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Hypothalamic–Pituitary–Adrenal Dysfunction in the Polycystic Ovary Syndrome

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SUMMARY

Between 20 and 30% of patients with polycystic ovary syndrome (PCOS) demonstrate adrenal androgen (AA) excess, detectable primarily by elevated dehydroepiandrosterone sulfate (DHEAS) levels. Generalized adrenocortical hyperresponsivity to adrenocorticotropic hormone (ACTH) stimulation is also observed and may be the principal mechanism determining AA excess in PCOS. The causes of this abnormality are unclear, but increased peripheral metabolism of cortisol, altered factors regulating glucose-mediated glucose disposal, and perhaps ovarian sex steroids may in different ways contribute to the AA excess in PCOS. Additionally, DHEAS levels and the response of AAs to ACTH are relatively constant over time and may be a genetically determined trait.

Key Words: Androgen; adrenal; hyperandrogenism; cortisol.

1. INTRODUCTION

The polycystic ovary syndrome (PCOS) is the most common endocrine disorder of reproductive-aged women, with an estimated prevalence of 6–7% *(1)*. Although the ovaries are the main source of androgen excess in PCOS, excess adrenal androgen (AA) levels (e.g., dehydroepiandrosterone [DHEA] and adrenal-secreted androsteredione [A4]) and adrenocortical dysfunction have also been observed in many patients with PCOS *(2–6)*. Proof of the role of AA excess in the development of PCOS is circumstantial at best. For example, peripubertal AA excess is linked to the development of PCOS-like symptomatology in patients with 21-hydroxylase (21-OH)-deficient classic (CAH) *(7)* and nonclassic adrenal hyperplasia (NCAH) *(8–11)*, including the development of polycystic-appearing ovaries on ultrasound, elevated luteinizing hormone levels, and ovarian hyperandrogenism. Other investigators have noted that patients with PCOS have a greater incidence of peripubertal stress, resulting in exaggerated AA secretion during this vulnerable period *(12)*. Finally, patients with premature adrenarche are at higher risk for the development of PCOS *(13–16)*. In this chapter we review the prevalence of AA excess and hypothalamic–pituitary–adrenal (HPA) axis dysfunction in PCOS and the potential underlying mechanisms.

2. BACKGROUND

2.1. Epidemiology of Adrenal Androgen Excess in PCOS

Clinically, the measurement of circulating levels of the AA metabolite DHEA sulfate (DHEAS) has been traditionally used as a marker for AA excess *(17–19)* because this steriod is (1) 97–99% of adrenocortical origin *(20–22)*, (2) the second most abundant steroid after cortisol (F), (3) relatively stable throughout the day and the menstrual cycle *(23–25)*, because of its relatively long half-life *(26–30)*, and (4) easily measured.

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Excess AA levels, particularly elevations in the levels of the dehydroepiandrosterone (DHEA) metabolite DHEAS and 11-hydroxyandrostenedione (11OHA4), were initially reported in 40–60% of patients with PCOS *(3–6)*. However, in most of these early studies criteria for the selection of PCOS patients were different from those currently used. Moreover, it is clear that several factors, including age and race, should be considered when estimating the prevalence of AA excess in PCOS, because AAs begin to decline after the age of 30 years in both normal women and women with PCOS *(31,32)*. In a retrospective study of 145 hyperandrogenic patients, we found that hyperandrogenic patients with high DHEAS levels were younger, in addition to being thinner and more hirsute, than hyperandrogenic women with lower DHEAS levels *(33)*. The impact of race on the prevalence of AA excess in PCOS is unclear. In one report the prevalence of AA excess among PCOS patients was found to be similar among Italian, US, Hispanic-American, and Japanese women *(34)*. However, only small groups of patients were compared.

