Adrenal Involvement in Polycystic Ovary Syndrome

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ABSTRACT—*The etiology of hyperandrogenic chronic anovulation is heterogeneous and relatively unknown in the majority of cases. Affected individuals in this latter segment are considered to have polycystic ovary syndrome (PCOS) of which 50 to 60% exhibit androgen excess of adrenal origin. An understanding of normal adrenal function provides insight into the factors that contribute to adrenal androgen excess in PCOS. Since pituitary ACTH secretion promotes developmental growth and overall steroidogenic efficiency within the adrenal cortex, it is probable that these actions of ACTH along with the adrenal's unique centripetal circulation play a major role in the induction of adrenarche. This latter phenomenon is characterized by alterations in adrenocortical morphology and steroidogenic enzyme activities culminating in increases in adrenal androgens to normal circulating adult levels. Thus, it is not surprising that adrenal dynamic testing has revealed increased 17,20 lyase activity or adrenal androgen hyper-responsiveness to ACTH as the two abnormalities leading to adrenal androgen excess in PCOS. Whereas 17,20 lyase hyperactivity diagnosed by defined criteria in response to pharmacological ACTH may be an intrinsic genetic defect, increases in 17,20 lyase activity and adrenal androgen hyper-responsiveness to ACTH in response to physiological ACTH may be promoted by the functional elevation of estrogen of ovarian origin in PCOS. The latest in vitro data suggest the estrogen may elicit its effect on the adrenal cortex through a receptor mediated mechanism. Therefore, the currently available data indicate that adrenal androgen excess in PCOS is also heterogeneous in etiology.*

KEYWORDS: Adrenal morphology, adrenal dynamic testing, 17,20 lyase hyperactivity, adrenal androgen hyper-responsiveness to ACTH, ovarian induced adrenal hyperandrogenism

Approximately 3–7% of women within the reproductive age group present with oligo-amenorrhea often in conjunction with hirsutism or acne consistent with the clinical entity known as hyperandrogenic chronic anovulation (HCA).[1,2] While the source of androgen excess can be the ovary alone or the adrenal alone, both glands frequently contribute to the pool of elevated serum androgens.[3] Only in the minority of cases has the cause of HCA been clearly elucidated. For instance, genetic or autoimmune impairment of insulin receptor function accounts for the excess in ovarian androgen production evident in the syndrome of hyperandrogenism, insulin resistance, and acanthosis nigracans (HAIR-AN syndrome).[4–6] In addition, a well-established genetic defect characterized by late onset 21α hydroxylase deficiency culminates in excess adrenal androgen production

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and is the most common form of congenital adrenal hyperplasia.[7] However, the cause of HCA in the majority of affected women is heterogeneous and relatively unknown. It is this latter segment that is considered to have polycystic ovary syndrome (PCOS).[8]

While the diagnosis of PCOS is currently based on clinical and biochemical criteria, the use of ultrasound for this purpose remains controversial. Women with PCOS often experience perimenarchal onset of signs and symptoms of HCA and exhibit a biochemical profile that can be distinguished from that of HAIR-AN syndrome or late onset 21αhydroxylase deficiency. Ultrasound examination of the ovary in PCOS often reveals gonadal enlargement, an increased amount of stroma and the presence of multiple antral follicles each measuring less than 10 mm in diameter.[9,10] However, these ultrasound findings may not be unique to PCOS because they have been described in women with other forms of HCA and in some normal ovulatory women.[2,11] Conversely, not all individuals with PCOS possess ovaries with a polycystic appearance on ultrasound.[12] Thus, it remains to be determined whether this specific ovarian morphology indicates a predisposition to hyperandrogenism or is merely a sign of a long-standing functional abnormality.[13,14]

A large subpopulation of women with PCOS exhibit adrenal androgen excess with a prevalence of approximately 50–60%.[15,16] It has recently become apparent that the cause of this adrenal involvement is heterogeneous.[17] Moreover, an intrinsic adrenal mechanism has been described but the influence of extrinsic factors, direct or indirect, must also be entertained. Animal and human studies indicate that circulating adrenal androgens serve as precursors of ovarian androgens by intraovarian conversion such that it is possible to induce polycystic ovaries functionally and morphologically if serum adrenal androgens are elevated.[18,19] Conversely, there is data to suggest that in PCOS, the ovary plays a role in promoting excess adrenal androgen production.[20–24] Therefore, in PCOS, the ovary and the adrenal may be capable of influencing each other in a bidirectional fashion aside from the traditional modulation exerted on either peripheral gland by the hypothalamus and the pituitary.

The awareness that there is etiological heterogeneity in PCOS, including that of the adrenal dysfunction, has facilitated the discovery of a number of pathophysiological mechanisms that culminate in clinically evident PCOS. Separate study of the various subpopulations of women with PCOS based on biochemical criteria has been the key to progress. This has been especially true in characterizing the contribution of the adrenal in PCOS. There are also several morphological, developmental, and physiological features of the adrenal cortex that aid in the understanding of these investigations. Consequently, a description of normal adrenal function and adrenal dynamic testing will serve as background for further discussion. A review of the scientific literature designed to attempt elucidation of adrenal androgen control, in general, and of adrenal androgen excess in PCOS will follow. Finally, some comments as to the direction of future investigation will be presented.

ADRENAL MORPHOLOGY

The morphology of the adrenal has been described previously in some detail[25] and will be summarized below.

