



Review

DHEA, DHEAS and PCOS

Mark O. Goodarzi^a, Enrico Carmina^b, Ricardo Azziz^{c,*}^a Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA^b University of Palermo, Palermo, Italy^c Georgia Regents University, Office of the President, 120 15th St., AA 311, Augusta, GA 30912, USA

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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.

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Abbreviations: 11 β -HSD1, 11 β -hydroxysteroid dehydrogenase type 1; 3 β -HSD, 3 β -hydroxysteroid dehydrogenase; 5 α -RA, 5 α -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, area-under-the-curve; BMI, body mass index; DHEA, dehydroepiandrosterone; ESHRE, European Society of Human Reproduction and Embryology; FSH, follicle stimulating hormone; FSGT, 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.

* Corresponding author. Tel.: +1 7067212301; fax: +1 7067212303.

E-mail addresses: razziz@gru.edu, Isherrouse@gru.edu (R. Azziz).

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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.

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

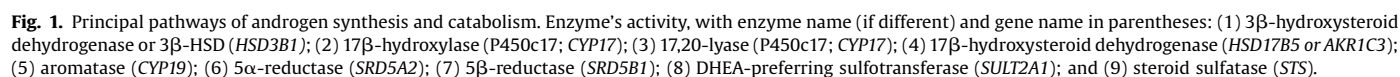
The adrenal cortices produce three steroids with variable degrees of androgenic activity, in descending order of adrenal production: dehydroepiandrosterone (DHEA), androstenedione (A4) and testosterone (T) (Fig. 1). In human adrenals, the primary biosynthetic pathway for androgenic steroidogenesis after cholesterol cleavage proceeds thru the Δ^5 pathway by way of pregnenolone and its subsequent hydroxylation to 17-hydroxypregnenolone (thru the 17 α -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 3 β -hydroxysteroid dehydrogenase (3 β -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 3 β -HSD) and then to 17 α -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].

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
Hyperandrogenism (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.



Although many of the factors regulating the production of APAs remain unclear, ACTH does appear to play a key role [30]. However, a

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 11 β -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

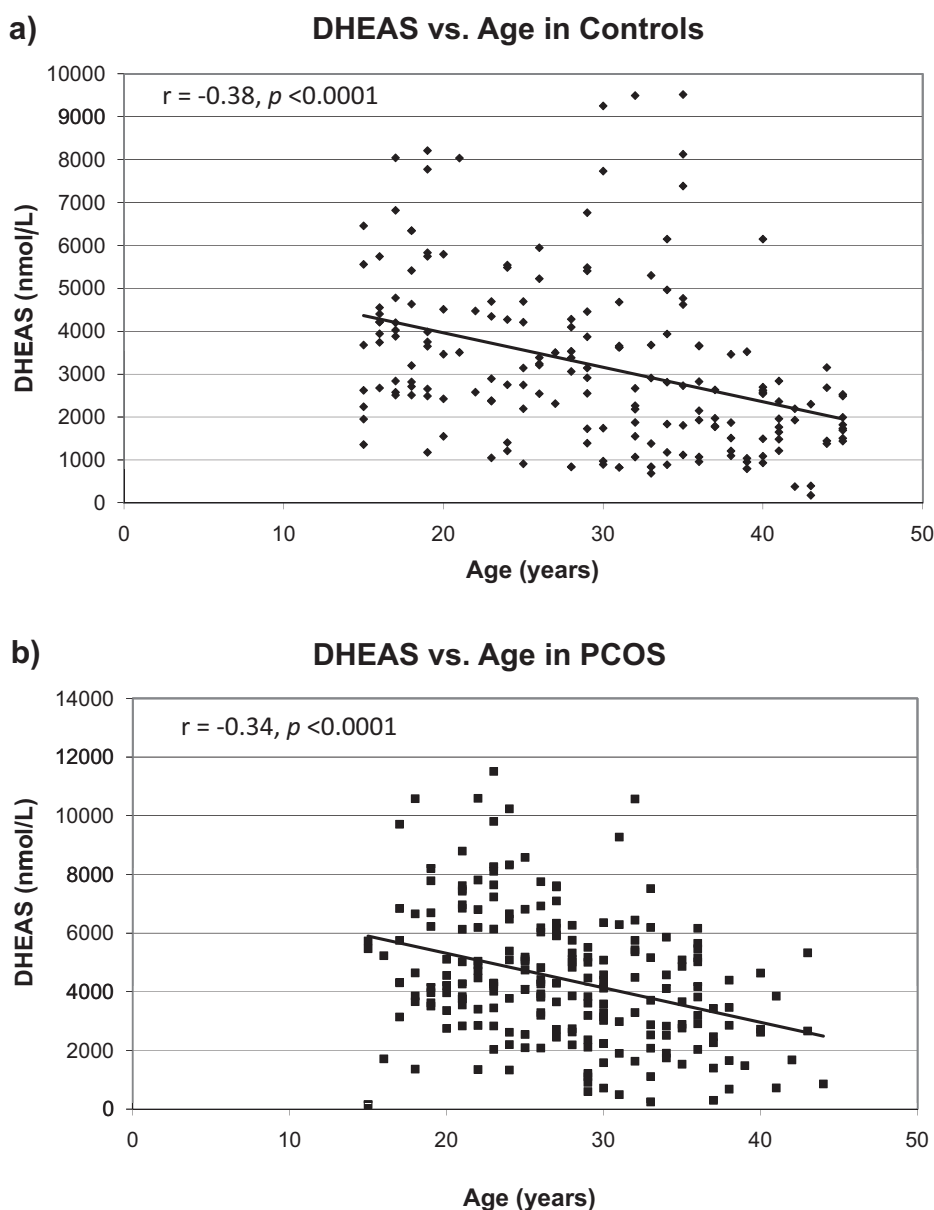


Fig. 2. DHEAS levels vs. age in control and PCOS women. Depicted are the scattergrams and linear regression trend lines for DHEAS levels according to age in healthy controls (top; $r = -0.38$, $p < 0.0001$) and PCOS (bottom; $r = -0.34$, $p < 0.0001$). Note that DHEAS levels in PCOS and controls decrease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

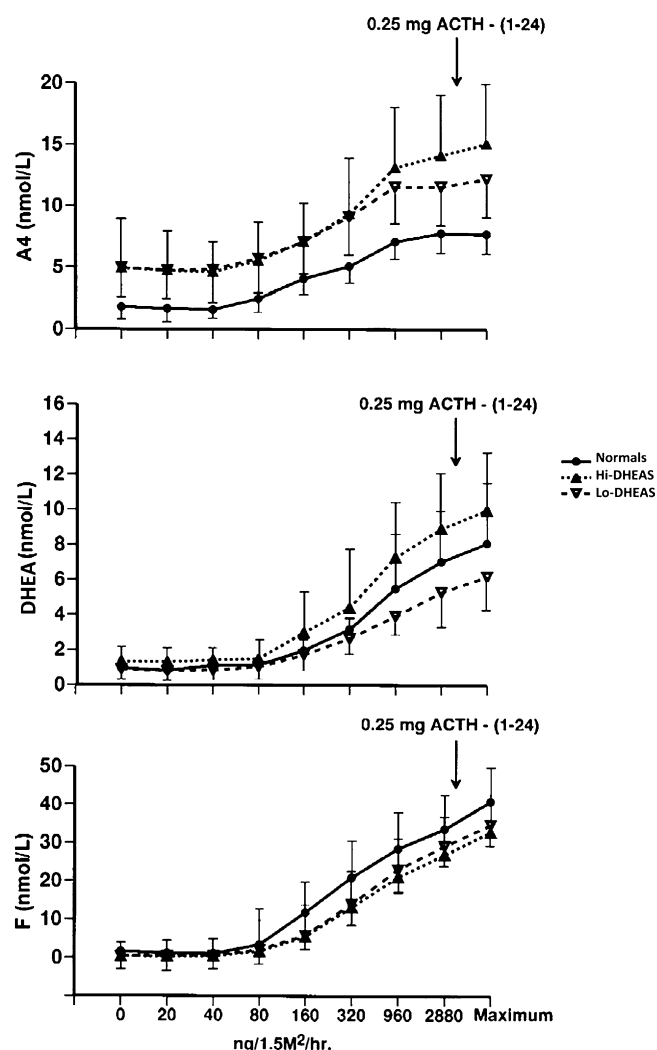


Fig. 3. Sensitivity and response of adrenal precursor androgens to ACTH stimulation in PCOS women with elevated (Hi-DHEAS) and normal (Lo-DHEAS) DHEAS levels and in matched controls. Depicted are the mean (\pm SD) A4, DHEA, and cortisol (F) response curves during incremental ACTH stimulation in Hi-DHEAS ($n=12$) and Lo-DHEAS ($n=12$) patients and in age- and weight-matched normal controls ($n=11$) (reprinted with permission from reference [64]).

