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Review

DHEA, DHEAS and PCOS

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ABSTRACT

Approximately 20–30% of PCOS women demonstrate excess adrenal precursor androgen (APA) production, primarily using DHEAS as a marker of APA in general and more specifically DHEA, synthesis. The role of APA excess in determining or causing PCOS is unclear, although observations in patients with inherited APA excess (e.g., patients with 21-hydroxylase deficient congenital classic or non-classic adrenal hyperplasia) demonstrate that APA excess can result in a PCOS-like phenotype. Inherited defects of the enzymes responsible for steroid biosynthesis, or defects in cortisol metabolism, account for only a very small fraction of women suffering from hyperandrogenism or APA excess. Rather, women with PCOS and APA excess appear to have a generalized exaggeration in adrenal steroidogenesis in response to ACTH stimulation, although they do not have overt hypothalamic–pituitary–adrenal axis dysfunction. In general, extra-adrenal factors, including obesity, insulin and glucose levels, and ovarian secretions, play a limited role in the increased APA production observed in PCOS. Substantial heritabilities of APAs, particularly DHEAS, have been found in the general population and in women with PCOS; however, the handful of SNPs discovered to date account only for a small portion of the inheritance of these traits. Paradoxically, and as in men, elevated levels of DHEAS appear to be protective against cardiovascular risk in women, although the role of DHEAS in modulating this risk in women with PCOS remains unknown. In summary, the exact cause of APA excess in PCOS remains unclear, although it may reflect a generalized and inherited exaggeration in androgen biosynthesis of an inherited nature.

Contents

Abbreviations: 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 5α-RA, 5α-reductase; 17-HP, 17-hydroxyprogesterone; 21-OH, 21-hydroxylase; A4, androstenedione; APA, adrenal precursor androgens; AE-PCOS Society, Androgen Excess and PCOS Society; ASRM, American Society of Reproductive Medicine; AUC, are-under-the-curve; BMI, body mass index; DHEA, dehydroepiandrosterone; ESHRE, European Society of Human Reproduction and Embryology; FSH, follicle stimulating hormone; FSIGT, frequently sampled intravenous glucose tolerance test; GnRH, gonadotropin releasing hormone; GWAS, genome-wide association studies; HOMA-IR, homeostatic model assessment of insulin resistance; NCAH, non-classic congenital adrenal hyperplasia; NIH, National Institutes of Health; PA, premature adrenarche; PCOS, polycystic ovary syndrome; SHBG, sex hormone-binding globulin; SNP, single nucleotide polymorphism; T, testosterone; TZD, thiazolidinedione.

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1. Introduction

1.1. Defining PCOS

The polycystic ovary syndrome (PCOS) is a heterogeneous disorder that, depending on how it is defined, affects between 10 and 20% of women of reproductive age. In its various forms PCOS is characterized by the presence of a combination of hyperandrogenic signs and symptoms, polycystic ovaries and ovulatory dysfunction. Women with PCOS also have an increased risk for insulin resistance, metabolic dysfunction, glucose intolerance and diabetes, and cardiovascular disease.

An easy way to understand the presentation of PCOS is to envision the disorder as a collection of various phenotypes or subphenotypes. For example, if we consider only two features we would have a total of four phenotypes to consider, if three features 8 phenotypes, if four features then 16 phenotypes, if five features then 32 phenotypes, and so on [1]. In general, we prefer to consider three, albeit somewhat inter-related, features when defining PCOS: (a) hyperandrogenism, either biochemical (i.e., hyperandrogenemia) or clinical (e.g., hirsutism), (b) oligo-anovulation (often reflected in menstrual dysfunction and or oligo-amenorrhea), and (c) polycystic ovarian morphology. Thus, using these features there appears to be four phenotypes of PCOS which would fit into one or more of the current diagnostic criterion for the disorder (Table 1) .

Today there are three widely used definitions for PCOS, which use a combination of the three aforementioned features: (a) that arising from the proceedings of a conference on PCOS co-sponsored by the National Institutes of Health (NIH) in 1990 (i.e., NIH, 1990), in which the attendees were surveyed [2]; (b) that arising from the proceedings of an expert conference held in Rotterdam and cosponsored by Erasmus University, the American Society of Reproductive Medicine (ASRM) and the European Society of Human Reproduction and Embryology (ESHRE) in 2003 (i.e., Rotterdam, 2003) [3,4]; and (c) that formulated by an expert task force established by the Androgen Excess and PCOS Society, published in 2006 (AE-PCOS Society, 2006) (Table 1).

It is worth noting that both the Rotterdam, 2003 and the AE-PCOS Society, 2006 definitions represent extensions or broadening of the original NIH, 1990 criteria, with this latter definition still defining the core group of subjects who unambiguously have the disorder. Recently, a consensus conference sponsored by the NIH suggested using the Rotterdam, 2003 criteria moving forward, as it captures the broadest population of PCOS subjects, with the proviso that a clear description of the phenotypes of the subjects included in any study be provided [5]. PCOS should be considered a diagnosis of exclusion, as all definitions proposed note that to make the diagnosis, related or mimicking disorders should be excluded by testing or clinical evaluation, including at a minimum androgen-secreting neoplasms, Cushing's syndrome, adrenal hyperplasia, and thyroid and prolactin dysfunction.

Table 1
Phenotypic criteria for diagnosis of PCOS according NIH 1990, AES 2006 and Rotterdam 2003 criteria.

Phenotypes	1	2	3	4	5	6	7	8
Hyperandrogenemism (biochemical or clinical)	Yes	Yes	Yes	No	Yes	No	No	No
Oligo-anovulation	Yes	Yes	No	Yes	No	Yes	No	No
Polycystic ovaries	Yes	No	Yes	Yes	No	No	Yes	No
NIH 1990 criteria	X	X						
Rotterdam 2003 criteria	X	X	X	X				
AE-PCOS Society 2006 criteria	X	X	X					

The number of possible phenotypes is equal to X^n, where X = number of possible responses for any particular feature (i.e., in this case 2, i.e., present or absent) and n = number of features considered. For example for two features the number of possible phenotypes would be $2^2 = 4$; for three features, $2^3 = 8$; for four, $2^4 = 16$; for five, $2^5 = 32$, etc.

1.2. Prevalence of PCOS

The prevalence of PCOS in the general population of reproductive-aged women will vary according to the definition used. The majority of studies have used the NIH 1990 definition, with prevalences world-wide generally ranging from 6% to 9% [6–20]. The prevalence of PCOS by the Rotterdam, 2003 or the AE-PCOS Society, 2006 definitions will be higher, with the Rotterdam definition identifying between 15 and 20% of unselected reproductive-aged women with the disorder [7,11–13,15,19,20]. And while we generally speak about the prevalence of the disorder in women of reproductive age (when the features of the disorder are most evident), as PCOS is primarily an inherited disorder (see below) it likely affects the same fraction of women at all ages. However, clinical features (hyperandrogenic and ovulatory dysfunction) will be less noticeable at each end of the reproductive spectrum. In prepubertal girls, adrenal and ovarian androgenic steroidogenesis is only beginning and ovulation has not yet started; in postmenopausal women, ovulation (and spontaneous menstruation) has ceased and androgenesis decreases with age.