Anatomic Description

The adrenal glands are paired organs located medial to the upper pole of each kidney. The right adrenal is pyramidal shaped while the left adrenal is more flattened and crescent shaped. Each adrenal gland weighs approximately 4 to 5 g in the normal unstressed adult and is composed of two developmentally unrelated tissues surrounded by a thin capsule. The outermost cortex is of ectodermal origin and constitutes 90% of the gland while the more centrally located medulla is of mesodermal origin and makes up only 10% of the gland.[26–28]

The adrenal cortex is classically divided into three zones known as the zona glomerulosa, the zona fasciculata, and the zona reticularis (Fig. 1). The zona glomerulosa is located immediately beneath the capsule and accounts for only 5% of cortical thickness. Glomerulosa cells are arranged in clumps that may lack continuity with each other. The zona fasciculata is located just below the zona glomerulosa and accounts for 70% of cortical thickness. Fasciculata cells are larger, contain lipid vacuoles for cholesterol storage and are arranged in a narrow cord-like columnar configuration. Superficially located fasciculata cells possess the largest and most numerous lipid vacuoles while the more deeply lying fasciculata cells have a high content of rough endoplasmic reticulum and mitochondria. The zona reticularis is the deepest zone located closest to the central medulla and accounts for the remaining 25% of cortical thickness. Reticularis cells have a size in between those of the glomerulosa and the fasciculata, possess a high content of smooth endoplasmic reticulum and mitochondria, and are arranged in an anastomotic

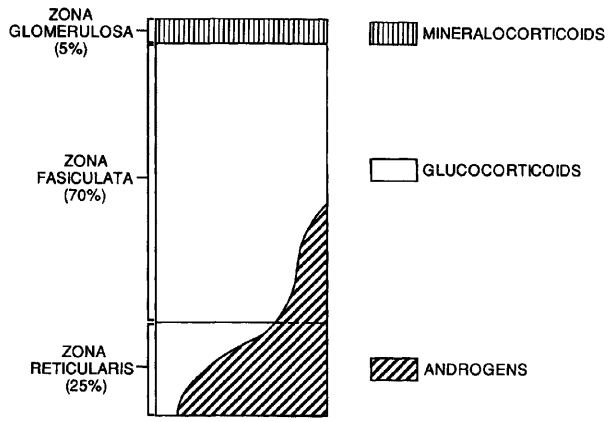

Figure 1. Diagrammatic representation of the adrenal cortex. The zonae glomerulosa, fasiculata, and reticularis occupy 5, 20, and 25% of cortical thickness, respectively. Mineralocorticoids are produced exclusively in the zona glomerulosa. However, the zona fasiculata and the zona reticularis produce glucocorticoids and androgens. While the majority of glucocorticoids are produced in the zona fasiculata, most adrenal androgens are produced in the zona reticularis.

network. Therefore, each zone has a characteristic appearance even though boundaries between zones are not always demarcated.[28,29]

The medulla is a direct extension of the sympathetic nervous system. Medullary cells arranged in clusters synthesize the catecholamines, epinephrine, and norepinephrine that are then stored in secretory granules. Functioning as postganglionic neurons, medullary cells are innervated by preganglionic fibers of the splanchnic nerve, the celiac ganglion, and the subsidiary plexus. Neuronal stimulation leads to release of catecholamines from the secretory granules into the medullary capillaries by exocytosis. Therefore, the medulla is responsible for instantaneous secretion of presynthesized neurohormones upon command.[28]

Adrenal Vasculature

The adrenal glands receive their blood supply from three arteries. The inferior phrenic artery, the abdominal aorta, and the renal artery supply the superior, middle, and inferior adrenal arteries, respectively, which coalesce to form a plexus over the capsule of each gland. The capsular plexus gives rise to approximately 40 to 60 vessels that penetrate the substance of the gland. A few of these penetrating arterioles pass directly through the cortex to supply the medullary capillaries. However, the vast majority of the vessels enter a cortical plexus that is continuous throughout all three zones and assumes the configuration of the respective zonal cellular arrangements. The cortical plexus empties into a large number of venules that either drain in medullary veins or continue into the capillary plexus of the medulla. Thus, the medullary plexus blood supply receives direct but minor input from capsular arterioles that bypass the cortex and a major contribution from the cortical plexus venules. The medullary plexus assumes the clustered configuration of medullary cells and ultimately drains into medullary veins, which coalesce into the large central vein that emerges from each adrenal gland. The right central vein is short (1-cm average) and enters directly into the inferior vena cava. The left central vein is somewhat longer (2-cm average) and enters into the left renal vein. Therefore, the adrenal vasculature exhibits continuity throughout the gland permitting centripetal blood flow through an intraglandular capillary network that extends from the cortex to the medulla.[26,28–30]

The adrenal gland receives the greatest amount of blood per gram of tissue than any other body organ except for possibly the thyroid gland. The intraglandular capillary network is composed of fenestrated endothelial cells that facilitate rapid passage of substances in or out of the blood stream. Adrenal blood flow is primarily controlled at the level of the medullary veins and the central vein both of which possess walls with prominent longitudinal smooth muscle bundles arranged in eccentric fascicles. Postganglionic sympathetic innervation controls the contractile state of these fascicles thereby regulating the proximal intradrenal blood flow. Therefore, the fenestrated capillary network and construction of venous outflow permits adequate intracellular exposure to the centripetal flow of intrinsic and extrinsic substances, which ultimately affect the intra-adrenal microenvironment.[28,29]

ADRENOCORTICAL STEROIDOGENESIS

Corticosteroid Descriptions

The adrenal cortex secretes five types of steroid hormones: mineralocorticoids, glucocorticoids, and androgens to a greater extent; estrogens and progestogens to a lesser extent.[27] The principal mineralocorticoid, aldosterone, is produced exclusively by the zona glomerulosa. Aldosterone plays a major role in electrolyte homeostasis and is primarily under the control of the renin-angiotensin system and the plasma concentration of potassium. The anterior pituitary hormone, adrenocorticotropin (ACTH), plays only a secondary role in the control of aldosterone that is limited to pathological situations associated with salt imbalance.[31]

Glucocorticoids and androgens are produced in both the zona fasciculata and the zona reticularis. However, the majority of glucocorticoid production occurs in the zona fasciculata (~93%), while the majority of androgen production occurs in the zona recularis (~66%). The glucocorticoids, primarily cortisol and to a lesser extent corticosterone, are involved in the overall regulation of carbohydrate metabolism and influence the synthesis of catecholamines in the medulla. In addition, glucocorticoids along with catecholamines aid in coordinating the body's metabolic response to stress.[28]

The principal adrenal androgens are dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), 11β hydroxyandrostenedione (11βA), and androstenedione. The adrenal accounts for greater than 90% of DHEA, DHEAS, and 11βA as well as slightly less than 50% of androstenedione found in the peripheral circulation. Intracortical conversion of androstenedione also leads to secretion of testosterone of adrenal origin that contributes roughly 25% to circulating levels.[32,33] Adrenal androgens play an important role in sexual maturation during adolescence after a series of morphological events within the adrenal called adrenarche.[34] In addition, peripheral conversion of adrenal androgens in muscle and adipose tissue provides the bulk of nongonadally derived estrogen.[35]

The majority of the body's source of estrogen and progestogens in women is the ovary. Secretion of estrogen by the adrenal cortex is quantitatively insignificant except in certain rare cortical neoplasms.[36] Progestogens of adrenal origin are generally precursors of the major corticosteroids and are not secreted in large amounts unless they are substrates of a congenitally deficient adrenal steroidogenic enzyme.