To revaluate the prevalence of AA excess in PCOS taking into account race and age-related changes in AAs, we undertook a study of 213 (27 black and 186 white) women with PCOS and 182 (88 black and 94 white) age-matched healthy eumenorrheic nonhirsute women (controls) *(31)*. The diagnosis of PCOS was based on hyperandrogenism and chronic anovulation, consistent with the National Institutes of Health 1990 criteria. DHEAS levels were significantly lower in black than white controls, whereas fasting insulin and body mass index (BMI) were higher in black controls, and DHEAS levels decreased similarly with age in control and PCOS women of either race (Fig. 1). Body mass and fasting insulin had little impact on circulating DHEAS levels in healthy women. Among PCOS patients, these parameters were negatively associated with circulating DHEAS levels among white, but not black patients. For each race and age group, the upper 95% normative values for log DHEAS was calculated, and the number of PCOS subjects with log DHEAS values above this level were assessed. The prevalence of supranormal DHEAS levels was 33 and 20% among black and white women with PCOS, respectively—not a significant difference.

These data indicate that AA excess, defined by the circulating level of DHEAS, is somewhat less common in PCOS than previously reported, affecting between 20 and 30% of affected women when using age- and race-adjusted normative values. However, it also appears that women with absolute DHEAS excess simply represent the upper edge of the normal DHEAS distribution in the general population, not a separate population. For example, cluster analysis failed to reveal any specific subpopulations of DHEAS levels among our patients with PCOS *(31)*. Finally, there may be significant differences in mean DHEAS levels between white and black control women. Whether these differences are also present among women of other racial or ethnic groups (e.g., Hispanics and Asians) remains to be deter- mined. However, because AA secretion appears to have a strong genetic component (*see* Subsection 2.7.), it is not surprising that race and ethnicity play a role in determining the prevalence of AA excess in PCOS.

Finally, we should note that DHEAS, does not uniformly reflect AA secretion in response to adrenocorticotropic hormone (ACTH) in normal or hyperandrogenic patients. For example, only 50% of patients with 21-OH-deficient NCAH have a supranormal DHEAS levels *(35)*. Witness also the profound suppression in DHEAS levels that occurs in NCAH patients treated with glucocorticoids despite the still elevated production of low-dose A4 *(36)*. Likewise, note should be taken of the increase in DHEAS in response to exogenous testosterone administration to oophorectomized women, despite the absence of any change in the AA response to acute ACTH stimulation *(37)*. Consequently, it is apparent that a number of factors may alter DHEAS levels without modifying adrenocortical AA production, most likely through regulation of DHEA sulfotransferase (DHEA-ST) activity. Hence, the investigation of those mechanisms underlying the AA excess of PCOS requires evaluation not only of DHEAS levels, but also of adrenocortical biosynthesis (usually measurable by the response to acute ACTH stimulation).

2.2. Hypothalamic–Pituitary Regulation and Adrenal Androgen Excess in PCOS

There appears to be no difference in basal morning plasma levels of ACTH *(38,39)* or the circadian or diurnal variation of this hormone *(39)* between PCOS or hyperandrogenic women and healthy