1.3. Adrenal precursor androgen (APA) steroidogenesis – a brief overview

The adrenal cortices produce three steroids with variable degrees of androgenic activity, in descending order of adrenal production: dehydroepiandrosterone (DHEA), androstenedione (A4) and testosterone (T) (Fig. 1). In human adrenals, the primary biosynthetic pathway for androgenic steroidogenesis after cholesterol cleavage proceeds thru the Δ^5 pathway by way of pregnenolone and its subsequent hydroxylation to 17-hydroxypregenolone (thru the 17α-hydroxylation action of cytochrome P450c17; *CYP17*), followed by cleavage of its C17 side chain to form DHEA (thru the 17,20-lyase action of P450c17; *CYP17*). This steroid is in part released into the blood stream and in part converted to A4 through the action of 3β-hydroxysteroid dehydrogenase (3β-HSD; *HSD3B2*). However, a significant portion of DHEA is sulfated through the action of DHEA sulfotransferase (DHEA-ST; *SULT2A1*) and the DHEA sulfate (DHEAS) is released into the circulation [21].

In some animals, A4 can be produced via the Δ^4 pathway, with conversion of pregnenolone to progesterone (via 3β-HSD) and then to 17α-hydroxyprogesterone (17-HP) via the action of P450c17. In these animals, 17-HP is then converted to A4 through the 17,20-lyase action of P450c17; however, in humans the Δ^4-17,20-lyase activity of P450c17 (which converts 17-HP to A4) is almost entirely absent [22,23]. Hence, in humans almost all A4 is produced from the transformation of DHEA [24].

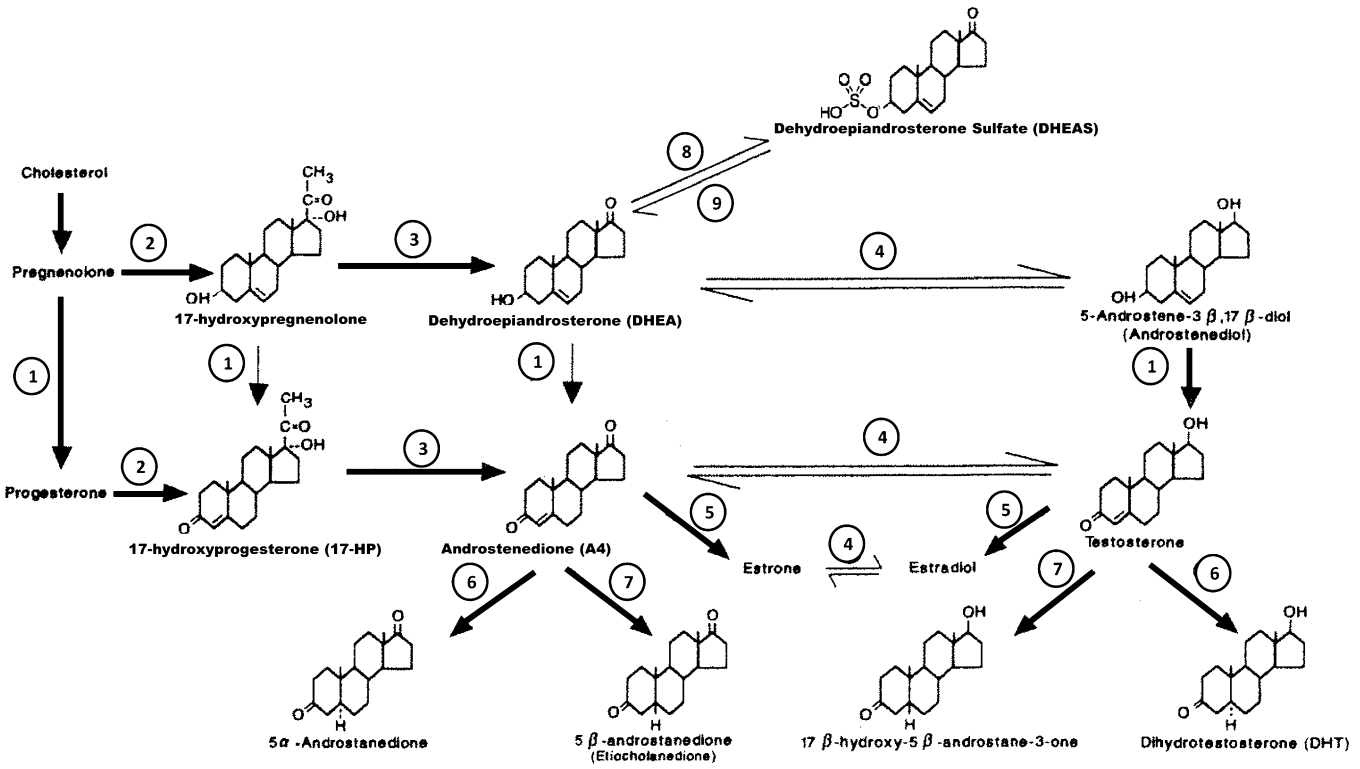

Fig. 1. Principal pathways of androgen synthesis and catabolism. Enzyme's activity, with enzyme name (if different) and gene name in parentheses: (1) 3β-hydroxysteroid dehydrogenase or 3β-HSD (*HSD3B1*); (2) 17β-hydroxylase (P450c17; *CYP17*); (3) 17,20-lyase (P450c17; *CYP17*); (4) 17β-hydroxysteroid dehydrogenase (*HSD17B5 or AKR1C3*); (5) aromatase (*CYP19*); (6) 5α-reductase (*SRD5A2*); (7) 5β-reductase (*SRD5B1*); (8) DHEA-preferring sulfotransferase (*SULT2A1*); and (9) steroid sulfatase (*STS*).

It has been generally assumed that the adrenal cortices do not directly produce T, but recent studies have demonstrated that small quantities of this potent androgen may be produced in human adrenals thru the action of 17β-hydroxysteroid-dehydrogenase type 5 (*HSD17B5 or AKR1C3*) on A4 [25,26]. However, the clinical significance of these small quantities of adrenal T is largely unknown and further studies are needed to understand whether it has a role in any pathologic conditions.

Overall, the androgenic potential of the adrenal cortices under normal circumstances is relatively small, at least in comparison to that of the ovaries and, even more, the testes. Adrenal precursor androgens primarily function as pre-hormones. For example, A4 can be converted in the liver into T, a pathway that is particularly important in women as it accounts for 50–60% of circulating T in these individuals, with approximately another 25–30% originating from the ovaries and the remainder from the adrenal cortex [24]. DHEA, either in its free form or as the product of DHEAS cleavage by steroid sulfatase (*STS*), can also serve as a pre-hormone for the formation of more potent androgens, including its conversion through hepatic and peripheral conversion to A4 [21,24]. Hepatic conversion of DHEA to A4 has been demonstrated in in vitro studies of hepatocytes of bovine and human origin [27,28]. In vivo, the greater levels of A4 resulting after oral versus transcutaneous administration of DHEA supports hepatic first pass transformation of DHEA to A4 [29]. In addition, several studies have indicated that DHEA can be converted to the most potent of androgens, dihydrotestosterone (DHT), in peripheral tissues once converted to A4 without first requiring formation of T [26]. The formation of powerful androgens from DHEA and DHEAS may be very important during postmenopausal years.

Although many of the factors regulating the production of APAs remain unclear, ACTH does appear to play a key role [30]. However, a special character of APAs is the fact that their production is strictly related to age and their levels start decreasing after age 30 [31,32]. This decline happens in spite of no changes in ACTH secretion. In part this is a consequence of a decline in selected enzymatic activity, in particular that of cytochrome b5 dependent 17,20 lyase (*CYP17*) activity in the reticularis zone of the adrenal [33], although other mechanisms remain unclear.

Substantial quantities of A4 (50%) and DHEA (20–30%) are produced in the ovary, while alternatively almost all DHEAS is produced by the adrenal cortices, although DHEA-ST activity (which could convert DHEA to DHEAS) is present in the liver and small intestine. Thus, circulating DHEAS appears to represent the readiest marker of APA production. Because of its high production rate and low metabolic clearance rate, DHEAS levels in blood are high and demonstrate little diurnal fluctuations. In addition, DHEAS is easily and accurately measured by direct commercial immunoassays [34].