Biosynthetic Mechanisms

Cholesterol Substrate

The building block for corticosteroid biosynthesis is cholesterol. The major source of adrenal cholesterol is low density lipoprotein (LDL) present in the circulation, Entry of LDL into cortical cells is accomplished by binding to a high-affinity receptor and subsequent internalization of the LDL-receptor complex. Upon arrival within the cell, the protein and cholesterol ester components of LDL undergo hydrolysis in lysosomes causing release of free cholesterol. While some of the free cholesterol is used for steroid synthesis, the remainder is re-esterified and stored in lipid vacuoles. When rapid hormone production is required, the pool of free cholesterol is expanded immediately by the mobilization and hydrolysis of stored cholesterol esters and to a lesser extend by de novo synthesis from acetate. However, uptake of LDL from the circulation is accelerated simultaneously and, thus, remains the prime source for hormone production.[37-39]

Molecular Enzyme Reactions and Pathways

In general, the corticosteroid biosynthetic pathway from cholesterol involves the following steps: (1) cholesterol side chain cleavage to form a C21 steroid; (2) hydroxylation of the steroid skeleton at variety of positions; (3) oxidation of the 3βhydroxyl group to a ketone with a subsequent double bond shift from the 5,6 position to the 4,5 position; (4) cleavage of the bond between carbons 17 and 20 to form a C19 steroid in some instances. The majority of these reactions are catalyzed by members of the cytochrome P450 group of enzymes (Fig. 2).[27,40]

Conversion of cholesterol to pregnenolone is the rate limiting step in steroidogenesis. The several reactions involved are mediated by the side chain cleavage cytochrome P450 enzyme (P450scc) encoded by a single gene and located within the mitochondria of adrenocortical cells. The carbons in position 20 and 22 are each hydroxylated to form 20α, 20∈-dehydrocholesterol with subsequent bond cleavage between carbons 20 and 22 to form pregnenolone and isocaproic aldehyde.[27,40,41]

Hydroxylation of a steroid at a number of positions and in a variety of stereoorientations is performed by 3 additional cytochrome P450 enzymes confined to specific organelles within the cells of certain adrenocortical cells. Throughout the cortex, glucocorticoids or mineralocorticoid precursors are hydroxylated at carbon 21 in the α orientation by P450 21 present in the endoplasmic reticulum and at carbon 11 in the β orientation by P450 11 present in the mitochondria. However, aldosterone synthesis requires an additional hydroxylation in the α orientation as well as methyl oxidation at the 18 position, which are also catalyzed by P450 11 but limited to the mitochondria of glomerulosa cells. On the other hand, synthesis of androgens and of the major glucocorticoid cortisol, requires 17α hydroxylation through the action of P450 17 limited to the endoplasmic reticulum of fasciculata and reticularis cells.[27,31,40,41] Therefore, steroid substrates must undergo intracellular transport between organelles possibly with the aid of sterol carrier proteins similar to the hepatic sterol carrier protein. In addition, some cytochrome P450 enzymes can metabolize multiple substrates mediating more than one reaction but often in a zone-specific manner that dictates zonal steroidogenic patterns.

ADRENOCORTICAL STEROIDOGENIC ENZYMES	INTRACELLULAR LOCATION	ADRENOCORTICAL CELL TYPE	STEROIDOGENIC FUNCTION
$P450_{SCC}$	MITOCHONDRIA	GLOMERULOSA FASICULATA RETICULARIS	CHOLESTEROL SIDECHAIN CLEAVAGE
P450 21	ENDOPLASMIC RETICULUM	GLOMERULOSA FASICULATA RETICULARIS	21α HYDROXYLATION
P450 11	MITOCHONDRIA	GLOMERULOSA FASICULATA RETICULARIS	11β HYDROXYLATION 18α HYDROXYLATION (GLOMERULOSA ONLY)
3βHSD	ENDOPLASMIC RETICULUM	GLOMERULOSA FASICULATA RETICULARIS	3β HYDROXYL OXIDATION 5,6 TO 4,5 DOUBLE BOND SHIFT
P450 17	ENDOPLASMIC RETICULUM	FASICULATA RETICULARIS	17α HYDROXYLATION 17, 20 BOND CISSION
SULFOTRANSFERASE	CYTOPLASM	RETICULARIS	DHEA SULFATION

Figure 2. Adrenocortical steroidogenic enzymes are confined to specific locations within certain adrenocortical cell types and have specific functions culminating in synthesis of mineralocorticoids, glucocorticoids, and androgens.

All cytochrome P450 enzymes are "mixed function" oxidases utilizing molecular oxygen and NADPH. Electrons are passed along an electron transport chain to reduce the cytochrome P450 enzymes, which, in time, reduces molecular oxygen to one molecule of water and an activated oxygen atom that is introduced between the appropriate hydrogen on the steroid backbone. The electron transport system of mitochondrial cytochrome P450 enzymes consist of a flavoprotein and adrenodoxin while that of their microsomal (endoplasmic reticulum) counterparts consist only of an NADPH-driven flavoprotein called P450 reductase.[27,40,41]

3β hydroxyl oxidation and the 5,6 to 4,5 double bond shift are sequentially accomplished by the action of the only noncytochrome P450 enzyme known as 3β hydroxysteroid dehydrogenase -Δ^5 3 ketosteroid isomerase (3βHSD), which is encoded by a single gene and is microsomal in origin. While the oxidation step requires NADP as a hydrogen acceptor, no cofactor is required for the isomerase step.[27,40]