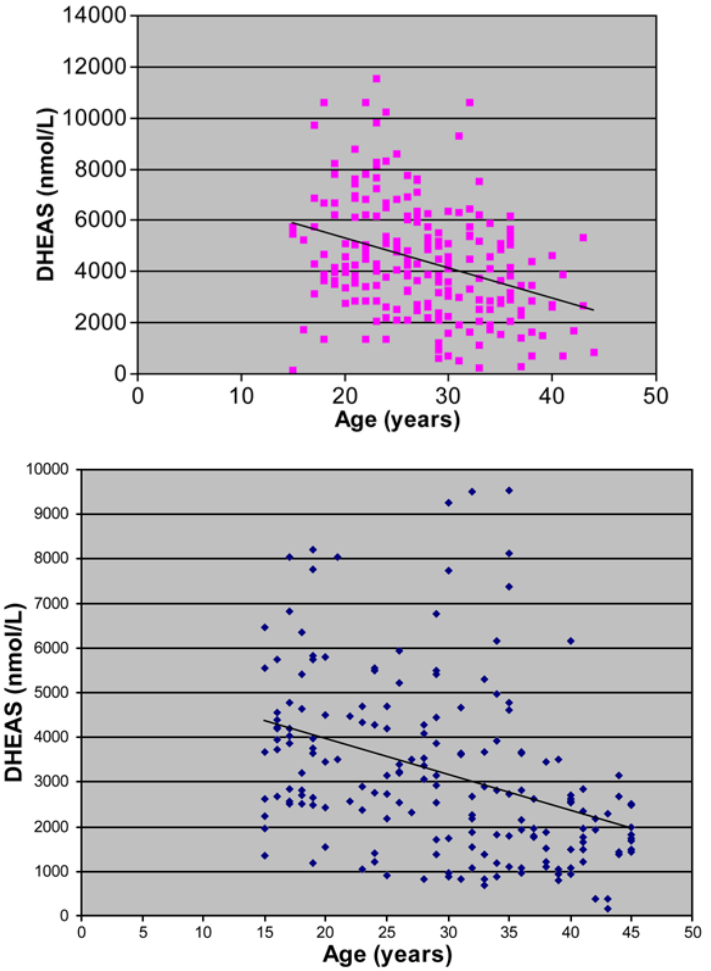

Fig. 1. Scatter grams and linear regression trend line for dehydroepiandrosterone sulfate (DHEAS) levels according to age in polycystic ovary syndrome (PCOS) (top) (r = –0.34, $p < 0.0001$) and healthy controls (bottom) (r = –0.38, $p < 0.0001$). Note that DHEAS levels in PCOS and controls decrease with age at similar rates. (From ref. *31*.)

controls. The response of ACTH to endogenous or exogenous corticotrophin-releasing hormone is also normal in PCOS *(40,41)*, with and without DHEAS excess *(41)*. These data suggest that pituitary responsivity is not altered in PCOS, regardless of the presence of AA excess.

Because hypothalamic–pituitary alterations appear to be mostly excluded, AA excess in PCOS may be a result of adrenocortical steroidogenic abnormalities, alterations in the sensitivity or responsivity of the adrenal to ACTH stimulation, or abnormalities in the metabolism of adrenal products, including DHEA and cortisol.

2.3. Alteration in Adrenocortical Biosynthesis in PCOS

Estimation of the relative adrenocortical enzymatic activities in vivo can be performed using acute adrenal stimulation by intravenous or intramuscular administration of ACTH(1-24). Acute administration of commercially available doses of 0.25 mg provides maximum adrenal stimulation, regardless of body weight *(42)*. Several studies have shown that PCOS women demonstrate a generalized hypersecretion of adrenocortical products, basally and in response to ACTH, including pregnenolone (PREG), 17-hydroxypregnenolone (17-OH-PREG), DHEA, A4, 11-deoxycortisol (S), and F *(40,41)*.

The observed exaggerated secretion in response to ACTH may be because of a change in the responsiveness or sensitivity of the adrenal cortex to ACTH stimulation. We observed that excess DHEAS levels in PCOS patients were associated with an exaggerated secretory response of the adrenal to ACTH stimulation for DHEA and A4 *(41)*. However, no differences in the sensitivity of AAs to ACTH stimulation was observed between PCOS women with and without DHEAS excess and controls.

Importantly, the exaggerated adrenocortical response to ACTH does not appear to be the result of genetic defects affecting 21-OH (P450c21, encoded by *CYP21*), 11β-hydroxylase (11-OH P450c11, encoded by *CYP11B1*), or 3β-hydroxysteroid dehydrogenase (3β-HSD; encoded by *HSD3B2*) *(42,43)*. Overall, the prevalence of NCAH resulting from 21-OH deficiency in the populations studied is 1–2% *(44)*, with NCAH as a result of 3β-HSD *(45)* or 11-OH *(46)* occurring very rarely, on the order of less than 1%.

Other investigators have observed defects in 3β-HSD activity among women with PCOS *(47–50)*, although these do not appear to be related to inherited defects of the *HSD3B2* gene *(45)*. Pang and colleagues suggested that the exaggerated 3β-HSD activity observed was secondary to a variant of insulin-resistant PCOS *(51)*. However, these investigators were not able to observe a difference in insulin resistance (measured by the frequently sampled intravenous glucose tolerance test [FSIVGTT]) between women with PCOS with and without 3β-HSD deficiency, suggesting that a mechanism other than a defect in insulin action was responsible for the perceived steroidogenic abnormality.