Top: Mean A4 measurements for normal subjects were significantly lower ($p < 0.05$) than for Hi-DHEAS or Lo-DHEAS subjects, while mean A4 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 3 β -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 3 β -HSD activity, possibly secondary to other alterations such as insulin resistance [79].

Finally, the exaggerated Δ^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 Δ^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 5 α -reductase (5 α -RA) [81,82]; or (b) impaired reactivation of cortisol from cortisone by 11 β -hydroxysteroid dehydrogenase type 1 (11 β -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 11 β -HSD1 overactivity in PCOS women is determined mainly by the obesity [82,87], while exaggerated 5 α -RA activity is partially dependent on excessive body weight [87,88]. In PCOS, increased 5 α -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 5 α -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 ($r = -0.20$, $p < 0.02$), 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 (6.8 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 3 β -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

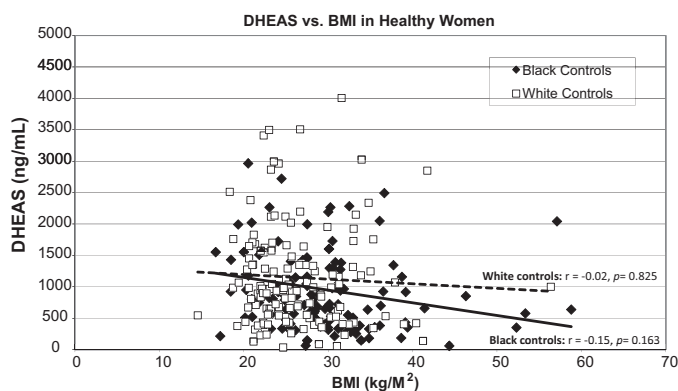


Fig. 4. DHEAS levels vs. BMI in control women, by race. Depicted are the scattergrams and linear regression trend lines for DHEAS levels according to BMI in black ($r = -0.15$, $p = 0.163$) and white ($r = -0.02$, $p = 0.825$) control women (adapted from reference [32]).

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.

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 needed 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

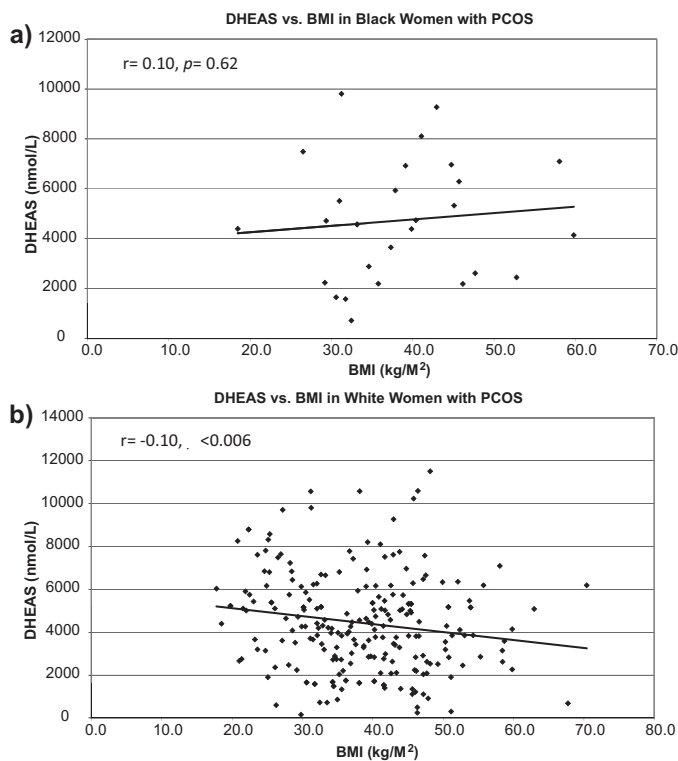


Fig. 5. DHEAS levels vs. BMI in PCOS women, by race. Depicted are the scattergrams and linear regression trend lines for DHEAS levels according to BMI in Black (top; $r = 0.10$, $p = 0.62$) and White (bottom; $r = -0.10$, $p < 0.006$) PCOS women (adapted from reference [32]).

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* (17 α -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, $p = 4.64 \times 10^{-9}$), *BCL2L1* (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 sample 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.

