study showed that women with PCOS were four times more likely to meet criteria for depression compared to the controls (OR 4.23, 95 % CI 1.49, 11.98), after adjusting for BMI, family history of depression, and a history of infertility. We rescreened the PCOS subjects after 18–24 months and found a similarly high risk of depression, with an overall prevalence of 40 % a conversion rate of 19 % of the 60 participants [57]. A systematic review and meta-analysis examining this risk included ten studies with a total of 997 subjects and found a fourfold increase in the prevalence of depressive symptoms in women with PCOS compared to age-matched controls (OR 4.03, 95 % CI 2.96–5.5) [50]. Notably, the individual studies included in the review had implemented one of five different validated depressive symptom-screening tools; the consistency in observed relationship highlights the strength of association between PCOS and depression.

Mechanisms that may explain the higher than expected prevalence of mood disturbances in women with PCOS are not entirely clear. There are conflicting data with regard to serum androgen levels and their correlation with mood disorders. Some smaller studies have reported an increase in serum androgen levels in premenopausal patients with depression compared to controls [61, 62]. However, in the PCOS population, there does not appear to be significant difference in serum androgen levels between women with and without depression [52, 57]. These findings are however limited by the variability in the testosterone assays and the questionable reproducibility of testosterone assays at the lower range of detection. Clinical hyperandrogenism, including hirsutism and acne, has been shown to be associated with an overall decrease in health-related quality of life measures, including a decrease in overall sexual satisfaction and increased emotional distress [63]. Interestingly, a small study reported a decrease in depression scores following treatment of facial hirsutism [64]. Overall, however, there does not appear to be a significant independent association between stigmata of hyperandrogenism and the presence of depression [58, 65].

Relationship between obesity and a diagnosis of mood disorders is recognized [66]. There is wide ethnic variation in prevalence of obesity in the PCOS population, with 25 and 80 % of women with PCOS reported to be overweight or obese. Obesity could also confer an added risk of depression within the PCOS population, with obese PCOS patients scoring higher on depression scales compared to their non-obese counterparts [67]. However, in our meta-analysis, a sub-analysis of BMI matched populations showed an increased risk of abnormal depression scores

among women with PCOS (OR 4.09, 95 % CI 2.62–6.41) suggesting that the relationship between depressed mood and PCOS is independent of body mass excess [50]. There are limited data on the effects of weight loss and depression scores in women with PCOS. One small study examined the effects of diet alone versus diet and either aerobic or aerobic-resistance exercise, and found that all three interventions resulted in similar degrees of weight loss and an improvement in depression and PCOS-health-related quality of life scores [69]. While future studies are needed to better evaluate the effects of weight-loss strategies, hormonal therapies to decrease androgens, anti-androgenic and antidepressant medications on mood and quality of life parameters in women with PCOS, in the meantime, PCOS must be appreciated as a potential state of psychological distress; screening for depressive symptoms and anxiety should be incorporated as a part of management paradigm for women with PCOS and available resources for support identified for those screening positive.

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Chapter 19
Emerging Concepts: Role of Vitamin D Deficiency in the Pathogenesis of PCOS

Sanam Lathief and Lubna Pal

Key Points

- Low vitamin D levels have been described in women with PCOS.
- Vitamin D deficiency is associated with obesity, insulin resistance, hyperandrogenemia, dyslipidemia, inflammation, and enhanced risks for diabetes and CVD.
- In women with PCOS, vitamin D deficiency has been related to menstrual irregularity, insulin resistance, and hyperandrogenemia.
- Small sample clinical trials have demonstrated improvement in insulin sensitivity and menstrual dysfunction, as well as reduction in hyperandrogenemia and CVD risks in women with PCOS.
- Limited observational data suggest compromised fertility treatment prognosis in women with evidence of vitamin D deficiency.
- Given the plausibility of a relevance of vitamin D signaling for processes underlying PCOS, there is a need for large-scale randomized controlled trials to further assess the relevance of vitamin D in PCOS.