However, DHEAS as a marker of APAs is not without its limitations. For example, in some forms of adrenal hyperandrogenism, such as 21-hydroxylase (21-OH) deficient non-classic adrenal hyperplasia, DHEAS blood levels are often normal [35], probably because most excessive DHEA is primarily diverted to A4 production. Therefore, the finding of a normal DHEAS level does not exclude the existence of APA excess, and in some conditions other APA markers, such as 11β-hydroxyandrostenedione [36] or dynamic testing (e.g., ACTH stimulation test), may be needed to detect APA excess. The common belief that DHEA and DHEAS are freely interconverted was challenged by a study finding no increase in DHEA levels after oral administration of DHEAS; this in vivo data were accompanied by in vitro experiments finding that hepatocytes were unable to convert DHEAS to DHEA, leading the investigators to conclude that DHEAS is not a reservoir DHEA and does not well represent bioavailable DHEA [28].

2. DHEA and APA excess in PCOS

2.1. Epidemiology of APA excess in PCOS

Adrenal precursor androgen excess measured by elevated serum levels of DHEAS and 11β-hydroxyandrostenedione has been reported to occur in up to 50% of patients with PCOS [36–40]. However, a more careful cluster analysis of 213 women with PCOS and 182 age-matched healthy eumenorrheic non-hirsute women, adjusting for age and race, indicated that the prevalence of supranormal DHEAS levels was 33.3% and 19.9%, respectively, among black and white women with PCOS [32]. Furthermore, this study did not reveal any specific subpopulations of DHEAS levels among PCOS patients, suggesting that in PCOS there is a generalized upregulation of APAs. It is worth noting that DHEAS declined similarly after the age of 30 years in both normal women and those with PCOS [35] (Fig. 2A and B). Thus, if age is not considered when establishing normative ranges for DHEAS, most women with PCOS and DHEAS excess will consequently be found to be lean teens.

Overall, the prevalence of DHEAS excess is 20–30% among PCOS patients, when using age and race-adjusted normative values. However, we should remember that while DHEAS levels are useful as a reflection of DHEA and other APA production (see Section 1.3), changes in DHEA-ST activity alone could result in changes (e.g., decline) in DHEAS levels without a concomitant change in DHEA production. A classic example of this divergence between DHEAS levels and APA production by the adrenal is seen when treating patients with classic adrenal hyperplasia with corticosteroids [41]. A final caveat is that many studies were performed using immunoassays that lack specificity for detection of the low APA levels in women.

2.2. APA excess as a risk factor for PCOS

Excess APA may be present in PCOS either because: (a) early onset APA excess increases the risk of developing PCOS; or (b) APA

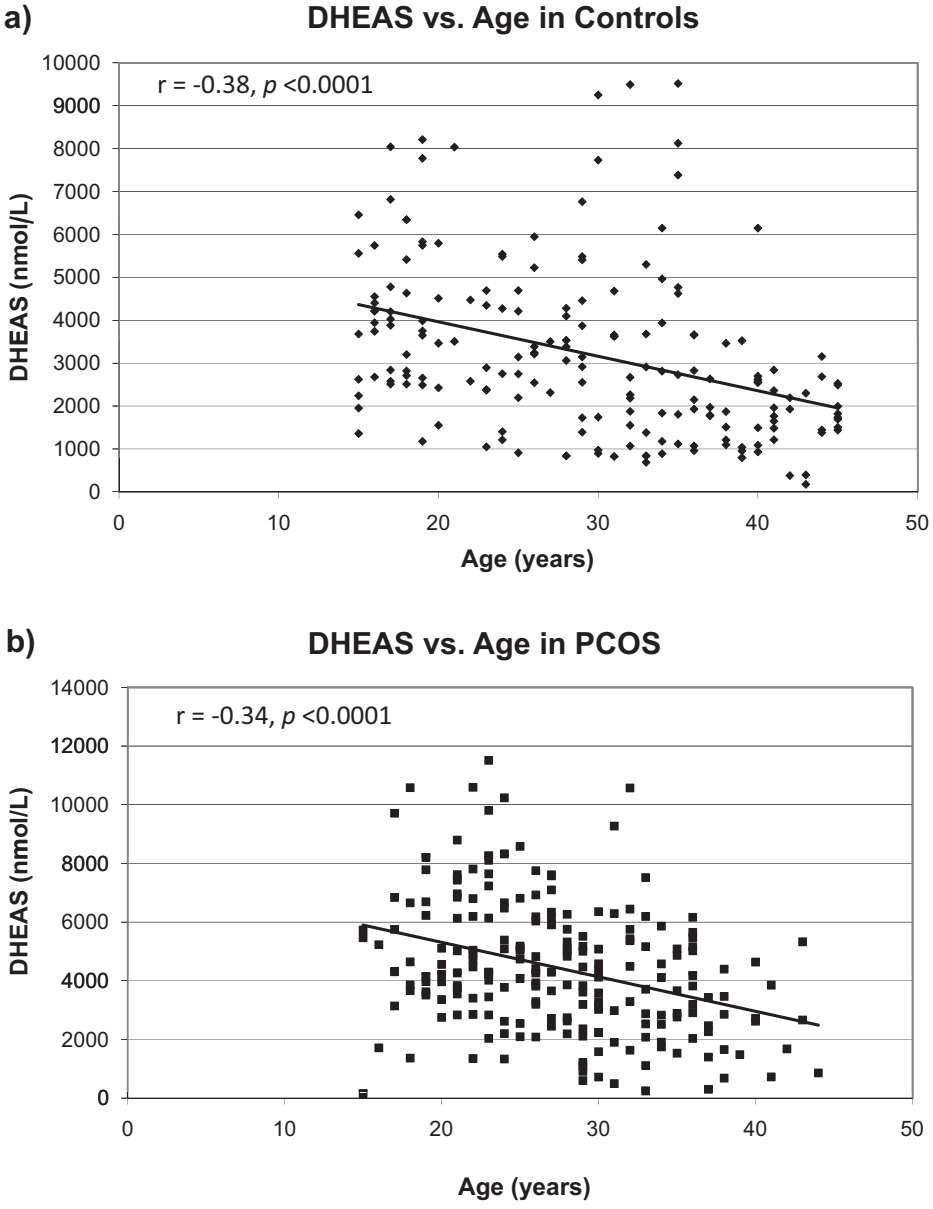

Fig. 2. DHEAS levels vs. age in control and PCOS women. Depicted are the scattergrams and linear regression trend lines for DHEAS levels according to age in healthy controls (top; $r = -0.38$, $p < 0.0001$) and PCOS (bottom; $r = -0.34$, $p < 0.0001$). Note that DHEAS levels in PCOS and controls decrease with age at similar rates (adapted from reference [32]).

excess is a reflection of the disorder, namely APA excess is the result of a generalized exaggeration in androgen steroidogenesis in PCOS. In fact, both processes appear to be operant. In this section we will review the data supporting the concept that APA excess, particularly when evident before or during pubertal development, increases the risk for developing PCOS stemming from observations in patients with 21-OH deficient non-classic congenital adrenal hyperplasia (NCAH) or premature adrenarche (PA). Below we will review the data indicating that APA excess in PCOS, at least in adult women, appears to reflect a generalized hyper-responsiveness of androgenic biosynthesis to stimulation.