References

- [1] R. Azziz, E. Carmina, D. Dewailly, E. Diamanti-Kandarakis, H.F. Escobar-Morreale, W. Futterweit, O.E. Janssen, R.S. Legro, R.J. Norman, A.E. Taylor, S.F. Witchel, Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an androgen excess society guideline, *J. Clin. Endocrinol. Metab.* 91 (2006) 4237–4245.
- [2] J.K. Zawadzki, A. Dunaif, Diagnostic criteria for polycystic ovary syndrome, in: A. Dunaif, J. Givens, F. Haseltine, G.R. Merriam (Eds.), *Polycystic Ovary Syndrome*, Blackwell Scientific Publications, Boston, 1992, pp. 377–384.
- [3] The 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.* 81 (2004) 19–25.
- [4] The Rotterdam ESHRE/ASRM Sponsored PCOS Consensus Workshop Group, Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS), *Hum. Reprod.* 19 (2004) 41–47.
- [5] NIH Office of Disease Prevention: Evidence-based Methodology Workshop on Polycystic Ovary Syndrome (PCOS), Dec. 3–5, 2012-Final report (<http://prevention.nih.gov/p2p/pcos/resources.aspx>)
- [6] E.S. Knochenhauer, T.J. Key, M. Kahsar-Miller, W. Waggoner, L.R. Boots, R. Azziz, Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study, *J. Clin. Endocrinol. Metab.* 83 (1998) 3078–3082.
- [7] K.F. Michelmores, A.H. Balen, D.B. Dunger, M.P. Vessey, Polycystic ovaries and associated clinical and biochemical features in young women, *Clin. Endocrinol. (Oxf.)* 51 (1999) 779–786.
- [8] E. Diamanti-Kandarakis, C.R. Kouli, A.T. Bergiele, F.A. Filandra, T.C. Tsianateli, G.G. Spina, E.D. Zapanti, M.I. Bartzis, A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile, *J. Clin. Endocrinol. Metab.* 84 (1999) 4006–4011.
- [9] M. Asunción, R.M. Calvo, J.L. San Millán, J. Sancho, S. Avila, H.F. Escobar-Morreale, A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain, *J. Clin. Endocrinol. Metab.* 85 (2000) 2434–2438.
- [10] R. Azziz, K.S. Woods, R. Reyna, T.J. Key, E.S. Knochenhauer, B.O. Yildiz, The prevalence and features of the polycystic ovary syndrome in an unselected population, *J. Clin. Endocrinol. Metab.* 89 (2004) 2745–2749.
- [11] C. Moran, G. Tena, S. Moran, P. Ruiz, R. Reyna, X. Duque, Prevalence of polycystic ovary syndrome and related disorders in Mexican women, *Gynecol. Obstet. Invest.* 69 (2010) 274–280.
- [12] W.A. March, V.M. Moore, K.J. Willson, D.I. Phillips, R.J. Norman, M.J. Davies, The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria, *Human. Reprod.* 25 (2010) 544–551.
- [13] F. Mehrabian, B. Khani, R. Kelishadi, E. Ghanbari, The prevalence of polycystic ovary syndrome in Iranian women based on different diagnostic criteria, *Endokrynol. Pol.* 62 (2011) 238–242.
- [14] J.A. Boyle, J. Cunningham, K. O'Dea, T. Dunbar, R.J. Norman, Prevalence of polycystic ovary syndrome in a sample of indigenous women in Darwin, Australia, *Med. J. Aust.* 196 (2012) 62–66.
- [15] B.O. Yildiz, G. Bozdag, Z. Yapici, I. Esinler, H. Yarali, Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria, *Hum. Reprod.* 27 (2012) 3067–3073.
- [16] H. Gill, P. Tiwari, P. Dabaddghao, Prevalence of polycystic ovary syndrome in young women from North India: a community-based study, *Indian J. Endocrinol. Metab.* 16 (2012) S389–S392.
- [17] R. Li, Q. Zhang, D. Yang, S. Li, S. Lu, X. Wu, Z. Wei, X. Song, X. Wang, S. Fu, J. Lin, Y. Zhu, Y. Jiang, H.L. Feng, J. Qiao, Prevalence of polycystic ovary syndrome in women in China: a large community-based study, *Hum. Reprod.* 28 (2013) 2562–2569.
- [18] S. Musmar, A. Afaneh, H. Mo'alla, Epidemiology of polycystic ovary syndrome a cross sectional study of university students at An-Najah national university-Palestine, *Reprod. Biol. Endocrinol.* 11 (2013) 47–53.
- [19] H. Rashidi, F. Ramezani Tehrani, M. Bahri Khomami, M. Tohidi, F. Azizi, To what extent does the use of the Rotterdam criteria affect the prevalence of polycystic ovary syndrome? A community-based study from the Southwest of Iran, *Eur. J. Obstet. Gynecol. Reprod. Biology* 174 (2014) 100–105.
- [20] M.P. Lauritsen, J.G. Bentzen, A. Pinborg, A. Loft, J.L. Forman, L.L. Thuesen, A. Cohen, D.M. Hougaard, A. Nyboe Andersen, The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Müllerian hormone, *Hum. Reprod.* (2014).
- [21] F. Labrie, V. Luu-The, A. Bélanger, S.X. Lin, J. Simard, G. Pelletier, C. Labrie, Is dehydroepiandrosterone a hormone? *J. Endocrinol.* 187 (2005) 169–196.
- [22] D. Lin, S.M. Black, Y. Nagahama, W.L. Miller, Steroid 17 α -hydroxylase and 17,20 lyase activities of P450c17: contributions of serine106 and P450 reductase, *Endocrinology* 132 (1993) 2498–2506.
- [23] D. Lin, T. Sugawara, J.F. Strauss 3rd, B.J. Clark, D.M. Stocco, P. Saenger, A. Rogol, W.L. Miller, Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis, *Science* 267 (1995) 1828–1831.
- [24] C. Longcope, Adrenal and gonadal androgen secretion in normal females, *Clin. Endocrinol. Metab.* 15 (1986) 213–228.
- [25] X.G. Hui, J. Akahira, T. Suzuki, M. Nio, Y. Nakamura, H. Suzuki, W.E. Rainey, H. Sasano, Development of the human adrenal zona reticularis: morphometric and immunohistochemical studies from birth to adolescence, *J. Endocrinol.* 203 (2009) 241–252.
- [26] Y. Nakamura, P.J. Hornsby, P. Casson, R. Morimoto, F. Satoh, Y. Xing, M.R. Kennedy, H. Sasano, W.E. Rainey, Type 5 17 β -hydroxysteroid dehydrogenase (AKR 1C3) contributes to testosterone production in the adrenal reticularis, *J. Clin. Endocrinol. Metab.* 94 (2009) 2192–2198.
- [27] J.C. Rijk, T.F. Bovee, A.A. Peijnenburg, M.J. Groot, I.M. Rietjens, M.W. Nielen, Bovine liver slices: a multifunctional in vitro model to study the prohormone dehydroepiandrosterone (DHEA), *Toxicol. In Vitro* 26 (2012) 1014–1021.
- [28] F. Hammer, S. Subtil, P. Lux, C. Maser-Gluth, P.M. Stewart, B. Allolio, W. Arlt, No evidence for hepatic conversion of dehydroepiandrosterone (DHEA) sulfate to DHEA: in vivo and in vitro studies, *J. Clin. Endocrinol. Metab.* 90 (2005) 3600–3605.
- [29] F. Labrie, A. Bélanger, C. Labrie, B. Candas, L. Cusan, J.L. Gomez, Bioavailability and metabolism of oral and percutaneous dehydroepiandrosterone in postmenopausal women, *J. Steroid Biochem. Mol. Biol.* 107 (2007) 57–69.