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Introduction

Polycystic ovary syndrome (PCOS), characterized by clinical and biochemical evidence of hyperandrogenism, menstrual irregularities, ovulatory dysfunction, and polycystic appearance of the ovaries, is the most common endocrinopathy of reproductive-age women [1–4]. Insulin resistance (IR) has emerged as being central to the pathophysiology of PCOS [5, 6], with resulting hyperinsulinemia as a mechanism contributory to both the ovulatory dysfunction and hyperandrogenism that characterize this disorder [6]. The endocrine and metabolic milieu of PCOS places this population at enhanced lifetime risk for a spectrum of morbidities including poor reproductive outcomes, type 2 diabetes mellitus (DM), cardiovascular disease (CVD), mood disorders including depression and anxiety, as well as endometrial cancer [1, 2]. Current therapeutic paradigms address the primary clinical concern(s) (e.g., ovulation induction by pharmacotherapy or surgery and assisted reproduction technologies target PCOS-related infertility, whereas hormonal interventions, such combined hormonal contraceptives and antiandrogens, are preferentially utilized to target symptoms of hyperandrogenism), although attention to the underlying IR through use of insulin-sensitizing agents is a common adjunctive strategy in PCOS management [7].

The role of vitamin D in calcium homeostasis and in the maintenance of skeletal health is well recognized [8]. In addition, a growing body of evidence relates deficiency of vitamin D to a number of nonskeletal sequelae, including obesity, DM, dyslipidemia, hypertension, inflammation, CVD, autoimmune disease, and cancers [8–13]. Genes that are critical for glucose and lipid metabolism are recognized to be downstream targets of vitamin D signaling [8–10]. A relationship between vitamin D deficiency and IR has additionally become apparent in recent years. Given that a role of calcium in oocyte activation and maturation is well understood [14–16] and since PCOS is a state of follicular developmental arrest, abnormalities in vitamin D metabolism and action could be theorized to be linked to the pathogenesis of PCOS. This latter argument is further strengthened by our current understanding of a pathophysiologic role for IR in PCOS, yet another aberration that has been linked to vitamin D deficiency. Thus, in PCOS, vitamin D deficiency has emerged as a plausible mechanism to explain some of the metabolic and endocrine features of PCOS. Indeed, multiple studies, observational as well as randomized controlled trials, have explored the relevance of vitamin D in PCOS [17–29]. Accruing data suggest low levels of vitamin D in PCOS [18, 20, 22, 29], and vitamin D deficiency is shown to be linked to PCOS pathophysiology through its associations with obesity, insulin resistance, hyperandrogenism, dyslipidemia, inflammation, as well as features of depression and risk for DM and CVD. While impaired folliculogenesis, steroidogenesis, and reproductive compromise are well described in the animal models of vitamin D deficiency, a relevance of vitamin D for human reproductive biology, however, is less well understood. Herein we attempt to provide an overview of our current understanding on the plausible relevance of vitamin D in the pathophysiology of PCOS.

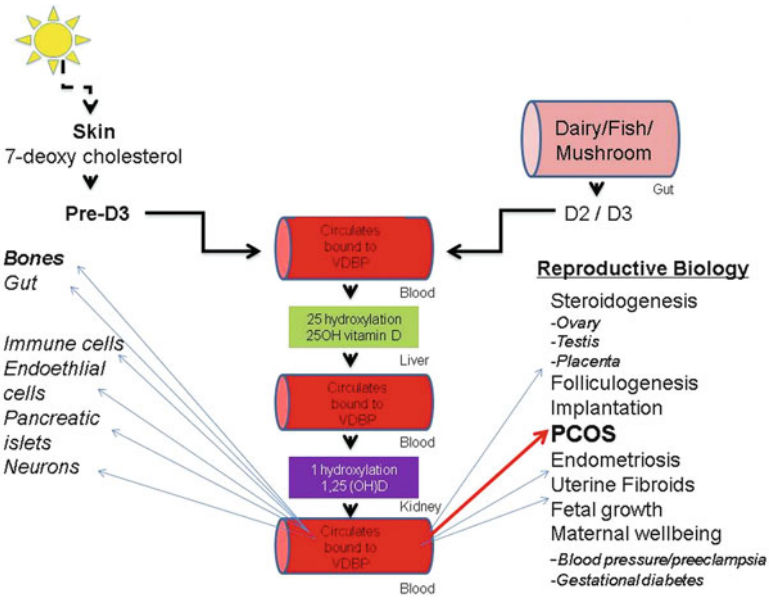

Fig. 19.1 An overview of vitamin D metabolism and salient actions. *VDBP* vitamin D-binding protein, *D2* ergocalciferol, *D3* cholecalciferol. Modified from [73]