The order of the enzyme action in the synthesis of glucocorticoids from pregnenolone can vary to yield two distinct pathways. Oxidation of the 3β hydroxyl group can occur either before or after the hydroxylations at the 21 and 11 positions generating steroid intermediates that are known as the Δ^4 and Δ^5 pathways, respectively. Although the actual in vivo pathway is unknown, there is evidence to suggest that the Δ^5 pathway is preferred. However, it would not be improper to assume that the possible pathways occur in a lattice configuration as opposed to a single route.[27]

Adrenal androgen synthesis is further mediated by P450 17 and sulfotransferase. Besides its 17α hydroxylase activity, P450 17 possesses 17,20 lyase activity to transform 17 hydroxylated C21 precursors to C19 androgens by bond scission between carbons 17 and 20. Although the activities of 17α hydroxylase and 17,20 lyase are both encoded by a single gene, a high ratio of P450 reductase to P45017 is necessary to promote 17,20 lyase activity.[40,42] Sulfotransferase attaches a sulfate group to DHEA to yield DHEA-S.[26] DHEA and its sulfate do not require the action of 3βHSD for their synthesis. Therefore, androgen production is predominant in an adrenocortical microenvironment where P45017 and sulfotransferase activities are high and 3βHSD activity is low.[43–46] Determinants of this microenvironment within the zona reticularis will be discussed later.

CONTROL OF ADRENOCORTICAL FUNCTION

The Modulating Role of ACTH

ACTH, a peptide of anterior pituitary origin, plays a dual role in the modulation of adrenocortical function. ACTH stimulates growth of the adrenal cortex during prenatal and postnatal life, and exerts primary control over the biosynthesis and secretion of glucocorticoids and androgens. The effects of ACTH can be generalized or specific, direct or indirect, and acute or chronic (Fig. 3). Therefore, the mechanisms by which ACTH performs its dual role are variable. Many of these mechanisms still require greater elucidation.

Effects on Adrenocortical Growth

The regulation of adrenocortical growth is achieved by actions that lead to cellular hypertrophy and tissue hyperplasia. ACTH promotes cellular hypertrophy by directly inhibiting the synthesis of DNA required for cell replication. On the other hand, ACTH indirectly promotes tissue hyperplasia by rapidly increasing adrenal blood flow and by inducing neovascularization of cortical tissue over time. These actions serve to increase exposure of the adrenal cortex to the nutrients and oxygen necessary for cell proliferation. It is postulated that the influence of ACTH on blood flow secondarily activates a neural reflex caused by stimulation of baroreceptors in response to hyperemia. This neural reflex is felt to play a potentiating, yet nonessential, role in cell proliferation of

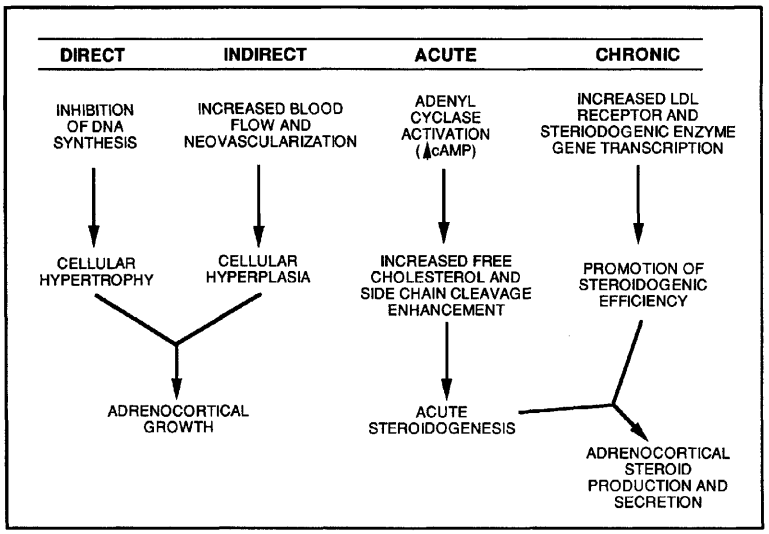

Figure 3. The variable actions of ACTH are suitable for its dual roles of inducing adrenocortical growth and stimulating adrenocortical steroidogenesis.

the contralateral adrenal cortex. Therefore, adrenocortical growth, in terms of hypertrophy versus hyperplasia, is the result of the delicate balance between the direct inhibitory and indirect stimulatory effects of ACTH.[43,47,48]

Effects on Corticosteroid Biosynthesis and Secretion

The immediate and delayed effects of ACTH provide significant impact on the biosynthesis of glucocorticoids and androgens. Both effects intertwine to make cholesterol substrate available to the steroidogenic pathway and to maintain a sufficient level of enzymatic activity for steroidogenic demands. More specifically, ACTH exhibits acute control over the rate limiting mitochondrial cholesterol side chain cleavage reaction. A brief description of the acute and chronic actions of ACTH is essential to understand biosynthetic regulation by ACTH.

Circulating ACTH binds to specific cortical cell membrane receptors causing stimulation of membrane bound adenylate cyclase and rapid cyclic AMP production. Cyclic AMP, in turn, immediately activates a protein kinase that stimulates a number of protein phosphorylations utilizing ATP for energy. One of the enzymes phosphorylated to its active form is cholesterol ester hydrolase, which liberates free cholesterol from cytoplasmic lipid vacuoles to ensure a continuous supply of substrate to the mitochondria. It has been postulated that other cyclic AMP-mediated phosphoproteins yet to be identified, increase cholesterol binding to P450scc to enhance cholesterol side chain cleavage.[31,41] Therefore, the acute action of ACTH is mediated by cyclic AMP to raise intracellular levels of free cholesterol and to drive the rate limiting step in steroidogenesis.