- [30] Y. Xing, M.A. Edwards, C. Ahlem, M. Kennedy, A. Cohen, C.E. Gomez-Sanchez, W.E. Rainey, The effect of adrenocorticotrophic hormone on steroid metabolomic profiles in human adrenal cells, *J. Endocrinol.* 209 (2011) 327–335.
- [31] F. Labrie, A. Bélanger, L. Cusan, J.L. Gomez, B. Candas, Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging, *J. Clin. Endocrinol. Metab.* 82 (1997) 2396–2402.
- [32] A. Kumar, K.S. Woods, A.A. Bartolucci, R. Azziz, Prevalence of adrenal androgen excess in patients with the polycystic ovary syndrome (PCOS), *Clin. Endocrinol. (Oxf.)* 62 (2005) 644–649.
- [33] S. Dharia, A. Slane, M. Jian, M. Conner, A.J. Conley, R.M. Brissie, C.R. Parker Jr., Effects of aging on cytochrome b5 expression in the human adrenal gland, *J. Clin. Endocrinol. Metab.* 90 (2005) 4357–4361.
- [34] F.Z. Stanczyk, Diagnosis of hyperandrogenism: biochemical criteria, *Best Pract. Res. Clin. Endocrinol. Metab.* 20 (2006) 177–191.
- [35] M. Pall, R. Azziz, J. Beires, D. Pignatelli, The phenotype of hirsute women: a comparison of polycystic ovary syndrome and 21-hydroxylase-deficient nonclassic adrenal hyperplasia, *Fertil. Steril.* 94 (2010) 684–689.
- [36] E. Carmina, F.Z. Stanczyk, L. Chang, R.A. Miles, R.A. Lobo, The ratio of androstenedione-11 β -hydroxyandrostenedione is an important marker of adrenal androgen excess in women, *Fertil. Steril.* 58 (1992) 1148–1152.
- [37] D.I. Hoffman, K. Klove, R.A. Lobo, The prevalence and significance of elevated dehydroepiandrosterone sulfate levels in anovulatory women, *Fertil. Steril.* 42 (1984) 76–81.
- [38] E. Steinberger, K.D. Smith, L.J. Rodriguez-Rigau, Testosterone, dehydroepiandrosterone, and dehydroepiandrosterone sulfate in hyperandrogenic women, *J. Clin. Endocrinol. Metab.* 59 (1984) 471–477.
- [39] E. Carmina, F. Rosato, A. Janni, Increased DHEAS levels in PCO syndrome: evidence for the existence of two subgroups of patients, *J. Endocrinol. Invest.* 9 (1986) 5–9.
- [40] C. Moran, E. Knochenhauer, L.R. Boots, R. Azziz, Adrenal androgen excess in hyperandrogenism: relation to age and body mass, *Fertil. Steril.* 71 (1999) 671–674.
- [41] L.A. Sánchez, C. Morán, R. Reyna, T. Ochoa, L.R. Boots, R. Azziz, Adrenal progesterone and androgen production in 21-hydroxylase-deficient non-classic adrenal hyperplasia is partially independent of adrenocorticotrophic hormone stimulation, *Fertil. Steril.* 77 (2002) 750–753.
- [42] D. Miller, S.J. Emans, I. Kohane, Follow-up study of adolescent girls with a history of premature pubarche, *J. Adolesc. Health* 18 (1996) 301–305.
- [43] L. Ibanez, N. Potau, R. Virdis, M. Zampolli, C. Terzi, M. Gussinye, A. Carrascosa, E. Vicens-Calvet, Postpubertal outcome in girls diagnosed of premature pubarche during childhood: increased frequency of functional ovarian hyperandrogenism, *J. Clin. Endocrinol. Metab.* 76 (1993) 1599–1603.
- [44] L. Ibanez, N. Potau, M. Zampolli, M.E. Street, A. Carrascosa, Girls diagnosed with premature pubarche show an exaggerated ovarian androgen synthesis from the early stages of puberty: evidence from gonadotropin-releasing hormone agonist testing, *Fertil. Steril.* 67 (1997) 849–855.
- [45] T. Meas, D. Chevenne, E. Thibaud, J. Leger, S. Cabrol, P. Czernichow, C. Levy-Marchal, Endocrine consequences of premature pubarche in post-pubertal Caucasian girls, *Clin. Endocrinol. (Oxf.)* 57 (2002) 101–106.
- [46] L. Ibanez, J. Dimartino-Nardi, N. Potau, P. Saenger, Premature adrenarche – normal variant or forerunner of adult disease? *Endocr. Rev.* 21 (2000) 671–696.
- [47] M. Maliqueo, T. Sir-Petermann, V. Perez, B. Echiburru, A.L. de Guevara, C. Galvez, N. Crisosto, R. Azziz, Adrenal function during childhood and puberty in daughters of women with polycystic ovary syndrome, *J. Clin. Endocrinol. Metab.* 94 (2009) 3282–3288.
- [48] R.A. Lobo, L.R. Granger, W.L. Paul, U. Goebelsmann, D.R. Mishell Jr., Psychological stress and increases in urinary norepinephrine metabolites, platelet serotonin, and adrenal androgens in women with polycystic ovary syndrome, *Am. J. Obstet. Gynecol.* 145 (1983) 496–503.
- [49] J.H. Levin, E. Carmina, R.A. Lobo, Is the inappropriate gonadotropin secretion of patients with polycystic ovary syndrome similar to that of patients with adult-onset congenital adrenal hyperplasia? *Fertil. Steril.* 56 (1991) 635–640.
- [50] E. Carmina, R.A. Lobo, Ovarian suppression reduces clinical and endocrine expression of late-onset congenital adrenal hyperplasia due to 21-hydroxylase deficiency, *Fertil. Steril.* 62 (1994) 738–743.
- [51] D. Dewailly, M.C. Vantyghem-Haudiquet, C. Sainsard, J. Buvat, J.P. Cappoen, K. Ardaens, A. Racadot, J. Lefebvre, P. Fossati, Clinical and biological phenotypes in late-onset 21-hydroxylase deficiency, *J. Clin. Endocrinol. Metab.* 63 (1986) 418–423.
- [52] C. Moran, R. Azziz, E. Carmina, D. Dewailly, F. Fruzzetti, L. Ibanez, E.S. Knochenhauer, J.A. Marcondes, B.B. Mendonca, D. Pignatelli, M. Pugeat, V. Rohmer, P.W. Speiser, S.F. Witchel, 21-hydroxylase-deficient nonclassic adrenal hyperplasia is a progressive disorder: a multicenter study, *Am. J. Obstet. Gynecol.* 183 (2000) 1468–1474.
- [53] H. Falhammar, M. Thoren, K. Hagenfeldt, A 31-year-old woman with infertility and polycystic ovaries diagnosed with non-classic congenital adrenal hyperplasia due to a novel CYP21 mutation, *J. Endocrinol. Invest.* 31 (2008) 176–180.
- [54] M.I. New, Extensive clinical experience: nonclassical 21-hydroxylase deficiency, *J. Clin. Endocrinol. Metab.* 91 (2006) 4205–4214.
- [55] W.M. Hague, J. Adams, C. Rodda, C.G. Brook, R. de Bruyn, D.B. Grant, H.S. Jacobs, The prevalence of polycystic ovaries in patients with congenital adrenal hyperplasia and their close relatives, *Clin. Endocrinol.* 33 (1990) 501–510.