Vitamin D: Mechanism of Action (Fig. 19.1)

Humans are primarily dependant on endogenous cutaneous synthesis of vitamin D through exposure to solar ultraviolet B (UVB); dietary sources account for less than 20 % of daily requirements of the vitamin. Exposure to solar UVB converts dehydrocholesterol in the skin to previtamin D_3 which is rapidly converted to vitamin D_3 (cholecalciferol). Dietary vitamin D gets incorporated in the chylomicrons and gets transported via the lymphatics to the circulation. Circulating vitamin D is transported to the liver where it undergoes the first step of activation wherein vitamin D-25-hydroxylase catalyzes the conversion to 25-hydroxy vitamin D (25(OH)D), the major circulating form that reflects the overall vitamin D status. Final activation of 25(OH)D occurs in the kidney as well as at target cell level, via 1-alpha (α-hydroxylase to 1,25-dihydroxyvitamin D (1,25(OH)2D), the active vitamin D metabolite that promotes intestinal calcium absorption through its interaction with the cognate vitamin D receptor (VDR)). Vitamin D acts as a transcription factor via signaling through the nuclear VDR–retinoic acid x-receptor (VDR-RXR) complex [8] and exerts actions across a host of tissues, including the skeleton, pancreas, parathyroid glands, and even ovary [30, 31]. Serum levels of calcium, phosphorus, parathyroid hormone (PTH), as well as fibroblast-derived growth factor-23 (FGF-23) are recognized modulators of 1α-hydroxylation of 25(OH)D [8].

Obesity, PCOS, and Vitamin D

There is increased prevalence of body mass excess in women with PCOS as compared to age-matched controls [1, 6, 32, 33]. A recent meta-analysis identified women with PCOS as having an increased prevalence of being overweight, obese, and centrally obese compared to non-PCO controls [33]. An inverse relationship between circulating 25(OH)D levels and parameters of body mass excess such as body mass index (BMI) and waist circumference (WC) is well described across populations including women with PCOS [18, 20–22, 24, 26, 34, 35]. In an observational study of 25 women with PCOS and 27 controls, Li et al. reported an inverse relationship between 25(OH)D levels and BMI, with as many as 72 % of the PCO group being vitamin D deficient, of which 44 % were severely deficient (defined as <25 ng/mL) [18]. A similar association was observed by Wehr et al. in an observational study involving 206 PCOS women wherein 72 % of PCOS population demonstrated evidence of vitamin D deficiency (serum 25(OH)D <30 ng/mL) and significant inverse correlation between 25(OH)D level with BMI was noted [20]. Of note, those meeting NCEP III criteria for metabolic syndrome [79] had significantly lower 25(OH)D levels compared to PCOS women without MS. Possible rationale for the lower circulating 25(OH)D levels in obesity may include increased sequestration of this fat-soluble vitamin in the adipose tissue [36], as well as decreased tendency of obese individuals to seek sunlight [37].

Vitamin D: A Modulator of Insulin Resistance in PCOS

Insulin secretion is a calcium-dependent process [38, 39] and IR and compensatory hyperinsulinemia are well described in the setting of PCOS [5, 6]. The precise mechanism of action whereby vitamin D influences insulin signaling appears to involve genomic stimulation of the insulin receptor mRNA via VDR signaling [40, 41]. Vitamin D signaling appears to promote insulin synthesis and release, enhance insulin receptor expression, and also inhibits pro-inflammatory cytokines that are recognized to play a role in the pathogenesis of IR [42]. The latter mechanisms may explain the observed associations between vitamin D deficiency with impaired glucose and insulin metabolism [43–46] and the recognized high prevalence of hypovitaminosis D in populations with type 2 DM [47–49]. Similar trends and associations have been described in women with PCOS in whom limited data suggest similar relationships between IR and vitamin D deficiency in women with PCOS [21, 22, 25, 26]. In an observational study of 120 women with PCOS, Hahn et al. observed inverse correlation between serum levels of 25(OH)D and indices of IR such as HOMA-IR [22]. On subgroup analysis stratified on BMI, significantly lower 25(OH)D levels were observed in the obese and the overweight phenotypes compared to the lean PCOS, suggesting a role for obesity in the observed relationship between vitamin D status and IR [22]. Interestingly, all three BMI-based subgroups