ACTH induces protein synthesis within cortical cells. The induction process is partially mediated by free cholesterol made available by the acute action of ACTH. An increase in the intracellular levels of free cholesterol is likely to stimulate synthesis of P450scc. Once the rate limiting step has been surmounted, the cascade of substrates made available may induce other enzymes within the steroidogenic pathway. ACTH also increases the number of LDL receptors to promote uptake of LDL from the circulation and then induces the enzymes required for de novo synthesis of cholesterol. Induction of protein synthesis is slow because proteins such as adrenocortical enzymes have long half-lives of 3 to 4 days. Therefore, the chronic action of ACTH efficiently maintains a high rate of steroidogenesis. This is accomplished by induction of enzymes and other proteins specific to not only the steroidogenic pathway from cholesterol, but also to those processes that optimize the supply of cholesterol to this pathway.[49]

The factors governing the secretion and regulation of ACTH have direct relevance to the control of glucocorticoid and androgen secretion by ACTH. Corticotropin Releasing Hormone (CRH) from the hypothalamus stimulates the release ACTH from the anterior pituitary. A long negative feedback loop exists by which glucocorticoids inhibit the secretion of ACTH and CRH. The source of glucocorticoids inducing suppression can be endogenous from the adrenal or an exogenously administered synthetic such as dexamethasone.[50] The feedback inhibition is biphasic, occurring rapidly within several minutes and after a delay of 2 or more hours. The delayed mechanism is characterized by a reduction in the transcription of ACTH

and CRH precursor molecules while the rapid mechanism is believed to be a nongenomic process. It is well established that glucocorticoids act directly on the pituitary to inhibit ACTH secretion. However, it remains to be established whether glucocorticoids act directly on the hypothalamus or indirectly through other parts of the brain to modulate CRH secretion.[51] Under physiological conditions, ACTH release occurs episodically in a diurnal 24-h cycle. This pattern of ACTH release is intrinsic to the hypothalmic control system and is independent of feedback control.[31] Secreted ACTH rapidly arrives in the adrenal cortex so that secretion of cortisol and adrenal androgens shows an episodic and circadian variation parallel to that of ACTH with certain exceptions. Testosterone does not demonstrate significant variation in women because of its derivation from several sources; namely, the ovary, the adrenal, and peripheral conversion of androstenedione.[29] However, the minor adrenal component does exhibit a circadian rhythm.[52] DHEA-S also shows negligible variation because it is present in plasma in large quantities and is metabolized slowly.[53]

Intraadrenal Determinants of Zonal Steroidogenic Patterns

Maintenance of Adrenocortical Mass

The acquisition and maintenance of adrenocortical mass throughout prenatal and postnatal life is determined by the relation between the rate of cell division and the rate of cell death (Fig. 4). Cell division is indirectly influenced by ACTH as described previously and is restricted to the outer regions of the cortex, namely the zona glomerulosa and the outer zona fasciculata. Considerable mitotic activity occurs in the outer cortex when compared to the inner regions and is probably a result of greater nutrient availability because the outer cortex is closer to the arterial blood supply. Cell death is age related, and limited to the inner cortex as demonstrated by the increased presence of age pigment (lipofuscin) in the zona reticularis. These observations clearly support the concept that adrenocortical cells are pushed en masse toward the medulla by cell division in the outer cortex, balanced by cell death in the inner cortex. Therefore, glomerulosa cells are pushed inward to become fasciculata cells and eventually end their life span as reticularis cells, indicating that a specific cell will secrete different types of corticosteroids over time through a process of interconversion.[54]

The concept of inward "escalator" migration suggests that cells from all three zones of the adrenal cortex are of the same basic cell types. Other findings also support this contention. Synthetically active adrenocortical cells from any zone exhibit a uniform characteristic during hormone production and secretion upon stimulation. Moreover, release of products into the circulation occurs after a short delay required for the synthesis of steroids from stored lipid precursors. Furthermore, the zona fasciculata and the zona reticularis are probably one functional unit. Clear cells of the outer fasciculata may be relatively inactive storage cells of surplus precursors while compact cells of the inner fasciculata and reticularis are most likely involved in active synthesis of glucocorticoids and androgens because they are abundant in endoplasmic reticulum and mitochondria. Acute ACTH stimulation causes a change in cellular configuration from clear to compact causing an apparent increase in the size of the zona reticularis at the expense of the zona fasciculata. Sustained ACTH stimulation converts the remaining clear cells to compact cells causing extreme precursor depletion and maximal steroidogenic output. Final support is provided by observation of adrenocortical cells placed in culture. Marked differences in corticosteroids produced by cells isolated from different zones disappear over time to yield identical patterns of steroidogenesis.[28,43,47] Therefore, cellular function within the adrenal cortex is determined by external stimulatory influences such as ACTH or the renin-angiotensin system and by the specific location of a given adrenocortical cell at the time of stimulation.

Maintenance of Functional Zonation: Reassessment of the Gradient Hypothesis

The mechanism for functional zonation of adrenocortical cells remains unknown. In the past, the gradient hypothesis provided a plausable explanation inclusive of proposed intrinsic factors responsible for zona reticularis formation and primary adrenal androgen secretion by late childhood. These latter events have collectively been termed the adrenarche.[34] The steroid gradient across the adrenal cortex created by the adrenal's centripetal blood flow formed the basis of the gradient hypothesis. Central to this hypothesis was the concept that specific corticosteroids termed gradient substances achieved the critical concentrations required to directly inhibit key adrenal enzymes as the flow of blood carried progressively higher amounts of these secreted steroids deeper within the cortex. Thus, it had been proposed that these enzyme inhibitions culminated in local shifts in steroidogenic patterns to establish boundaries between zones.[43,47]