In fact, the single steroidogenic difference that we were able to observe between PCOS women and healthy controls was a greater estimated $\Delta^5$17-α-hydroxylase (17-OH) activity, primarily observed in PCOS patients with high DHEAS levels *(52)*. We should note that $\Delta^5$17-OH in vivo activity is estimated from the ratio of the 17-OH-PREG to PREG (i.e., poststimulated levels), such that women with PCOS and DHEAS excess demonstrated a supranormal 17-HPREG$_{60}$:PREG$_{60}$ ratio. Consequently, if only 17α-hydroxylated (17-OH-PREG) and not 17-deoxy (e.g., PREG) products are measured, the higher 17-HPREG levels observed could suggest 3β-HSD deficiency, as observed by some investigators *(51)*.

The exaggerated $\Delta^5$17-OH activity observed in women with PCOS and DHEAS excess may reflect an abnormality in P450c17 function, the enzyme determining 17-OH and 17,20-lyase activity. The *CYP17* gene encodes this enzyme. However, we were unable to demonstrate a difference in the prevalence of a common polymorphism of *CYP17*, hypothesized to increase transcription of the gene, between women with PCOS with and without DHEAS excess *(53)*. We should note, however, that this does not necessarily exclude the presence of other mutations or variants that may affect the function of this gene. What factors may result in the exaggerated $\Delta^5$17-OH activity remain to be elucidated, although potential candidates include various extra-adrenal factors (*see* Subsections 2.5. and 2.6.).

2.4. Alterations in Cortisol Metabolism in PCOS

Increased peripheral metabolism of F has been observed in some patients with PCOS. An increase in F metabolism would potentially result in decreased negative feedback of ACTH, such that hypothalamic–pituitary–adrenal (HPA) axis activity would increase to maintain normal F levels at the expense of AA excess *(54,55)*. The increased metabolism of F may be a result of enhanced inactivation of this steroid by 5α-reductase (5α-RA) *(55,56)* or impaired reactivation of F from cortisone by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) *(54,55)*.

Studying 11 PCOS patients, Stewart and colleagues observed that total urinary cortisol metabolites were higher in patients than controls, suggesting increased breakdown of this steroid *(55)*. They also observed a higher ratio of 5α- to 5β-cortisol metabolites in the urine of these women, consistent with enhanced activity of 5α-RA. Tsilchorozidou and colleagues also noted enhanced 5α-RA and reduced 11β-HSD1 activities, as assessed by the urinary profile, in 18 lean PCOS women compared with 19 lean controls *(56)*. In this study, insulin seemed to enhance 5α-reduction of steroids in PCOS, but was not associated with the elevated F production rate or with enhanced 11β-HSD1 activity observed.

Taken together, these data suggest that increased F metabolism may be present in PCOS and appears independent of the presence of obesity, but may be enhanced by hyperinsulinemia. However, the role of these metabolic abnormalities in the AA excess of PCOS remains to be demonstrated.

2.5. The Role of Extra-Adrenal Sex Steroids in the HPA Dysfunction of PCOS

Adrenocortical dysfunction in PCOS patients may represent an acquired defect secondary to abnormal ovarian secretion. In general, most investigators have observed a 20–25% decrease in mean DHEAS levels following long-acting GnRH analog (GnRHa) suppression in women with PCOS and elevated levels of this metabolite, although the elevated AA levels rarely normalize *(57,58)*. Consequently, it is possible that ovarian factors, possibly including androgens and estrogens, may increase AA secretion in PCOS.

Ditkoff et al. observed that transdermal estradiol (E2) administration restored the exaggerated responses of 11OHA4 and DHEA to ACTH stimulation following GnRHa suppression in women with PCOS *(59)*. Although estrogen may be operant in maintaining the exaggerated adrenocortical function observed in PCOS, we were unable to observe a similar effect on the adrenocortical steroidogenesis of postmenopausal women receiving transdermal E2 *(60)*.