of women with PCOS demonstrated evidence of vitamin D insufficiency, adding further evidence that PCOS is a state associated with hypovitaminosis D [22]. In another observational study, Kotsa et al. reported significant increase, compared to baseline, in the first phase of insulin secretion during an oral glucose tolerance test (OGTT) following 1 μg/day treatment with alpha-calcidiol over 3 months in 15 obese women with PCOS [25]. In a case–control study of 85 women with PCOS and 115 controls, Mahmoudi et al. reported significant positive association of the PCOS state with indices of IR such as HOMA-IR and insulin levels that persisted after adjustment for age and BMI [26]. The controls did not demonstrate this association, but there were positive correlations between parameters of IR and serum levels of PTH [26]. In yet another case–control study involving 301 women with PCOS and 113 controls, Panidis et al. also reported inverse correlations between HOMA-IR and 25(OH)D levels [21]. In the latter study, the associations were BMI dependent as the obese patients had significantly lower 25(OH)D levels across both populations [21]. In conclusion, a growing body of data accrued in women with PCOS suggests a relationship between IR and vitamin D deficiency [22, 25]; as is evident, however, the existing literature is limited by predominantly observational designs and small study samples. Despite the tantalizing associations, a valid interpretation of "cause-and-effect" nature to the observed relationship between vitamin D deficiency and IR cannot be construed, thus highlighting a need for appropriately powered randomized controlled trials to substantiate if amelioration of vitamin D deficiency through supplementation can mitigate the dysmetabolic milieu of PCOS.

Vitamin D: Relationship with Hyperandrogenemia of PCOS

Vitamin D status can be hypothesized to modulate circulating androgen levels through interactions with sex hormone-binding globulin (SHBG), and PTH. Recognized as a modulator of circulating levels of free androgens, the hepatic SHBG is the dominant carrier protein that binds circulating androgens including testosterone and androstenedione and, hence, minimizes percentage of free androgen available to act at the target tissue; a decline in SHBG levels, as seen in states of IR, is associated with increase in circulating free androgen levels and, hence, worsening features of hyperandrogenism.

Serum levels of 25(OH)D are shown to correlate positively with SHBG levels [50]. Conversely, hyperinsulinemia, as in PCOS, promotes hyperandrogenism through inhibition of hepatic synthesis of SHBG [51]. In an observational study on 206 women with PCOS, Wehr et al. reported positive correlation of 25(OH)D levels with SHBG; the association, however, was abolished on adjustment for BMI [20]. These authors additionally observed a negative correlation between serum 25(OH)D levels with hirsutism scores; hirsute PCOS women were reported to have significantly lower 25(OH)D levels compared to the non-hirsute, and the association between 25(OH)D and hirsutism score remained statistically significant after adjusting for BMI [20]. Hahn et al. reported a positive correlation between 25(OH)D and SHBG levels, and

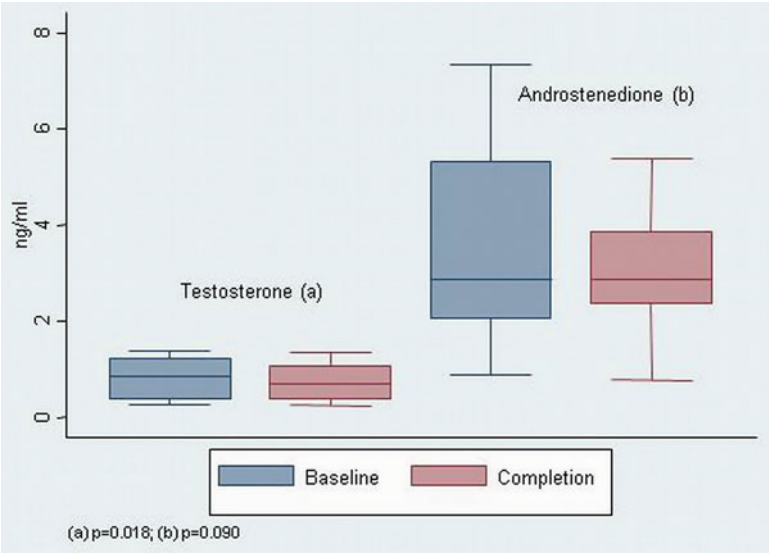

Fig. 19.2 Effects of vitamin D and calcium supplementation on total testosterone (**a**) and androstenedione (**b**) levels in overweight women with PCOS ($n = 12$). Baseline values are compared to those achieved following a 3-month supplementation. Based on data presented in ref. [23]

