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An International Consortium Update: Pathophysiology, Diagnosis, and Treatment of Polycystic Ovarian Syndrome in Adolescence

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Abstract

This paper represents an international collaboration of paediatric endocrine and other societies (listed in the Appendix) under the International Consortium of Paediatric Endocrinology (ICPE) aiming to improve worldwide care of adolescent girls with polycystic ovary syndrome (PCOS)[1]. The manuscript examines pathophysiology and guidelines for the diagnosis and management of PCOS during adolescence. The

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complex pathophysiology of PCOS involves the interaction of genetic and epigenetic changes, primary ovarian abnormalities, neuroendocrine alterations, and endocrine and metabolic modifiers such as anti-Müllerian hormone, hyperinsulinemia, insulin resistance, adiposity, and adiponectin levels. Appropriate diagnosis of adolescent PCOS should include adequate and careful evaluation of symptoms, such as hirsutism, severe acne, and menstrual irregularities 2 years beyond menarche, and elevated androgen levels. Polycystic ovarian morphology on ultrasound without hyperandrogenism or menstrual irregularities should not be used to diagnose adolescent PCOS. Hyperinsulinemia, insulin resistance, and obesity may be present in adolescents with PCOS, but are not considered to be diagnostic criteria. Treatment of adolescent PCOS should include lifestyle intervention, local therapies, and medications. Insulin sensitizers like metformin and oral contraceptive pills provide short-term benefits on PCOS symptoms. There are limited data on anti-androgens and combined therapies showing additive/synergistic actions for adolescents. Reproductive aspects and transition should be taken into account when managing adolescents.

Introduction

Polycystic ovary syndrome (PCOS) is a long-term recognized, complex heterogeneous familial disorder [1, 2]. Yet, despite decades of research, the etiology of PCOS remains elusive [3]. This collaborative effort, initiated by Pediatric Endocrine Societies, was undertaken because of persistent questions in three areas: pathophysiology, diagnosis, and treatment[1]. This is attested to increased focus and number of publications related to PCOS, both in general and in the adolescent female (Fig. 1a, b).

The clinical symptoms, including hyperandrogenism and chronic anovulation, typically develop during adolescence. Further, the early onset of adrenarche may represent the initial clinical feature of PCOS for some girls [4]. By the time patients present for medical attention, this multisystem disorder often has become a self-perpetuating derangement in which identification of initiating factors are difficult. Recent insights from genetic epidemiology support long-standing clinical investigations indicating a broad etiopathology of PCOS.

[1] Note that each of the societies designated one or more experts regarding aspects of PCOS to participate in this endeavor. This is intended to be an update of the current status of knowledge for the perspective that etiologic factors, diagnostic criteria and treatment guidelines continue to be elucidated.

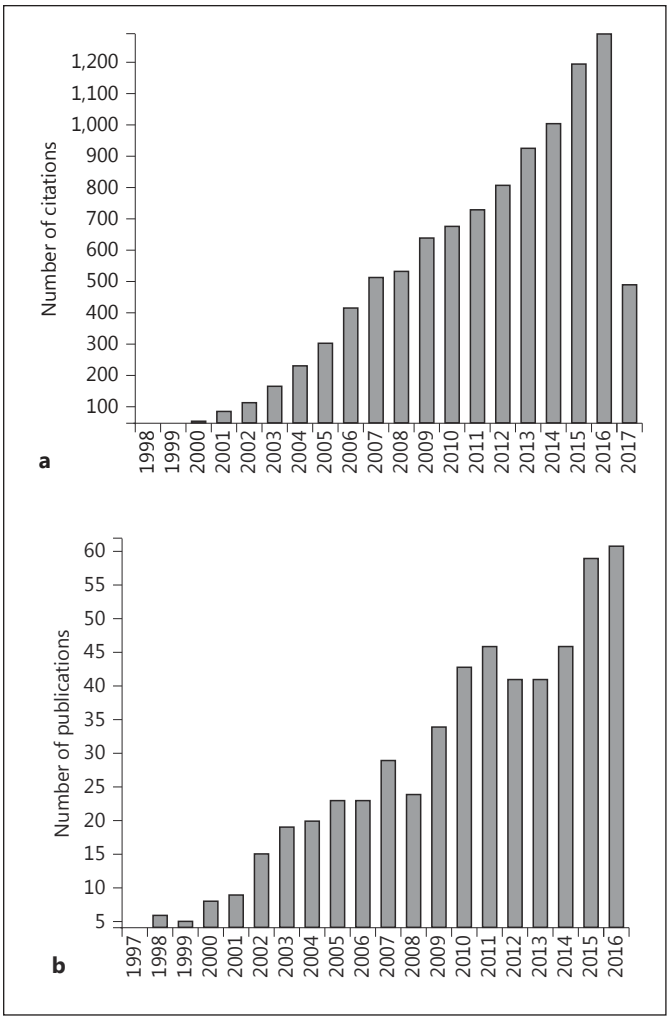

Fig. 1. a Annual number of citations for "adolescent PCOS" over the past 2 decades. **b** Annual number of publications for "adolescent PCOS" over the past 2 decades. Web of Science, Thomson Reuters, 2017.

Since this is a review of published manuscripts and existing diagnostic and clinical practices and meets ethical guidelines, it is exempt from Human Rights Review Committees and none have indicated any conflict of interest.

A. Pathophysiology

Androgen excess, observed in approximately 60–80% of patients with PCOS, is a key feature of the disorder. Hirsutism and hyperandrogenism are manifestations of the excessive androgen production. Indeed, hyperandrogenism, commonly demonstrated by elevated free

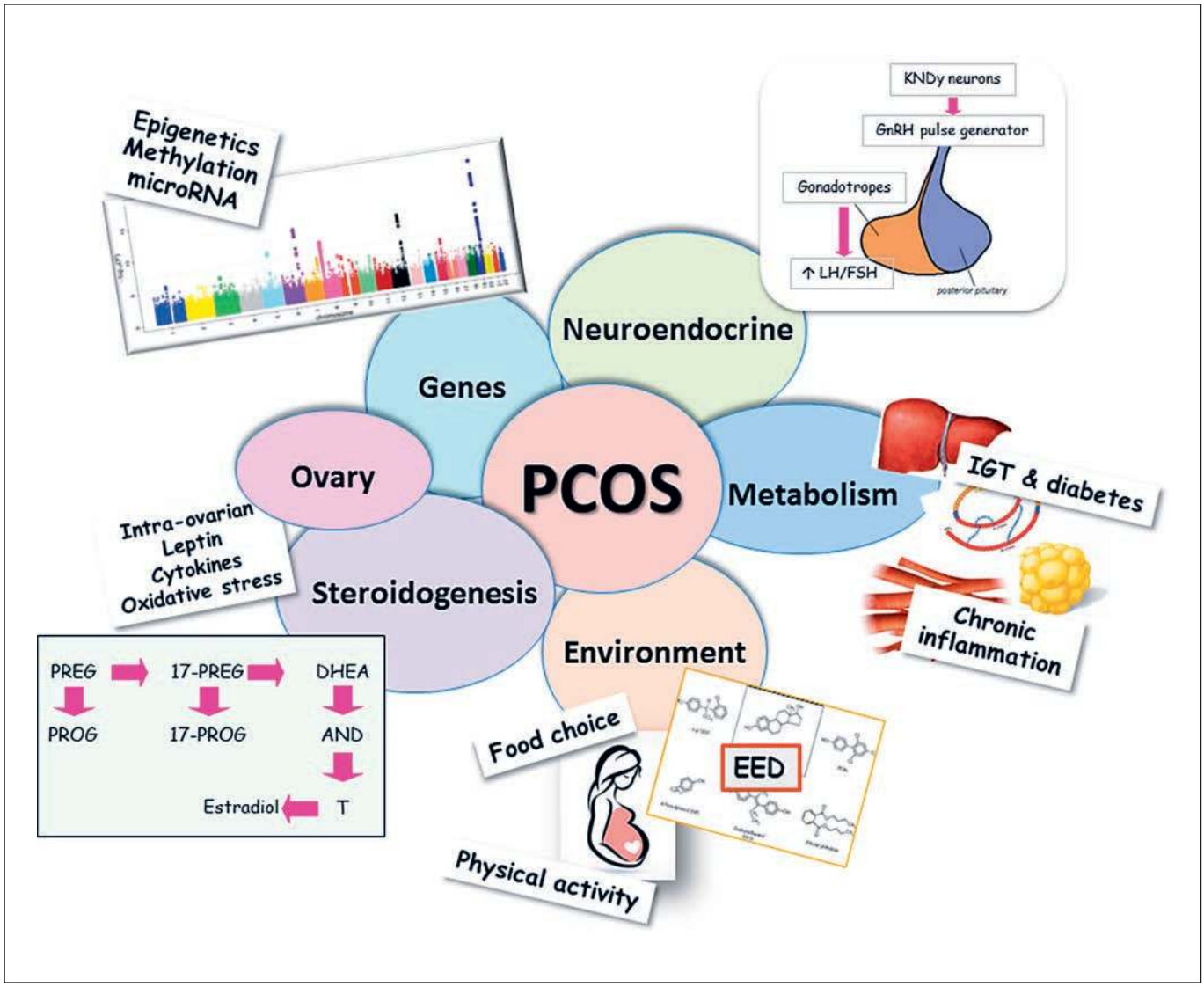

Fig. 2. Potential factors involved in pathophysiology of PCOS. Alterations in steroidogenesis, ovarian folliculogenesis, neuroendocrine function, metabolism, insulin secretion, insulin sensitivity, adipose cell function, inflammatory factors, and sympathetic nerve function contribute to the pathogenesis of this disorder. Not all factors play roles in individual patients. Environment factors such as food choice, exercise, and endocrine disruptors influence the development of clinical features. Genome-wide association studies have identified loci of interest in close proximity to genes involved in gonadotropin secretion, gonadotropin action, ovarian follicular development, and insulin sensitivity.