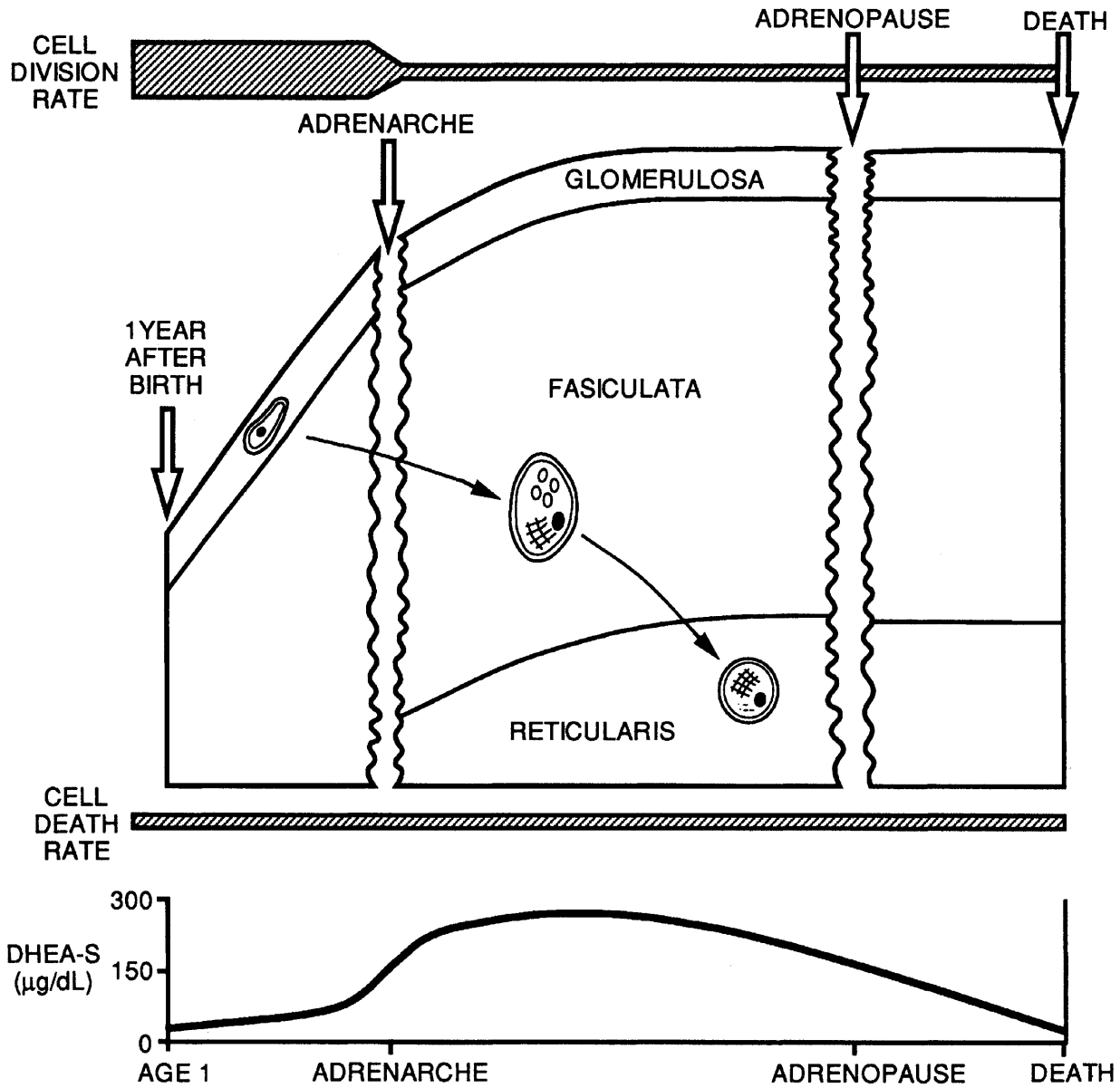

Figure 4. Adrenocortical thickness is believed to be related to the ratio of new cell formation in the outer cortex to that of old cell death in the inner cortex. This ratio is increased before adrenarche and proportional after adrenarche. In the process, it is proposed that a given adrenocortical cell will undergo inward "escalator" migration resulting in interconversion to different cell types during its lifespan. The persistence of the zona reticularis, despite the decline in its production of androgens during the so-called adrenopause, is an unexplained phenomenon.

Until recently, the data accumulated was consistent with the gradient hypothesis. Quantitation of the steroid concentrations within normal adrenal tissue in vitro confirmed the presence of an intra-adrenal steroid gradient by demonstrating progressive increases in the steroid concentrations from the outer layers to the inner layers of the adrenal cortex.[54] Kinetic studies performed on purified adrenal enzyme extracts indicated efficacious inhibition by specific corticosteroids at concentrations equal to those found within the appropriate cortical layers where zonal boundaries are located. As a consequence, the glomerulosa/fasciculata boundary was thought to be established when gradient substance candidates such as corticosterone or cortisol achieved the critical concentration required to directly inhibit P450 11 18α hydroxylation resulting in suspension of aldosterone production.[43,47] In addition, the fasciculata/reticularis boundary was thought to be established with the attainment of sufficient cortical thickness by late childhood through the direct and indirect actions of ACTH to permit corticosterone or another gradient substance candidate, andro-

stenedione, to achieve the critical concentration required for direct inhibition of 3βHSD activity. This latter phenomenon along with a concomitant increase in the activity of highly ACTH dependent P450 17 by the chronic action of ACTH would culminate in development of the zona reticularis and initiation of an adult pattern of adrenal androgen secretion.[43,54,55]

The latest investigations are inconsistent with direct enzyme inhibition as the mechanism for decreased 3βHSD activity during adrenarche. Moreover, lower levels of 3βHSD messenger RNA (mRNA) and enzyme are present in the zona reticularis compared to the zona fasciculata in the postadrenarchal adrenal cortex.[56] This suggests that the decrease in 3βHSD activity observed during adrenarche is a result of lowered 3βHSD gene expression. It is possible that the intra-adrenal steroid gradient plays a role in lowering 3βHSD gene expression or increasing P450 17 gene expression for that matter. In this instance, the appropriate gradient substance could impose its effects through a receptor mediated mechanism upon attaining the necessary critical concentrations. However, support for this postulate remains to be demonstrated.

Contribution to the induction of adrenarche by a hormonal factor distinct from ACTH and the intra-adrenal steroid gradient has been proposed. To date, none of the hormones implicated in this regard such as estrogen, prolactin or the gonadotropins, to name a few, exhibit plasma levels that change in parallel with the adrenarche.[34,36] In the case of estrogen, adrenarche occurs before gonadarche indicating that any possible modulating role played by rises in estrogen during gonadarche would be contributory rather than primary. Beyond adrenarche, however, there are a number of situations to suggest that another factor participates in the regulation of adrenal androgen synthesis. For instance, the stress of anorexia nervosa, other chronic illnesses, and burn trauma promote ACTH hypersecretion, which causes serum cortisol levels to rise while DHEA and DHEA-S concentrations fall.[57–59] With advancing age, during the so-called adrenopause, ACTH and cortisol secretion is minimally altered while adrenal androgen secretion declines without a reduction in adrenal gland weight or a corresponding involution of the zona reticularis.[60] Thus, there is a divergence in the secretion of cortisol and the adrenal androgens despite similar ACTH exposure, unaltered adrenal morphology, or both. A human pituitary factor localized to the 18 amino acid joining peptide fragment of propiomelanocortin (POMC) has been called the cortical androgen stimulating hormone (CASH) due to its ability to bind adrenal cells and stimulate DHEA secretion synergistically with ACTH in vitro (Fig. 5).[61] Because the activity of this substance has been shown to be small or undetectable,[62,63] its contribution to adrenal androgen regulation in vivo remains controversial. However, this area of research is still in its infancy such that an increase in the potency of this POMC fragment by dimerization, amidation or variable POMC processing may still be possible.[64]