statistically nonsignificant inverse correlations between 25(OH)D with total testosterone, androstenedione, and DHEAS levels, and with the free androgen index (FAI) in 120 women with PCOS [22]. Li et al. described positive correlations between 25(OH)D and SHBG, and inverse correlation between 25(OH)D and FAI [18]. The SHBG levels were lower in the severely vitamin D-deficient women with PCOS, but the above relationship was rendered insignificant on BMI adjustment [18].

Serum PTH levels are intimately related to vitamin D status, and are known to be higher in the obese individuals, secondary to vitamin D deficiency [52–56]. Panidis et al. reported significantly higher PTH levels and lower 25(OH)D levels in the obese compared to normal-weight women with and without PCOS as well as significant positive correlation between PTH and total testosterone levels independent of both age and BMI; these observations are suggestive of mechanistic implications of vitamin D deficiency-related secondary hyperparathyroidism for the hyperandrogenemia of PCOS [21].

Consistent with the observational data discussed earlier, in a single-arm intervention trial of vitamin D and calcium supplementation in 13 hyperandrogenic women with PCOS, Thys-Jacobs et al. described clinical improvement in acne following a 6-month intervention (50,000 IU vitamin D weekly and 1,500 mg of elemental calcium daily), suggesting therapeutic potential of vitamin D supplementation against symptoms of hyperandrogenism [17]. This latter impression was corroborated in pilot study undertaken by our group wherein over a 3-month period of

vitamin D and calcium supplementation undertaken in 12 overweight women with PCOS, a significant reduction in serum levels of total testosterone and androstenedione levels was observed (Fig. 19.2) [23].

To summarize, existing data, while sparse and based on small study samples [23] as well as predominantly comprising observational studies [20, 22, 23], are consistent in the directionality of observations that suggest vitamin D insufficiency to relate to hyperandrogenemia of PCOS. Yet again, as discussed under section on IR, any meaningful interpretation of a "cause-and-effect" nature to the observed associations between vitamin D deficiency and hyperandrogenemia may not be arrived at, yet again highlighting a need for appropriately powered randomized controlled trials to establish if correction of vitamin D deficiency through supplementation can improve the androgenic milieu of PCOS.

Vitamin D: Implications for Ovarian Physiology and Relevance for PCOS

Animal studies have established the role of calcium in oocyte activation and maturation [14–16]. An increase in intracellular free calcium is responsible for progression of oocyte meiosis [14–16]. Given the known association of PCOS with ovulatory dysfunction [57], studies were conducted to investigate the contribution of altered calcium homeostasis in PCOS pathophysiology [17, 20, 27]. In a single-arm intervention trial undertaken in 13 anovulatory, oligo/amenorrheic women with PCOS, Thys-Jacobs et al. reported improvement in menstrual disturbances following vitamin D and calcium supplementation over a 6-month period [17]. All participants were normocalcemic, whereas 5 of the 13 exhibited elevated PTH levels suggestive of secondary hyperparathyroidism. Within 2 months of supplementation with 1,500 mg/day of elemental Ca and ergocalciferol (D2) 50,000 IU weekly or biweekly, resumption of normal menstrual cyclicity is described in 7/9 with oligomenorrhea (78 %) and improvement in dysfunctional bleeding patterns described in an additional two participants; the authors surmise these phenomenon as being secondary to facilitatory influences of vitamin D and calcium on ovarian physiology [17]. Wehr et al., in an interventional trial, supplemented 46 PCOS women with weekly 20,000 IU cholecalciferol for 24 weeks and reported resolution of menstrual disturbances in 50 % of the cohort by week 24 [58]. In the only randomized controlled trial that compared spontaneous ovarian follicular development in women with PCOS-related infertility (mean age 26.16 ± 3.95), Rashidi et al. randomized participants to calcium 1,000 mg plus vitamin D 400 IU alone (group 1, referent population), calcium 1,000 mg plus vitamin D 400 IU plus metformin 1,500 mg/day (group 2), and metformin 1,500 mg/day alone (group 3) [27]. Subjects were treated with respective agents for 3 months and underwent additional observation for another 3 months (total time under observation was 6 months). After the third month of intervention, more women in the combined group (group 2) were likely to acknowledge menstrual regularity (50 % in group 2 compared to 30 and 35 % in