(unbound) testosterone in circulation, is the most common abnormality observed in the syndrome and plays a major role in perpetuating the aberrant hormone contributors to the pathophysiology of PCOS. Excessive ovarian androgen production is present in the majority of cases, but excessive adrenal androgen production can occur among some. The elevated androgen concentrations suppress sex hormone-binding globulin (SHBG) concentrations contributing to higher free testosterone concentrations [5]. Herein, we deconstruct this complex disorder into its major pathophysiologic components. Although we discuss specific elements, PCOS represents an example of systems biology with multiple interconnected signaling networks, which in individual instances may not involve all networks (Fig. 2).

1.1 Primary Ovarian Pathophysiology

In humans, the factors influencing follicular growth are coordinated such that typically there is only a single follicle selected for terminal maturation and ovulation in a sequential fashion. The maximum number of ovarian follicles, approximately 6–7 million, exist during mid-gestation and decrease to roughly 2–3 million primordial follicles at birth. Subsequently, primordial follicles are continuously recruited from this pool, with mechanisms to control the rate of entry of primordial follicles into the growing pool being essential to maintain the ovarian reserve to preserve fertility [6]. These poorly understood initial phases of follicular growth are gonadotropin-independent and influenced by autocrine, paracrine, and local endocrine factors.

There is a dynamic balance between growing and dormant follicles. In PCOS, the balance between androgens, anti-Müllerian hormone (AMH), and FSH is disrupted leading to follicular arrest [7]. Abundant LH drives the theca cells to produce androgens, but FSH concentrations and conversion of androgens to estradiol are insufficient, resulting in failure to select a dominant follicle, thus chronic anovulation [8]. AMH, secreted by granulosa cells, plays a major role in governing this balance because it inhibits transition from primordial to primary follicles. Hence, PCOS is characterized by increased growth of small follicles but subsequent growth arrest leading to the typical polycystic morphology. It has been suggested that the follicles in a PCOS ovary inherently differ from follicles in a normal ovary [9].

Theca cells obtained from women with PCOS retain their phenotype with increased androgen secretion from increased *CYP17A1* expression or P450c17 activity [10]. Immunohistochemical studies have indicated that proteins involved in the alternate "backdoor pathway" of steroidogenesis are more highly expressed in PCOS theca cells [11]. Genome-wide association studies (GWAS) directed investigation to a specific locus, *DENND1A*, alternative splicing of the *DENND1A* transcript generates several variants. Expression of one variant, *DENND1A.V2*, is greater in PCOS theca cells. Curiously, knockdown of this variant recapitulates a normal theca cell phenotype in PCOS ovaries, whereas overexpression in theca cells from normal women recapitulates PCOS phenotype [12]. The mechanism governing the regulation of the alternative splicing appears to reside outside of the *DENND1A* gene [13].

Many steroidogenic enzymes are expressed in both the adrenal cortex, especially the zona reticularis, and the theca cell. Hormones secreted by the zona reticularis include dehydroepiandrosterone (DHEA), DHEA sulfate, and androstenedione. It is becoming apparent that the steroidogenic repertoire of the adrenal and, perhaps, the theca cell include 11-hydroxyandrostenedione, which is ultimately converted to the potent androgen 11-ketotestosterone [14]. Women with PCOS showed higher serum concentrations of the 11-oxygenated androgens 11β-hydroxyandrostenedione, 11-ketoandrostenedione, 11β-hydroxytestosterone, and 11-ketotestosterone concentrations than control women [15].

2. Insulin Resistance/Hyperinsulinemia

Insulin resistance (IR) and hyperinsulinemia are common findings in women with PCOS independent of their degree of adiposity, body fat topography, and androgen levels [16]. Women with PCOS have a high risk of developing impaired glucose tolerance and type 2 diabetes mellitus [17, 18] The pathogenesis of IR in PCOS reflects the interaction of genetic influences, non-heritable intra- and extrauterine environmental factors, and alternative adaptations to energy excess. However in the context of PCOS, puberty per se might play an important role in the molecular origins of IR and hyperinsulinemia. During puberty, adolescents experience a temporary decline in insulin sensitivity with a nadir in mid-puberty [19–21]. This was first described in an effort to understand the deterioration of glycemic control in type 1 diabetes during adolescence [20]. This transient IR and hyperinsulinemia have been attributed to the increases in growth hormone and IGF-1 concentrations in this period of growth to provide more amino acids [20]. Pubertal IR appears to be selective for glucose metabolism, whereas protein metabolism seems to respond normally to insulin action [22].

Importantly, IR in PCOS women is tissue-selective. Resistance to the metabolic actions of insulin has been reported primarily in skeletal muscle, adipose tissue, and liver; while sensitivity to insulin actions on steroidogenesis persists in the adrenal gland and ovary. Hence the paradox: whereas some tissues manifest IR in women with PCOS, steroid-producing tissues remain insulin sensitive [23].

While early studies attributed the IR in PCOS to obesity, subsequent studies including euglycemic-hyperinsulinemic clamp studies demonstrated the existence of IR in lean PCOS women [16, 24]. However, Stepto et al. [24] included patients diagnosed using the Rotterdam criteria in their study (2 of the 3 criteria: oligo/anovulation, hyperandrogenism, and polycystic ovaries on ultrasound were used). Nevertheless, another large study found that only 53 out of 201 (26.3%) lean PCOS women (body mass

index [BMI] less than 25) had IR, suggesting that ethnic background and dietary composition might play a role in the metabolic factors among these women [25].

Both in vivo and in vitro studies suggest that insulin as well as IGF-1 can synergize with LH to increase theca cell androgen production [26]. Insulin can also decrease the hepatic synthesis of SHBG increasing circulating free androgens [27]. Additionally, insulin may directly stimulate the activity of ovarian P450c17 and P450scc enzymes to promote ovarian androgen steroidogenesis [28]. In addition, pancreatic beta cell secretory dysfunction has been described in a subset of women with PCOS; this subset probably has the highest risk of developing carbohydrate intolerance and type 2 diabetes [29].

Other potential mechanisms, including pubertal increase in androgen production are hypothesized to contribute to IR and hyperinsulinemia. The association between IR and androgen excess in women has long been recognized because of the association of hyperandrogenic features with the rare syndromes of extreme IR due to mutations of the insulin receptor or autoantibodies targeting the insulin receptor [30–32]. Insulin may also potentiate the steroidogenic response to gonadotropins indirectly, by acting at the pituitary to increase gonadotrope sensitivity to GnRH [33]. Furthermore, increased androgen levels have been associated with decreased adiponectin secretion by adipocytes in PCOS women, thereby further reducing insulin sensitivity and consequently increasing compensatory insulin levels [34]. In addition, insulin may also drive adipose androgen generation by increasing aldo-keto reductase 1C3 (AKR1C3) activity in female subcutaneous adipose tissue [35].

Obesity alone is associated with IR and compensatory hyperinsulinemia. Although the prevalence rates of obesity vary widely across different geographic regions and ethnicities, a large proportion of PCOS patients are overweight or obese [36, 37]. Among obese adolescents, obesity-associated IR may exacerbate the IR associated with puberty during this period of life, predisposing this group of individuals to develop prediabetes and type 2 diabetes [38].

Several studies have reported associations for visceral obesity, proinflammatory markers, elevated fasting and glucose-stimulated insulin levels, and greater IR among women with PCOS [39–42]. Endothelial dysfunction has been described and may promote chronic inflammation [43]. Although the mechanisms responsible for obesity-related IR are not completely clear, ectopic accumulation of fatty acids in organs and tissue that are not meant to store large amounts of fat appears to play a role [44]. Ectopic fat accumulation can also occur in the absence of obesity, i.e., when there has been reduced prenatal growth and thus a reduction in subcutaneous fat storage capacity that is followed by rapid postnatal catch-up and a relative excess of fat, which is stored in the same ectopic depots [45].

Molecular mechanisms responsible for IR in PCOS include defective post-receptor insulin activity, increased free fatty acids, increased cytokine secretion, and increased androgens [46–50]. Intra-abdominal adipocytes show increased release of free fatty acids and increased cytokine secretion, e.g., TNF-α, IL-6, leptin, and resistin [51, 52]. The increased free fatty acids drain via the portal vein to the liver and subsequently affect the secretion, metabolism, and peripheral actions of insulin. Hence, the distribution of fat, rather than the mere presence of obesity or increased BMI, may be highly relevant in PCOS [53, 54]. Some studies have also suggested that IR in subjects with PCOS might be driven by alternative mechanisms differing from those occurring in obesity. In fact, women with PCOS are reported to have a higher degree of serine phosphorylation of the insulin receptor and insulin receptor substrate-1 resulting in impaired insulin signal transduction and intrinsic IR independent of total or fat-free body mass [55]. In addition, a proinflammatory milieu amplified by PCOS and obesity has been described in ovarian granulosa cells and stroma [56, 57].