ADRENAL DYNAMIC TESTING

A description of the adrenal dynamic testing modalities used extensively to elucidate adrenal dysfunction in PCOS is merited to facilitate an understanding of the findings of past investigators (Fig. 6). The status of adrenocortical function can be determined by comparing serum corticosteroid levels or their urinary metabolites before and after adrenal suppression, adrenal stimulation, or both.[27] Adrenal suppression can be induced with the use of dexamethasone, a potent synthetic glucocorticoid that causes negative feedback inhibition of endogenous ACTH.[50] Adrenal stimulation can be accomplished directly by administration of cortrosyn, a synthetic fragment of ACTH or indirectly by administration of CRH usually of ovine origin.[27,65] Adrenal dynamic testing has classically been used as a diagnostic tool for disease states associated with glucocorticoid hypo- or hypersecretion. However, this form of testing has also been used to determine the source of androgen excess and the nature, if any of adrenal abnormality in hyperandrogenic states.[66–71] The extensive utility of dexamethasone suppression and ACTH stimulation has been previously reviewed in great detail.[25] Therefore, a discussion of these modalities will be limited to testing protocols pertinent to PCOS. The use of CRH in the study of PCOS is a more recent development worthy of additional discussion.

Dexamethasone Suppression

Acute low dose dexamethasone suppression offers a unique advantage in diagnosis. Because negative feedback inhibition of ACTH is elicited for only a short period of time, the adrenocortical growth-promoting and enzyme-inducing properties of ACTH are unaffected.[50] As a result, the structural and/or functional identity of the zona reticularis remains intact and the androgen-producing capability of the adrenal is maintained. Any underlying pathophysiology is thereby preserved for further testing. When performed in an overnight fashion, a dose of 1 mg of dexametha-

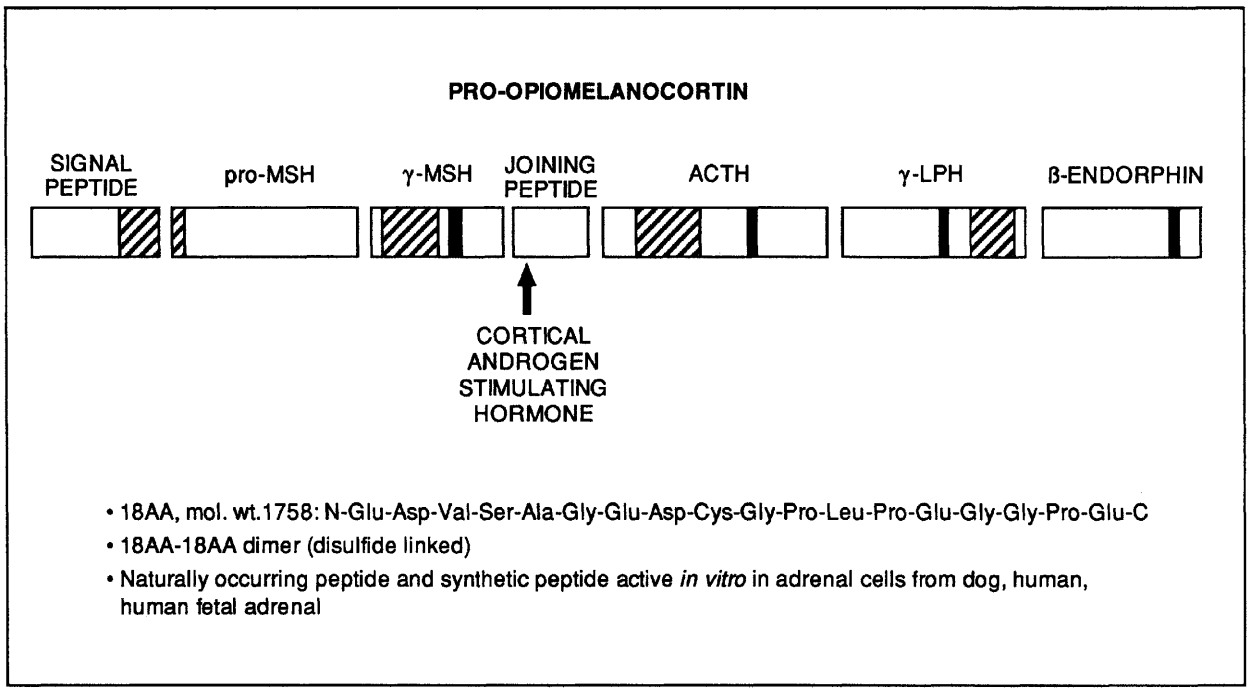

Figure 5. The location within the pro-opiomelanocortin precursor molecule as well as the amino acid sequence and characteristics of the peptide proposed as the cortical androgen-stimulating hormone (CASH). The putative role of this peptide in vivo still remains controversial. (Adapted with permission from Blackwell Science, Inc. and based on results from Parker et al.[61])

sone is administered orally at 11 PM and plasma corticosteroid levels are checked for suppression at 8 AM. Overnight dexamethasone suppression is useful in eliminating adrenal stimulation by excessive ACTH secretion in response to stress, which may contribute to elevated adrenal androgen levels independent of an underlying hyperandrogenic disease.[27] Therefore, dexamethasone pretreatment overnight provides a standardized stress-free baseline prior to investigation of adrenal responsiveness.

