

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

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Keywords

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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

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.

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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 treatment¹. 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.

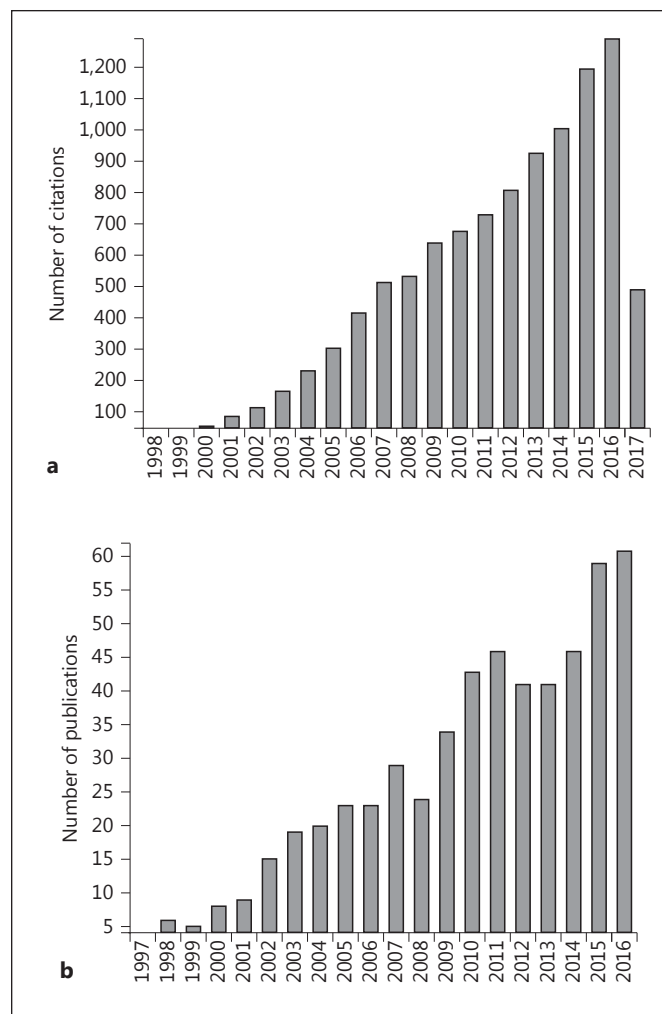


Fig. 1. a Annual number of citations for “adolescent PCOS” over the past 2 decades. **b** Annual number of publications for “adolescent PCOS” over the past 2 decades. Web of Science, Thomson Reuters, 2017.

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

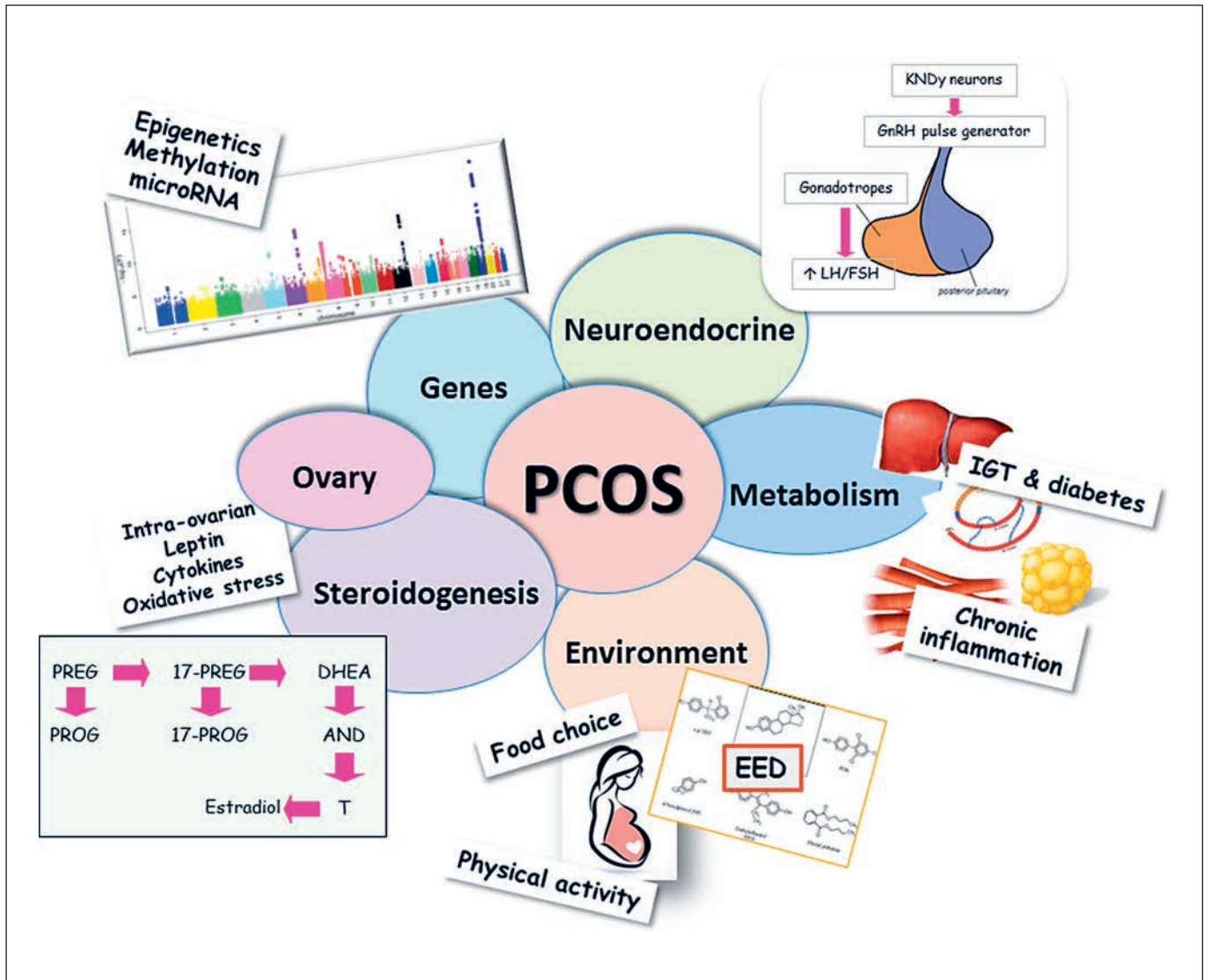


Fig. 2. Potential factors involved in pathophysiology of PCOS. Alterations in steroidogenesis, ovarian folliculogenesis, neuroendocrine function, metabolism, insulin secretion, insulin sensitivity, adipose cell function, inflammatory factors, and sympathetic nerve function contribute to the pathogenesis 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) con-

centrations 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 11 β -hydroxyandrostenedione, 11-ketoandrostenedione, 11 β -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]. Ec-

topic 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 (ER β), 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 preclinical

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 co-workers 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 3 α - and 3 β -androstane diols, 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 as 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 ad-

ipose 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 expres-

sion 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 (*CYP11B1*), 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 (*DMAPI1*) [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	1. PCOM	1. Obesity	1. Must generally be 2 years post-menarche
2. Evidence of hyperandrogenism:	2. Severe cystic acne	2. Insulin resistance	2. Must rule out other disorders of hyperandrogenism (e.g., NC-CAH, Cushing syndrome)
a. Biochemical		3. Hyperinsulinemia	
b. Clinical (e.g., progressive hirsutism)		4. Biomarkers (e.g., AMH, T/DHT ratio)	
		5. Acanthosis nigricans	

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]. In-

deed, 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 chromatography 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]. How-

ever, 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 5 α -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
Spirolactone	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 5 α -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 5 α -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 im-

provements 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 (CSPM); 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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References