groups 1 and 3, respectively), although this difference was not of statistical significance ($p=0.400$). On multivariate analyses, follicular growth parameters were significantly better ($p=0.037$) in group 2 (metformin plus calcium and vitamin D) compared to the reference group 1 (calcium and vitamin D alone); no significant impact of metformin alone (group 3) was observed on the follicular response ($p=0.290$). The authors conclude that addition of vitamin D and calcium to metformin regimen is more effective in correcting menstrual disorders and follicular growth than either metformin or calcium and vitamin D alone. The randomized controlled trial design is a laudable strength of this pilot study.

Vitamin D: Potential Implications for Cardiovascular Health in PCOS

Epidemiological data identify vitamin D deficiency as a risk factor for enhanced cardiovascular morbidity and mortality [59–61]. Vitamin D receptors are located in the vascular smooth muscle [62] and endothelium [63], and inflammation, dyslipidemia, hypertension, coronary artery disease, cardiac failure, and accelerated carotid atherosclerosis have been described in association with vitamin D insufficiency across populations [59–61]. Limited data are additionally available on the relationship between vitamin D status and CVD risk in the PCOS population [18, 20, 22]. Li et al. identified 25(OH)D levels to relate inversely with C-reactive protein (CRP), a known risk factor for CVD [18], whereas both Li et al. and Hahn et al. identified positive correlations between 25(OH)D and HDL levels [18, 22]. Wehr et al. demonstrated inverse correlations between vitamin D status and serum levels of triglycerides, total cholesterol, and systolic and diastolic blood pressure [20]. In the previously mentioned pilot study undertaken by our group, improvements in blood pressure (BP) parameters (systolic blood pressure [SBP], diastolic blood pressure [DBP], and mean arterial blood pressure [MAP]) followed a 3-month period of vitamin D and calcium supplementation undertaken in 12 overweight and vitamin D-defiant women with PCOS [23]. Significant lowering in BP was observed in those with baseline SBP $\geq$120 mmHg and/or baseline DBP $\geq$80 mmHg ($n=8$, Fig. 19.3); significant lowering in SBP was seen in those with baseline serum 25(OH)D $\leq$20 ng/mL ($n=9$, SBP 122.22 ± 11.19 at baseline versus 112.56 ± 10.42 mmHg at completion, $p=0.033$). Notably, at baseline, 17 % (2/12) met the criteria for hypertension (SBP>140/DBP>80 mmHG) compared to 0 % at the completion of the clinical trial ($p<0.001$) [23]. Reduction in SBP by as little as 2 mm has been shown to reduce CVD-related mortality by 6 % [64]. The magnitude of reduction in SBP achieved through vitamin D and calcium supplementation (i.e., by an average of 7 mmHg) thus is clinically meaningful, although given that the study design lacked a control arm; this observation merits substantiation in a future randomized controlled trial.