Investigating the effect of extra-adrenal testosterone, we administered exogenous parenteral testosterone for 3 weeks to seven healthy oophorectomized women and did not observe significant changes in adrenocortical steroidogenesis, measured by the response to acute ACTH stimulation *(37)*. Alternatively, we were able to observe an increase in the DHEAS-to-DHEA ratio, suggesting increased DHEA-ST activity. Alternatively, studying 31 male-to-female and 22 female-to-male transsexual patients before and during sex steroid treatment, Polderman and colleagues reported that basal AA levels decreased by 27–48% in males treated with ethinylestradiol and cyproterone acetate and increased by 23–70% in females treated with testosterone *(61)*. Alternatively, studying four female-to-male transsexuals before and after 12 months of testosterone enanthate treatment, Futterweit did not observe any difference in the 17-OH-PREG, 17-hydroxyprogesterone (17-HP), DHEA, A4, S, and F levels, basally and after acute ACTH stimulation *(62)*.

In conclusion, available data suggest that extra-adrenal sex steroids may alter AA secretion in response to ACTH stimulation, and DHEA-ST activity and circulating DHEAS levels. However, there is significant conflict in the published data, and better designed and longer-term studies are needed to definitively answer this question.

2.6. Glucose/Insulin Axis and HPA Dysfunction in PCOS

Approximately 50–70% of PCOS patients have insulin resistance and hyperinsulinemia *(63,64)*. Insulin resistance in young women generally results in the development of compensatory hyperinsulinemia, which stimulates androgen secretion by the ovary *(65)* and perhaps the adrenal *(66)*. Studying minced human adrenal tissues, we observed that insulin (0.2–5.0 nmol/L) decreased DHEA and increased DHEAS, suggesting that at a minimum insulin stimulates DHEA-ST activity *(57)*. Additional evidence that hyperinsulinism plays a role in regulating adrenocortical biosynthesis arises from studies examining the effect of insulin sensitizers. We studied 305 women with PCOS randomized to receive placebo or the insulin sensitizer troglitazone in doses of 150, 300, or 600 mg/day for 20 weeks *(67)*. In these women, circulating DHEAS levels decreased 18–26% with troglitazone 600 mg/day, but not placebo, regardless of basal DHEAS level (Fig. 2). Nevertheless, troglitazone alters androgen biosynthesis directly *(68)* and ovarian androgen secretion indirectly *(69)*, which may have contributed to the observed results.

In contrast, studying 213 consecutive untreated women with PCOS and 165 age- and race-matched healthy eumenorrheic nonhirsute controls, Kumar et al. were unable to detect a significant association between circulating DHEAS and fasting insulin levels *(31)*. Moreover, PCOS patients with increased DHEAS tend to be leaner than patients with normal DHEAS levels *(33)*, a finding that seems to exclude a major role for circulating insulin in AA excess in PCOS.