- Azziz R, Dumesic DA, Goodarzi MO: Polycystic ovary syndrome: an ancient disorder? *Fertil Steril* 2011;95:1544–1548.
- Unluturk U, Sezgin E, Yildiz BO: Evolutionary determinants of polycystic ovary syndrome: part 1. *Fertil Steril* 2016;106:33–41.
- Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, Lizneva D, Natterson-Horowitz B, Teede HJ, Yildiz BO: Polycystic ovary syndrome. *Nat Rev Dis Primers* 2016;2:16057.
- Oberfield SE, Sopher AB, Gerken AT: Approach to the girl with early onset of pubic hair. *J Clin Endocrinol Metab* 2011;96:1610–1622.
- Chang AY, Abdullah SM, Jain T, Stanek HG, Das SR, McGuire DK, Auchus RJ, de Lemos JA: Associations among androgens, estrogens, and natriuretic peptides in young women: observations from the Dallas Heart Study. *J Am Coll Cardiol* 2007;49:109–116.
- Hsueh AJ, Kawamura K, Cheng Y, Fauser BC: Intraovarian control of early folliculogenesis. *Endocr Rev* 2015;36:1–24.
- Franks S, Stark J, Hardy K: Follicle dynamics and anovulation in polycystic ovary syndrome. *Hum Reprod Update* 2008;14:367–378.
- Lebbe M, Woodruff TK: Involvement of androgens in ovarian health and disease. *Mol Hum Reprod* 2013;19:828–837.
- Webber LJ, Stubbs S, Stark J, Trew GH, Margara R, Hardy K, Franks S: Formation and early development of follicles in the polycystic ovary. *Lancet* 2003;362:1017–1021.
- Nelson VL, Legro RS, Strauss JF 3rd, McAllister JM: Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. *Mol Endocrinol* 1999;13:946–957.
- Marti N, Galván JA, Pandey AV, Trippel M, Tapia C, Müller M, Perren A, Flück CE: Genes and proteins of the alternative steroid backdoor pathway for dihydrotestosterone synthesis are expressed in the human ovary and seem enhanced in the polycystic ovary syndrome. *Mol Cell Endocrinol* 2017;441:116–123.
- McAllister JM, Modi B, Miller BA, Biegler J, Bruggeman R, Legro RS, Strauss JF 3rd: Overexpression of a DENND1A isoform produces a polycystic ovary syndrome theca phenotype. *Proc Natl Acad Sci USA* 2014;111:E1519–E1527.
- Tee MK, Speek M, Legeza B, Modi B, Teves ME, McAllister JM, Strauss JF 3rd, Miller WL: Alternative splicing of DENND1A, a PCOS candidate gene, generates variant 2. *Mol Cell Endocrinol* 2016;434:25–35.
- Turcu A, Smith JM, Auchus R, Rainey WE: Adrenal androgens and androgen precursors—definition, synthesis, regulation and physiologic actions. *Compr Physiol* 2014;4:1369–1381.
- O'Reilly MW, Kempegowda P, Jenkinson C, Taylor AE, Quanson JL, Storbeck KH, Arlt W: 11-oxygenated C19 steroids are the predominant androgens in polycystic ovary syndrome. *J Clin Endocrinol Metab* 2017;102:840–848.
- Dunaif A, Segal KR, Shelley DR, Green G, Dobrjansky A, Licholai T: Evidence for distinctive and intrinsic defects in insulin action in polycystic ovary syndrome. *Diabetes* 1992;41:1257–1266.
- Dunaif A, Graf M, Mandeli J, Laumas V, Dobrjansky A: Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperinsulinemia. *J Clin Endocrinol Metab* 1987;65:499–507.
- Diamanti-Kandarakis E, Dunaif A: Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocr Rev* 2012;33:981–1030.
- Moran A, Jacobs DR Jr, Steinberger J, Hong CP, Prineas R, Luepker R, Sinaiko AR: Insulin resistance during puberty: results from clamp studies in 357 children. *Diabetes* 1999;48:2039–2044.
- Amiel SA, Sherwin RS, Simonson DC, Lauritano AA, Tamborlane WV: Impaired insulin action in puberty. A contributing factor to poor glycemic control in adolescents with diabetes. *N Engl J Med* 1986;315:215–219.
- Ball GD, Huang TT, Gower BA, Cruz ML, Shaibi GQ, Weigensberg MJ, Goran MI: Longitudinal changes in insulin sensitivity, insulin secretion, and beta-cell function during puberty. *J Pediatr* 2006;148:16–22.
- Saenger P: Metabolic consequences of growth hormone treatment in paediatric practice. *Horm Res* 2000;53(suppl 1):60–69.
- Geffner ME, Golde DW: Selective insulin action on skin, ovary, and heart in insulin-resistant states. *Diabetes Care* 1988;11:500–505.
- Stepito NK, Cassar S, Joham AE, Hutchison SK, Harrison CL, Goldstein RF, Teede HJ: Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulinaemic clamp. *Hum Reprod* 2013;28:777–784.
- Morciano A, Romani F, Sagnella F, Scarinci E, Palla C, Moro F, Tropea A, Policola C, Della Casa S, Guido M, Lanzone A, Apa R: Assessment of insulin resistance in lean women with polycystic ovary syndrome. *Fertil Steril* 2014;102:250–256.
- Willis D, Franks S: Insulin action in human granulosa cells from normal and polycystic ovaries is mediated by the insulin receptor and not the type-I insulin-like growth factor receptor. *J Clin Endocrinol Metab* 1995;80:3788–3790.
- Nestler JE, Powers LP, Matt DW, Steingold KA, Plymate SR, Rittmaster RS, Clore JN, Blackard WG: A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome. *J Clin Endocrinol Metab* 1991;72:83–89.
- Adashi EY, Hsueh AJW, Yen SSC: Insulin enhancement of luteinizing hormone and follicle-stimulating hormone release by cultured pituitary cells. *Endocrinology* 1981;108:1441–1449.
- Torchen LC, Fogel NR, Brickman WJ, Papanodis R, Dunaif A: Persistent apparent pancreatic β -cell defects in premenarchal PCOS relatives. *J Clin Endocrinol Metab* 2014;99:3855–3862.
- Taylor SI, Cama A, Accili D, Barbetti F, Quon MJ, de la Luz Sierra M, Suzuki Y, Koller E, Levy-Toledano R, Wertheimer E, Moncada VY, Kadowaki H, Kadowaki T: Mutations in the insulin receptor gene. *Endocr Rev* 1992;13:566–595.
- Moller DE, Flier JS: Insulin resistance – mechanisms, syndromes, and implications. *N Engl J Med* 1991;325:938–948.
- Corbould A: Effects of androgens on insulin action in women: is androgen excess a component of female metabolic syndrome? *Diabetes Metab Res Rev* 2008;24:520–532.
- Soldani R, Cagnacci A, Yen SSC: Insulin-like growth factor I (IGF-I) and IGF-II enhance basal and gonadotrophin-releasing hormone-stimulated luteinizing hormone release from rat anterior pituitary cells in vitro. *Eur J Endocrinol* 1994;131:641–645.