Accumulation of lipids, i.e., diacylglycerol (DAG) and ceramides, in muscle and liver interferes with insulin signaling [58]. Intra-cellular ceramides can also impair insulin signaling by blocking the translocation of Akt, an important mediator of insulin sensitivity, to the plasma membrane [59, 60]. Interestingly, animal data have shown that disrupted insulin signaling in the central nervous system is associated with the development of obesity and impaired ovarian follicular maturation [61], suggesting another link between IR, hyperinsulinemia, obesity, and PCOS.

3. Neuroendocrine Alterations

3.1. Changes in GnRH and Gonadotropin Secretion in PCOS

Although not mandatory for diagnosis, a hallmark of PCOS is the presence of deregulated secretion of the gonadotropins, LH and FSH, which control ovarian steroidogenesis, follicular dynamics, and ovulation [3, 62–64]. Hence, it is reasonable to hypothesize that altered gonadotropin secretory profiles could impact the cardinal features of PCOS, including hyperandrogenism and

ovulatory dysfunction [3, 4]. In fact, increased circulating LH levels, increased LH:FSH ratios, elevated LH pulse frequency and/or amplitude, as well as relatively decreased FSH levels have been typically described in women with PCOS [3, 65]. Yet, a fraction of PCOS patients with hyperandrogenism, especially when associated with obesity, display non-elevated basal or stimulated LH levels, which further attests the heterogeneity of presentations (and pathophysiology) of the syndrome. Although LH is considered to be the biomarker of GnRH pulses, dissociation between GnRH and LH has been reported in several models which may contribute to the lower LH secretion in some obese women with PCOS [66].

The alterations in gonadotropin secretory profiles are compatible with changes in the profiles of GnRH pulsatility, presumably reflecting an increase in the activity of the GnRH pulse generator. Indeed, classical neuroendocrine studies established that a pattern of GnRH secretion defined by increased number of pulses favors LH over FSH secretion by the pituitary [67]. While it is possible that primary (e.g., genetically determined) alterations at the GnRH pulse generator network might drive such changes in some patients, data from different clinical and experimental studies have pointed out contributing roles of perturbations of key modulators of GnRH neurosecretion, including insulin and androgens, whose levels are reportedly altered in PCOS [68].

Considering that hyperandrogenism is a hallmark of PCOS, considerable attention has been devoted to the investigation of potential mechanisms through which deregulated androgen secretion contributes to the neuroendocrine alterations of the syndrome [3]. Indeed, compelling evidence suggests that elevated androgens disrupt the capacity of sex steroids to regulate GnRH/LH secretion via classical feedback loops. This would result in diminished negative feedback actions of ovarian steroids (estrogens and progesterone) that would contribute to and perpetuate the LH hypersecretion characteristic of PCOS [67]. In fact, clinical data point out that diminished progesterone- and estrogen-negative feedback, linked to androgen excess, has a role in the reported elevation of LH pulsatility in patients with PCOS [69]. Furthermore, reduced sensitivity to progesterone-negative feedback, due to early-onset hyperandrogenism, has been mechanistically linked to elevated LH secretion in women with PCOS, although only half of the patients seem to display overtly impaired negative feedback of progesterone [69]. From a mechanistic standpoint, it is notable that GnRH neurons appear to be devoid of the major sex steroid receptors responsible for negative feedback [70], while the estrogen receptor-β (ERβ), whose role in feedback control of GnRH neurons remains unclear, is present. Accordingly, it is tenable that the primary impact of androgen excess on the feedback regulatory loops during different developmental windows occurs at neuronal sites other than (and likely upstream of) GnRH cells.

3.2. Altered Kisspeptin Signaling in PCOS

Among the various afferent neurons to GnRH neurons, Kiss1 neurons, which produce kisspeptins (encoded by the *KISS1* gene), have emerged in the last decade as master regulators of GnRH neurosecretion and ovulation. Kisspeptins are among the most potent activators of GnRH neurons identified to date [70]. Various KISS1/Kiss1 neuronal populations have been identified in different mammalian species, including humans, rodents, and non-human primates. A prominent and highly conserved population of KISS1 neurons has been reported at the arcuate nucleus (ARC) of the mediobasal hypothalamus, or its equivalent infundibular region in humans [71]. In rodents, this ARC Kiss1 neuronal population has been proposed to operate as a major hub for mediating the negative feedback effects of sex steroids, as sex steroids consistently suppress *Kiss1* expression at this site. In contrast, a second more rostral hypothalamic population of Kiss1 neurons may participate in positive feedback, as estrogen enhances *Kiss1* expression at this site [70].

One interesting feature of the population of Kiss1 neurons in the ARC is that at least a fraction of them co-express other neurotransmitters that also play major roles in the control of GnRH/gonadotropin secretion [72]. These other neurotransmitters include neurokinin B (NKB) and dynorphin. The NKB receptor NK3R is also expressed in Kiss1 neurons. This population of neurons which co-express kisspeptins, NKB, and dynorphin has been called KNDy neurons [73]. Based on the reported actions of NKB and dynorphin, which predominantly stimulate and inhibit LH secretion, respectively, and the dense interconnection of KNDy neurons within the ARC, it has been proposed that NKB and dynorphin participate in a Ying-Yang fashion in the (auto)regulation of kisspeptin output to GnRH neurons, and hence in the generation of GnRH pulses. As KNDy neurons are sensitive to sex steroids and modulate GnRH pulse generation, it is reasonable to speculate that deregulated function of this neuronal population might contribute to the neuroendocrine alterations of PCOS.

However, limited experimental evidence is available to support or refute this possibility. Despite the meager data for human and non-human primates, review of preclini-

cal rodent models can provide insights into the neuroendocrinology of PCOS. To date, some studies have reported alterations in *Kiss1* expression and/or the number of Kiss1 neurons in the hypothalamus of various preclinical animal models of PCOS, generated by excessive androgen exposure at different developmental windows. Studies in rodent models of PCOS due to postnatal exposure to androgens have reported persistent suppression of hypothalamic *Kiss1* expression [74]; a finding that is consistent with the proven inhibitory action of sex steroids on *Kiss1* expression in the ARC and compatible with similar observations in models of neonatal estrogenization of female rats [75]. However, different models of androgenization have been reported to cause variable deregulation of *Kiss1*/kisspeptin expression in the hypothalamus. Thus, it is likely that the actual change (up- or downregulation) of the Kiss1 system depends on the developmental window and regimen of exposure to androgens.

Although supportive evidence is sparse, the other KNDy neuropeptides might also be involved in the pathophysiology of neuroendocrine alterations of PCOS [70, 72]. NKB has been suggested to operate as stimulatory drive for kisspeptin neurosecretion onto GnRH neurons. Hence, alterations of central NKB levels might impact GnRH and LH secretory profiles. This corresponds with recent evidence showing that oral administration of the antagonist of NKB receptor, ESN364, to intact female monkeys lowered LH concentrations and blocked the LH surge [76]. Another NK3R antagonist (AZD4901) was administered to 67 women with PCOS for 28 days; treatment with the highest dose was associated with decreased LH pulse frequency and decreased testosterone concentrations [77]. Interestingly, pharmacological studies in humans have also shown that while administration of NKB alone did not alter circulating gonadotropin levels, NKB partially suppressed gonadotropin responses to kisspeptin [78]. Thus, multidimensional interactions likely modulate the actions of the KNDy peptides in the control of gonadotropin secretion. This complexity has also been documented in equivalent preclinical studies [79, 80]. Whether such interactions are appreciably perturbed in PCOS remains to be clarified.

3.3. Altered GABA Signaling and PCOS

In addition to perturbed kisspeptin/KNDy signaling, evidence for deregulation of other key central neuroendocrine pathways governing GnRH neuron function has been presented in preclinical models of PCOS. Among those, elegant studies conducted by Campbell and coworkers have convincingly demonstrated a multi-factorial alteration of gamma-aminobutyric acid (GABA) signaling following early androgenization in a mouse model of PCOS, which might explain part of the neuroendocrine alterations of the syndrome [81].

While GABA is generally regarded as inhibitory transmitter, different studies have documented that under certain conditions, acting via GABA-A receptors, GABA can evoke depolarization (activation) responses directly in GnRH neurons [82]. Moreover, a GABA neuronal pathway originating from the ARC is likely to play a role in transmitting the feedback actions of sex steroids. In this context, studies in a mouse PCOS model of prenatal exposure to dihydrotestosterone (DHT) has documented an increase in the GABAergic drive to GnRH neurons, as evidenced by functional (increased postsynaptic currents) and morphological (increased number of appositions of GABA fibers) data. This state of enhanced GABA input would derive from suppressed progesterone receptor expression in ARC GABA neurons projecting to GnRH neurons, thus resulting in diminished restraint of GABA signaling to GnRH neurons with consequent elevated GnRH neurosecretion. In addition, androgenic metabolites generated following inappropriate exposures to DHT, such as 3α- and 3β-androstanediols, may also contribute to activation of GABA-A receptors and suppression of the negative feedback machinery in GnRH neurons [81]. Admittedly, the experimental data supporting such a pathogenic GABA pathway derive from a single mouse PCOS model, which does not mimic the obese phenotype that is commonly seen in at least half of PCOS patients. Hence, it remains unclear whether GABAergic deregulation is commonplace in the wide spectrum of clinical cases of PCOS.