- [56] N.M. Stikkelbroeck, A.R. Hermus, D. Schouten, H.M. Suliman, G.J. Jager, D.D. Braat, B.J. Otten, Prevalence of ovarian adrenal rest tumours and polycystic ovaries in females with congenital adrenal hyperplasia: results of ultrasonography and MR imaging, *Eur. Radiol.* 14 (2004) 1802–1806.
- [57] G.E. Jones, J.E. Howard, H. Langford, The use of cortisone in follicular phase disturbances, *Fertil. Steril.* 4 (1953) 49–62.
- [58] R. Azziz, V.Y. Black, E.S. Knochenhauer, G.A. Hines, L.R. Boots, Ovulation after glucocorticoid suppression of adrenal androgens in the polycystic ovary syndrome is not predicted by the basal dehydroepiandrosterone sulfate level, *J. Clin. Endocrinol. Metab.* 84 (1999) 946–950.
- [59] R.J. Chang, F.P. Mandel, A.R. Wolfson, H.L. Judd, Circulating levels of plasma adrenocorticotropin in polycystic ovary disease, *J. Clin. Endocrinol. Metab.* 54 (1982) 1265–1267.
- [60] P.M. Horrocks, F.R. Kandeel, D.R. London, W.R. Butt, S.S. Lynch, G. Holder, R. Logan-Edwards, ACTH function in women with the polycystic ovarian syndrome, *Clin. Endocrinol.* 19 (1983) 143–150.
- [61] A. Mongioi, M. Macchi, E. Vicari, M.C. Fornito, A.E. Calogero, C. Riccioli, G. Minacapilli, M.L. Moncada, R. D'Agata, Pituitary and adrenal response to ovine corticotropin-releasing hormone in women with polycystic ovarian syndrome, *J. Endocrinol. Invest.* 11 (1988) 637–640.
- [62] S.K. Cunningham, T. Loughlin, X. Bertagna, F. Girard, T.J. McKenna, Plasma propiomelanocortin fragments and adrenal steroids following administration of metyrapone to normal and hirsute women, *J. Endocrinol. Invest.* 11 (1988) 247–253.
- [63] E. Carmina, J.H. Levin, G. Malizia, R.A. Lobo, Ovine corticotropin-releasing factor and dexamethasone responses in hyperandrogenic women, *Fertil. Steril.* 54 (1990) 245–250.
- [64] E. Carmina, R.A. Lobo, Pituitary adrenal responses to ovine corticotropin releasing hormone in polycystic ovary syndrome and other hyperandrogenic patients, *Gynecol. Endocrinol.* 4 (1990) 225–231.
- [65] P.M. Stewart, R. Penn, R. Holder, A. Parton, J.G. Ratcliffe, D.R. London, The hypothalamo-pituitary-adrenal axis across the normal menstrual cycle and in polycystic ovary syndrome, *Clin. Endocrinol. (Oxf.)* 38 (1993) 387–391.
- [66] A. Lanzone, M. Ciampelli, F. Petraglia, A. Caruso, A.M. Fulghesu, S. Mancuso, Corticotropin-releasing hormone induces and exaggerated response of adrenocorticotrophic hormone and cortisol in polycystic ovary syndrome, *Fertil. Steril.* 63 (1995) 1195–1199.
- [67] R. Azziz, V. Black, G.A. Hines, L.M. Fox, L.R. Boots, Adrenal androgen excess in the polycystic ovary syndrome: sensitivity and responsivity of the hypothalamic-pituitary-adrenal axis, *J. Clin. Endocrinol. Metab.* 83 (1998) 2317–2323.
- [68] G. Gennarelli, J. Holte, M. Stridsberg, U. Lundqvist, M. Massobrio, T. Bäckström, C. Berne, Response of the pituitary-adrenal axis to hypoglycemic stress in women with the polycystic ovary syndrome, *J. Clin. Endocrinol. Metab.* 84 (1999) 76–81.
- [69] C. Invitti, M. De Martin, G. Delitala, J.D. Veldhuis, F. Cavagnini, Altered morning and nighttime pulsatile corticotropin and cortisol release in polycystic ovary syndrome, *Metabolism* 47 (1998) 143–148.
- [70] N. Cinar, A. Harmanci, D.Y. Aksoy, K. Aydin, B.O. Yildiz, Adrenocortical steroid response to ACTH in different phenotypes of non obese polycystic ovary syndrome, *J. Ovarian Res.* 5 (2012) 42.
- [71] D. Glintborg, A.P. Hermann, K. Brusgaard, J. Hangaard, C. Hagen, M. Andersen, Significantly higher adrenocorticotropin-stimulated cortisol and 17-hydroxyprogesterone levels in 337 consecutive, premenopausal, Caucasian, hirsute patients compared with healthy controls, *J. Clin. Endocrinol. Metab.* 90 (2005) 1347–1353.
- [72] R. Azziz, Abnormalities of adrenocortical steroidogenesis in PCOS, in: R. Azziz, J. E. Nestler, D. Dewailly (Eds.), *Androgen Excess Disorders in Women*, Lippincott-Raven, Philadelphia, 1997, pp. 403–414.
- [73] R.A. Lobo, U. Goebelsmann, Evidence for reduced 3 beta-ol-hydroxysteroid dehydrogenase activity in some hirsute women thought to have polycystic ovary syndrome, *J. Clin. Endocrinol. Metab.* 53 (1981) 394–400.
- [74] S.Y. Pang, A.J. Lerner, E. Stoner, L.S. Levine, S.E. Oberfield, I. Engel, M.I. New, Late-onset adrenal steroid 3 beta-hydroxysteroid dehydrogenase deficiency. I. A cause of hirsutism in pubertal and postpubertal women, *J. Clin. Endocrinol. Metab.* 60 (1985) 428–439.
- [75] S.F. Siegel, D.N. Finegold, R. Lanes, P.A. Lee, ACTH stimulation tests and plasma dehydroepiandrosterone sulfate levels in women with hirsutism, *N. Engl. J. Med.* 323 (1990) 849–854.
- [76] R. Azziz, E.L. Bradley Jr, H.D. Potter, L.R. Boots, 3 beta-hydroxysteroid dehydrogenase deficiency in hyperandrogenism, *Am. J. Obstet. Gynecol.* 168 (1993) 889–895.
- [77] C. Moran, H.D. Potter, R. Reyna, L.R. Boots, R. Azziz, Prevalence of 3beta-hydroxysteroid dehydrogenase-deficient nonclassic adrenal hyperplasia in hyperandrogenic women with adrenal androgen excess, *Am. J. Obstet. Gynecol.* 181 (1999) 596–600.
- [78] C. Lutfallah, W. Wang, J.I. Mason, Y.T. Chang, A. Haider, B. Rich, M. Castro-Magana, K.C. Copeland, R. David, S. Pang, Newly proposed hormonal criteria via genotypic proof for type II 3beta-hydroxysteroid dehydrogenase deficiency, *J. Clin. Endocrinol. Metab.* 87 (2002) 2611–2622.
- [79] G. Carbanaru, P. Prasad, B. Scoccia, P. Shea, N. Hopwood, F. Ziai, Y.T. Chang, S.E. Myers, J.I. Mason, S. Pang, The hormonal phenotype of Nonclassic 3 beta-hydroxysteroid dehydrogenase (*HSD3B*) deficiency in hyperandrogenic females is associated with insulin-resistant polycystic ovary syndrome and is not a variant of inherited *HSD3B2* deficiency, *J. Clin. Endocrinol. Metab.* 89 (2004) 783–794.