- 34 O'Connor A, Phelan N, Tun TK, Boran G, Gibney J, Roche HM: High-molecular-weight adiponectin is selectively reduced in women with polycystic ovary syndrome independent of body mass index and severity of insulin resistance. *J Clin Endocrinol Metab* 2010;95:1378–1385.
- 35 O'Reilly M, Gathercole L, Capper F, Arlt W, Tomlinson J: Effect of insulin on AKR1C3 expression in female adipose tissue: in-vivo and in-vitro study of adipose androgen generation in polycystic ovary syndrome. *Lancet* 2015; 385(suppl 1):S16.
- 36 Lim SS, Davies MJ, Norman RJ, Moran LJ: Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod Update* 2012;18:618–637.
- 37 Hoeger KM: Obesity and lifestyle management in polycystic ovary syndrome. *Clin Obstet Gynecol* 2007;50:277–294.
- 38 Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, Savoye M, Rieger V, Tak-sali S, Barbetta G, Sherwin RS, Caprio S: Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. *N Engl J Med* 2002;346:802–810.
- 39 Hughan KS, Tfayli H, Warren-Ulanch JG, Barinas-Mitchell E, Arslanian SA: Early biomarkers of subclinical atherosclerosis in obese adolescent girls with polycystic ovary syndrome. *J Pediatr* 2016;168:104–111.
- 40 Puder JJ: Central fat excess in polycystic ovary syndrome: relation to low-grade inflammation and insulin resistance. *J Clin Endocrinol Metab* 2005;90:6014–6021.
- 41 Pasquali R, Casimirri F, Venturoli S, Antonio M, Morselli L, Reho S, Pezzoli A, Paradisi R: Body fat distribution has weight-independent effects on clinical, hormonal, and metabolic features of women with polycystic ovary syndrome. *Metabolism* 1994;43:706–713.
- 42 Ojeda-Ojeda M, Murri M, Insenser M, Escobar-Morreale HF: Mediators of low-grade chronic inflammation in polycystic ovary syndrome (PCOS). *Curr Pharm Des* 2013;19: 5775–5791.
- 43 Lambert EA, Teede H, Sari CI, Jona E, Shor-akae S, Woodington K, Hemmes R, Eikelis N, Straznicki NE, De Courten B, Dixon JB, Schlaich MP, Lambert GW: Sympathetic activation and endothelial dysfunction in polycystic ovary syndrome are not explained by either obesity or insulin resistance. *Clin Endocrinol (Oxf)* 2015;83:812–819.
- 44 de Zegher F, López-Bermejo A, Ibáñez L: Adipose tissue expandability and the early origins of PCOS. *Trends Endocrinol Metab* 2009;20:418–423.
- 45 de Zegher F, Reinher T, Malpique R, Darendeliler F, López-Bermejo A, Ibáñez L: Reduced prenatal weight gain and/or augmented postnatal weight gain precede polycystic ovary syndrome in adolescent girls. *Obesity (Silver Spring)* 2017;25:1486–1489. DOI: 10.1002/oby.21935.
- 46 Escobar-Morreale HF, Luque-Ramírez M, González F: Circulating inflammatory markers in polycystic ovary syndrome: a systematic review and metaanalysis. *Fertil Steril* 2011;95:1048–1058.
- 47 Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boeing H, Pfeiffer AF: Inflammatory cytokines and the risk to develop type 2 diabetes: Results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* 2003;52:812–817.
- 48 Ciaraldi TP, Carter L, Nikoulina S, Mudaliar S, McClain DA, Henry RR: Glucosamine regulation of glucose metabolism in cultured human skeletal muscle cells: divergent effects on glucose transport/phosphorylation and glycogen synthase in non-diabetic and type 2 diabetic subjects. *Endocrinology* 1999;140: 3971–380.
- 49 Previs SF, Withers DJ, Ren JM, White MF, Shulman GI: Contrasting effects of IRS-1 versus IRS-2 gene disruption on carbohydrate and lipid metabolism. *J Biol Chem* 2000;275: 38990–38994.
- 50 Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB 3rd, Kaestner KH, Bartolomei MS, Shulman GI, Birnbaum MJ: Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKBb). *Science* 2001;292:1728–1731.
- 51 Carpentier AC: Postprandial fatty acid metabolism in the development of lipotoxicity and type 2 diabetes. *Diabetes Metab* 2008;34: 97–107.
- 52 Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R: Obesity and the polycystic ovary syndrome. *Int J Obes Relat Metab Disord* 2002;26:883–96.
- 53 Lord J, Wilkin T: Polycystic ovary syndrome and fat distribution: the central issue? *Hum Fertil (Camb)* 2002;5:67–71.
- 54 Ibáñez L, Ong KK, López-Bermejo A, Dunger DB, de Zegher F: Hyperinsulinaemic androgen excess in adolescent girls. *Nat Rev Endocrinol* 2014;10:499–508.
- 55 Dhindsa G, Bhatia R, Dhindsa M, Bhatia V: Insulin resistance, insulin sensitization and inflammation in polycystic ovarian syndrome inflammation in polycystic ovarian syndrome inflammation in polycystic ovarian syndrome. *J Postgrad Med* 2004;50:140–144.
- 56 Adams J, Liu Z, Ren YA, Wun WS, Zhou W, Kenigsberg S, Librach C, Valdes C, Gibbons W, Richards J: Enhanced inflammatory transcriptome in the granulosa cells of women with polycystic ovarian syndrome. *J Clin Endocrinol Metab* 2016;101:3459–3468.
- 57 Schmidt J, Weijdegard B, Mikkelsen AL, Lindenberg S, Nilsson L, Brannstrom M: Differential expression of inflammation-related genes in the ovarian stroma and granulosa cells of PCOS women. *Mol Hum Reprod* 2014;20:49–58.
- 58 Jornayvaz FR, Shulman GI: Diacylglycerol activation of protein kinase C ϵ and hepatic insulin resistance. *Cell Metab* 2012;15:574–584.
- 59 Badin PM, Langin D, Moro C: Dynamics of skeletal muscle lipid pools. *Trends Endocrinol Metab* 2013;24:607–615.
- 60 Chavez JA, Summers SA: A ceramide-centric view of insulin resistance. *Cell Metab* 2012;15: 585–594.
- 61 Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, Klein R, Krone W, Muller-Wieland D, Kahn CR: Role of brain insulin receptor in control of body weight and reproduction. *Science* 2000;289:2122–2125.
- 62 Fauser BC, Tarlatzis BC, Rebar RW, Legro RS, Balen AH, Lobo R, Carmina E, Chang J, Yildiz BO, Laven JS, Boivin J, Petraglia F, Wijeyeratne CN, Norman RJ, Dunaif A, Franks S, Wild RA, Dumesic D, Barnhart K: Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril* 2012;97:28–38.e25.
- 63 Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS: Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. *Endocr Rev* 2015;36:487–525.
- 64 Rosenfield RL, Ehrmann DA: The pathogenesis of polycystic ovary syndrome (PCOS): the hypothesis of PCOS as functional ovarian hyperandrogenism revisited. *Endocr Rev* 2016; 37:467–520.
- 65 Taylor AE, McCourt B, Martin KA, Anderson EJ, Adams JM, Schoenfeld D, Hall JE: Determinants of abnormal gonadotropin secretion in clinically defined women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 1997;82 2248–56.
- 66 Moenter SM: Leap of faith: does serum luteinizing hormone always accurately reflect central reproductive neuroendocrine activity? *Neuroendocrinology* 2015;102:256–266.
- 67 Thompson IR, Kaiser UB: GnRH pulse frequency-dependent differential regulation of LH and FSH gene expression. *Mol Cell Endocrinol* 2014;385:28–35.
- 68 Moore AM, Campbell RE: The neuroendocrine genesis of polycystic ovary syndrome: a role for arcuate nucleus GABA neurons. *J Steroid Biochem Mol Biol* 2016;160:106–117.
- 69 Eagleson CA, Gingrich MB, Pastor CL, Arora TK, Burt CM, Evans WS, Marshall JC: Polycystic ovarian syndrome: evidence that flutamide restores sensitivity of the gonadotropin-releasing hormone pulse generator to inhibition by estradiol and progesterone. *J Clin Endocrinol Metab* 2000;85:4047–52.
- 70 Pinilla L, Aguilar E, Dieguez C, Millar RP, Tena-Sempere M: Kisspeptins and reproduction: physiological roles and regulatory mechanisms. *Physiol Rev* 2012;92:1235–316.
- 71 Oakley AE, Clifton DK, Steiner RA: Kisspeptin signaling in the brain. *Endocr Rev* 2009;30:713–43.
- 72 Navarro VM, Tena-Sempere M: Neuroendocrine control by kisspeptins: role in metabolic regulation of fertility. *Nat Rev Endocrinol* 2012;8:40–53.