3.4. Other Endocrine and Metabolic Modifiers of GnRH Secretion in PCOS: AMH, Insulin, and Adiponectin

Recent data has revealed a previously unknown role of AMH in the stimulatory control of GnRH neurons [83]. Central injection of AMH has been shown to increase the pulsatile secretion of LH in female mice in a dose-dependent manner. This GnRH-dependent effect was associated to an increase in the firing of GnRH neurons, which express the AMH receptor AMHR2 [83]. In this context, it has been proposed that deregulated AMH levels in PCOS might contribute to the state of LH hypersecretion. However, it is important to note that the stimulatory actions of AMH on GnRH neurosecretion have been observed in control mice, not in PCOS models or patients; hence, although very appealing, the potential central role

of AMH in the neuroendocrine dysfunction associated with PCOS remains to be verified.

Although hyperandrogenism and possibly other ovarian factors are major factors contributing to the increased GnRH/LH secretion, the elevated insulin levels and IR are also putatively involved in such neuroendocrine alterations. In fact, central insulin actions are indispensable for proper functioning of the gonadotropic axis in mice; lack of brain insulin signaling decreases LH levels and disturbs follicular maturation [60]. In good agreement, insulin infusion in control women increased LH pulse frequency, reminiscent of secretory profiles of women with PCOS [84]. In fact, lean patients with PCOS have been shown to display increased basal LH levels and LH:FSH ratios. Yet, another study involving women with PCOS reported that insulin administration failed to alter LH pulsatility [85].

The mechanisms responsible for the effects of high insulin levels on the GnRH pulse generator need further elucidation. Insulin receptors in GnRH neurons appear dispensable for proper pubertal maturation and fertility, therefore pointing to a primary action of insulin at other brain targets, likely occurring upstream of the GnRH neurons [86]. Studies in sheep and rodents suggest that insulin signaling may modulate Kiss1 neuron function, thereby regulating GnRH neurosecretion [87, 88]. In fact, analyses in a sheep model of PCOS generated by gestational androgenization revealed a decrease in IR expression in ARC KNDy neurons [87]. However, the functional relevance of such direct actions of insulin in Kiss1 neurons, in terms of control of gonadotropin secretion and fertility appear to be modest, if any, according to rodent studies [89]. This would suggest that insulin operates at other elements of the GnRH pulse generator to modulate GnRH secretion. Alternatively, related factors, such a IGF-I, known to act directly at GnRH neurons to control the reproductive axis might contribute to deregulated gonadotropin secretory profiles in women with PCOS.

Another metabolic regulator with putative pathophysiological roles in PCOS is adiponectin, an adipokine negatively correlated with IR and adiposity. Although conflicting results have been reported on changes in circulating adiponectin in women with PCOS, systematic analyses of published data suggest that women with PCOS display lower adiponectin levels, which correlate with IR [90]. While the pathogenic relevance of such alterations remains to be established in humans, an experimental rat model of PCOS associated with DHEA administration revealed that adiponectin administration was largely sufficient to reverse the PCOS-like phenotypes of DHEA-treated rats [91]. Moreover, transplantation of brown adipose tissue (BAT) in this model, which caused an increase in circulating adiponectin, equally corrected the metabolic and ovarian abnormalities of this preclinical model of PCOS [91]. It must be stressed, however, that the therapeutic benefits of adiponectin administration and/or BAT transplantation in women with PCOS are yet to be demonstrated.

4. Genetics

Studies of monozygotic and dizygotic twins have indicated a moderate heritability of PCOS. Other epidemiological studies have indicated the likely importance of considering risk factors and biological processes acting throughout the life-course: low birth weight and fetal exposure to androgens; postnatal rapid weight gain; precocious adrenarche and early age at pubertal development; adult weight status and lifestyle.

Until recently, candidate gene studies have been underpowered leading to poorly reproducible results. The advent of large-scale GWAS with their stringent statistical thresholds has brought robust new insights, although as yet these have been limited to adult PCOS cases and their direct relevance to adolescent PCOS is yet to be established. The first GWAS for PCOS were performed in Han Chinese populations [92, 93]; while the identified genomic loci were replicable in that population, their effects estimates are consistently smaller in Caucasian PCOS cases, possibly due to population differences in genetic architecture or even PCOS sub-phenotypes [94, 95].

Several of the individual genomic signals for PCOS have provided new insights into its pathophysiology. As noted above, the role of *DENND1A* splice transcripts in ovarian theca cell steroidogenesis is being investigated. The PCOS susceptibility allele in the *FSHB* gene is also associated robustly with lower circulating FSH levels [94, 95], and with other phenotypes indicative of diminished ovarian follicle stimulation: later onset of puberty, and lower risk for dizygotic twinning [96]. Together, these genetic findings indicate a co-primary neuroendocrine pathogenesis of PCOS, alongside its likely ovarian etiology. These genetic studies and the pharmacologic studies involving NK3R antagonists encourage further investigation into the neuroendocrine features of PCOS. GWAS findings also suggest the importance of future studies of the possible role of epidermal growth factor receptors on ovarian follicle development/steroidogenesis [94].

Another powerful use of the genomic data is to test combinations of signals that indicate the potential causal influences of biological pathways. Such Mendelian ran-

domization analyses have indicated causal roles in PCOS etiology for higher BMI, higher IR, and lower serum SHBG concentrations, which could act by increasing the bioactivity of androgens or other sex steroids [94]. Finally, a highly robust yet unexplained association between genetic variants that confer a later age at menopause and higher susceptibility to PCOS is intriguing [94]. It suggests that perhaps the evolutionary incongruity of this common heritable disorder impacting fertility might be explained by its co-susceptibility to preserved fecundity at older age.

5. Epigenetics

A number of GWAS as well as replication studies in Chinese and Caucasian subjects have identified the LH/choriogonadotropin receptor (*LHCGR*) (locus 2p16.3) as a susceptibility gene for PCOS [97]. Increased LH activity is a common feature in PCOS and may contribute to the defective folliculogenesis and hyperandrogenism commonly seen in these patients. Hypomethylation of the *LHCGR* was first described in a mouse model of PCOS and has been recently confirmed in human peripheral blood cells and granulosa cells from PCOS subjects [98, 99]. Decreased *LHCGR* methylation is known to increase gene expression [100]. Hypomethylation of *LHCGR*, by causing hypersensitivity to LH pulses, may thus be a plausible mechanism underlying susceptibility to PCOS.

Aromatase, encoded by *CYP19A1*, is another candidate gene in PCOS. As estrogens are required for follicle selection and growth, decreased aromatase may contribute to the defective folliculogenesis observed in PCOS patients. In Chinese women with PCOS, *CYP19A1* was hypermethylated in ovarian tissue, which correlated with decreased mRNA and protein levels [101]. In another study, *EPHX1*, which encodes for epoxide hydrolase 1, an enzyme necessary for the degradation of aromatic compounds, was hypomethylated in peripheral blood cells from women with PCOS. In human granulosa-like tumor cells, it was also demonstrated that EPHX1 regulated estradiol concentrations, indicating a role for *EPHX1* hypomethylation in ovarian steroidogenesis [102]. Alterations in the methylation pattern and expression of peroxisome proliferator-activated receptor gamma 1 (PPARG1), which is involved in the regulation of ovarian function, and of its co-repressors has also been described in granulosa cells from women with PCOS and in animal models of PCOS [103].

Besides gene-targeted studies, genome-wide methylation studies in ovaries of women with PCOS have revealed alterations in DNA methylation and gene expression in pathways such as the type 1 diabetes mellitus pathway, p53 signaling pathway and NOD-like receptor signaling pathway (involved in immune responses), as well as in metabolic pathways involved in ovarian function (IGFBP2, INSR, SLC2A8, NRIP1) and in ovarian steroidogenesis (CYP19A1, AMH and its receptor AMHR2) [104, 105].

In peripheral blood cells, differential methylation was observed in pathways related to the immune response and to cancer pathways (cellular survival, proliferation, pluripotency, invasion, metastasis, and angiogenesis) [106]. Interestingly, the association with immune pathways was also described in another report relating PCOS with epigenetic changes in pathways involved in autoimmune and allergic diseases, such as type 1 diabetes mellitus, thyroid disease, and asthma [107], and was consistent with the abovementioned results in ovarian tissue [104].

In addition to the ovary and peripheral blood cells, genome-wide methylation studies have been performed in adipose tissue from women with PCOS and in a primate model of PCOS. In women, differential methylation was observed in genes involved in steroid metabolism (*CYP1B1*), liver function (*GPT*), in a candidate gene for PCOS (*RAB5B*, which participates in intracellular vesicle transport), in two genes related to type 2 diabetes mellitus (*PPARG, SVEP1*) and in one gene involved in DNA methylation (*DMAP1*) [108]. In prenatally androgenized female rhesus monkeys, differential methylation in adipose tissue was observed for two anti-proliferative gene signaling pathways: *TOB* (involved in T-cell signaling) and transforming growth factor-β (*TGFB*) [109]. The available genome-wide methylation studies in women with PCOS are summarized in a recent review by Li et al. [110]. The authors highlight the significant association of PCOS phenotype with immune responses both in ovaries and in peripheral blood cells.

Regulation of gene expression by microRNAs (miRNAs) is considered to be an additional layer of epigenetic regulation. A genome-wide circulating miRNA expression profile identified a number of miRNAs dysregulated in women with PCOS. These miRNA species are involved in glycometabolism and ovarian follicle development pathways [111, 112]. Interestingly, miRNA-592 has been shown to be downregulated and to be inversely related to LHCGR levels in PCOS patients [113].