- [80] C. Moran, R. Reyna, L.S. Boots, R. Azziz, Adrenocortical hyperresponsiveness to corticotropin in polycystic ovary syndrome patients with adrenal androgen excess, *Fertil. Steril.* 81 (2004) 126–131.
- [81] P.M. Stewart, C.H. Shackleton, G.H. Beastall, C.R. Edwards, 5 alpha-reductase activity in polycystic ovary syndrome, *Lancet* 335 (1990) 431–433.
- [82] D.A. Vassiliadi, T.M. Barber, B.A. Hughes, M.I. McCarthy, J.A. Wass, S. Franks, P. Nightingale, J.W. Tomlinson, W. Arlt, P.M. Stewart, Increased 5 alpha-reductase activity and adrenocortical drive in women with polycystic ovary syndrome, *J. Clin. Endocrinol. Metab.* 94 (2009) 3558–3566.
- [83] A. Rodin, H. Thakkar, N. Taylor, R. Clayton, Hyperandrogenism in polycystic ovary syndrome: evidence of dysregulation of 11 β -hydroxysteroid dehydrogenase, *N. Engl. J. Med.* 330 (1994) 460–465.
- [84] R. Pasquali, D. Biscotti, G. Spinucci, V. Vicennati, A.D. Genazzani, L. Sgarbi, F. Casimirri, Pulsatile secretion of ACTH and cortisol in premenopausal women: effect of obesity and body fat distribution, *Clin. Endocrinol.* 48 (1998) 603–612.
- [85] P.M. Stewart, A. Boulton, S. Kumar, P.M. Clark, C.H. Shackleton, Cortisol metabolism in human obesity: impaired cortisone-cortisol conversion in subjects with central adiposity, *J. Clin. Endocrinol. Metab.* 84 (1999) 1022–1027.
- [86] R. Andrew, D.I. Phillips, B.R. Walker, Obesity and gender influence cortisol secretion and metabolism in man, *J. Clin. Endocrinol. Metab.* 83 (1998) 1806–1809.
- [87] A. Gambineri, G. Forlani, A. Munarini, F. Tomasconi, G.E. Cognigni, W. Ciampaglia, U. Pagotto, B.R. Walker, R. Pasquali, Increased clearance of cortisol by 5 β -reductase in a subgroup of women with adrenal hyperandrogenism in polycystic ovary syndrome, *J. Endocrinol. Invest.* 32 (2009) 210–218.
- [88] T. Tsilchorozidou, J.W. Honour, G.S. Conway, Altered cortisol metabolism in polycystic ovary syndrome: insulin enhances 5 α -reduction but not the elevated adrenal steroid production rates, *J. Clin. Endocrinol. Metab.* 88 (2003) 5907–5913.
- [89] F. Roelfsema, P. Kok, A.M. Pereira, H. Pijl, Cortisol production rate is similarly elevated in obese women with or without the polycystic ovary syndrome, *J. Clin. Endocrinol. Metab.* 95 (2010) 3318–3324.
- [90] E. Carmina, R.A. Lobo, Prevalence and metabolic characteristics of adrenal androgen excess in hyperandrogenic women with different phenotypes, *J. Endocrinol. Invest.* 30 (2007) 111–116.
- [91] R. Azziz, F.L. Gay, S.R. Potter, E. Bradley Jr., L.R. Boots, The effects of prolonged hypertestosteronemia on adrenocortical biosynthesis in oophorectomized women, *J. Clin. Endocrinol. Metab.* 72 (1991) 1025–1030.
- [92] G.A. Hines, E.R. Smith, R. Azziz, Influence of insulin and testosterone on adrenocortical steroidogenesis in vitro: preliminary studies, *Fertil. Steril.* 76 (2001) 730–735.
- [93] A. Kumar, D. Magoffin, I. Munir, R. Azziz, Effect of insulin and testosterone on androgen production and transcription of *SULT2A1* in the NCI-H295R adrenocortical cell line, *Fertil. Steril.* 92 (2009) 793–797.
- [94] R. Azziz, R.S. Rittmaster, L.M. Fox, E.L. Bradley Jr., H.D. Potter, L.R. Boots, Role of the ovary in the adrenal androgen excess of hyperandrogenic women, *Fertil. Steril.* 69 (1998) 851–859.
- [95] F. Gonzalez, D.A. Hatala, L. Speroff, Adrenal and ovarian steroid hormone responses to gonadotropin-releasing hormone agonist treatment in polycystic ovary syndrome, *Am. J. Obstet. Gynecol.* 165 (1991) 535–545.
- [96] E. Carmina, F. Gonzalez, L. Chang, R.A. Lobo, Reassessment of adrenal androgen secretion in women with polycystic ovary syndrome, *Obstet. Gynecol.* 6 (1995) 26.
- [97] M.I. Cedars, K.A. Steingold, D. de Ziegler, P.S. Lapolt, R.J. Chang, H.L. Judd, Long-term administration of gonadotropin-releasing hormone agonist and dexamethasone: assessment of the adrenal role in ovarian dysfunction, *Fertil. Steril.* 57 (1992) 495–500.
- [98] R. Azziz, W.Y. Chang, F.Z. Stanczyk, K. Woods, Effect of bilateral oophorectomy on adrenocortical function in women with polycystic ovary syndrome, *Fertil. Steril.* 99 (2013) 599–604.
- [99] G.A. Burghen, J.R. Givens, A.E. Kitabchi, Correlation of hyperandrogenism with hyperinsulinism in polycystic ovarian disease, *J. Clin. Endocrinol. Metab.* 50 (1980) 113–116.
- [100] J.E. Nestler, L.P. Powers, D.W. Matt, K.A. Steingold, S.R. Plymate, R.S. Rittmaster, J.N. Clore, W.G. Blackard, A direct effect of hyperinsulinemia on serum sex hormone-binding globulin levels in obese women with the polycystic ovary syndrome, *J. Clin. Endocrinol. Metab.* 72 (1991) 83–89.
- [101] K. Brennan, A. Huang, R. Azziz, Dehydroepiandrosterone sulfate and insulin resistance in patients with polycystic ovary syndrome, *Fertil. Steril.* 91 (2009) 1848–1852.
- [102] R.P. Kauffman, V.M. Baker, P. DiMarino, V.D. Castracane, Hyperinsulinemia and circulating dehydroepiandrosterone sulfate in white and Mexican American women with polycystic ovary syndrome, *Fertil. Steril.* 85 (2006) 1010–1016.
- [103] R.P. Buyalos, E.L. Bradley Jr., H.L. Judd, H.A. Zacur, R. Azziz, No acute effect of physiological insulin increase on dehydroepiandrosterone sulfate in women with obesity and/or polycystic ovarian disease, *Fertil. Steril.* 56 (1991) 1179–1182.
- [104] R.P. Buyalos, M.E. Geffner, R. Azziz, H.L. Judd, Impact of overnight dexamethasone suppression on the adrenal androgen response to an oral glucose tolerance test in women with and without polycystic ovary syndrome, *Hum. Reprod.* 12 (1997) 1138–1141.
- [105] R. Azziz, E.L. Bradley Jr., H.D. Potter, C.R. Parker Jr., L.R. Boots, Chronic hyperinsulinemia and the adrenal androgen response to acute corticotropin-(1–24) stimulation in hyperandrogenic women, *Am. J. Obstet. Gynecol.* 172 (1995) 1251–1256.