Patients with PA (early onset of pubic/axillary hair and apocrine sweat gland development) are at an increased risk for developing PCOS compared to unaffected peers [42–45]. For example, up to 50% of individuals with PA subsequently develop PCOS [46]. Furthermore, daughters (age 4–13 years) of women with PCOS were found to have elevated DHEAS and biochemical evidence of PA more frequently than daughters of control women [47]. Investigators have also suggested that stress resulting in exaggerated APA secretion during the peripubertal time period increases the risk of developing PCOS [48]. However, whether the development of PCOS in patients with PA is the result of prepubertal hyperandrogenism or the latter is simply a sign of developing PCOS remains unclear.

Finally, a compelling model illustrating the impact of peripubertal APA excess as a risk factor for PCOS is 21-OH deficient NCAH. This disorder is the result of a primary abnormality in adrenocortical dysfunction, namely the excessive production of APAs due to a congenital defect in CYP21A2, the gene encoding for the enzyme P450c21 which determines 21-OH activity. 21-OH deficient NCAH is linked to the development of PCOS-like features, including the development of polycystic ovarian morphology on ultrasound, ovarian hyperandrogenism, and elevated LH levels [49–54]. In turn, in classic congenital adrenal hyperplasia (CAH), the prevalence of polycystic ovarian morphology on imaging has ranged from rates similar to the general population to as high as two-thirds of postmenarcheal patients [55,56].

Overall, evidence suggests that when present in the peripubertal period APA excess is associated with an increased risk for developing PCOS. Alternatively, whether APA excess is necessary to maintain the disorder once it has manifested itself fully in postpuberty is much less clear. While a number of studies dating back to the 1950s [57] have suggested that suppression of APA production using glucocorticoids may help improve ovulatory dysfunction in PCOS, we were unable to demonstrate this beneficial effect in a prospective study of 36 women with PCOS treated with up to four cycles with dexamethasone 0.5 mg/day [58]. Of the 138 cycles monitored, 78% remained anovulatory, and of the 36 patients studied, 50% had none, 28% had one, 14% had two, and 8% had three ovulatory cycles during treatment. There were no significant differences either in physical features, basal hormones, adrenal response to ACTH stimulation, or DHEAS levels between patients responding or not to treatment. Taken together, these data suggest that, while APA excess may be an important risk factor for PCOS, once the syndrome is established, it plays a limited role in the associated ovulatory dysfunction.

2.3. Mechanisms underlying APA excess in PCOS

2.3.1. Hypothalamic–pituitary–adrenal axis dysfunction in APA excess and PCOS

Most studies on hypothalamic–pituitary–adrenal function in PCOS have found that circulating ACTH levels are similar to those found in normal women matched for weight and age [59–67]. Also the ACTH response to various stimuli, including corticotrophin releasing hormone (CRH), has been generally found to be similar in PCOS women and in controls matched for age and body weight [61–65,67,68], although the response of APAs to ACTH stimulation is exaggerated in women with PCOS and DHEAS excess (Fig. 3) [67]. Only one study, assessing ACTH values in a small group of lean PCOS, found increased values of this pituitary hormone in women with the disorder [69]. Taken together, these data suggest that the increased APA secretion found in a fraction of PCOS patients does not depend on an increased hypothalamic–pituitary drive. Alternatively, most studies have observed an increased APA response to ACTH stimulation in PCOS [61,64,67,70], particularly in patients with DHEAS excess [67], suggesting that adrenocortical androgen biosynthesis is heightened in PCOS, whether due to intra-adrenal or secondary to peripheral influences (see Section 2.3.3).

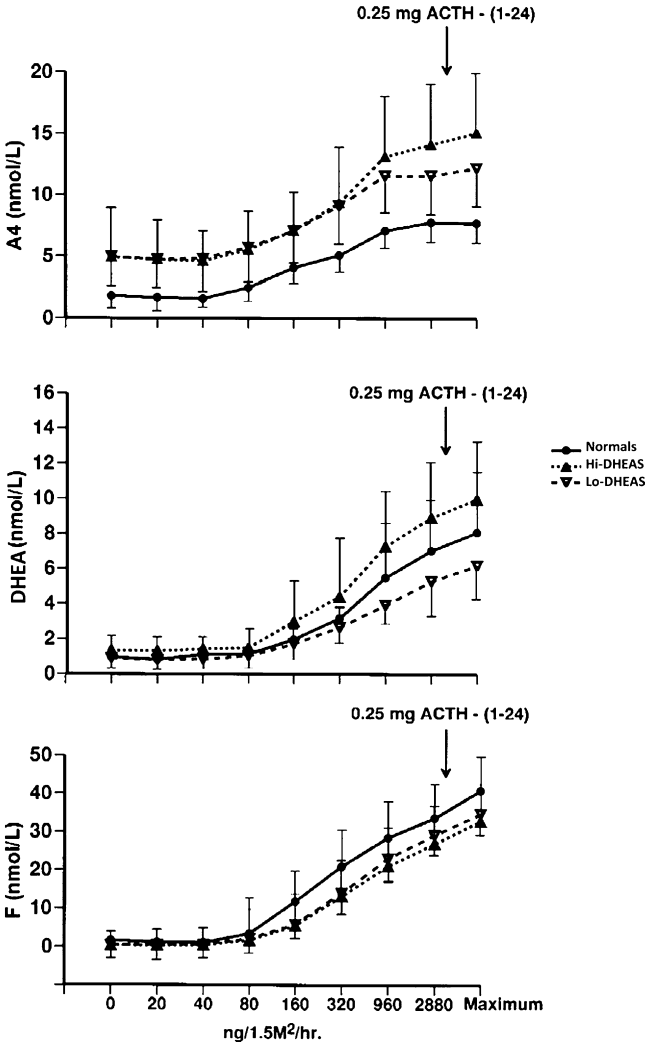

Fig. 3. Sensitivity and response of adrenal precursor androgens to ACTH stimulation in PCOS women with elevated (Hi-DHEAS) and normal (Lo-DHEAS) DHEAS levels and in matched controls. Depicted are the mean (±SD) A4, DHEA, and cortisol (F) response curves during incremental ACTH stimulation in Hi-DHEAS ($n = 12$) and Lo-DHEAS ($n = 12$) patients and in age- and weight-matched normal controls ($n = 11$) (reprinted with permission from reference [64]).
Top: Mean A_4 measurements for normal subjects were significantly lower ($p < 0.05$) than for Hi-DHEAS or Lo-DHEAS subjects, while mean A_4 values for Hi-DHEAS and Lo-DHEAS subjects were not significantly different.
Middle: Mean DHEA measurements for Hi-DHEAS subjects were significantly higher ($p < 0.05$) than for Lo-DHEAS patients, while mean DHEA values for normal women were not significantly different from Hi-DHEAS or Lo-DHEAS subjects.
Bottom: Mean cortisol (F) measurements for normal subjects were significantly higher ($p < 0.05$) than for Lo-DHEAS or Hi-DHEAS women, and mean F values for Hi-DHEAS and Lo-DHEAS subjects not significantly different.

2.3.2. Abnormalities of adrenocortical steroidogenesis in PCOS

2.3.2.1. Alteration in adrenocortical biosynthesis in PCOS. Women with PCOS and APA excess demonstrate a generalized hypersecretion of adrenocortical products in response to ACTH that includes not only androgens (DHEA and A4) but also pregnenolone, 17-hydroxypregnenolone, 11-deoxycortisol, and cortisol [64,67,71]. However, myriad studies indicate that there are no specific adrenal steroidogenic defects among women with PCOS, particularly after 21-OH deficient NCAH is excluded per the various diagnostic protocols noted above [72].