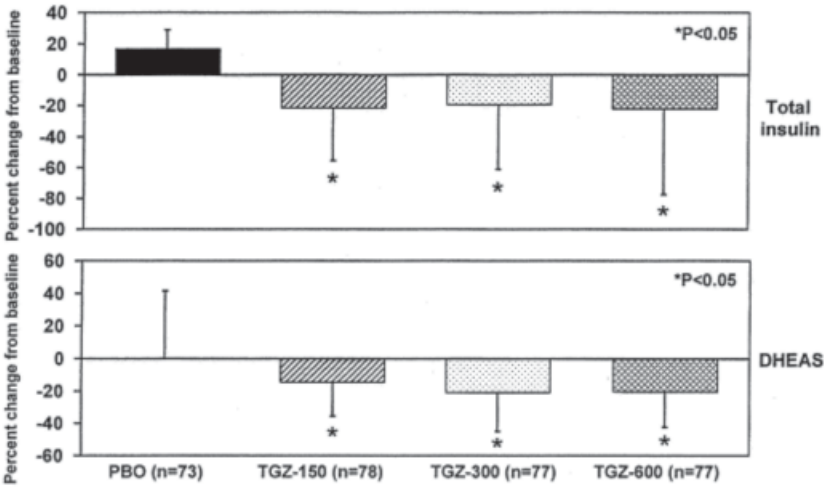

Fig. 2. Change in basal fasting total insulin and dehydroepiandrosterone sulfate (DHEAS) levels with placebo (PBO) or troglitazone 150 mg/day (TGZ-150), 300 mg/day (TGZ-300), or 600 mg/day (TGZ-600). Mean ± SD noted. (From ref. *67*.)

In order to determine whether more subtle abnormalities of insulin action are related to the adrenocortical dysfunction of PCOS, Falcone and colleagues studied 19 women with PCOS and nine age- and weight-matched controls using a tolbutamide-modified FSIVGTT analyzed by the minimal model *(70)*. A significant decline in DHEA levels was observed in control subjects and in PCOS women with normal insulin sensitivity 3 hours after glucose administration. Alternatively, no change in DHEA was observed in insulin-resistant PCOS subjects. DHEAS levels were not measured in this study. The investigators hypothesized that failure of glucose-stimulated endogenous insulin secretion to significantly depress DHEA levels in insulin-resistant women with PCOS may account in part for their androgen excess.

Farah-Eways and colleagues studied nine reproductive-aged patients with PCOS and nine age-, race-, and body mass indexed-matched controls with an insulin-modified FSIVGTT and an acute 60-minute ACTH(1-24)-stimulation test *(71)*. The fasting insulin and fasting glucose levels were not correlated to any of the adrenal parameters studied, with the exception of a positive association between the basal 17-OHP and the fasting glucose level ($r = 0.85$, $p < 0.004$). The insulin sensitivity index (S_I) and the acute insulin response to glucose (AIR_G) also had a limited correlation with adrenocortical parameters in both groups. Alternatively, glucose effectiveness (S_G), a measure of the ability of glucose to control its own production/uptake (i.e., glucose- or non-insulin-mediated glucose disposal), was strongly and positively associated in PCOS patients, but not controls, with the basal levels of F, DHEA, and DHEAS, the ACTH-stimulated peak levels of F, DHEA, and 17-OH-PREG, and the net increment following ACTH administration for F, DHEA, and 17-OH-PREG. These results suggest that adrenocortical biosynthesis, basally and in response to ACTH, may be more closely associated with glucose-mediated glucose disposal, or the mechanisms determining it, than with the degree of hyperinsulinemia or the sensitivity of insulin-mediated glucose disposal.

Overall, available data suggest that there may be a complex interaction between the glucose–insulin axis and HPA dysfunction in PCOS. Insulin itself may have a modest stimulatory effect on DHEA-ST, which may increase the correct levels of DHEAS at the expense of DHEA. It is also possible that AA excess may be the result of a decreased ability of insulin to suppress DHEA production. Finally, our data suggest that adrenocortical dysfunction in PCOS may be closely linked to those mechanisms underlying glucose-mediated glucose disposal (i.e., glucose effectiveness).

2.7. Heritability of Adrenal Androgen Excess and HPA Dysfunction in PCOS

Inheritance plays a significant role in determining the circulating AA levels in normal individuals *(72)*. In addition, circulating AA levels and their response to ACTH are highly individualized, compared to their secretion of glucocorticoids in normal women *(73)*. For example, both basal and ACTH-stimulated levels of DHEA demonstrated a high level of between-subject variability (60–70%) compared to the between-subject variability for F of 15–40% *(73)*. Essentially, the population could be divided into significant numbers of "high and low AA secretors." Furthermore, in PCOS the within-subject responses of DHEA, A4, or F to ACTH stimulation remain relatively unchanged over time *(74)*. Taken together, these data suggest that ACTH-stimulated AA secretion is highly individualized but relatively constant within individuals, consistent with the behavior of an inherited factor. Whether this represents the inheritance of factors regulating adrenocortical biosynthesis or of factors determining AA metabolism or clearance remains unclear.