- 73 Lehman MN, Coolen LM, Goodman RL: Minireview: kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: a central node in the control of gonadotropin-releasing hormone secretion. *Endocrinology* 2010;151:3479–3489.
- 74 Brown RE, Wilkinson DA, Imran SA, Caraty A, Wilkinson M: Hypothalamic kiss1 mRNA and kisspeptin immunoreactivity are reduced in a rat model of polycystic ovary syndrome (PCOS). *Brain Res* 2012;1467:1–9.
- 75 Navarro VM, Sánchez-Garrido MA, Castellano JM, Roa J, García-Galiano D, Pineda R, Aguilar E, Pinilla L, Tena-Sempere M: Persistent impairment of hypothalamic KiSS-1 system after exposures to estrogenic compounds at critical periods of brain sex differentiation. *Endocrinology* 2009;150:2359–2367.
- 76 Fraser GL, Hoveyda HR, Clarke IJ, Ramaswamy S, Plant TM, Rose C, Millar RP: The NK3 receptor antagonist ESN364 interrupts pulsatile LH secretion and moderates levels of ovarian hormones throughout the menstrual cycle. *Endocrinology* 2015;156:4214–4225.
- 77 George JT, Kakkar R, Marshall J, Scott ML, Finkelman RD, Ho TW, Veldhuis J, Skorupskaite K, Anderson RA, McIntosh S, Webber L: Neurokinin B receptor antagonism in women with polycystic ovary syndrome: a randomized, placebo-controlled trial. *J Clin Endocrinol Metab* 2016;101:4313–4321.
- 78 Narayanaswamy S, Prague JK, Jayasena CN, Papadopoulou DA, Mizamtsidi M, Shah AJ, Bassett P, Comminos AN, Abbara A, Bloom SR, Veldhuis JD, Dhillon WS: Investigating the KNDy hypothesis in humans by coadministration of kisspeptin, neurokinin B, and naltrexone in men. *J Clin Endocrinol Metab* 2016;101:3429–3436.
- 79 Ruiz-Pino F, García-Galiano D, Manfredi-Lozano M, Leon S, Sánchez-Garrido MA, Roa J, Pinilla L, Navarro VM, Tena-Sempere M: Effects and interactions of tachykinins and dynorphin on FSH and LH secretion in developing and adult rats. *Endocrinology* 2015;156:576–588.
- 80 García-Galiano D, Pineda R, Roa J, Ruiz-Pino F, Sánchez-Garrido MA, Castellano JM, Aguilar E, Navarro VM, Pinilla L, Tena-Sempere M: Differential modulation of gonadotropin responses to kisspeptin by aminoacidic, peptidergic, and nitric oxide neurotransmission. *Am J Physiol Endocrinol Metab* 2012;303:E1252–E1263.
- 81 Moore AM, Prescott M, Marshall CJ, Yip SH, Campbell RE: Enhancement of a robust arcuate GABAergic input to gonadotropin-releasing hormone neurons in a model of polycystic ovarian syndrome. *Proc Natl Acad Sci USA* 2015;112:596–601.
- 82 Herbison AE, Moenter SM: Depolarising and hyperpolarising actions of GABA(A) receptor activation on gonadotropin-releasing hormone neurones: towards an emerging consensus. *J Neuroendocrinol* 2011;23:557–569.
- 83 Cimino I, Casoni F, Liu X, Messina A, Parkash J, Jamin SP, Catteau-Jonard S, Collier F, Baroncini M, Dewailly D, Pigny P, Prescott M, Campbell R, Herbison AE, Prevot V, Giacobini P: Novel role for anti-Müllerian hormone in the regulation of GnRH neuron excitability and hormone secretion. *Nat Commun* 2016;7:10055.
- 84 Moret M, Stettler R, Rodieux F, Gaillard RC, Waeber G, Wirthner D, Giusti V, Tappy L, Pralong FP: Insulin modulation of luteinizing hormone secretion in normal female volunteers and lean polycystic ovary syndrome patients. *Neuroendocrinology* 2009;89:131–139.
- 85 Patel K, Coffler MS, Dahan MH, Yoo RY, Lawson MA, Malcom PJ, Chang RJ: Increased luteinizing hormone secretion in women with polycystic ovary syndrome is unaltered by prolonged insulin infusion. *J Clin Endocrinol Metab* 2003;88:5456–5461.
- 86 Divall SA, Williams TR, Carver SE, Koch L, Brüning JC, Kahn CR, Wondisford F, Radovick S, Wolfe A: Divergent roles of growth factors in the GnRH regulation of puberty in mice. *J Clin Invest* 2010;120:2900–2909.
- 87 Cernea M, Phillips R, Padmanabhan V, Coolen LM, Lehman MN: Prenatal testosterone exposure decreases colocalization of insulin receptors in kisspeptin/neurokinin B/dynorphin and agouti-related peptide neurons of the adult ewe. *Eur J Neurosci* 2016;44:2557–2568.
- 88 Qiu X, Dao H, Wang M, Heston A, Garcia KM, Sangal A, Dowling AR, Faulkner LD, Molitor SC, Elias CF, Hill JW: Insulin and leptin signaling interact in the mouse Kiss1 neuron during the peripubertal period. *PLoS One* 2015;10:e0121974.
- 89 Evans MC, Rizwan M, Mayer C, Boehm U, Anderson GM: Evidence that insulin signaling in gonadotrophin-releasing hormone and kisspeptin neurones does not play an essential role in metabolic regulation of fertility in mice. *J Neuroendocrinol* 2014;26:468–479.
- 90 Toulis KA, Goulis DG, Farmakiotis D, Georgopoulos NA, Katsikis I, Tarlatzis BC, Papanicolaou I, Panidis D: Adiponectin levels in women with polycystic ovary syndrome: a systematic review and a meta-analysis. *Hum Reprod Update* 2009;15:297–307.
- 91 Yuan X, Hu T, Zhao H, Huang Y, Ye R, Lin J, Zhang C, Zhang H, Wei G, Zhou H, Dong M, Zhao J, Wang H, Liu Q, Lee HJ, Jin W, Chen ZJ: Brown adipose tissue transplantation ameliorates polycystic ovary syndrome. *Proc Natl Acad Sci USA* 2016;113:2708–2713.
- 92 Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, Li Z, You L, Zhao J, Liu J, Liang X, Zhao X, Zhao J, Sun Y, Zhang B, Jiang H, Zhao D, Bian Y, Gao X, Geng L, Li Y, Zhu D, Sun X, Xu JE, Hao C, Ren CE, Zhang Y, Chen S, Zhang W, Yang A, Yan J, Li Y, Ma J, Zhao Y: Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. *Nat Genet* 2011;43:55–59.
- 93 Shi Y, Zhao H, Shi Y, Cao Y, Yang D, Li Z, Zhang B, Liang X, Li T, Chen J, Shen J, Zhao J, You L, Gao X, Zhu D, Zhao X, Yan Y, Qin Y, Li W, Yan J, Wang Q, Zhao J, Geng L, Ma J, Zhao Y, He G, Zhang A, Zou S, Yang A, Liu J, Li W, Li B, Wan C, Qin Y, Shi J, Yang J, Jiang H, Xu JE, Qi X, Sun Y, Zhang Y, Hao C, Ju X, Zhao D, Ren CE, Li X, Zhang W, Zhang Y, Zhang J, Wu D, Zhang C, He L, Chen ZJ: Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. *Nat Genet* 2012;44:1020–1025.
- 94 Hayes MG, Urbanek M, Ehrmann DA, Armstrong LL, Lee JY, Sisk R, Karaderi T, Barber TM, McCarthy MI, Franks S, Lindgren CM, Welt CK, Diamanti-Kandarakis E, Panidis D, Goodarzi MO, Azziz R, Zhang Y, James RG, Olivier M, Kissebah AH: Reproductive Medicine Network, Stener-Victorin E, Legro RS, Dunaif A: Genome-wide association of polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. *Nat Commun* 2015;18:7502.
- 95 Day FR, Hinds DA, Tung JY, Stolk L, Strykarsdottir U, Saxena R, Bjornnes A, Broer L, Dunger DB, Halldorsson BV, Lawlor DA, Laval G, Mathieson I, McCardle WL, Louwers Y, Meun C, Ring S, Scott RA, Sulem P, Uitterlinden AG, Wareham NJ, Thorsteinsdottir U, Welt C, Stefansson K, Laven JS, Ong KK, Perry JR: Causal mechanisms and balancing selection inferred from genetic associations with polycystic ovary syndrome. *Nat Commun* 2015;6:8464.
- 96 Mbarek H, Steinberg S, Nyholt DR, Gordon SD, Miller MB, McRae AF, Hottenga JJ, Day FR, Willemsen G, de Geus EJ, Davies GE, Martin HC, Penninx BW, Jansen R, McAlooney K, Vink JM, Kaprio J, Plomin R, Spector TD, Magnusson PK, Reversade B, Harris RA, Aagaard K, Kristjansson RP, Olafsson I, Eyjolfsson GI, Sigurdardottir O, Iacono WG, Lambalk CB, Montgomery GW, McGue M, Ong KK, Perry JR, Martin NG, Stefansson H, Stefansson K, Boomsma DI: Identification of common genetic variants influencing spontaneous dizygotic twinning and female fertility. *Am J Hum Genet* 2016;98:898–908.
- 97 Mutharasan P, Galdones E, Peñalver Bernabé B, García OA, Jafari N, Shea LD, Woodruff TK, Legro RS, Dunaif A, Urbanek M: Evidence for chromosome 2p16.3 polycystic ovary syndrome susceptibility locus in affected women of European ancestry. *J Clin Endocrinol Metab* 2013;98:E185–E190.
- 98 Zhu JQ, Zhu L, Liang XW, Xing FQ, Schatten H, Sun QY: Demethylation of LHR in dehydroepiandrosterone-induced mouse model of polycystic ovary syndrome. *Mol Hum Reprod* 2010;16:260–266.
- 99 Wang P, Zhao H, Li T, Zhang W, Wu K, Li M, Bian Y, Liu H, Ning Y, Li G, Chen ZJ: Hypomethylation of the LH/choriogonadotropin receptor promoter region is a potential mechanism underlying susceptibility to polycystic ovary syndrome. *Endocrinology* 2014;155:1445–1452.