Previously, in some PCOS patients presenting with high DHEAS levels and an exaggerated 17-hydroxypregnenolone/17-HP ratio after ACTH stimulation, the presence of a genetic defect of 3β-HSD activity was postulated [73–75], although not all agreed [76,77], In fact, subsequent studies in these patients demonstrated the absence of inherited defects in their *HSD3B2* gene [78], and it is likely that these patients have a functional deficiency of 3β-HSD activity, possibly secondary to other alterations such as insulin resistance [79].

Finally, the exaggerated $\Delta^5$17-hydroxylase activity observed in PCOS women with DHEAS excess [80] may also reflect a functional abnormality in P450c17 function, the enzyme determining both 17-hydroxylase and 17, 20-lyase activity. What factors may result in the exaggerated $\Delta^5$17-hydroxylase activity remain to be elucidated, although potential candidates include various extra-adrenal modulators (see Section 2.3.3).

2.3.2.2. Abnormalities in cortisol metabolism in PCOS. Increased peripheral metabolism of cortisol has been observed in patients with PCOS. Studying 11 PCOS patients, Stewart et al. observed that total urinary cortisol metabolites were higher in patients than controls suggesting increased breakdown of this steroid [81]. These authors hypothesized that an elevated cortisol metabolism would result in increased ACTH production to maintain normal cortisol levels at the expense of APA excess. Two different mechanisms have been suggested to explain the increased cortisol metabolism: (a) enhanced inactivation of this steroid by 5α-reductase (5α-RA) [81,82]; or (b) impaired reactivation of cortisol from cortisone by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) [82,83].

The interpretation of these data has been complicated by the confounding influence of obesity [84–86] and by the fact that frank increased ACTH secretion in PCOS women is generally absent (see before). In fact, obese women demonstrate altered cortisol metabolism without APA excess or increased ACTH secretion [85,86]. More recent studies have shown that 11β-HSD1 overactivity in PCOS women is determined mainly by the obesity [82,87], while exaggerated 5α-RA activity is partially dependent on excessive body weight [87,88]. In PCOS, increased 5α-RA activity may be the result of hyperinsulinemia or even androgen excess itself [88].

The actual clinical significance of these findings is unclear, as an effect of increased 5α-RA activity on APA production has not yet been demonstrated [87]. In addition, total cortisol production rate in PCOS is generally normal [89] and ACTH secretion is not significantly increased (Section 1.3). Finally, PCOS patients with increased DHEAS levels generally have lower insulin levels [90], while the prevalence of increased APA androgens in classic PCOS (who demonstrate a higher prevalence of hyperinsulinemia) is similar to that in other forms of hyperandrogenism with normal levels of insulin (ovulatory PCOS, idiopathic hyperandrogenism) [70,90].

2.3.3. Role of extra-adrenal factors on adrenal steroidogenesis

2.3.3.1. Effect of the ovary on APA excess and dysregulated adrenocortical steroidogenesis in PCOS. It is possible that ovarian-secreted factors, such as the androgen T, may contribute to the adrenal hyperandrogenism of these patients. For example, elevated T levels in men, and the administration of T to oophorectomized women, have been associated with elevations in the ratio of DHEAS to DHEA [91], suggesting that ovarian hyperandrogenemia may increase DHEA sulfation, and consequently the circulating levels of DHEAS. However, in our hands T administration does not significantly alter the response of A4, DHEA, or cortisol to ACTH stimulation, although it does appear to increase the sulfation of DHEA to DHEAS [91]. In addition, we found that in vitro exogenous T had no predictable effect on the production of DHEA, DHEAS or cortisol in human adrenal tissue [92], although in NCI-H295R adrenocortical cell lines T increased the production of DHEA and decreased the secretion of DHEAS [93]. Overall, external T appears to have only a modest effect on APA secretion, primarily increasing the sulfation of DHEA to DHEAS, and does not result in an exaggerated secretion of active androgens.

Some investigators [94–97], but not all [97], evaluating the effect of ovarian suppression with a long-acting gonadotropin releasing hormone (GnRH) analogue reported decreased DHEAS levels, suggesting an effect of the ovary on the adrenal in PCOS. However, to determine whether adrenocortical dysfunction in PCOS results from ovary-secreted products, excluding estrogen, we examined the effects of oophorectomy on APA secretion in women with PCOS [98]. We found that patients experienced the expected decreases in ovarian androgen levels (total and free T), and increases in FSH levels, following oophorectomy. However, there was no effect on basal DHEAS levels or DHEA levels, or on the secretion of DHEA in response to ACTH stimulation. However, as the group of patients studied was older, as would be expected for women undergoing an elective oophorectomy, and hence had lower DHEAS levels, it is possible that an ovarian effect may be observable in PCOS patients at the highest levels of DHEAS excess.

Taken together, these data suggest that the ovary, either through the production of T, estrogens or other products, has at best only a limited effect on adrenocortical function and APA production in normal or PCOS women.

2.3.3.2. Effect of abnormalities of the glucose/insulin axis on APA excess and dysregulated adrenocortical steroidogenesis in PCOS. Insulin resistance and the consequent compensatory hyperinsulinemia are well recognized as factors contributing to the development and/or severity of PCOS, given that insulin stimulates ovarian androgen production as well as lowers hepatic production of sex hormone-binding globulin (SHBG) [99,100]. Whether abnormalities in glucose homeostasis contribute to APA excess in PCOS is less well established.

In our study of 213 women with PCOS and 182 controls assessing the prevalence of APA excess in PCOS [32], we observed that DHEAS levels were not significantly associated with fasting insulin levels in either black ($r = -0.06$, $p = 0.58$) or white controls ($r = -0.103$, $p = 0.325$). In contrast, among PCOS patients a negative association between DHEAS and fasting insulin levels was observed in white ($r = -020$, $p < 0.02$), but not black ($r = 0.12$, $p = 0.57$) PCOS patients.

In a cross-sectional analysis in over 350 women with PCOS, DHEAS levels and the homeostatic model assessment of insulin resistance (HOMA-IR), a variable that mainly reflects fasting insulin, were observed to be negatively correlated [101]. This relationship remained statistically significant in multivariable models that adjusted for adiposity, age, and free T. While no relationship was observed between circulating insulin and DHEAS levels in a study of 111 non-Hispanic white and 50 Mexican–American individuals, Mexican Americans, who were more insulin resistant, and presumably more hyperinsulinemic, tended to manifest lower DHEAS levels [102].

During oral glucose tolerance testing, in women with PCOS acute glucose-induced hyperinsulinemia was found either to have

no effect [103] or to decrease DHEAS levels [104]. Another study found no difference in the adrenal steroid (cortisol, DHEA, A4) response to ACTH stimulation between hyperandrogenic women with or without chronic hyperinsulinemia [105].

Ludwig et al. studied seven patients with PCOS and 20 healthy controls using a hyperinsulinemic glucose clamp study with stepwise reduction of the plasma glucose level from hyperglycemia to hypoglycemia [106]. These investigators observed that neither hyperglycemia nor hypoglycemia influenced T, A4, DHEAS, or 17-HP levels in PCOS patients and healthy controls. The concentrations of DHEA increased during hypoglycemia in PCOS patients and in controls, but the reduced glucose tolerance of PCOS patients did not influence the response of androgen levels to alterations in plasma glucose levels in comparison with healthy controls.

Diamond et al. [107] demonstrated that neither short-term hyperinsulinemia nor mild hyperglycemia had a significant influence on T, A4, and DHEA levels in healthy, non-obese women. A hyperinsulinemic euglycemic clamp and a hyperinsulinemic hyperglycemic clamp (with a glucose concentration of 125 mg/dL (68 mmol/L), corresponding to a mild hyperglycemic state) was performed in healthy women over a period of 2 h each. The same investigators also assessed pancreatic insulin secretion in response to hyperinsulinemia and hyperglycemia in healthy, non-obese women and found no significant correlation between serum androgens and either glucose uptake or insulin-mediated glucose utilization.