6. Altered Sympathetic Nerve Activity

An alteration in sympathetic nerve activity has been proposed to contribute to the etiology of PCOS. Indeed,

Table 1. Suggested criteria for the diagnosis of PCOS in adolescence

Required	Optional[a]	Not recommended[b]	Comments
1. Irregular menses/ oligomenorrhea 2. Evidence of hyperandrogenism: a. Biochemical b. Clinical (e.g., progressive hirsutism)	1. PCOM 2. Severe cystic acne	1. Obesity 2. Insulin resistance 3. Hyperinsulinemia 4. Biomarkers (e.g., AMH, T/DHT ratio) 5. Acanthosis nigricans	1. Must generally be 2 years post-menarche 2. Must rule out other disorders of hyperandrogenism (e.g., NC-CAH, Cushing syndrome)

PCOS; polycystic ovary syndrome; PCOM, polycystic ovarian morphology; AMH, anti-Müllerian hormone; T/DHT, testosterone to dihydrotestosterone; NC-CAH, non-classical congenital adrenal hyperplasia. [a] These criteria are often used in concert with the required criteria, but should not be used independently as diagnostic features. [b] These criteria have been associated with PCOS but are not diagnostic.

many of the common clinical symptoms of PCOS, including central obesity, hyperinsulinemia, and hyperandrogenemia, are associated with chronic increased activity of the sympathetic nervous system [114, 115]. Direct assessment of sympathetic activity in PCOS women revealed an association between high muscle sympathetic nerve activity and PCOS independently of BMI [116]. Additional indirect markers of autonomic activity including heart rate variability and heart rate recovery after exercise have demonstrated that young PCOS women exhibit increased sympathetic and decreased parasympathetic responses to these challenges [117–119].

Increased ovarian sympathetic tone in PCOS is supported by the finding of a greater density of catecholaminergic nerve fibers in polycystic ovaries [120] and additional studies in a rat PCOS model that demonstrated increased sympathetic outflow previous to the appearance of ovarian cysts [121]. Additional studies in this rat model showed an association between the development of follicular cysts and chronic increased production of nerve growth factor in the ovary [122], a hallmark of sympathetic hyperactivity. The association between the neurotrophins and PCOS was strengthened by the finding that ovarian nerve growth factor production is increased in PCOS women [123].

B. Diagnosis

As previously reviewed [124], diagnostic criteria for PCOS in adolescence remain controversial, primarily because the diagnostic pathological features used in adult women may be normal pubertal physiological events. These features include irregular menses, cystic acne, and polycystic ovarian morphology (PCOM) [125, 126]. Indeed, it is possible that adolescent hyperandrogenemia is a consequence of the lack of full maturation of the hypothalamic-pituitary-ovarian axis during this time of life. Similarly, prolonged anovulatory cycles are simply typical of pubertal development rather than an early manifestation of PCOS. Most importantly, it remains unclear when persistence of adolescent oligomenorrhea becomes a significant clinical finding (Table 1).

As noted above, IR and hyperinsulinemia are often noted in women with PCOS and may influence the development of PCOS in some patients. However, current definitions of PCOS do not include obesity, IR, or hyperinsulinemia as diagnostic criteria [127–135]. Nevertheless, we will discuss as to whether adolescents with these findings should be considered as being at risk for PCOS, since they may carry an additional risk for manifestation of metabolic disease in adult life.

1. Clinical Features

As in adults, signs of hyperandrogenism in adolescents can be clinical or biochemical. Hirsutism is defined as excessive, coarse, terminal hairs distributed in a male fashion, and PCOS is the most common cause of hirsutism in adolescence [136]. The severity of hirsutism may not correlate with serum androgen levels; moreover, there are ethnic/genetic differences that may affect the degree of hirsutism [137–139]. Hirsutism must be distinguished from hypertrichosis defined as excessive vellus hair distributed in a non-sexual pattern. Mild hirsutism may not be a sign of hyperandrogenemia [140], but the likelihood of androgen excess is increased when associated with other findings such as menstrual irregularities [141, 142]. Moderate or severe hirsutism may be a sign of androgen excess in early postmenarcheal years. In adults, the evaluation and grading of hirsutism can be done using the

Ferriman-Gallwey scoring system, which may not be suitable for adolescents (modified Ferriman-Gallwey) [132]. Adult terminal hair distribution is usually achieved by 2 years after menarche. The original report of Ferriman and Gallwey included females starting from the age of 15 years [143]. Ethnic and racial variation in the extent of hair growth influences this semi-subjective cutaneous sign of androgen excess [131].

Although acne is a common problem in adolescence, it is usually transient and may not be indicative of hyperandrogenism [144, 145]. Moderate or severe inflammatory acne, especially if unresponsive to topical therapy, however, may require investigation of androgen excess [124, 146]. In a 5-year longitudinal analysis, development of moderate to severe inflammatory acne has been reported to be associated with androgen excess [147]. Alopecia is rare and not well studied in adolescents [148]. Isolated acne and alopecia should not be considered to be diagnostic criteria of PCOS in adolescence.

Premature adrenarche (PA), defined in girls as the appearance of pubic hair before 8 years of age with Tanner II–III levels of adrenal androgens, may herald PCOS in childhood [149]. However, PA does not precede PCOS in all girls [148] and not all girls with PA will develop PCOS [4, 150]. Persistent hyperandrogenemia in girls with PA may lead to PCOS, especially if accompanied by obesity [151]. Continued prospective monitoring of girls with PA should be performed. The diagnosis of non-classic congenital adrenal hyperplasia should be excluded based on history, examination, and hormone levels including ACTH stimulation tests if warranted [152]. Similarly, patients should be screened for Cushing syndrome, if clinical features are suggestive.

Irregular menses should also not be used as the only criterion for PCOS in adolescence because menstrual irregularities are typical for at least 2 years after menarche [124]. In adolescence, irregular menses that persist 2 years after menarche may be a sign of PCOS, although irregular menses may continue up to the 5th year after menarche without development of PCOS [153, 154]. About 85% of cycles are anovulatory during the 1st year after menarche, 59% during the 3rd year, and 25% in the 6th year [154]. Moreover, irregular cycles may not necessarily be associated with clinical or biochemical hyperandrogenism [155]. The Endocrine Society Guidelines required persistent oligomenorrhea (menstrual cycles longer than 45 days) for the diagnosis of PCOS in adolescents [134]. Based on the expected variation of menstrual cycles in normal girls [156, 157], the persistence of oligomenorrhea, secondary amenorrhea (absence of cycles for more than 3 months), or primary amenorrhea in girls with completed puberty may suggest androgen excess [158, 159]. Ovulatory dysfunction may also present as dysfunctional uterine bleeding (cycles shorter than 21 days or lasting more than 7 days) [160–162]. These menstrual disturbances may all be reflective of androgen excess [154]. One should keep in mind that age at menarche may differ in girls with PCOS due to variable presentation, including early puberty and primary amenorrhea. Age at menarche may be inversely correlated to obesity [163, 164].

Confirmation of biochemical hyperandrogenism is important in symptomatic adolescents before a definitive diagnosis of PCOS can be considered. As described in prior publications on PCOS in adolescents, measurements of total and/or free testosterone have been the most recommended hormone determinations to document hyperandrogenemia [165]. Methodological problems regarding testosterone determinations include the following: (1) inadequate assay sensitivity to measure low testosterone concentrations in girls and women; (2) assay interference due to simultaneous presence of other steroid molecules with similar structure; (3) lack of well-defined normative values; (4) binding of testosterone to SHBG and other proteins in the peripheral circulation, and (5) technical aspects of testosterone assays [166–168].

Most recommendations advocate utilization of high-quality liquid chromatography/tandem mass spectrometry (LC-MS/MS) to measure testosterone. However, until this technology is universally available, high-quality RIA with extraction and chromotography should be employed. Available guidelines have suggested total testosterone concentrations >55 ng/dL (1.91 nmol/L) are likely consistent with hyperandrogenism. Further, Gambineri et al. [169] defined hyperandrogenism during the follicular phase as total testosterone concentrations >42 ng/dL (1.45 nmol/L) using a LC-MS/MS assay. Because of the variability in the results of testosterone assays and the limited data on the normal development fluctuations in testosterone levels during adolescence, no clear cutoff testosterone concentrations can be given.

2. Polycystic Ovary on Ultrasound: PCOM

The presence of enlarged ovaries with increased stroma and multiple small peripheral cysts is known as PCOM. PCOM is associated with hyperandrogenism but is not always included as a diagnostic element of PCOS. PCOM is an inconsistent finding in healthy girls [170] and adults [171], but a higher persistence of PCOM over time is observed in hyperandrogenic adolescents [172].

Furthermore, the criteria to define the ultrasonographic pattern of PCOS continue to be modified [173].

The anatomic appearance of the ovary changes with age [174]. Ovarian volume increases during puberty and reaches the adult volume in the years following menarche. It remains stable in young adulthood and decreases after the middle of the fourth decade of life. [175]. Follicle size also changes with age, and the greatest number of small follicles is observed during adolescence and young adulthood, with a significant decrease in follicle count with age [176].