- 100 Zhang Y, Fatima N, Dufau ML: Coordinated changes in DNA methylation and histone modifications regulate silencing/derepression of luteinizing hormone receptor gene transcription. *Mol Cell Biol* 2005;25:7929–7939.
- 101 Yu YY, Sun CX, Liu YK, Li Y, Wang L, Zhang W: Promoter methylation of *CYP19A1* gene in Chinese polycystic ovary syndrome patients. *Gynecol Obstet Invest* 2013;76:209–213.
- 102 Sang Q, Li X, Wang H, Wang H, Zhang S, Xu N, Kwon S, Abbott DH, Geller DH, Dumesic DA, Azziz R, Guo X, Goodarzi MO: Quantitative methylation level of the *EPHX1* promoter in peripheral blood DNA is associated with polycystic ovary syndrome. *PLoS One* 2014;9:e88013.
- 103 Qu F, Wang FF, Yin R, Ding GL, El-Prince M, Gao Q, Shi BW, Pan HH, Huang YT, Jin M, Leung PC, Sheng JZ, Huang HF: A molecular mechanism underlying ovarian dysfunction of polycystic ovary syndrome: hyperandrogenism induces epigenetic alterations in the granulosa cells. *J Mol Med* 2012;90:911–923.
- 104 Wang XX, Wei JZ, Jiao J, Jiang SY, Yu DH, Li D: Genome-wide DNA methylation and gene expression patterns provide insight into polycystic ovary syndrome development. *Oncotarget* 2014;5:6603–6610.
- 105 Yu YY, Sun CX, Liu YK, Li Y, Wang L, Zhang W: Genome-wide screen of ovary-specific DNA methylation in polycystic ovary syndrome. *Fertil Steril* 2015;104:145–153.e6.
- 106 Shen HR, Qiu LH, Zhang ZQ, Qin YY, Cao C, Di W: Genome-wide methylated DNA immunoprecipitation analysis of patients with polycystic ovary syndrome. *PLoS One* 2013;8:e64801.
- 107 Li S, Zhu D, Duan H, Ren A, Glinborg D, Andersen M, Skov V, Thomassen M, Kruse T, Tan: Differential DNA methylation patterns of polycystic ovarian syndrome in whole blood of Chinese women. *Oncotarget* 2017;8:20656–20666.
- 108 Kokosar M, Benrick A, Perfilyev A, Fornes R, Nilsson E, Maliqueo M, Behre CJ, Sazonova A, Ohlsson C, Ling C, Stener-Victorin E: Epigenetic and transcriptional alterations in human adipose tissue of polycystic ovary syndrome. *Sci Rep* 2016;6:22883.
- 109 Xu N, Kwon S, Abbott DH, Geller DH, Dumesic DA, Azziz R, Guo X, Goodarzi MO: Epigenetic mechanism underlying the development of polycystic ovary syndrome (PCOS)-like phenotypes in prenatally androgenized rhesus monkeys. *PLoS One* 2011;6:e27286.
- 110 Li S, Zhu D, Duan H, Tan Q: The epigenomics of polycystic ovarian syndrome: from pathogenesis to clinical manifestations. *Gynecol Endocrinol* 2016;32:942–946.
- 111 Jiang L, Huang J, Chen Y, Yang Y, Li R, Li Y, Chen X, Yang D: Identification of several circulating microRNAs from a genome-wide circulating microRNA expression profile as potential biomarkers for impaired glucose metabolism in polycystic ovarian syndrome. *Endocrine* 2016;53:280–90.
- 112 Wu HL, Heneidi S, Chuang TY, Diaond MP, Layman LC, Azziz R, Chen YH: The expression of the miR-25/93/106b family of microRNAs in the adipose tissue of women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 2014;99:E2754–E2761.
- 113 Song J, Luo S, Li SW: miRNA-592 is down-regulated and may target *LHCGR* in polycystic ovary syndrome patients. *Reprod Biol* 2015;15:229–237.
- 114 Lansdown A, Rees DA: The sympathetic nervous system in polycystic ovary syndrome: a novel therapeutic target? *Clin Endocrinol (Oxford)* 2012;77:791–801.
- 115 Rahmouni K, Morgan DA, Morgan GM, Liu X, Sigmund CD, Mark AL, Haynes WG: Hypothalamic PI3K and MAPK differentially mediate regional sympathetic activation to insulin. *J Clin Invest* 2004;114:652–658.
- 116 Rahmouni K, Morgan DA, Morgan GM, Liu X, Sigmund CD, Mark AL, Haynes WG: Abnormal heart rate recovery after maximal cardiopulmonary exercise stress testing in young overweight women with polycystic ovary syndrome. *Clin Endocrinol (Oxford)* 2008;68:88–93.
- 117 Tekin G, Tekin A, Kiliçarslan EB, Haydardedeoğlu B, Katircibaşı T, Koçum T, Erol T, Cölkesen Y, Sezgin AT, Müderrisoğlu H: Altered autonomic neural control of the cardiovascular system in patients with polycystic ovary syndrome. *Int J Cardiol* 2008;130:49–55.
- 118 Yildirim A, Aybar F, Kabakci G, Yarali H, Oto A: Heart rate variability in young women with polycystic ovary syndrome. *Ann Non-invasive Electrocardiol* 2006;11:306–312.
- 119 Sverrisdottir YB, Mogren T, Kataoka J, Janson PO, Stener-Victorin E: Is polycystic ovary syndrome associated with high sympathetic nerve activity and size at birth? *Am J Physiol Endocrinol Metab* 2008;294:E576–E581.
- 120 Heider U, Pedal I, Spanel-Borowski K: Increase in nerve fibers and loss of mast cells in polycystic and postmenopausal ovaries. *Fertil Steril* 2001;75:1141–1147.
- 121 Lara HE, Ferruz JL, Luza S, Bustamante DA, Borges Y, Ojeda SR: Activation of ovarian sympathetic nerves in polycystic ovary syndrome. *Endocrinology* 1993;133:2690–2695.
- 122 Lara HE, Dissen GA, Leyton V, Paredes A, Fuenzalida H, Fiedler JL, Ojeda SR: An increased intraovarian synthesis of nerve growth factor and its low affinity receptor is a principal component of steroid-induced polycystic ovary in the rat. *Endocrinology* 2000;141:1059–1072.
- 123 Dissen GA, Garcia-Rudaz C, Paredes A, Mayer C, Mayerhofer A, Ojeda SR: Excessive ovarian production of nerve growth factor facilitates development of cystic ovarian morphology in mice and is a feature of polycystic ovarian syndrome in humans. *Endocrinology* 2009;150:2906–2914.
- 124 Witchel SF, Oberfield S, Rosenfield RL, Codner E, Bonny A, Ibáñez L, Pena A, Horikawa R, Gomez-Lobo V, Joel D, Tfayli H, Arslanian S, Dabadghao P, Garcia Rudaz C, Lee PA: The diagnosis of polycystic ovary syndrome during adolescence. *Horm Res Paediatr* 2015;83:376–389.
- 125 Carmina E, Oberfield SE, Lobo R: The diagnosis of polycystic in adolescents. *Am J Obstet Gynecol* 2010;203:201.e1–e5.
- 126 Di Fede G, Mansueto P, Pepe G, Rini B, Carmina E: High prevalence of polycystic ovary syndrome in women with mild hirsutism and no other significant clinical symptoms. *Fertil Steril* 2010;94:194–197.
- 127 Reinehr T, Bosse C, Lass N, Rothermel J, Knop C, Roth CL: Effect of weight loss on puberty onset in overweight children. *J Pediatr* 2017;184:143–50.
- 128 Reinehr T, Kulle A, Rothermel J, Knop-Schmenn C, Lass N, Bosse C, Holtherus PM: Longitudinal analyses of the steroid metabolome in obese girls with weight loss. *Endocr Connect* 2017;6:213–224.
- 129 Zawadzki J, Dunaif A: Diagnostic criteria for polycystic ovary syndrome: towards a rational approach; in Dunaif A, Givens JR, Haseltine FP, Merriam GR (eds): *Polycystic Ovary Syndrome*. Boston, Blackwell Scientific Publications, 1992, vol 4, pp 377–384.
- 130 Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group: Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004;81:19–25.
- 131 Yildiz BO, Bolour S, Woods K, Moore A, Azziz R: Visually scoring hirsutism. *Hum Reprod Update* 2010;16:51–64.
- 132 Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF; Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society: The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril* 2009;91:456–488.
- 133 Johnson T, Kaplan L, Ouyang P, Rizza R: National Institutes of Health evidence-based methodology workshop on polycystic ovary syndrome (PCOS). NIH EBMW Report. Bethesda, National Institutes of Health, 2012, vol 1, pp 1–14.
- 134 Legro RS, Arslanian SA, Ehrmann DA, Hoeger KM, Murad MH, Pasquali R, Welt CK; Endocrine Society: Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2013;98:4565–4592.
- 135 Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, Janssen OE, Legro RS, Norman RJ, Taylor AE, Witchel SF; Androgen Excess Society: Position statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J Clin Endocrinol Metab* 2006;91:4237–4245.