The relationship between insulin levels and APA excess has also been interrogated in smaller sample sizes using detailed physiologic phenotyping. In a study of nine women with PCOS and nine BMI-, age- and race-matched controls, phenotyped using the frequently sampled intravenous glucose tolerance test (FSIGT) and ACTH stimulation tests, neither insulin sensitivity nor acute insulin response to glucose were found to correlate with basal DHEAS or basal or ACTH-stimulated APAs [108]. The main finding was that glucose effectiveness (the ability of glucose to promote its own removal from the bloodstream) was correlated with DHEAS and basal and stimulated steroids in women with PCOS. Another study examined T, A4, and DHEA levels during the three hours of FSIGT, finding that DHEA levels decreased in controls and insulin sensitive women with PCOS, but did not change in insulin resistant women with PCOS [109].

Studies utilizing insulin sensitizing therapies in PCOS have also addressed the relationship between insulin resistance and APA excess. In a large multi-center trial, treatment with the thiazolidinedione (TZD) troglitazone was found to decrease DHEAS levels [110]. In overweight women with PCOS, rosiglitazone treatment reduced both DHEA and DHEAS [111,112]. A smaller study treating PCOS women with pioglitazone found no effect on basal or ACTH-stimulated DHEAS, whereas ACTH-stimulated A4 and 17-HP were reduced after treatment [113]. Similar results were observed in a study evaluating APA responses to corticotrophin releasing factor before and after pioglitazone [114]. While other individual studies also found no effect of pioglitazone on DHEAS levels [115,116], a meta-analysis of ten trials suggested that TZDs are more potent than metformin in reduction of DHEAS [117].

That TZDs and metformin reduce both fasting insulin and DHEAS suggest that insulin may stimulate DHEAS production; however, this conflicts with several reports of negative correlation between insulin and DHEAS or DHEA levels [101,109], suggesting that insulin sensitizer-mediated reduction in DHEAS levels may occur through mechanisms independent of insulin lowering. Changes in adipocytokines have been suggested as potential mediators of the effect of TZDs to lower APAs [118,119]. In vitro studies found that TZDs but not metformin may directly inhibit steroidogenic enzymes such as P450c17 and 3β-HSD [120]. This may explain the observation that metformin-induced lowering of APAs has been less consistently observed than with TZDs [121,122].

To mimic the hormonal milieu of PCOS, an adrenocortical cell line was treated with T and insulin. In this model, T stimulated DHEA production and inhibited DHEAS production; the inhibitory effect of T on DHEAS output being augmented by insulin, suggesting that these hormones do not contribute to the DHEAS elevation frequently found in women with PCOS [93]. This contrasted with an earlier pilot study of fresh normal adrenal tissue from organ donors, in which insulin treatment, while having no clear effect in individual tissue samples, appeared to suppress DHEA and stimulate DHEAS production in the aggregate [92]. These data primarily point out the difficulty of doing in vitro studies on adrenocortical tissue.

In summary, the relationship between the insulin axis and APA excess is complex and incompletely understood. Several studies suggested that hyperinsulinemia may suppress adrenal steroid production or that lower insulin levels may be associated with higher DHEAS levels, while others appeared to link insulin resistance with higher APA levels. Further work is needed to clarify these relationships, although the negative relationship that DHEAS levels have with cardiovascular risk in women (see below) suggests that higher levels of APA may actually be protective against metabolic and cardiovascular dysfunction.

2.3.3.3. Effect of obesity on DHEA/APA excess and dysregulated adrenocortical steroidogenesis in PCOS. Obesity may impact on adrenocortical function by decreasing insulin sensitivity and increasing circulating insulin levels. However, obesity may also alter adrenal function through the secretion of adipocytokines and other inflammatory products [123], and by increasing the circulating levels of estrogens, through increased aromatization by adipose tissue stromal cells [124–126]. However, studies assessing the effect of obesity in otherwise healthy women have provided conflicting results.

In our study of 213 women with PCOS and 182 controls assessing the prevalence of APA excess in PCOS [32], we observed that DHEAS levels were not significantly associated with BMI ($r = -0.15$, $p = 0.163$ and $r = -0.02$, $p = 0.825$) in either black controls or white controls, respectively (Fig. 4). Among PCOS patients a negative association between DHEAS levels and BMI was observed among white ($r = -0.20$, $p < 0.006$), but not black ($r = 0.10$, $p = 0.62$) women (Fig. 5A and B).

In order to determine the effect of obesity on adrenocortical biosynthesis in vivo, we studied 30 normal-weight and 27 overweight healthy, eumenorrheic, non-hirsute female volunteers using acute ACTH stimulation [127]. Obese women demonstrated higher free T levels and a higher DHEAS/DHEA ratio, but no significant difference in basal or APA responses to ACTH stimulation, except for a 2-fold greater increment in A4 with ACTH stimulation in obese women. These results contrast with those of Vicennati et al., who studied 12 women with abdominal and 13 with peripheral obesity, and seven healthy normal-weight women, did not observe significant differences in basal and ACTH-stimulated levels of DHEA, A4 and 17-HP among the three groups, and no significant correlation between basal and stimulated APA levels and body mass [128]. In another study in healthy women, the A4 response to ACTH stimulation was also not correlated with BMI in [129].

In contrast, Brody et al. noted among 29 postmenopausal women that the degree of obesity was correlated to the net response of DHEA to ACTH stimulation [130]. In agreement, Komindr et al. studied 10 normal and 16 obese eumenorrheic non-hirsute women matched for age, and reported that the mean DHEA response slope was significantly greater, and the minimal ACTH

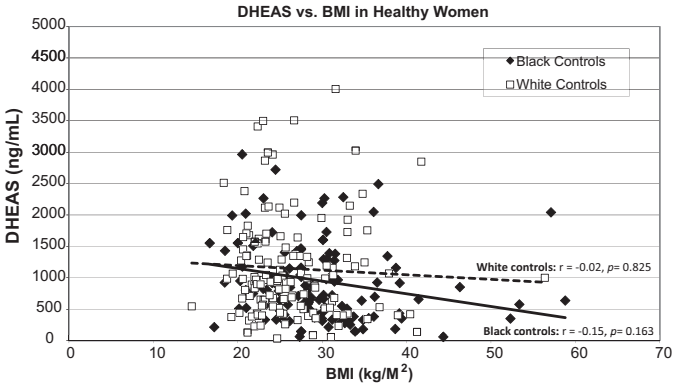

Fig. 4. DHEAS levels vs. BMI in control women, by race. Depicted are the scattergrams and linear regression trend lines for DHEAS levels according to BMI in black ($r = -0.15$, $p = 0.163$) and white ($r = -0.02$, $p = 0.825$) control women (adapted from reference [32]).

threshold dose for A4 was significantly lower (increased sensitivity), in obese women [131].

Rittmaster et al. attempted to study the inter-relation between adrenal hyperandrogenism, insulin resistance, and obesity in PCOS [129]. APA secretion (defined as the A4 response to ACTH) and insulin resistance (estimated by calculating the area-under-the-curve [AUC] for serum insulin levels in response to a 75 g oral glucose load) were quantified in oligomenorrheic women with PCOS and in three groups of eumenorrheic women: weight-matched hirsute women, obese nonhirsute women, and thin nonhirsute women. The study results indicated that mean A4 response to ACTH and the insulin AUC in PCOS was greater than that in eumenorrheic hirsute women, obese nonhirsute women, and lean nonhirsute women. The A4 response was not correlated with BMI, while the insulin response was highly correlated with BMI. Overall, there was no correlation between APA secretion and insulin resistance in any of the groups, leading the investigators to conclude that adrenal hyperandrogenism and insulin resistance are independent predictors of anovulation in hirsute women.