The ultrasonographic diagnosis of PCOM has been standardized for adults using the transvaginal route. In adolescents, however, most exams are performed by the transabdominal route, where the high physiologic follicle number may render the follicle count an unreliable criterion for the diagnosis of PCOM. The importance of using appropriate diagnostic criteria of PCOM in adolescents is emphasized because application of the adult criteria can lead to a falsely elevated prevalence of PCOM (30–40% range) [177, 178]. Therefore, ovarian volume is better suited than follicle count to determine the presence of PCOM in adolescence [179]. The Androgen Excess and PCOS Society suggested that an ovarian volume of 10 mL be recommended for the diagnosis of PCOM in adolescents [132]. Later, based on an ovarian volume larger than 2 SD above the mean in the healthy adolescent population [180], an enlarged ovarian volume of 12 mL was recommended by an international consensus [179].

Available data suggest that among non-obese, non-hirsute girls with regular menstrual cycles, PCOM is not associated with hyperandrogenism or IR. Similar levels of androgens and indexes of insulin sensitivity were observed in healthy girls with and without PCOM [181]. Nevertheless, persistence of enlarged ovaries and menstrual irregularities may foretell the future development of PCOS [176, 182, 183].

3. Biomarkers for PCOS

Limited data are available regarding newer biomarkers, except for AMH, nor has their utility to aid in the establishment of the diagnosis of PCOS in adolescence been completely verified. AMH is a glycoprotein secreted by the granulosa cells of small, growing follicles. As noted above, animal studies have inferred a possible role for AMH in the ontogeny of PCOS. AMH serum levels correlate with the number of small antral follicles (2–5 mm) identified by transvaginal ultrasound in adult women [184, 185]. Elevated AMH levels have been a consistent hormone finding in women with PCOS [186, 187]. However, in adolescents, AMH should not be used as a criterion of PCOS since there is a weaker association of AMH levels with the disorder [188, 189]. This divergence may be due to the presence of higher AMH serum levels in healthy adolescents compared to adult women, with a wide normal range [178, 180, 190, 191].

Besides AMH, several biomarkers may be associated with PCOS. A high ratio of total testosterone to dihydrotestosterone (T/DHT) is associated with an adverse metabolic phenotype in PCOS patients [192]. Munzker et al. [192] found that T/DHT was significantly higher in PCOS patients than in non-PCOS patients, and T/DHT was even higher in obese PCOS patients than in non-obese PCOS patients. This phenomenon may be linked to conversion of testosterone to DHT by the 5α-reductase enzymes, and may ultimately be useful to assess for the diagnosis of PCOS.

Proteomic profiling studies have indicated specific proteins to be used as biomarkers for PCOS. Sarray and Almawi [193] detected significantly elevated sCD40L in women with PCOS. They posited that sCD40L, a transmembrane glycoprotein that regulates several cell types in the inflammatory network, can be used as a predictor for PCOS in a Bahraini Arab population [193]. Though this result cannot be generalized across ethnic groups, it is an important finding for future replication and validation. HSP90B1, a stress-inducible chaperone protein associated with the growth of cancerous cells, has also been identified as a potential biomarker for PCOS [194]. HSP90B1 may have a role in promoting granulosa cellular activity in the ovary, leading to PCOS. Further study is necessary to confirm this action [194].

Alongside proteomics and hormone discoveries, promising preliminary work in the use of microRNA for PCOS diagnosis is underway [195]. Circulating or ovarian miRNAs could potentially modulate steroidogenesis and ovarian function in women with PCOS [195]. Biomarkers are useful tools in general, and progress continues in the discovery of newer biomarkers to assist in making the diagnosis of PCOS.

4. IR in the Context of PCOS

IR and compensatory hyperinsulinemia are not considered to be diagnostic criteria for PCOS. Yet, IR and hyperinsulinemia have been documented in women with PCOS since the late 1980s, when some studies showed that obese women with PCOS had significantly increased glucose levels during an oral glucose tolerance test compared to age- and weight-matched ovulatory women with elevated plasma androgen levels and control women.

Moreover, the presence of some degree of IR in subjects with PCOS is corroborated by the high prevalence of glucose intolerance in obese PCOS adolescents. Estimated at ~40% [37], glucose intolerance in obese PCOS adolescents is much higher than in the general US population of obese adolescents in which the prevalence of impaired glucose tolerance is about 15–20% [196].

The diagnosis of IR in PCOS is unfortunately confounded by the variety of definitions used in different studies [16, 18, 37, 197]. IR may be measured directly using a euglycemic insulin clamp (requiring an intravenous line), but is usually measured indirectly, through the oral glucose tolerance test, or most commonly through fasting levels of glucose and insulin [197]. Though the derived indices obtained from indirect measures may be somewhat less accurate than direct, whole-body measurement, their utility as non-invasive measures of IR is vital. Indirect measurements of IR may be calculated in a variety of ways. These include fasting glucose to insulin ratio, early insulin response, homeostatic model assessment (HOMA), the Matsuda Index, and oral Sg index [197–201]. These methods are particularly useful in individual or population studies.

B. Diagnosis: Conclusions with Level of Evidence

1. Clinical Features of PCOS

- Moderate to severe hirsutism constitutes clinical evidence of androgen excess (Level B).
- Mild hirsutism may be a sign of androgen excess when associated with menstrual irregularities (Level C).
- Moderate or severe inflammatory acne unresponsive to topical therapy may require investigation of androgen excess (Level C).
- Isolated acne and/or alopecia should not be considered diagnostic criteria for PCOS in adolescence (Level C).
- Persistent menstrual disturbances (oligomenorrhea and secondary amenorrhea) beyond 2 years after menarche or primary amenorrhea in girls with completed puberty may suggest androgen excess (Level B).
- Biochemical hyperandrogenism should be defined based on the methodology used, as no clear cutoff for testosterone concentrations exists for adolescents (Level A).
- Biochemical evidence of hyperandrogenism based on elevations of total and/or free testosterone measured in a reliable reference laboratory documents hyperandrogenemia in a symptomatic adolescent (Level B).

2. Polycystic Ovarian Morphology

- The presence of PCOM in an adolescent who does not have hyperandrogenism/oligo-anovulation does not indicate a diagnosis of PCOS (Level A).
- The measurement of ovarian volume, follicle number and size, and uterine dimensions may be useful in the evaluation of amenorrhea, but is not needed for PCOS diagnosis in adolescents (Level A).

3. Biomarkers of PCOS

- The use of AMH, T/DHT ratios, and specific proteins or microRNA as biomarkers of PCOS has not been validated in adolescents (Level C.).

4. Insulin Resistance

- IR, compensatory hyperinsulinemia, or obesity should not be considered as diagnostic criteria for PCOS in adolescents (Level A).

C. Treatment of PCOS

No pharmacological treatment has been approved so far by FDA/EMA for use in adolescents with PCOS; however, some pharmacological interventions have been used to manage PCOS symptoms. In the following sections, the baseline and additive pharmacological treatments and their potential benefits, as well as reproductive aspects in PCOS adolescents are discussed. Doses and sequences of intervention combinations need to be individualized.

1. Baseline Treatment

1.1. Lifestyle Intervention

Weight loss and increased physical exercise are generally recommended as the first-line therapy in overweight or obese girls [134]. Two small randomized controlled trials (RCTs) [202, 203] and one well-controlled clinical study [204] in overweight PCOS girls have shown that the combination of weight loss and intensified exercise decreases testosterone levels and the free androgen index, increases SHBG concentrations, and normalizes menstrual regularity comparably to drug therapy, and is devoid of side effects. The combination of lifestyle intervention with medications normalized more androgen levels and menses in one of these studies [204]. However, long-term data reporting sustained benefits on cycle regularity or on pregnancy outcomes after weight loss in adolescent girls are lacking.

Cardiovascular risk factors such as hypertension, dyslipidemia, and impaired glucose tolerance, as well as early markers of atherosclerosis such as carotid intima-media thickness also improved after lifestyle intervention [134]. Weight loss, but not participation in lifestyle intervention itself, predicted the amelioration of components of PCOS [134]. Extremely obese adolescents often respond poorly to lifestyle intervention [205]. A reduction of BMI SDS of 0.25 or greater [206] and/or 30 min per day of moderate to vigorous physical activity resulted in an improvement of cardiovascular risk factors in adolescents with PCOS [207].

Lifestyle intervention should be based on the combination of calorie-restricted diets (with no evidence that one type of diet is superior for adolescents), behavioral treatment, and exercise [208]. Along these lines, a meta-analysis has demonstrated the benefits of dietary modification in young women with PCOS [209]. Increasing physical activity from moderate to vigorous is effective in reducing the development of metabolic syndrome in normal-weight girls [209]. However, no large RCTs support the benefits of exclusive weight loss in normal-weight PCOS adolescents.

Decreasing sedentary behavior is at least as important as increasing physical activity [210]. Furthermore, family treatment is an essential component in lifestyle intervention since parents' readiness to change habits affects the outcome [208, 210].

1.2 Local Therapies/Cosmetics

Cosmetic hair-removal methods for hirsutism include bleaching, chemical epilation, plucking, waxing, shaving, electrolysis, and laser hair removal. Although only the latter result in permanent – albeit partial – hair removal, efficacy and safety of electrolysis is not supported by any RCT.

Evidence based on 11 RCTs [211] and 21 controlled trials [212] supports the efficacy for up to 6 months of partial hair removal with laser or intense pulsed light (IPL), despite a great variability following photoepilation [212]. Partial long-term hair removal efficacy (beyond 6 months) has been observed for all laser therapies after repetitive treatments, although the data are limited [212]. The data comparing different laser methods are few and contradictory; however, the available studies show that diode and alexandrite offer the higher success rate, whereas Nd:Yag provides the lowest [213]. The studies comparing laser and IPL devices are few and of low quality; all have been performed in adults with mixed forms of hirsutism, hyperandrogenism, or unwanted hair growth. Only 2 RCTs have evaluated the effect of photoepilation in selected PCOS patients aged 16 years or older, showing the benefits of laser therapy on facial hirsutism [214] and the superiority of alexandrite laser over IPL [215].