- 136 Plouffe L Jr: Disorders of excessive hair growth in the adolescent. *Obstet Gynecol Clin North Am* 2000;27:79–99.
- 137 Engmann L, Jin S, Sun F, Legro RS, Polotsky AJ, Hansen KR, Coutifaris C, Diamond MP, Eisenberg E, Zhang H, Santoro N; Reproductive Medicine Network: Racial and ethnic differences in the polycystic ovary syndrome (PCOS) metabolic phenotype. *Am J Obstet Gynecol* 2017;216:493.e1–e13.
- 138 Yildiz BO, Bolour S, Woods K, Moore A, Azziz R: Visually scoring hirsutism. *Hum Reprod Update* 2010;16:51–64.
- 139 Li R, Qiao J, Yang D, Li S, Lu S, Wu X, Wei Z: Epidemiology of hirsutism among women of reproductive age in the community: a simplified scoring system. *Eur J Obstet Gynecol Reprod Biol* 2012;163:165–169.
- 140 Martin KA, Chang RJ, Ehrmann DA, Ibanez L, Lobo RA, Rosenfield RL, Shapiro J, Montori VM, Swiglo BA: Evaluation and treatment of hirsutism in premenopausal women: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2008;98:1105–1120.
- 141 Souter I, Sanchez A, Perez M, Bartolucci A, Azziz R: The prevalence of androgen excess among patients with minimal unwanted hair growth. *Am J Obstet Gynecol* 2004;191:1914–1920.
- 142 Hawryluk EB, English JC 3rd: Female adolescent hair disorders. *J Pediatr Adolesc Gynecol* 2009;22:271–281.
- 143 Ferriman D, Gallwey JD: Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab* 1961;21:1440–1447.
- 144 Dilutunmbi Y, Paley K, English JC: Adolescent female acne: etiology and management. *J Pediatr Adolesc Gynecol* 2008;21:171–176.
- 145 Chang RJ, Coffler MS: Polycystic ovary syndrome: early detection in the adolescent. *Clin Obstet Gynecol* 2007;50:178–187.
- 146 Chen WC, Zouboulis CC: Hormones and the pilosebaceous unit. *Dermatoendocrinol* 2009;1:81–86.
- 147 Lucky AW, Biro FM, Simbartl LA, Morrison JA, Sorg NW: Predictors of severity of acne vulgaris in young adolescent girls: results of a five-year longitudinal study. *J Pediatr* 1997;130:30–39.
- 148 Merino PM, Codner E, Cassorla F: A rational approach to the diagnosis of polycystic ovarian syndrome during adolescence. *Arq Bras Endocrinol Metabol* 2011;55:590–598.
- 149 Ibañez L, Potau N, Virdis R, Zampolli M, Terzi C, Gussinyé M, Carrascosa A, Vicens-Calvet E: Postpubertal outcome in girls diagnosed of premature pubarche during childhood: increased frequency of functional ovarian hyperandrogenism. *J Clin Endocrinol Metab* 1993;76:1599–1603.
- 150 Witchel SF: Puberty and polycystic ovary syndrome. *Mol Cell Endocrinol* 2006;254–255:146–153.
- 151 McCartney CR, Blank SK, Prendergast KA, Chhabra S, Eagleson CA, Helm KD, Yoo R, Chang RJ, Foster CM, Caprio S, Marshall JC: Obesity and sex steroid changes across puberty: evidence for marked hyperandrogenemia in pre- and early pubertal obese girls. *J Clin Endocrinol Metab* 2007;92:430–436.
- 152 Carmina E, Dewailly D, Escobar-Morreale HF, Kelestimur F, Moran C, Oberfield S, Witchel SF, Azziz R: Non-classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency revisited: an update with a special focus on adolescent and adult women. *Hum Reprod Update* 2017;5:1–20.
- 153 Metcalf MG, Skidmore DS, Lowry GF, Mackenzie JA: Incidence of ovulation in the years after the menarche. *J Endocrinol* 1983;97:213–219.
- 154 Apter D: Endocrine and metabolic abnormalities in adolescents with a PCOS-like condition: consequences for adult reproduction. *Trends Endocrinol Metab* 1998;9:58–61.
- 155 Hickey M, Doherty DA, Atkinson H, Sloboda DM, Franks S, Norman RJ, Hart R: Clinical, ultrasound and biochemical features of polycystic ovary syndrome in adolescents: implications for diagnosis. *Hum Reprod* 2011;26:1469–1477.
- 156 Diaz A, Laufer MR, Breech LL: Menstruation in girls and adolescents: using the menstrual cycle as a vital sign. *Pediatrics* 2006;118:2245–2250.
- 157 World Health Organization multicenter study on menstrual and ovulatory patterns in adolescent girls. I. A multicenter cross-sectional study of menarche. World Health Organization Task Force on Adolescent Reproductive Health. *J Adolesc Health Care* 1986;7:229–235.
- 158 Southam AL, Richart RM: The prognosis for adolescents with menstrual abnormalities. *Am J Obstet Gynecol* 1966;94:637–645.
- 159 Franks S: Adult polycystic ovary syndrome begins in childhood. *Best Pract Res Clin Endocrinol Metab* 2002;16:263–272.
- 160 van Hooff MH, Voorhorst FJ, Kaptein MB, Hirasig RA, Koppelaar C, Schoemaker J: Predictive value of menstrual cycle pattern, body mass index, hormone levels and polycystic ovaries at age 15 years for oligo-amenorrhoea at age 18 years. *Hum Reprod* 2004;19:383–392.
- 161 Wiksten-Almströmer M, Hirschberg AL, Hagenfeldt K: Prospective follow-up of menstrual disorders in adolescence and prognostic factors. *Acta Obstet Gynecol Scand* 2008;87:1162–1168.
- 162 Rosenfield RL, Ehrmann DA, Littlejohn EE: Adolescent polycystic ovary syndrome due to functional ovarian hyperandrogenism persists into adulthood. *J Clin Endocrinol Metab* 2015;100:1537–1543.
- 163 Carroll J, Saxena R, Welt CK: Environmental and genetic factors influence age at menarche in women with polycystic ovary syndrome. *J Pediatr Endocrinol Metab* 2012;25:459–466.
- 164 Dahlgren E, Johansson S, Lindstedt G, Knutsson F, Odén A, Janson PO, Mattson LA, Crona N, Lundberg PA: Women with polycystic ovary syndrome wedge resected in 1956 to 1965: a long-term follow-up focusing on natural history and circulating hormones. *Fertil Steril* 1992;57:505–513.
- 165 Escobar-Morreale HF, Carmina E, Dewailly D, Gambineri A, Kelestimur F, Moghetti P, Pugeat M, Qiao J, Wijayarathne CN, Witchel SF, Norman RJ: Epidemiology, diagnosis and management of hirsutism: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome Society. *Hum Reprod Update* 2012;18:146–170.
- 166 Rosner W, Vesper H: Toward excellence in testosterone testing: a consensus statement. *J Clin Endocrinol Metab* 2010;95:4542–4548.
- 167 Legro RS, Schlaff WD, Diamond MP, Coutifaris C, Casson PR, Brzyski RG, Christman GM, Trussell JC, Krawetz SA, Snyder PJ, Ohl D, Carson SA, Steinkampf MP, Carr BR, McGovern PG, Cataldo NA, Gosman GG, Nestler JE, Myers ER, Santoro N, Eisenberg E, Zhang M, Zhang H; Reproductive Medicine Network: Total testosterone assays in women with polycystic ovary syndrome: precision and correlation with hirsutism. *J Clin Endocrinol Metab* 2010;95:5305–5313.
- 168 Auchus RJ: Steroid assays and endocrinology. Best practices for basic scientists. *Endocrinology* 2014;155:2049–2051.
- 169 Gambineri A, Fanelli F, Prontera O, Repaci A, Di Dalmazi G, Zanolli L, Pagotto U, Flacco ME, Guidi J, Fava GA, Manzoli L, Pasquali R: Prevalence of hyperandrogenic states in late adolescent and young women: epidemiological survey on Italian high-school students. *J Clin Endocrinol Metab* 2013;98:1641–1650.
- 170 Codner E, Villarroel C, Eyzaguirre FC, López P, Merino PM, Pérez-Bravo F, Iñiguez G, Cassorla F: Polycystic ovarian morphology in postmenarchal adolescents. *Fertil Steril* 2011;95:702–6.e1–e2.
- 171 Murphy MK, Hall JE, Adams JM, Lee H, Welt CK: Polycystic ovarian morphology in normal women does not predict the development of polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006;91:3878–3884.
- 172 Venturoli S, Porcu E, Fabbri R, Pluchinotta V, Ruggeri S, Macrelli S, Paradisi R, Flamigni C: Longitudinal change of sonographic ovarian aspects and endocrine parameters in irregular cycles of adolescence. *Pediatr Res* 1995;38:974–980.
- 173 Dewailly D: Diagnostic criteria for PCOS: is there a need for a rethink? *Best Pract Res Clin Obstet Gynaecol* 2016;37:5–11.
- 174 Holm K, Laursen EM, Brocks V, Muller J: Pubertal maturation of the internal genitalia: an ultrasound evaluation of 166. *Ultrasound Obstet Gynecol* 1995;6:175–181.
- 175 Kelsey TW, Dodwell SK, Wilkinson AG, Greve T, Andersen CY, Anderson RA, Wallace WH: Ovarian volume throughout life: a validated normative model. *PLoS One* 2013;8:e71465.
- 176 Bentzen JG, Forman JL, Johannsen TH, Pinborg A, Larsen EC, Andersen AN: Ovarian antral follicle subclasses and anti-mullerian hormone during normal reproductive aging. *J Clin Endocrinol Metab* 2013;98:1602–1611.