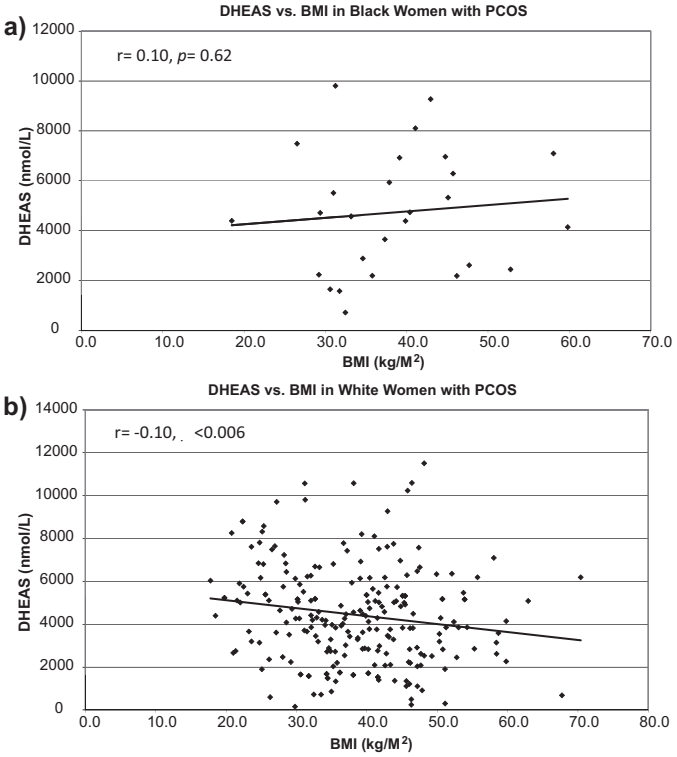

Fig. 5. DHEAS levels vs. BMI in PCOS women, by race. Depicted are the scattergrams and linear regression trend lines for DHEAS levels according to BMI in Black (top; $r = 0.10$, $p = 0.62$) and White (bottom; $r = -0.10$, $p < 0.006$) PCOS women (adapted from reference [32]).

Similarly conflicting results are observed for cortisol secretion. While we [124], Brody et al. [130], and Komindr et al. [131], observed that cortisol responses, overall, were no different between obese and normal weight subjects, a number of other investigators have also observed a greater degree of cortisol secretion and metabolism in those obese subjects with visceral adiposity [84,85,128,132,133]. More recently, Roelfsema et al. investigated 15 obese PCOS women and 15 healthy obese controls with a regular menstrual cycle, using 24 h blood sampling at 20 min intervals [89]. Basal, pulsatile, and total cortisol production was similar in PCOS patients and matched healthy control women. In addition, the regularity of cortisol secretion and the diurnal properties were identical. Compared with 10 lean control women, mean cortisol production per liter distribution volume was similar in the three groups, although the total 24 h cortisol production was increased in obese control women and PCOS women. Overall, this study demonstrates equally increased cortisol production in PCOS women and obese healthy control women.

Taken together, these studies suggest while obesity is not associated with increased DHEAS levels, it may stimulate increased secretion of F, and possibly A4 and DHEA, in response to ACTH, although there is wide disagreement. Whether this observation, if true, is due to the higher circulating levels of insulin (see above) in obese individuals, or whether this reflects the effects of adipocyte-generated adipocytokines or similar factors, remains unknown. Future studies are need to more clearly document the effect of obesity on adrenocortical function, including the production and metabolism of glucocorticoids, the effect of body fat distribution, the effect of weight loss, the mechanisms underlying such relationships, and its role in the adrenocortical hyper-responsivity of PCOS.

2.3.4. Heritability and genetic factors in the regulation of APAs in general and in the APA excess of PCOS

That APA levels are under significant genetic control has been established by heritability studies. Heritability is the proportion of trait variance that arises from inherited factors, and ranges from 0 (no genetic contribution) to 100% (completely determined by genetics). A caveat is that heritability calculations typically cannot resolve genetic influences from shared family environmental influences, such that heritability can be considered an upper bound of the genetic effect. An early family study found the heritability of DHEAS to be 65%; this study adjusted for the well-known effect of age on DHEAS [134]. In a twin-study of middle-aged men, the heritability of DHEAS was found to be 37–46%; the time of day at which DHEAS was measured affected the heritability estimates [135]. Analysis in the Cincinnati Myocardial Infarction and Hormone (CIMIH) family study found that the correlation of DHEAS between family members was stronger in women (heritability 74%) than in men (29%) [136]. The heritability of DHEAS was estimated as 58% in white families, 66% in African Americans, and 29–43% in Mexican Americans [136–140]. In twin studies, the heritability estimates of DHEAS ranged from 26% (European American adult men) to 65% (Chinese girls) [135,141–145]. No reports of the heritability of DHEA have been published.

In PCOS cohorts, significant heritability of APAs has also been observed. Among 62 women with PCOS and their 69 sisters, the heritability of DHEAS was found to be 44% [146]. A study of 125

women with PCOS and 214 of their sisters found a high heritability (67%) of A4 among affected sibling pairs, and no heritability (0%) among discordant sibling pairs; the overall heritability was 58% [147]. Significant correlations were observed in ACTH-stimulated cortisol and DHEA levels between women with PCOS and their sisters [148]. Brothers of women with PCOS had significantly elevated DHEAS levels compared to control men, and there was a significant positive correlation in DHEAS levels between PCOS women and their brothers [149]. The heritability observed may be related to the presence of similar environments and environmental effects in families with PCOS, the inheritance of genetic factors that may indirectly influence APA production or metabolism, or more likely, considering the limited role that the environment and extra-adrenal factors play in modulating APAs in PCOS, is primarily due to the inheritance of genetic factors directly affecting APA biosynthesis.

Several candidate gene studies focusing on APA excess have been carried out in PCOS cohorts. Sulfonation of DHEA to produce DHEAS is carried out by the enzyme DHEA sulfotransferase (*SULT2A1*); the reverse reaction is carried out by steroid sulfatase (*STS*); these genes are therefore logical candidate genes for study in PCOS. A genetic association study in 287 PCOS cases and 187 healthy controls found that single nucleotide polymorphism (SNP) rs182420 in *SULT2A1* was associated with DHEAS levels in cases but not controls; no associations with variants in *STS* were observed [150]. A subsequent study of 582 patients and 2017 controls examined variation in *SULT2A1*, *STS*, and *PAPSS2* (the gene for 3-phosphoadenosine 5-phosphosulfate, which contributes a sulfate group in the conversion of DHEA to DHEAS); nominal associations of variants in *SULT2A1* were associated with DHEAS (but not DHEA) in the PCOS women, with the strongest finding being association of SNP rs2910397 with the DHEAS to DHEA ratio [151].

Neither of these studies found association of *SULT2A1* variants with PCOS diagnosis itself, suggesting that genetic variation in *SULT2A1* may modify the APA excess phenotype in affected women without altering overall PCOS risk. The cytochrome P450 enzyme CYP3A7 metabolizes DHEA and DHEAS. *CYP3A7* is expressed in fetal tissues and sharply downregulated at birth [152]; however, a particular promoter haplotype results in persistent expression in adulthood [153]. This haplotype was associated with reduced DHEAS levels in general population cohorts [154] and in women with PCOS [155]. Similar to *SULT2A1*, the *CYP3A7* haplotype associated with DHEAS levels was not associated with PCOS risk; this variant thus represents another genetic modulator of the PCOS APA excess phenotype. Studies of the genes coding the enzymes involved in DHEA synthesis, *CYP11A1* (cholesterol side-chain cleavage enzyme) and *CYP17A1* (17α-hydroxylase/17,20-lyase), have generally been negative for association with PCOS or DHEAS levels [66–70]. Likewise, variants of *CYP21A2* (encoding for P450c21) have not found to be predictive of ovarian or adrenal hyperandrogenism in PCOS [156,157].