- [106] A.K. Ludwig, L.G. Goharian, T. Dietze, S. Tauchert, S. Rudolf, K. Diedrich, U. Schweiger, K.M. Oltmanns, Impact of glycemic variations on the regulation of androgen metabolism in obese women with polycystic ovary syndrome, *Fertil. Steril.* 92 (2009) 271–276.
- [107] M.P. Diamond, D. Grainger, A.J. Laudano, K. Starick-Zych, R.A. DeFronzo, Effect of acute physiological elevations of insulin on circulating androgen levels in nonobese women, *J. Clin. Endocrinol. Metab.* 72 (1991) 883–887.
- [108] L. Farah-Ewais, R. Reyna, E.S. Knochenhauer, A.A. Bartolucci, R. Azziz, Glucose action and adrenocortical biosynthesis in women with polycystic ovary syndrome, *Fertil. Steril.* 81 (2004) 120–125.
- [109] T. Falcone, D.T. Finegood, I.G. Fantus, D. Morris, Androgen response to endogenous insulin secretion during the frequently sampled intravenous glucose tolerance test in normal and hyperandrogenic women, *J. Clin. Endocrinol. Metab.* 71 (1990) 1653–1657.
- [110] R. Azziz, D.A. Ehrmann, R.S. Legro, A.G. Fereshtian, M. O'Keefe, M.N. Ghazzi, Troglitazone decreases adrenal androgen levels in women with polycystic ovary syndrome, *Fertil. Steril.* 79 (2003) 932–937.
- [111] K. Rautio, J.S. Tapanainen, A. Ruokonen, L.C. Morin-Papunen, Endocrine and metabolic effects of rosiglitazone in overweight women with PCOS: a randomized placebo-controlled study, *Hum. Reprod.* 21 (2006) 1400–1407.
- [112] V. Sepilian, M. Nagamani, Effects of rosiglitazone in obese women with polycystic ovary syndrome and severe insulin resistance, *J. Clin. Endocrinol. Metab.* 90 (2005) 60–65.
- [113] M. Guido, D. Romualdi, R. Suriano, M. Giuliani, B. Costantini, R. Apa, A. Lanzone, Effect of pioglitazone treatment on the adrenal androgen response to corticotropin in obese patients with polycystic ovary syndrome, *Hum. Reprod.* 19 (2004) 534–539.
- [114] D. Romualdi, M. Giuliani, G. Draisci, B. Costantini, F. Cristello, A. Lanzone, M. Guido, Pioglitazone reduces the adrenal androgen response to corticotropin-releasing factor without changes in ACTH release in hyperinsulinemic women with polycystic ovary syndrome, *Fertil. Steril.* 88 (2007) 131–138.
- [115] E. Aigner, N. Bachofner, K. Klein, C. De Geyter, F. Hohla, W. Patsch, C. Datz, Retinol-binding protein 4 in polycystic ovary syndrome – association with steroid hormones and response to pioglitazone treatment, *J. Clin. Endocrinol. Metab.* 94 (2009) 1229–1235.
- [116] D. Glinborg, A.P. Hermann, C. Hagen, L.T. Jensen, J. Frystyk, P. Bennett, A. Flyvbjerg, M. Andersen, A randomized placebo-controlled study on the effects of pioglitazone on cortisol metabolism in polycystic ovary syndrome, *Fertil. Steril.* 91 (2009) 842–850.
- [117] X.J. Li, Y.X. Yu, C.Q. Liu, W. Zhang, H.J. Zhang, B. Yan, L.Y. Wang, S.Y. Yang, S.H. Zhang, Metformin vs thiazolidinediones for treatment of clinical, hormonal and metabolic characteristics of polycystic ovary syndrome: a meta-analysis, *Clin. Endocrinol. (Oxf.)* 74 (2011) 332–339.
- [118] A. Majuri, M. Santaniemi, K. Rautio, A. Kunnari, J. Vartiainen, A. Ruokonen, Y.A. Kesaniemi, J.S. Tapanainen, O. Ukkola, L. Morin-Papunen, Rosiglitazone treatment increases plasma levels of adiponectin and decreases levels of resistin in overweight women with PCOS: a randomized placebo-controlled study, *Eur. J. Endocrinol.* 156 (2007) 263–269.
- [119] V.P. Sepilian, J.R. Crochet, M. Nagamani, Serum soluble leptin receptor levels and free leptin index in women with polycystic ovary syndrome: relationship to insulin resistance and androgens, *Fertil. Steril.* 85 (2006) 1441–1447.
- [120] W. Arlt, R.J. Auchus, W.L. Miller, Thiazolidinediones but not metformin directly inhibit the steroidogenic enzymes P450c17 and 3 β -hydroxysteroid dehydrogenase, *J. Biol. Chem.* 276 (2001) 16767–16771.
- [121] S.A. Arslanian, V. Lewy, K. Danadian, R. Saad, Metformin therapy in obese adolescents with polycystic ovary syndrome and impaired glucose tolerance: amelioration of exaggerated adrenal response to adrenocorticotropin with reduction of insulinemia/insulin resistance, *J. Clin. Endocrinol. Metab.* 87 (2002) 1555–1559.
- [122] A. la Marca, G. Morgante, T. Paglia, L. Ciotta, A. Cianci, V. De Leo, Effects of metformin on adrenal steroidogenesis in women with polycystic ovary syndrome, *Fertil. Steril.* 72 (1999) 985–989.
- [123] M. Ehrhart-Bornstein, J.P. Hinson, S.R. Bornstein, W.A. Scherbaum, G.P. Vinson, Intraadrenal interactions in the regulation of adrenocortical steroidogenesis, *Endocr. Rev.* 19 (1998) 101–143.
- [124] J.P. Forney, L. Milewich, G.T. Chen, J.L. Garlock, B.E. Schwarz, C.D. Edman, P.C. MacDonald, Aromatization of androstenedione to estrone by human adipose tissue in vitro. Correlation with adipose tissue mass, age, and endometrial neoplasia, *J. Clin. Endocrinol. Metab.* 53 (1981) 192–199.
- [125] G.E. Ackerman, M.E. Smith, C.R. Mendelson, P.C. MacDonald, E.R. Simpson, Aromatization of androstenedione by human adipose tissue stromal cells in monolayer culture, *J. Clin. Endocrinol. Metab.* 53 (1981) 412–417.
- [126] S.E. Bulun, E.R. Simpson, Competitive reverse transcription-polymerase chain reaction analysis indicates that levels of aromatase cytochrome p450 transcripts in adipose tissue of buttocks, thighs, and abdomen of women increase with advancing age, *J. Clin. Endocrinol. Metab.* 78 (1994) 428–432.
- [127] R. Azziz, H.A. Zacur, C.R. Parker Jr., E.L. Bradley Jr., L.R. Boots, Effect of obesity on the response to acute adrenocorticotropin stimulation in eumenorrheic women, *Fertil. Steril.* 56 (1991) 427–433.
- [128] V. Vicennati, F. Calzoni, A. Gambineri, L. Gagliardi, A.M. Morselli Labate, F. Casimirri, R. Pasquali, Secretion of major adrenal androgens following ACTH