- 177 Mortensen M, Rosenfield RL, Littlejohn E: Functional significance of polycystic-size ovaries in healthy adolescents. *J Clin Endocrinol Metab* 2006;91:3786–3790.
- 178 Villarroel C, Merino PM, López P, Eyzaguirre FC, Van Velzen A, Iniguez G, Codner E: Polycystic ovarian morphology in adolescents with regular menstrual cycles is associated with elevated anti-Mullerian hormone. *Hum Reprod* 2011;26:2861–2868.
- 179 Dewailly D, Lujan ME, Carmina E, Cedars MI, Laven J, Norman RJ, Escobar-Morreale HF: Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. *Hum Reprod Update* 2014;20:334–352.
- 180 Rosenfield RL: The polycystic ovary morphology-polycystic ovary syndrome spectrum. *J Pediatr Adolesc Gynecol* 2014;28:412–419.
- 181 van Hooff MH, Voorhorst FJ, Kaptein MB, Hirasing RA, Koppenaal C, Schoemaker J: Polycystic ovaries in adolescents and the relationship with menstrual cycle patterns, luteinizing hormone, androgens, and insulin. *Fertil Steril* 2000;74:49–58.
- 182 Venturoli S, Porcu E, Fabbri R, Magrini O, Gammì L, Paradisi R, Flamigni R: Longitudinal evaluation of the different gonadotropin pulsatile patterns in anovulatory cycles of young girls. *J Clin Endocrinol Metab* 1992;74:836–841.
- 183 Mortensen M, Ehrmann DA, Littlejohn E, Rosenfield RL: Asymptomatic volunteers with a polycystic ovary are a functionally distinct but heterogeneous population. *J Clin Endocrinol Metab* 2009;94:1579–1586.
- 184 Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S, Dewailly D: Elevated serum level of anti-mullerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. *J Clin Endocrinol Metab* 2003;88:5957–5962.
- 185 Pigny P, Jonard S, Robert Y, Dewailly D: Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2006;91:941–945.
- 186 Hart R, Doherty DA, Norman RJ, Franks S, Dickinson JE, Hickey M, Sloboda DM: Serum antimullerian hormone (AMH) levels are elevated in adolescent girls with polycystic ovaries and the polycystic ovarian syndrome (PCOS). *Fertil Steril* 2010;94:1118–1121.
- 187 Rosenfield RL, Wroblewski K, Padmanabhan V, Littlejohn E, Mortensen M, Ehrmann DA: Antimullerian hormone levels are independently related to ovarian hyperandrogenism and polycystic ovaries. *Fertil Steril* 2012;98:242–249.
- 188 Villarroel C, Lopez P, Merino PM, Iniguez G, Sir-Petermann T, Codner E: Hirsutism and oligomenorrhea are appropriate screening criteria for polycystic ovary syndrome in adolescents. *Gynecol Endocrinol* 2015;31:625–629.
- 189 Lie Fong S, Visser JA, Welt CK, de Rijke YB, Eijkemans MJ, Broekmans FJ, Roes EM, Peters WH, Hokken-Koelega AC, Fauser BC, Themmen AP, de Jong FH, Schipper I, Laven JS: Serum anti-mullerian hormone levels in healthy females: a nomogram ranging from infancy to adulthood. *J Clin Endocrinol Metab* 2012;97:4650–4655.
- 190 Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WH: A validated model of serum anti-mullerian hormone from conception to menopause. *PLoS One* 2011;6:e22024.
- 191 Sopher AB, Grigoriev G, Laura D, Cameo T, Lerner JP, Chang RJ, McMahon DJ, Oberfield SE: Anti-Mullerian hormone may be a useful adjunct in the diagnosis of polycystic ovary syndrome in nonobese adolescents. *J Pediatr Endocrinol Metab* 2014;27:1175–1179.
- 192 Munzker J, Hofer D, Trummer C, Ulbing M, Harger A, Pieber T, Owen L, Keevil B, Brabant G, Lerchbaum E, Obermayer-Pietsch B: Testosterone to dihydrotestosterone ratio as a new biomarker for an adverse metabolic phenotype in the polycystic ovary syndrome. *J Clin Endocrinol Metab* 2015;100:653–660.
- 193 Sarray S, Almawi WY: Levels of CD40L and other inflammatory biomarkers in obese and non-obese women with polycystic ovary syndrome. *Am J Reprod Immunol* 2016;76:285–291.
- 194 Li Li, Zhang J, Deng Q, Li J, Li Z, Xiao Y, Hu S, Li T, Tan Q, Li X, Luo B, Mo H: Proteomic profiling for identification of novel biomarkers differentially expressed in human ovaries from polycystic ovary syndrome patients. *PLoS One* 2016;11:e0164538.
- 195 Sorensen AE, Udesen PB, Wissing ML, Englund AM, Dalgaard LT: MicroRNAs related to androgen metabolism and polycystic ovary syndrome. *Chem Biol Interact* 2016;259:8–16.
- 196 Harris MI, Hadden WC, Knowler WC, Bennett PH: Prevalence of diabetes and impaired glucose tolerance and plasma glucose levels in U.S. population aged 20–74 yr. *Diabetes* 1987;36:523–34.
- 197 Matsuda M: Measuring and estimating insulin resistance in clinical and research settings. *Nutr Metab Cardiovasc Dis* 2010;20:79–86.
- 198 Burgert TS, Vuguin PM, DiMartino-Nardi J, Attie KM, Saenger P: Assessing insulin resistance: application of a fasting glucose to insulin ratio in growth hormone-treated children. *Horm Res* 2002;57:37–42.
- 199 Jean AM, Hassoun A, Hughes J, Pomeranz C, Fennoy I, McMahon DJ, Oberfield SE: Utility of insulin response and proinsulin to assess insulin resistance. *J Pediatr* 2009;155:893–899.
- 200 Nagasaka S, Kusaka I, Yamashita K, Funase Y, Yamauchi K, Katakura M, Ishibashi S, Aizawa T: Index of glucose effectiveness derived from oral glucose tolerance test. *Acta Diabetol* 2012;49:S195–S204.
- 201 Matsuda M, DeFronzo R: Insulin sensitivity indices obtained from oral glucose tolerance test: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22:1462–1470.
- 202 Hoeger K, Davidson K, Kochman L, Cherry T, Kopin L, Guzik DS: The impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women in two randomized, placebo-controlled clinical trials. *J Clin Endocrinol Metab* 2008;93:4299–4306.
- 203 Ornstein RM, Copperman NM, Jacobson MS: Effect of weight loss on menstrual function in adolescents with polycystic ovary syndrome. *J Pediatr Adolesc Gynecol* 2011;24:161–165.
- 204 Lass N, Kleber M, Winkel K, Wunsch R, Reinehr T: Effect of lifestyle intervention on features of polycystic ovarian syndrome, metabolic syndrome, and intima-media thickness in obese adolescent girls. *J Clin Endocrinol Metab* 2011;96:3533–3540.
- 205 Knop C, Singer V, Uysal Y, Schaefer A, Wolters B, Reinehr T: Extremely obese children respond better than extremely obese adolescents to lifestyle interventions. *Pediatr Obes* 2015;10:7–14.
- 206 Reinehr T, Lass N, Toschke C, Rothermel J, Lanzinger S, Holl RW: Which amount of BMI-SDS reduction is necessary to improve cardiovascular risk factors in overweight children? *J Clin Endocrinol Metab* 2016;101:3171–3179.
- 207 Harrison CL, Lombard CB, Moran LJ, Teede HJ: Exercise therapy in polycystic ovary syndrome: a systematic review. *Hum Reprod Update* 2011;17:171–183.
- 208 Oude Luttikhuis H, Baur L, Jansen H, Shrewsbury VA, O'Malley C, Stolk RP, Summerbell CD: Interventions for treating obesity in children. *Cochrane Database Syst Rev* 2009;1:CD001872.
- 209 Moran LJ, Ko H, Misso M, Marsh K, Noakes M, Talbot M, Frearson M, Thondan M, Stepto N, Teede HJ: Dietary composition in the treatment of polycystic ovary syndrome: a systematic review to inform evidence-based guidelines. *J Acad Nutr Diet* 2013;113:520–545.
- 210 Reinehr T: Lifestyle intervention in childhood obesity: changes and challenges. *Nat Rev Endocrinol* 2013;9:607–614.
- 211 Haedersdal M, Gotsche PC: Laser and photoepilation for unwanted hair growth. *Cochrane Database Syst Rev* 2006;4:CD004684.
- 212 Haedersdal M, Wulf HC: Evidence-based review of hair removal using lasers and light sources. *J Eur Acad Dermatol Venereol* 2006;20:9–20.
- 213 Sadighha A, Mohaghegh Zahed G: Meta-analysis of hair removal laser trials. *Lasers Med Sci* 2009;24:21–25.
- 214 Clayton WJ, Lipton M, Elford J, Rustin M, Sherr L: A randomized controlled trial of laser treatment among hirsute women with polycystic ovary syndrome. *Br J Dermatol* 2005;152:986–992.