Two RCTs performed in hirsute patients aged 16 years or older reported the benefits of topical eflornithine HCl 13.9% cream applied twice daily in reducing facial hirsutism [216]. The safety profile was good and percutaneous absorption was minimal. Drawbacks included non-response in 30% of users and regrowth to pretreatment levels within 8 weeks of discontinuation. Three other RCTs performed in hirsute women showed the ability of topical eflornithine when added to photoepilation to promote faster and more complete laser removal of facial hirsutism and to reduce hair regrowth between laser sessions and after cessation of IPL use [216, 217]. Laser epilation is most effective when used to treat areas of full, dark hair on light-skinned people. The studies reporting the effects of topical finasteride on idiopathic hirsutism are limited and contradictory.

We suggest photoepilation as first-line management of localized hirsutism in PCOS; diode and alexandrite lasers are preferred. Topical eflornithine is recommended as an adjuvant to photoepilation in cases with laser-resistant facial hirsutism or as monotherapy in patients with facial hirsutism where photoepilation is not indicated. The use of topical finasteride is not recommended based on the existing data.

2. Additive Pharmaceuticals

Pharmacological interventions that have been used in adolescent PCOS are included in Table 2.

2.1. Metformin

Metformin is the only insulin sensitizer that has been evaluated in double-blind RCTs as single medication for adolescent PCOS; metformin use has increased over the last 10 years despite not being licensed for PCOS [218].

A meta-analysis of metformin use with and without lifestyle changes in PCOS up to August 2014 showed beneficial effects on BMI and menstrual cycles [219]. Of the 12 RCTs included, 2 were performed in adolescents [202, 220]. The meta-analysis also highlighted the many limitations of the RCTs such as small sample size, short duration (most trials had a duration of 6 months), and a moderate risk for bias.

Observational studies and 6 randomized trials [202, 220–224] (Table 2) have demonstrated short-term beneficial effects of metformin in PCOS adolescents who were mostly overweight or obese. There are only 2 small

Table 2. Medications used in the treatment of polycystic ovary syndrome in adolescent girls

Medication	Mechanism(s) of action	Dosage	Side effects	Contraindications
Estroprogestagen OCP	Inhibition of ovarian androgen secretion and increase in hepatic SHBG production, resulting in less circulating free androgens	21 out of 28 days/month	Breast tenderness, headache, increased risk of venous thromboembolism, tend to increase insulin resistance	Pregnancy, uncontrolled hypertension, liver dysfunction, complicated valvular heart disease, migraines with aura or focal neurologic symptoms, thromboembolism, diabetes complications, organ transplantation
Metformin	Upregulation of the energy sensors STK11 and AMPK Improvement of insulin sensitivity in muscle and adipose tissue Downregulation of hepatic gluconeogenesis (improves fasting blood glucose) Increase of GLP-1 secretion and GLP-1 receptor expression (improves postprandial blood glucose) Decrease of ovarian and adrenal androgen production	850 mg/day up to 1 g b.i.d.	Gastrointestinal discomfort[1], lactic acidosis[2]	Renal and liver dysfunction, surgery, use of contrast agents, heart failure, alcoholism, metabolic acidosis, dehydration, hypoxemia
Pioglitazone	Peroxisome proliferator-activated receptor-γ activator At low dose, inhibition of CDK5-mediated phosphorylation of peroxisome proliferator-activated receptor-γ	7.5 mg/day up to 30 mg/day	Weight gain (higher doses), bladder cancer risk inconclusive results; studies include only male diabetic patients >40 years, risk with cumulative doses >28,000 mg	Pregnancy, liver dysfunction, bladder cancer
Flutamide	Androgen receptor blockade	62.5 mg/day up to 250 mg/day	Dose-dependent hepatotoxicity Absent at doses of 1 mg/kg/day Feminization of male fetuses	Pregnancy, renal and liver dysfunction
Spironolactone	Aldosterone antagonism Androgen receptor blockade	50–200 mg/day	Mostly dose-dependent: irregular menstrual bleeding, headache, hypotension, nausea, decreased libido, feminization of male fetuses	Pregnancy, renal failure, hyperkalemia
Cyproterone acetate	Competition with dihydrotestosterone at receptor level Inhibition of 5α-reductase, prevents conversion of testosterone to dihydrotestosterone	50–100 mg/day Combined with OCP 2 mg/day	Liver toxicity, irregular menstrual bleeding, nausea, decreased libido, feminization of male fetuses	Pregnancy, renal and liver dysfunction
Finasteride	Inhibition of 5α-reductase, prevents conversion of testosterone to dihydrotestosterone	1–5 mg/day	Feminization of male fetuses, liver dysfunction (rare)	Pregnancy

OCP, oral contraceptive pill; SHBG, sex hormone-binding globulin; STK11, serine/threonine protein kinase; AMPK, adenosine monophosphate-activated protein kinase; b.i.d., bis in die. [1] Gradually increasing doses minimizes the appearance of gastrointestinal symptoms. [2] Older patients with type 2 diabetes and renal failure.

observational studies in non-obese PCOS adolescents with hyperinsulinemia showing improvement in ovulation and testosterone levels with doses as low as 850 mg/day [225, 226]. Most studies failed to accurately report side effects and adherence to interventions. Overall, metformin was associated with gastrointestinal discomfort, but no serious adverse effects have been reported.

A recent meta-analysis of metformin versus oral contraceptive pills (OCP) including 4 RCTs [202, 221, 224] and a total of 170 adolescents showed that metformin and OCP had similar benefits on hirsutism, triglycerides, and HDL cholesterol. Metformin was accompanied by a greater improvement of BMI, while the use of OCP was associated with improvement in menstrual regularity (modest) and acne (mild). The conclusion was that these

estimates were derived from low-quality evidence involving small studies and that further research is required [227].

2.2 Anti-Androgens

Two types of anti-androgens are used in the management of PCOS: androgen receptor blockers like spironolactone, flutamide, and the third generation progestin, cyproterone acetate, and inhibitors of 5-alpha reductase such as finasteride, which prevents the conversion of testosterone to DHT. In adolescents with PCOS, direct comparisons of the various anti-androgens or RCTs are not available [228, 229]. Spironolactone is the most commonly used because of its availability and safety profile, with an initial dose of 25 mg/day gradually increasing up to 200 mg/day. At initiation, spironolactone may be associated with transient menstrual irregularity or spotting, breast tenderness, and occasionally fatigue or orthostasis from volume depletion. Flutamide is not available in some countries and is used sparingly because of concerns regarding its potential hepatotoxicity at high doses (>250 mg/day). Evidence indicates that 1 mg/kg/day is effective and not hepatotoxic, even with extended use [230]. Data on efficacy of spironolactone compared to flutamide are limited, and the methodological quality of the studies is low [231]. Anti-androgens significantly reduce hirsutism compared with placebo [232] and normalize menstrual cyclicity and endocrine-metabolic variables better than monotherapy with metformin [231]. The efficacy is enhanced when combined with OCP, metformin, or other anti-androgens [231–234]. In sexually active adolescents, anti-androgens should only be used when adequate contraceptive measures are ensured, to avoid incomplete virilization of male fetuses.

2.3. Oral Contraceptive Pills

Combination OCP containing an estrogen component (typically ethinylestradiol) and a progestin component address multiple concerns in adolescents with PCOS. An increase in SHBG and decreased LH release due to the estrogen component leads to a decreased free androgen index, and the progestin component allows for suppression of endometrial proliferation and regular withdrawal bleeding. As such, there is improvement in acne and hirsutism and reduction in menstrual irregularity with OCP. Unfortunately, there are few RCTs comparing the relative efficacy or metabolic impact of the different formulations of hormonal contraceptives in adolescents. An RCT comparing the progestins desogestrel and cyproterone acetate in combination with ethinylestradiol found equal improvements in hirsutism, but total and LDL cholesterol were increased by both formulations [235]. Additionally, there was evidence for worsening of HOMA-IR and fasting glycemia with both preparations [236]. Metabolic changes overall, however, did not result in significant concentrations outside the normal ranges. In young women with PCOS (aged 20–25 years) treated with an OCP containing drospirenone versus a combined contraceptive vaginal ring, an RCT suggested that both methods worsened the lipid profile, but OCP significantly worsened triglycerides while remaining within the normal range [237]. In adult women, an RCT involving OCP with 3 different progestins (desogestrel, drospirenone, and cyproterone acetate) showed identical metabolic impact [238]. Overall, high-quality RCTs of specific OCP formulations for adolescents with PCOS are lacking to fully inform decision-making in this population; no specific formulation can be recommended over another.

2.4. Combination Treatments

Combination treatments under development for PCOS in adolescent girls aim at improving the function of multiple pathways and at obtaining additive/synergistic actions that lead collectively to a profile with high benefit and low risk. Lifestyle improvement is the baseline treatment for most adolescent girls with PCOS, particularly if overweight or obese (see Lifestyle Intervention section C.1.1.). In most adolescents with PCOS, the addition of an OCP will be followed by a reduction of PCOS symptoms via normalization of circulating free androgens (primarily due to increased circulating SHBG concentrations) and via pseudo-normalization of the menstrual pattern within a state of anovulatory infertility (see Oral Contraceptive Pills section C.2.3.).