Genome-wide association studies (GWAS) in large cohorts have been the mainstay of gene discovery in common complex disorders for the last several years. A number of GWAS have focused on circulating levels of sex hormones such as DHEAS, T, and SHBG [158]. A meta-analysis of GWAS from seven cohorts, totaling over 14,000 Caucasian individuals each with ~2.5 million SNP genotypes, identified eight independent SNPs highly associated with DHEAS levels [159]. These SNPs are in or near the genes for *ZKSCAN5* (rs11761528, $p = 3.15 \times 10{-}36$), *ARPC1A* (rs740160, $p = 1.56 \times 10{-}16$), *SULT2A1* (rs2637125, $p = 2.61 \times 10{-}19$), *CYP3A43/TRIM4* (rs17277546, $p = 4.50 \times 10{-}11$), *BMF* (rs7181230, $p = 5.44 \times 10{-}11$), *HHEX* (rs2497306, $p = 4.64 \times 10{-}9$), *BCL2L11* (rs6738028, $p = 1.72 \times 10{-}8$), and *CYP2C9* (rs2185570, $p = 2.29 \times 10{-}8$). These SNPs explain only a small amount of the variation in DHEAS between subjects, about 4% of the total variance in DHEAS and 7% of the genetic variance [159].

Given the considerable heritability of DHEAS described above, it is clear that a substantial number of genetic determinants remain to be discovered. It is notable that *SULT2A1*, identified as a genetic determinant of DHEAS in much smaller PCOS cohorts [150,151], was one of the top signals in the GWAS. Furthermore, the GWAS signal was mainly driven by association in women ($p = 7.65 \times 10{-}14$ for rs2637125); in men, no signals in the *SULT2A1* region were associated with DHEAS at genome-wide significance levels ($p < 5 \times 10^{-8}$). This greater participation of genetic variation in *SULT2A1* to DHEAS levels in women has clear relevance to the development of APA excess in many women with PCOS. In addition, the signal near the *CYP3A43* and *TRIM4* genes on chromosome 7 is close to the gene for *CYP3A7*, previously studied as a genetic determinant of DHEAS [154,155].

Similar to the candidate gene studies in PCOS, the GWAS meta-analysis did not identify *CYP11A1* or *CYP17A1* as genetic regulators of DHEAS; *STS* was not studied because it is on the X chromosome [159]. Two published GWAS for PCOS have identified 11 susceptibility loci, none of which has a known relationship to APA production [160,161]; to date, the limited studies examining GWAS-derived PCOS genetic variants for association with quantitative traits have not included DHEA or DHEAS [162].

In summary, several studies have documented that DHEAS is highly regulated by genetic factors. Candidate gene and GWAS studies, both in PCOS cohorts and large general population cohorts have identified less than ten independent genetic regions that regulate DHEAS levels, leaving a substantial amount of its heritability unexplained. Large-scale genetic investigation of other APAs (DHEA, A4) or ACTH-stimulated APAs are badly needed. More work is needed to fully characterize the genetic control of APAs levels. Such knowledge may lead to new therapeutic measures in PCOS and other conditions impacted by adrenal sex hormones.

3. Metabolic consequences of DHEAS excess in PCOS

As noted above, the relationship between APAs and DHEAS levels and predictors of metabolic dysfunction, such as insulin resistance, hyperglycemia, or obesity, is unclear and weak at best. However and more clearly, as for men [163,164] among postmenopausal women with coronary risk factors undergoing coronary angiography for suspected myocardial ischemia, lower DHEAS levels were linked with higher cardiovascular and all-cause mortality [165]. In PCOS, Carmina and Lobo observed that patients with increased DHEAS levels generally presented with lower insulin levels and more favorable metabolic and cardiovascular parameters [90].

4. Summary

Approximately 20–30% of PCOS women demonstrate excess APA production, using DHEAS as a marker of APA in general and more specifically DHEA synthesis, although we should recognize that DHEAS levels do not always reflect adrenal hyperandrogenism. The role of APA excess in determining or causing PCOS is unclear, although observations in patients with inherited APA excess (e.g., patients with 21-OH deficient congenital classic or non-classic adrenal hyperplasia) demonstrate that APA excess can result in a PCOS-like phenotype. Studies in daughters of women with PCOS suggest that an exaggerated adrenarche and or DHEAS levels precedes the development of PCOS symptoms. Furthermore, anecdotal reports suggest that peripubertal stress, and concomitant APA excess, can increase the risk of PCOS.

Inherited defects of the enzymes responsible for steroid biosynthesis, or defects in cortisol metabolism, account for only

a very small fraction of women suffering from hyperandrogenism or APA excess. Women with PCOS and APA excess appear to have a generalized exaggeration in adrenal steroidogenesis in response to ACTH stimulation, although they do not have overt abnormality in hypothalamic–pituitary–adrenal axis function. In general, extra-adrenal factors, including obesity, insulin and glucose levels, and ovarian secretions, play a limited role in the increased APA production observed in PCOS, although excess extra-adrenal T may increase the conversion of DHEA to DHEAS, elevating the circulating levels of the latter.

Substantial heritabilities of APAs, particularly DHEAS, have been found in the general population as well as studies in women with PCOS; however, the handful of SNPs discovered to date account only for a small portion of the inheritance of these traits. This may reflect the current technology-driven focus on SNPs, which are common variations in the genome. The many other types of genetic and epigenetic variation have not been explored for a role in the genetic control of APAs. Overall, there appears to be strong evidence of heritability in APA production in women, healthy and PCOS, although no specific associated genetic defects have yet been widely demonstrated.

In summary, the exact cause of APA excess in PCOS remains unclear, although it may reflect a generalized and inherited exaggeration in androgen biosynthesis. As for men, elevated levels of DHEAS appear to be protective against cardiovascular risk in women, although the role of DHEAS in modulating this risk in women with PCOS remains unknown.

5. Key unanswered questions: a guide to future research

The field of APA, including DHEA and DHEAS, excess in PCOS remains fertile, as the exact cause and clinical implications remain unknown. Prospective longitudinal studies of girls suffering from peripubertal stress or exaggerated adrenarche are needed to determine the role that APA excess plays in the risk for the disorder. Additional studies investigating the role of emerging endocrine/paracrine signals (e.g., free fatty acids, adipocytokines, myokines, other inflammatory factors, etc.,) in modulating APA secretion in normal and PCOS women are still needed.

Genetic studies, with increasing samples sizes achieved by development of multi-center consortia, will provide greater power to discover genes that regulate APA levels. Greater sample sizes would also allow interrogation of rare, coding variation in addition to the common and generally non-coding variation interrogated by GWAS. In addition, advances in technology and growing resources such as the Encyclopedia of DNA Elements (ENCODE) and the NIH Epigenomics Roadmap, will increase the ability to examine epigenetic markers such as DNA methylation and histone modification for their roles in PCOS and APA excess. These genetic studies should include sufficient numbers of well-defined and well-phenotyped PCOS women with and without APA excess. Finally, studies of PCOS women with well-defined APA excess are required to determine their risk of metabolic dysfunction and cardiovascular risk, compared to the general population and to women with PCOS but without APA excess.

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