The heritability of AA secretion was also demonstrated by Legro and colleagues, who studied 119 brothers of 87 unrelated women with PCOS and 68 weight- and ethnicity-comparable unrelated control men *(75)*. Brothers of women with PCOS had significantly higher DHEAS levels compared to controls, with a significant positive linear relationship in DHEAS levels between PCOS probands and their brothers. These data suggested a familial clustering of elevated DHEAS levels in the families of PCOS women, suggesting that this may reflect an inherited abnormality in the disorder.

Overall, these data indicate that a wide variation in the ability of the adrenal to secrete DHEA, basally and in response to ACTH and compared to that of F, exists in the normal population. Consequently, it is possible that those women with a greater ability to secrete AAs may also be at greater risk for developing PCOS. Consequently, AA excess in PCOS may result from overrepresentation by those individuals who are "high AA secretors" among PCOS women. Overall, AA secretion, basally and in response to ACTH stimulation, is relatively constant over time, potentially representing an inherited trait and a risk factor for PCOS.

3. CONCLUSIONS

AA excess affects approximately 20–30% of PCOS patients, as reflected by the circulating DHEAS levels using age-matched normative values. Patients with PCOS demonstrate a generalized hypersecretion of adrenocortical products in response to ACTH stimulation, primarily because of hyperresponsivity of AAs to ACTH stimulation and to increased $\Delta^5$17-OH activity. The mechanisms underlying these abnormalities remain unclear. However, several factors, including altered F metabolism, increased ovarian steroids and hyperinsulinemia, or factors regulating glucose-mediated glucose disposal (glucose effectiveness) may play a role. Finally, we should note that circulating AA levels and their response to ACTH are highly individualized and relatively constant over time, such that the population has significant numbers of "high and low AA secretors." Consequently, AA excess and HPA axis dysfunction in PCOS may reflect inherited factors and hypothetically may reflect an overrepresentation by high AA secretors in this population, essentially indicating that individuals who are genetically determined to secrete high levels of AAs will be at greater risk for developing PCOS.

4. FUTURE AVENUES OF INVESTIGATION

Despite extensive work by many investigators, this field remains fertile for new and innovative research. Areas that should be explored include (1) ethnic and racial differences in AA secretion, (2) extra-adrenal regulation of AA biosynthesis and metabolism (e.g., DHEA-ST), including the long-term effect of sex steroids on HPA and AA secretion, (3) the role of insulin, insulin-mediated glucose disposal, and glucose-mediated glucose disposal (i.e., glucose effectiveness) in AA steroidogenesis in normal and PCOS individuals, (4) the role that altered F metabolism plays in the AA excess and HPA axis dysfunction observed in PCOS, (5) the molecular mechanism underlying the steroidogenic

abnormalities observed in vivo (e.g., exaggerated $\Delta^5$17-OH activity observed), (6) the heritability of adrenocortical function in normal and PCOS individuals, and (7) the role of peripubertal AA excess in the development of PCOS.

KEY POINTS

- HPA dysfunction in the form of AA excess (generally indicated by elevated circulating DHEAS levels) is present in 20–30% of PCOS patients compared to age- and race-matched healthy women.
- PCOS women with AA excess have a generalized adrenocortical hypersecretion of adrenocortical products, basally and in response to ACTH stimulation, similar to the hypersecretion in response to human chorionic gonadotropin or GnRH analogs observed in the ovaries of these patients.
- AA excess in PCOS appears to be partially related to multiple extra-adrenal factors, including factors regulating glucose-mediated glucose disposal (glucose effectiveness), cortisol metabolism dysregulation, extra-adrenal sex steroids, or factors regulating insulin and glucose homeostasis.
- Circulating AA levels and their response to ACTH stimulation are highly individualized and relatively constant over time, suggesting that AA hypersecretion may be an inherited trait. Whether AA hypersecretion represents an inherited factor and whether it predisposes to PCOS remain to be determined.

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