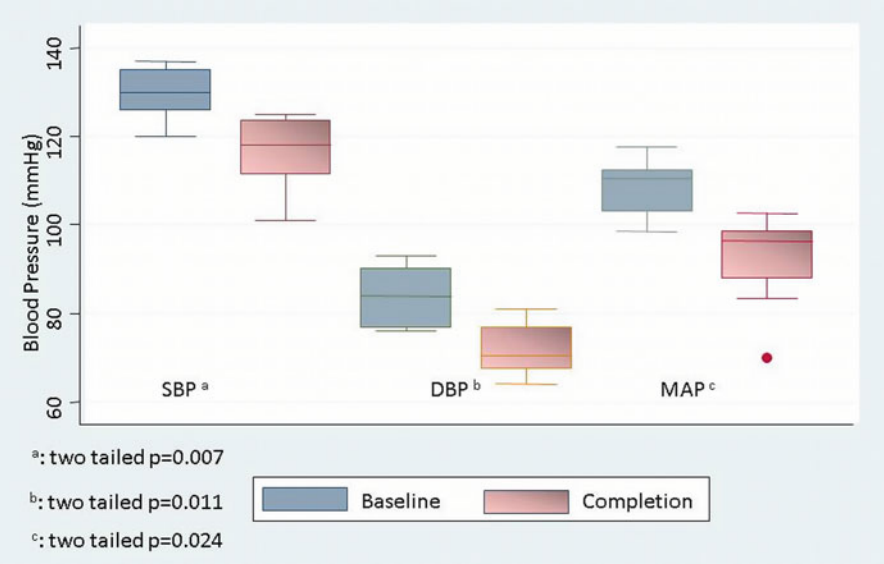

Fig. 19.3 Effects of vitamin D and calcium supplementation on blood pressure (BP) parameters in overweight and vitamin D-deficient women with PCOS whose baseline BP was greater than 120/80 mmHg ($n=8$). Baseline values are compared to those achieved following a 3-month supplementation. Based on data presented in [23]

Vitamin D: Potential Implications for Psychological Well-Being in Women with PCOS

Vitamin D deficiency has been linked to depression in the non-PCOS populations [65, 66]. Women with PCOS carry a substantial psychological burden; although the exact underpinnings remain unclear, altered physical appearance (obesity, acne, alopecia, hirsutism), menstrual irregularity, and difficulties in conceiving are recognized as potential contributors to the prevalent issues of depression, anxiety disorders, body image dissatisfaction, and sexual dysfunction encountered in women with PCOS [67–70]. In a prospective observational study, our group had identified a negative correlation between serum levels of 25(OH)D with depressive symptoms manifest in women with PCOS [71]. Patients with PCOS seeking clinical care ($n=103$) were administered a validated screening tool (personal health questionnaire [PHQ]) [72]; these data were presented at the annual meeting of the American Society for Reproductive Medicine, 2011 [71], and a manuscript is under peer review. The observational study design and lacking controls do not allow determination of a cause–effect relationship to the observed association between vitamin D deficiency and depressive symptoms in the studied population of women with PCOS.

Vitamin D: Potential Implications for Reproductive Success in Women with PCOS

Animal experimental models have identified vitamin D as essential for procreative success, with recognized effects on folliculogenesis, spermatogenesis, steroidogenesis, and implantation [73–75]. Although VDR has been identified in the human endometrium, myometrium, ovarian, cervical, and breast tissues [76], data supportive of relevance of vitamin D signaling for reproductive biology in humans are sparse. Vitamin D is recognized to enhance action of the enzyme aromatase, which is responsible for conversion of androgens to estrogens in the ovarian granulosa cells [77], and interruption of estrogen signaling is suggested as a mechanism for reproductive disturbances in the setting of vitamin D deficiency [77, 78]. In a study of infertile women undergoing in vitro fertilization, our group had observed significantly higher 25(OH)D levels in the ovarian follicular fluid of women who achieved clinical pregnancy following fresh embryo transfer [79], and this finding was subsequently corroborated by others [80–82].

In the context of PCOS-related infertility, spontaneous conception was achieved by 2/13 women completing the 6-month clinical trial of vitamin D and calcium supplementation undertaken by Thys Jacobs et al. Given an absence of details regarding the proportion of participants who were attempting to conceive in this latter study, it is, however, difficult to interpret the two spontaneous conceptions as evidence of therapeutic success of vitamin D and calcium in PCOS-related subfertility. More recently, Ott et al. [81] reported in a prospective cohort study wherein serum 25(OH)D levels were assessed in 91 anovulatory women with PCOS undergoing ovulation induction treatment with clomiphene citrate (CC). Serum 25(OH)D levels were positively predictive of likelihood for achieving a dominant follicle in response to CC treatment ($p=0.014$) and of successful pregnancy ($p<0.001$). Conversely, the likelihood for ovarian responsiveness to CC was reduced by 77 % (OR 0.33, 95 % CI 0.13–0.85) and for CC treatment-related pregnancy was reduced by 76 % (OR 0.24, 95 % CI 0.07–0.84) in women with evidence of severe vitamin D deficiency (serum 25(OH)D level <25 nmol/L or <10 ng/mL) [81].