- 215 McGill DJ, Hutchison C, McKenzie E, McSherry E, Mackay IR: A randomized, split-face comparison of facial hair removal with the alexandrite laser and intense pulsed light system. *Lasers Surg Med* 2007;39:767–772.
- 216 Somani N, Turvy D: Hirsutism: an evidence-based treatment update. *Am J Clin Dermatol* 2014;15:247–266.
- 217 Vissing AC, Taudorf EH, Haak CS, Philipssen PA, Hædersdal M: Adjuvant eflornithine to maintain IPL-induced hair reduction in women with facial hirsutism: a randomized controlled trial. *J Eur Acad Dermatol Venereol* 2016;30:314–319.
- 218 Wang T, McNeill AM, Chen Y, Senderak M, Shankar RR: Metformin prescription patterns among US adolescents aged 10–19 years: 2009–2013. *J Clin Pharm Ther* 2016;41:229–236.
- 219 Naderpoor N, Shorakae S, de Courden B, Misso ML, Moran LJ, Teede HJ: Metformin and lifestyle modification in polycystic ovary syndrome: systematic review and meta-analysis. *Hum Reprod Update* 2015;21:560–574.
- 220 Ladson G, Dodson WC, Sweet SD, Archibong AE, Kunselman AR, Demers LM, Lee PA, Williams NI, Coney P, Legro RS: Effects of metformin in adolescents with polycystic ovary syndrome undertaking lifestyle therapy: a pilot randomized double-blind study. *Fertil Steril* 2011;95:2595–2598.e1–e6.
- 221 Allen HF, Mazzoni C, Heptulla RA, Murray MA, Miller N, Koenigs L, Reiter EO: Randomized controlled trial evaluating response to metformin versus standard therapy in the treatment of adolescents with polycystic ovary syndrome. *J Pediatr Endocrinol Metab* 2005;18:761–768.
- 222 Al-Zubeidi H, Klein KO: Randomized clinical trial evaluating metformin versus oral contraceptive pills in the treatment of adolescents with polycystic ovarian syndrome. *J Pediatr Endocrinol Metab* 2015;28:853–858.
- 223 Bridger T, MacDonald S, Baltzer F, Rodd C: Randomized placebo-controlled trial of metformin for adolescents with polycystic ovary syndrome. *Arch Pediatr Adolesc Med* 2006;160:241–246.
- 224 El Maghraby H, Nafee T, Guiziry D, El-nashar A: Randomized controlled trial of the effects of metformin versus combined oral contraceptives in adolescent PCOS women through a 24 month follow up period. *Middle East Fertil Soc J* 2015;20:131–137.
- 225 Ibáñez L, Potau N, Ferrer A, Rodríguez-Hierro F, Marcos MV, de Zegher F: Anovulation in eumenorrheic, nonobese adolescent girls born small for gestational age: insulin sensitization induces ovulation, increases lean body mass, and reduces abdominal fat excess, dyslipidemia, and subclinical hyperandrogenism. *J Clin Endocrinol Metab* 2002;87:5702–5705.
- 226 Ibáñez L, Valls C, Ferrer A, Marcos MV, Rodríguez-Hierro F, de Zegher F: Sensitization to insulin induces ovulation in nonobese adolescents with anovulatory hyperandrogenism. *J Clin Endocrinol Metab* 2001;86:3595–3598.
- 227 Al Khalifah RA, Florez ID, Dennis B, Thabane L, Bassilious E: Metformin or oral contraceptives for adolescents with polycystic ovarian syndrome: a meta-analysis. *Pediatrics* 2016;137:e20154089.
- 228 Swiglo BA, Cosma M, Flynn DN, Kurtz DM, Labella ML, Mullan RJ, Erwin PJ, Montori VM: Clinical review: Antiandrogens for the treatment of hirsutism: a systematic review and meta-analyses of randomized controlled trials. *J Clin Endocrinol Metab* 2008;93:1153–1160.
- 229 Moghetti P, Tosi F, Tosti A, Negri C, Misciali C, Perrone F, Caputo M, Muggeo M, Castello R: Comparison of spironolactone, flutamide, and finasteride efficacy in the treatment of hirsutism: a randomized, double blind, placebo-controlled trial. *J Clin Endocrinol Metab* 2000;85:89–94.
- 230 de Zegher F, Ibáñez L: Low-dose flutamide for hirsutism: into the limelight, at last. *Nat Rev Endocrinol* 2010;6:421–422.
- 231 Ganie MA, Khurana ML, Eunice M, Gupta N, Gulati M, Dwivedi SN, Ammini AC: Comparison of efficacy of spironolactone with metformin in the management of polycystic ovary syndrome: an open-labeled study. *J Clin Endocrinol Metab* 2004;89:2756–2762.
- 232 Ganie MA, Khurana ML, Nisar S, Shah PA, Shah ZA, Kulshrestha B, Gupta N, Zargar MA, Wani TA, Mudasar S, Mir FA, Taing S: Improved efficacy of low-dose spironolactone and metformin combination than either drug alone in the management of women with polycystic ovary syndrome (PCOS): a six-month, open-label randomized study. *J Clin Endocrinol Metab* 2013;98:3599–3607.
- 233 Ibáñez L, Valls C, Ferrer A, Ong K, Dunger DB, de Zegher F: Additive effects of insulin-sensitizing and anti-androgen treatment in young, nonobese women with hyperinsulinism, hyperandrogenism, dyslipidemia, and anovulation. *J Clin Endocrinol Metab* 2002;87:2870–2874.
- 234 Keleştimur F, Everest H, Unlühizarci K, Bayram F, Sahin Y: A comparison between spironolactone and spironolactone plus finasteride in the treatment of hirsutism. *Eur J Endocrinol* 2004;150:351–354.
- 235 Mastorakos G, Koliopoulos C, Creatsas G: Androgen and lipid profiles in adolescents with polycystic ovary syndrome who were treated with two forms of combined oral contraceptives. *Fertil Steril* 2002;77:919–927.
- 236 Mastorakos G, Koliopoulos C, Deligeorglou E, Diamanti-Kandarakis E, Creatsas G: Effects of two forms of combined oral contraceptives on carbohydrate metabolism in adolescents with polycystic ovary syndrome. *Fertil Steril* 2006;85:420–427.
- 237 Battaglia C, Mancini F, Fabbri R, Persico N, Busacchi P, Facchinetti F, Venturoli S: Polycystic ovary syndrome and cardiovascular risk in young patients treated with drospirenone-ethinylestradiol or contraceptive vaginal ring. A prospective, randomized, pilot study. *Fertil Steril* 2010;94:1417–1425.
- 238 Bhattacharya SM, Jha A: Comparative study of the therapeutic effects of oral contraceptive pills containing desogestrel, cyproterone acetate, and drospirenone in patients with polycystic ovary syndrome. *Fertil Steril* 2012;98:1053–1059.
- 239 Ibáñez L, del Río L, Díaz M, Sebastiani G, Pozo OJ, López-Vermejo A, de Zegher F: Normalizing ovulation rate by preferential reduction of hepato-visceral fat in adolescent girls with polycystic ovary syndrome. *J Adolesc Health* 2017, DOI: 10.1016/j.jadohealth.2017.04.010.
- 240 Ibáñez L, de Zegher F, Potau N: Anovulation after precocious pubarche: early markers and time course in adolescence. *J Clin Endocrinol Metab* 1999;84:2691–2695.
- 241 US Medical Eligibility Criteria for Contraceptive Use, 2010. *MMWR Recomm Rep* 2010;59:1–86.
- 242 Bonny AE, Ziegler J, Harvey R, Debanne SM, Secic M, Cromer BA: Weight gain in obese and nonobese adolescent girls initiating depot medroxyprogesterone, oral contraceptive pills, or no hormonal contraceptive method. *Arch Pediatr Adolesc Med* 2006;160:40–45.
- 243 Harel Z, Johnson CC, Gold MA, Cromer B, Peterson E, Burkman R, Stager M, Brown R, Bruner A, Coupey S, Hertweck P, Bone H, Wolter K, Nelson A, Marshall S, Bachrach LK: Recovery of bone mineral density in adolescents following the use of depot medroxyprogesterone acetate contraceptive injections. *Contraception* 2010;81:281–291.
- 244 Francis JKR, Gold MA: Long-acting reversible contraception for adolescents. A review. *JAMA Pediatr* 2017;171:694–701.
- 245 Palomba S, de Wilde MA, Falbo A, Koster MP, La Sala GB, Fauser BC: Pregnancy complications in women with polycystic ovary syndrome. *Hum Reprod Update* 2015;21:575–592.
- 246 Kuchenbecker WK, Groen H, van Asselt SJ, Bolster JH, Zwerver J, Slart RH, Vd Jagt EJ, Muller Kobold AC, Wolffenbuttel BH, Land JA, Hoek A: In women with polycystic ovary syndrome and obesity, loss of intra-abdominal fat is associated with resumption of ovulation. *Hum Reprod* 2011;26:2505–2512.
- 247 Jones H, Sprung VS, Pugh CJ, Daousi C, Irwin A, Aziz N, Adams VL, Thomas EL, Bell JD, Kemp GJ, Cuthbertson DJ: Polycystic ovary syndrome with hyperandrogenism is characterized by an increased risk of hepatic steatosis compared to nonhyperandrogenic PCOS phenotypes and healthy controls, independent of obesity and insulin resistance. *J Clin Endocrinol Metab* 2012;97:3709–3716.