- administration in obese women with different body fat distribution, *Hormone Metab. Res.* 30 (1998) 133–136.
- [129] R.S. Rittmaster, N. Deshwal, L. Lehman, The role of adrenal hyperandrogenism, insulin resistance, and obesity in the pathogenesis of polycystic ovarian syndrome, *J. Clin. Endocrinol. Metab.* 76 (1993) 1295–1300.
- [130] S. Brody, K. Carlstrom, A. Lagrelus, N.O. Lunell, G. Mollerstrom, Adrenal steroids in post-menopausal women relation to obesity and to bone mineral content, *Maturitas* 9 (1987) 25–32.
- [131] S. Komindr, B.R. Kurtz, M.D. Stevens, J.G. Karas, J.B. Bittle, J.R. Givens, Relative sensitivity and responsiveness of serum cortisol and two adrenal androgens to alpha-adrenocorticotropin-(1–24) in normal and obese, nonhirsute, eumenorrheic women, *J. Clin. Endocrinol. Metab.* 63 (1986) 860–864.
- [132] V. Vicennati, R. Pasquali, Abnormalities of the hypothalamic-pituitary-adrenal axis in nondepressed women with abdominal obesity and relations with insulin resistance: evidence for a central and a peripheral alteration, *J. Clin. Endocrinol. Metab.* 85 (2000) 4093–4098.
- [133] E. Carmina, S. Bucchieri, A. Esposito, A. Del Puente, P. Mansueto, G. Di Fede, G. B. Rini, Abdominal fat quantity and distribution in women with polycystic ovary syndrome and extent of its relation to insulin resistance, *J. Clin. Endocrinol. Metab.* 92 (2007) 2500–2505.
- [134] J.I. Rotter, F.L. Wong, E.T. Lifrak, L.N. Parker, A genetic component to the variation of dehydroepiandrosterone sulfate, *Metabolism* 34 (1985) 731–736.
- [135] E.C. Prom-Wormley, T.P. York, K.C. Jacobson, L.J. Eaves, S.P. Mendoza, D. Hellhammer, N. Maninger, S. Levine, S. Lupien, M.J. Lyons, R. Hauger, H. Xian, C.E. Franz, W.S. Kremen, Genetic and environmental effects on diurnal dehydroepiandrosterone sulfate concentrations in middle-aged men, *Psychoneuroendocrinology* 36 (2011) 1441–1452.
- [136] T. Rice, D.L. Sprecher, I.B. Borecki, L.E. Mitchell, P.M. Laskarzewski, D.C. Rao, The Cincinnati Myocardial Infarction and Hormone Family study: family resemblance for dehydroepiandrosterone sulfate in control and myocardial infarction families, *Metabolism* 42 (1993) 1284–1290.
- [137] P. An, T. Rice, J. Gagnon, Y. Hong, A.S. Leon, J.S. Skinner, J.H. Wilmore, C. Bouchard, D.C. Rao, Race differences in the pattern of familial aggregation for dehydroepiandrosterone sulfate and its responsiveness to training in the HERITAGE Family Heart Study, *Metabolism* 50 (2001) 916–920.
- [138] C.E. Jaquish, J. Blangero, S.M. Haffner, M.P. Stern, J.W. MacCluer, Quantitative genetics of dehydroepiandrosterone sulfate and its relation to possible cardiovascular disease risk factors in Mexican Americans, *Hum. Hered.* 46 (1996) 301–309.
- [139] B.D. Mitchell, C.M. Kammerer, J. Blangero, M.C. Mahaney, D.L. Rainwater, B. Dyke, J.E. Hixson, R.D. Henkel, R.M. Sharp, A.G. Comuzzie, J.L. VandeBerg, M.P. Stern, J.W. MacCluer, Genetic and environmental contributions to cardiovascular risk factors in Mexican Americans. The San Antonio Family Heart Study, *Circulation* 94 (1996) 2159–2170.
- [140] C.E. Jaquish, M.C. Mahaney, J. Blangero, S.M. Haffner, M.P. Stern, J.W. MacCluer, Genetic correlations between lipoprotein phenotypes and indicators of sex hormone levels in Mexican Americans, *Atherosclerosis* 122 (1996) 117–125.
- [141] H. Li, C. Ji, Change of dehydroepiandrosterone in serum of 6–18 year-old twin girls in Qingdao city, *Wei Sheng Yan Jiu* 36 (2007) 41–42.
- [142] A.W. Meikle, J.D. Stringham, M.G. Woodward, D.T. Bishop, Heritability of variation of plasma cortisol levels, *Metabolism* 37 (1988) 514–517.
- [143] A.W. Meikle, R.A. Stephenson, C.M. Lewis, G.A. Wiebke, R.G. Middleton, Age genetic, and nongenetic factors influencing variation in serum sex steroids and zonal volumes of the prostate and benign prostatic hyperplasia in twins, *Prostate* 33 (1997) 105–111.
- [144] J.E. Nestler, J.B. Whitfield, T.Y. Williams, G. Zhu, J. Condon, K.M. Kirk, A.C. Heath, G.W. Montgomery, N.G. Martin, Genetics of serum dehydroepiandrosterone sulfate and its relationship to insulin in a population-based cohort of twin subjects, *J. Clin. Endocrinol. Metab.* 87 (2002) 682–686.
- [145] Y. Akamine, K. Kato, H. Ibayashi, Studies on changes in the concentration of serum adrenal androgens in pubertal twins, *Acta Endocrinol. (Copenh.)* 93 (1980) 356–364.
- [146] B.O. Yildiz, M.O. Goodarzi, X. Guo, J.I. Rotter, R. Azziz, Heritability of dehydroepiandrosterone sulfate in women with polycystic ovary syndrome and their sisters, *Fertil. Steril.* 86 (2006) 1688–1693.
- [147] S. Franks, L.J. Webber, M. Goh, A. Valentine, D.M. White, G.S. Conway, S. Wiltshire, M.I. McCarthy, Ovarian morphology is a marker of heritable biochemical traits in sisters with polycystic ovaries, *J. Clin. Endocrinol. Metab.* 93 (2008) 3396–3402.
- [148] M.O. Goodarzi, X. Guo, B.O. Yildiz, F.Z. Stanczyk, R. Azziz, Correlation of adrenocorticotropin steroid levels between women with polycystic ovary syndrome and their sisters, *Am. J. Obstet. Gynecol.* 196 (398) (2007) e391–e395.
- [149] R.S. Legro, A.R. Kunselman, L. Demers, S.C. Wang, R. Bentley-Lewis, A. Dunaif, Elevated dehydroepiandrosterone sulfate levels as the reproductive phenotype in the brothers of women with polycystic ovary syndrome, *J. Clin. Endocrinol. Metab.* 87 (2002) 2134–2138.
- [150] M.O. Goodarzi, H.J. Antoine, R. Azziz, Genes for enzymes regulating dehydroepiandrosterone sulfonation are associated with levels of dehydroepiandrosterone sulfate in polycystic ovary syndrome, *J. Clin. Endocrinol. Metab.* 92 (2007) 2659–2664.
- [151] Y.V. Louwers, F.H. de Jong, N.A. van Herwaarden, L. Stolk, B.C. Fauser, A.G. Uitterlinden, J.S. Laven, Variants in *SULT2A1* affect the DHEA sulphate to DHEA ratio in patients with polycystic ovary syndrome but not the hyperandrogenic phenotype, *J. Clin. Endocrinol. Metab.* 98 (2013) 3848–3855.
- [152] M. Komori, K. Nishio, M. Kitada, K. Shiramatsu, K. Muroya, M. Soma, K. Nagashima, T. Kamataki, Fetus-specific expression of a form of cytochrome P-450 in human livers, *Biochemistry* 29 (1990) 4430–4433.
- [153] P. Kuehl, J. Zhang, Y. Lin, J. Lamba, M. Assem, J. Schuetz, P.B. Watkins, A. Daly, S. A. Wrighton, S.D. Hall, P. Maurel, M. Relling, C. Brimer, K. Yasuda, R. Venkataramanan, S. Strom, K. Thummel, M.S. Boguski, E. Schuetz, Sequence diversity in *CYP3A* promoters and characterization of the genetic basis of polymorphic *CYP3A5* expression, *Nat. Genet.* 27 (2001) 383–391.
- [154] P. Smit, R.H. van Schaik, M. van der Werf, A.W. van den Beld, J.W. Koper, J. Lindemans, H.A. Pols, A.O. Brinkmann, F.H. de Jong, S.W. Lamberts, A common polymorphism in the *CYP3A7* gene is associated with a nearly 50% reduction in serum dehydroepiandrosterone sulfate levels, *J. Clin. Endocrinol. Metab.* 90 (2005) 5313–5316.
- [155] M.O. Goodarzi, N. Xu, R. Azziz, Association of *CYP3A7*1C* and serum dehydroepiandrosterone sulfate levels in women with polycystic ovary syndrome, *J. Clin. Endocrinol. Metab.* 93 (2008) 2909–2912.
- [156] H.F. Escobar-Morreale, J.L. San Millan, R.R. Smith, J. Sancho, S.F. Witchel, The presence of the 21-hydroxylase deficiency carrier status in hirsute women: phenotype-genotype correlations, *Fertil. Steril.* 72 (1999) 629–638.
- [157] S.F. Witchel, M. Kahsar-Miller, C.E. Aston, C. White, R. Azziz, Prevalence of *CYP21* mutations and *IRS1* variant among women with polycystic ovary syndrome and adrenal androgen excess, *Fertil. Steril.* 83 (2005) 371–375.
- [158] L. Vandenput, C. Ohlsson, Genome-wide association studies on serum sex steroid levels, *Mol. Cell. Endocrinol.* 382 (2014) 758–766.
- [159] G. Zhai, A. Teumer, L. Stolk, J.R. Perry, L. Vandenput, A.D. Coviello, A. Koster, J.T. Bell, S. Bhasin, J. Eriksson, A. Eriksson, F. Ernst, L. Ferrucci, T.M. Frayling, D. Glass, E. Grundberg, R. Haring, A.K. Hedman, A. Hofman, D.P. Kiel, H.K. Kroemer, Y. Liu, K.L. Lunetta, M. Maggio, M. Lorentzon, M. Mangino, D. Melzer, I. Miljkovic, A. Nica, B.W. Penninx, R.S. Vasan, F. Rivadeneira, K.S. Small, N. Soranzo, A.G. Uitterlinden, H. Volzke, S.G. Wilson, L. Xi, W.V. Zhuang, T.B. Harris, J.M. Murabito, C. Ohlsson, A. Murray, F.H. de Jong, T.D. Spector, H. Wallaschofski, Eight common genetic variants associated with serum DHEAS levels suggest a key role in ageing mechanisms, *PLoS Genet.* 7 (2011) e1002025.
- [160] Z.J. Chen, H. Zhao, L. He, Y. Shi, Y. Qin, Z. Li, L. You, J. Zhao, J. Liu, X. Liang, X. Zhao, Y. Sun, B. Zhang, H. Jiang, D. Zhao, Y. Bian, X. Gao, L. Geng, Y. Li, D. Zhu, X. Sun, J.E. Xu, C. Hao, C.E. Ren, Y. Zhang, S. Chen, W. Zhang, A. Yang, J. Yan, J. Ma, Y. Zhao, Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3, *Nat. Genet.* 43 (2011) 55–59.
- [161] Y. Shi, H. Zhao, Y. Shi, Y. Cao, D. Yang, Z. Li, B. Zhang, X. Liang, T. Li, J. Chen, J. Shen, J. Zhao, L. You, X. Gao, D. Zhu, X. Zhao, Y. Yan, Y. Qin, W. Li, J. Yan, Q. Wang, J. Zhao, L. Geng, J. Ma, Y. Zhao, G. He, A. Zhang, S. Zou, A. Yang, J. Liu, W. Li, B. Li, C. Wan, Y. Qin, J. Shi, J. Yang, H. Jiang, J.E. Xu, X. Qi, Y. Sun, Y. Zhang, C. Hao, X. Ju, D. Zhao, C.E. Ren, X. Li, W. Zhang, Y. Zhang, J. Zhang, D. Wu, C. Zhang, L. He, Z.J. Chen, Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome, *Nat. Genet.* 44 (2012) 1020–1025.
- [162] L. Cui, H. Zhao, B. Zhang, Z. Qu, J. Liu, X. Liang, X. Zhao, J. Zhao, Y. Sun, P. Wang, T. Li, Y. Shi, Z.J. Chen, Genotype-phenotype correlations of PCOS susceptibility SNPs identified by GWAS in a large cohort of Han Chinese women, *Hum. Reprod.* 28 (2013) 538–544.
- [163] K.T. Khaw, Dehydroepiandrosterone, dehydroepiandrosterone sulphate and cardiovascular “warning” [Some lines or texts are hidden inside the Frame|Please check and proceed|-|1e| Accept |scular disease, *J. Endocrinol.* 150 (1996) S149–S153.
- [164] C. Ohlsson, F. Labrie, E. Barrett-Connor, M.K. Karlsson, O. Ljunggren, L. Vandenput, D. Mellström, A. Tivesten, Low serum levels of dehydroepiandrosterone sulfate predict all-cause and cardiovascular mortality in elderly Swedish men, *J. Clin. Endocrinol. Metab.* 95 (2010) 4406–4414.
- [165] C. Shufelt, P. Bretsky, C.M. Almeida, B.D. Johnson, L.J. Shaw, R. Azziz, G.D. Braunstein, C.J. Pepine, V. Bittner, D.A. Vido, F.Z. Stanczyk, C.N. Bairey Merz, DHEA-S levels and cardiovascular disease mortality in postmenopausal women: results from the National Institutes of Health? National Heart, Lung, and Blood Institute (NHLBI)-sponsored Women’s Ischemia Syndrome Evaluation (WISE), *J. Clin. Endocrinol. Metab.* 95 (2010) 4985–4992.