More recently, in a retrospective cohort approach, our group reported on vitamin D status of women with PCOS who had participated in the PPCOS1 (Pregnancy in PCOS 1) randomized controlled trial that compared the efficacy of CC versus metformin versus a combination of CC plus metformin for managing PCOS-related ovulatory infertility [82]; serum 25(OH)D levels were assayed in stored serum samples in 540 of the original 620 participants who had completed the PPCOS1 clinical trial and implications of vitamin D status for cycle outcome were analyzed. These data were presented at the annual meeting of the American Society for Reproductive Medicine, and our findings were supportive of facilitatory effects of higher 25(OH)D levels for treatment success [83]; a manuscript is under preparation. Yet again, a need for appropriately designed and adequately powered prospective clinical trials is appreciated to establish if infertility treatment success can be improved through optimization of vitamin D status.

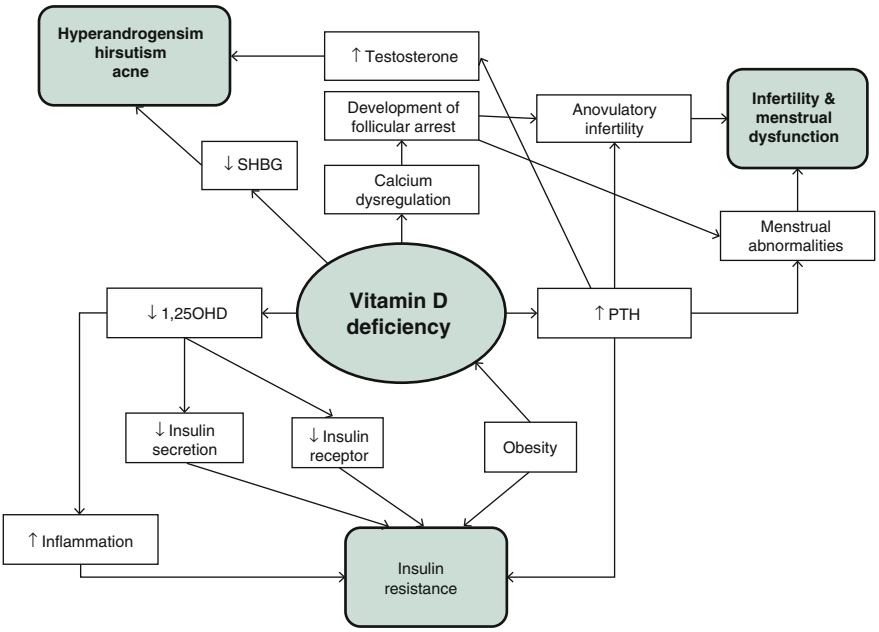

Fig. 19.4 The role of vitamin D deficiency in the pathology of PCOS. Adapted from Thomson RL, Spedding S, Buckley JD. Vitamin D in the aetiology and management of polycystic ovary syndrome. Clin Endocrinol 2012;77:343–350, with permission from John Wiley and Sons. © 2012 Blackwell Publishing Ltd

Summary

A vast body of literature links low vitamin D status to obesity, insulin resistance, menstrual irregularity, depression, and increased CVD risk (Fig. 19.4), and a growing body of literature suggests a relevance of vitamin D insufficiency for the pathophysiology of PCOS. The overwhelming majority of studies linking vitamin D insufficiency with PCOS are, however, underpowered and observational, and hence do not allow interpretation of a cause-and-effect nature to the observed associations. Although a limited number of interventional trials have demonstrated improvement of insulin sensitivity, menstrual dysfunction, a reduction in circulating androgens, and improvement in CVD risk parameters such as blood pressure in women with PCOS, the small sample sizes and lacking controls limit any conclusive interpretation. A need for appropriately powered double-blind randomized controlled trials is underscored by the currently existing data so as to definitively address if vitamin D insufficiency may be a modifiable mechanism in the pathophysiology of PCOS, and if normalization of vitamin D status could mitigate the endocrine, metabolic, and clinical stigmata of PCOS.

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