Slower reductions of PCOS symptoms can be obtained with combinations of insulin-sensitizing and anti-androgenic generics, the most promising low-dose combination nowadays perhaps being that of metformin (850 mg/day), spironolactone (50 mg/day), and pioglitazone (7.5 mg/day) [239]. This triple combination appears to normalize cardiovascular risk and body composition more than combinations of only metformin and an anti-androgen [54, 234] and to result in a more favorable post-treatment pattern of circulating androgens and ovulation rates than oral contraceptive intake [239].

3. Reproductive Aspects

3.1. Ovulation

Ovulation may occur in about 10% of adult women with PCOS. The frequency of ovulation in adolescent

PCOS is unknown. In normal puberty, menarche is followed by an interval of anovulatory bleeding of variable length. During this interval, synchronization of hypothalamic-pituitary-ovarian activity takes place that leads to ovulation and regular menstrual cycles. In adolescent PCOS, there is persisting anovulation in most but not all individuals. Notably, girls with premature pubarche (PP) that are at risk for PCOS may exhibit ovulatory frequency (25%) during early postmenarche (1–3 years), which is indistinguishable from non-PP individuals [240]. Beyond 3 years after menarche, the ovulatory rate in PP was reduced. It was also noted that some early postmenarchal adolescents (<3 years) with irregular menstruation and elevated androgen levels followed for 3 years developed regular ovulatory cycles [182]. These findings suggest that in some adolescents with or at risk for PCOS, normal ovulatory function may exist or emerge with time and present as ovulatory adolescent PCOS.

3.2 Contraception

There is no evidence to suggest a decreased pregnancy risk in adolescents with PCOS compared to that of adult women with PCOS. Given that ovulation may occur spontaneously despite a pattern of chronic menstrual irregularity, contraceptive decision-making is important in sexually active adolescents with this disorder. Menstrual irregularity and menorrhagia in adolescents with PCOS can be difficult to manage without hormonal intervention. Accordingly, OCP are recommended as a first-line therapy for adolescents with PCOS consistent with published guidelines [134]. The anti-androgenic properties of OCP and the benefit of menstrual control make them an excellent contraceptive choice in young women with PCOS. Medical contraindications for the use of OCP are outlined in the 2010 Center for Disease Control guidelines [241]. The potential metabolic impact of OCP in PCOS is outlined in the above section (see Oral Contraceptives section C.2.3.). The use of progestin-only contraception, such as depot medroxyprogesterone acetate, is associated with weight gain in adolescents and possibly bone loss, although this is recoverable [242, 243]. Progestin-only therapy does not raise SHBG as do OCP containing ethinylestradiol. The progestin-only intrauterine device may be an alternative first-line therapy given the low systemic impact and overall high contraceptive effectiveness [244]. Overall, there is a lack of high-quality RCTs of contraceptive treatment options for adolescents with PCOS to fully inform decision-making in this population.

4. Transition

Management of girls with PCOS should focus on appropriate diagnosis, reduction of symptoms in adolescence, and improvement of post-treatment health in adulthood. Specific therapeutic goals include attenuation of pregestational oligo-anovulation (thus the need for assisted reproduction) and reduction of gestational complications such as diabetes mellitus, preeclampsia, and preterm delivery [245]. Given the apparent role of hepato-visceral fat excess in the pathogenesis of anovulatory androgen excess [246, 247], PCOS therapy in adolescence should also aim at reducing hepato-visceral adiposity via lifestyle measures leading to weight loss in obese girls (see Lifestyle Intervention section C.1.1.) and via pharmacological measures. These approaches would enhance the preferential loss of central fat in non-obese girls with a low subcutaneous fat storage capacity, such as girls with a lipodystrophy, girls with ethnic backgrounds associated with a high risk of developing diabetes, and girls with a history of prenatal growth restraint [54]. The more low-risk and/or low-cost interventions for PCOS during adolescence, the fewer high-risk and/or high-cost treatments will be needed during adulthood, and the better the outlook will be for the offspring of PCOS mothers.

C. Treatment: Conclusions with Level of Evidence

1. Baseline Treatments

1.1. Lifestyle Intervention

- Lifestyle intervention should be based on the combination of calorie-restricted diets, behavioral treatment, and exercise (Level A).
- Combined weight loss and physical exercise are the first-line therapy in overweight and obese girls (Level C). They decrease androgen levels, normalize menstrual cycles (Level A), and improve markers of cardiometabolic health (Level B).
- Extremely obese adolescents respond poorly to lifestyle intervention (Level B).
- In normal-weight girls, increasing physical activity is effective in reducing the development of metabolic syndrome (Level C). However, the benefits of exclusive weight loss in these adolescents are not supported by RCTs (Level C).

1.2 Local Therapies/Cosmetic

- Photoepilation is the first-line management of localized hirsutism in PCOS (Level B). Diode and alexandrite la-

sers are preferred (Level C). The alexandrite laser is superior to IPL methods in facial hirsutism (Level B).
- Topical eflornithine is recommended as an adjuvant to photoepilation in girls with laser-resistant facial hirsutism aged 16 years or older, or as monotherapy in those where photoepilation is not indicated (Level A).
- The use of topical finasteride is not recommended based on existing data (Level C).

2. Additive Pharmaceuticals

2.1 Metformin
- Metformin has beneficial effects in overweight or obese adolescents with PCOS, but only short-term data are available (Level A).
- In non-obese adolescents with PCOS and hyperinsulinemia, metformin improves ovulation and testosterone levels (Level B).

2.2. Anti-Androgens
- Anti-androgens reduce androgen excess features more than metformin in monotherapy (Level B). Spironolactone is the most commonly used albeit data on efficacy compared to flutamide are limited (Level C).
- Anti-androgens should only be used when contraceptive measures are guaranteed.

2.3. Oral Contraceptive Pills
- There are no high-quality RCTs of specific OCP formulations for adolescents with PCOS to help decision-making in this population, and no specific formulation can be recommended over another (Level B).

2.4. Combination Treatments
- Where available, triple low-dose combinations of insulin-sensitizing and anti-androgenic generics normalize cardiovascular risk and body composition more than combinations of only metformin and an anti-androgen and result in a more favorable post-treatment pattern of circulating androgens and ovulation rates than OCP intake (Level A).

3. Reproductive Aspects
- In some adolescents with or at risk for PCOS, normal ovulatory function may exist or emerge with time and present as ovulatory adolescent PCOS (Level A).

4. Transition
- PCOS therapy in adolescence should aim at decreasing hepato-visceral adiposity, enhancing central fat loss, and thus, attenuating pregestational oligo-anovulation, and reducing gestational complications such as diabetes mellitus, preeclampsia, and preterm delivery (Level B).

Conclusion

This first global update on the pathophysiology, diagnosis, and treatment of adolescent PCOS is the outcome of an international collaborative effort initiated by Pediatric Endocrine Societies.

One aim of this update was to offer a more developmental perspective than previous reports on adolescent PCOS. The authors have attempted to merge many opinions on much evidence, and they realize that there may be apparent inconsistencies between consecutive sections. Hence, this report discloses the many uncertainties and knowledge gaps persisting at the time of writing.

A second aim of this initiative was to document the main directions of past, present, and future investigations into adolescent PCOS. In the past, the keywords for pathogenesis, diagnosis, and treatment may have been, respectively, ovarian and adrenal steroidogenesis, IR and LH hypersecretion; hirsutism and menstrual pattern; cosmetics and oral contraceptives. Current focuses have shifted to include (epi)genetics and body adiposity; androgens (by LC-MS/MS) and ovulatory function; lifestyle measures, insulin sensitization and anti-androgens. In the near future, the keywords are expected to include ectopic lipids and microbiome; miRNAs, metabolomics, and adipo-, hepato-, myo-, and osteo-kines. Treatment will aim at a slow but steady return to an overall healthy state with combination therapies that may vary over time and allow for spontaneous ovulations, uncomplicated pregnancies, and healthy offspring.

Finally and most importantly, this global update should contribute to improvement of the care worldwide for adolescent girls with PCOS.

Appendix

S.E.O., S.F.W., and P.A.L. are members of the Pediatric Endocrine Society (PES); L.I., S.F.W., F.D., A.G., A.L.-B., K.O., T.R., N.S., F.Z., and P.A.L. are members of the European Society for Paediatric Endocrinology (ESPE); C.G.R. and A.S.P. are members of the Australasian Paediatric Endocrine Group (APEG); P.D. is a member of the Asia Pacific Paediatric Endocrine Society (APPES); D.J. is a member of the African Society for Paediatric and Adolescent Endocrinology (ASPAE); Xiao-Ping Luo is a member of the Chinese Society of Pediatric Endocrinology and Metabolism (CSPEM); R.H. is a mem-

ber of the Japanese Society for Pediatric Endocrinology (JSPE); E.C. is a member of the Sociedad Latinoamericana de Endocrinología Pediátrica (SLEP); N.S.E., H.A., and A.D. are members of the Arab Society of Paediatric Endocrinology and Diabetes (ASPED); P.D. is a member of the Indian Society for Pediatric and Adolescent Endocrinology (ISPAE); S.F.W., S.E.O., R.J.C., and K.M.H. are members of The Androgen Excess and PCOS Society (AE-PCOS); and R.J.A., and M.T.-S. are members of the Endocrine Society (ES).

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