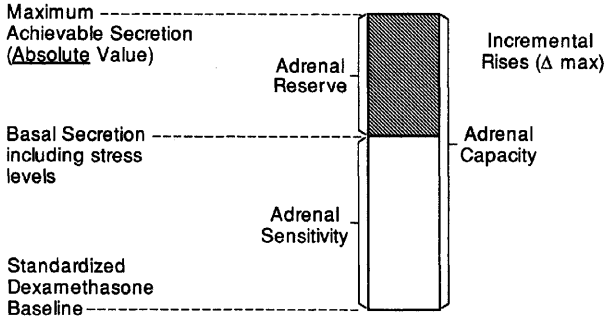

Figure 6. Diagrammatic representation of adrenal dynamic testing. The bar graph illustrates the incremental rises of any corticosteroid associated with determination of adrenal sensitivity, adrenal capacity and adrenal reserve. See text for definitions of these distinct incremental rises. (Reproduced with permission from Gonzalez et al.[25])

ACTH Stimulation

ACTH stimulation can be performed in several fashions to study the various aspects of adrenocortical function. When a physiological dose of ACTH is administered intravenously after overnight dexamethasone suppression, the incremental rise (Δ max) between the pre- and poststimulation values of the various corticosteroids is an indicator of adrenal sensitivity.[72,73] The 200-ng pulse of cortrosyn utilized for this purpose is analogous to circadian variation.[74] In contrast, ACTH can also be given in pharmacological doses either by constant infusion[74,75] or by bolus administration of 0.25 mg of cortrosyn[27,73] to elicit maximal adrenocortical response. If pretreatment with dexamethasone overnight is administered in conjunction, the incremental rise following stimulation is indicative of adrenal capacity.[73,74] If dexamethasone is omitted, the incremental rise following stimulation is indicative of adrenal reserve. For the majority of corticosteroids such as cortisol and androgens, maximally achievable levels plateau within 60 min of ACTH administration. However, the DHEA-S level must be drawn no earlier than 180 min post

ACTH to detect a maximum increase due to the slower metabolic clearance and the large serum pool of DHEA-S, which causes its apparent slower response to stimulation.[68] Therefore, adrenal sensitivity, capacity, and reserve are determined by varying the dose of ACTH and/or the use of dexamethasone pretreatment and the timing for blood drawing following ACTH stimulation is longer for DHEA-S than for other corticosteroids.

Overnight dexamethasone suppression prior to ACTH stimulation may or may not offer some advantage depending on the intended purpose. In the diagnosis of adult onset 21α hydroxylase deficiency, adrenal reserve is as predictive as adrenal capacity and the absolute plasma corticosteroid levels post-ACTH are more important as an index of adrenal reserve than the respective increments.[76] On the other hand, adrenal capacity determinations provide more specific information about steroidogenic enzyme efficiencies because hormone biosynthesis must be initiated from a resting unstressed baseline in the face of maximal stimulation. Post-ACTH substrate-product ratios of the absolute plasma levels offer additional information about enzyme efficiencies in either testing regimen.[68,76] Therefore, detection of mild congenital enzyme defects requires less vigorous scrutiny of adrenocortical function than what may be desired for scientific investigation.

CRH Stimulation

CRH stimulation can be performed as an alternative determinant of adrenal sensitivity. When 100 ug or 1 ug/kg of ovine CRH is administered intravenously, a physiological quantity of endogenous ACTH is released from the pituitary into the circulation. The plasma levels of most corticosteroids except DHEA-S subsequently peak within 15 to 30 min and are also physiological in magnitude.[77] Ovine CRH has a slower metabolic clearance than endogenous CRH. As a result, endogenous ACTH secretion is more prolonged following stimulation with ovine CRH compared to the short pulse of ACTH released in response to endogenous CRH or administered exogenously in a physiological cortrosyn dose.[65] Therefore, adrenal sensitivity following CRH stimulation is best expressed as the ratio of the incremental rise between the pre- and poststimulation values of corticosteroid compared to those of ACTH.[24]

THE ADRENAL CONTRIBUTION IN PCOS

Adrenal androgen excess in PCOS is well documented. Several studies have demonstrated that at least half of individuals with PCOS have adrenal involvement characterized by elevations in the serum levels of DHEA-S or 11 βA[15,16] established peripheral markers of adrenal androgen production.[78,79] Although 11βA is considered to be the more sensitive marker for adrenal dynamic testing,[79–81] DHEA-S is more convenient to measure in the basal circumstance due to its minimal diurnal variation.[53] There is a lack of correlation between levels of DHEA-S and 11βA in women with PCOS[80] such that either adrenal androgen can be oversecreted independently of the other. This not only suggests differential regulation of DHEA-S and 11βA but both of these markers may need to be measured to avoid missing the presence of adrenal androgen excess in a given individual with PCOS. Currently, the clinical availability of the assay to measure 11βA is not widespread.

Two types of adrenal abnormalities have been identified in PCOS; namely, adrenal enzyme dysfunction and adrenal androgen hyper-responsiveness to ACTH. Each of these abnormalities will be discussed separately.

Adrenal Enzyme Dysfunction

Dysfunction of either of the two key branch point enzymes involved in the synthesis of adrenal androgens has been proposed as part of the mechanism for adrenal involvement in PCOS. Moreover, underactivity of 3βHSD can cause increases in DHEA-S and overactivity of P450 17 can cause increases in DHEA-S and 11βA. Adrenal enzyme dysfunction characterized by deficiency, including that of 3βHSD, is considered to be a relatively rare phenomenon.[70] In fact, the reliability of the published criteria for mild 3βHSD deficiency[20,82,83] has recently come into question because these criteria, based on historical controls, can lead to overdiagnosis of this enzyme defect. Dysregulation of P450 17 as described in the ovary[83] has been assumed to also exist in the adrenal in PCOS.[84] Investigators detecting markedly increased 17 hydroxyprogesterone (17OHP) and Δ^4 androgen responses to ovarian dynamic testing have defined dysregulation as an increase in both activities within the P450 17 enzyme complex with 17,20 lyase activity being increased to a lesser extent than that of 17αhydroxylase.[83] However, several studies utilizing various adrenal dynamic testing modalities have revealed variability in 17α hydroxylase activity, a normal 17 OHP dynamic response and an increase in 17,20 lyase activity in PCOS.[17,24,85,86] These latter findings are at variance with the concept of P450 17 dysregulation and instead consistently point to overactivity of the 17,20 lyase arm of this

complex. Moreover, a subgroup of women with PCOS appear to have 17,20 lyase hyperactivity by criteria based on prospective comparison of pharmacological ACTH responses to those of normal ovulatory women (Fig. 7).[17] Therefore, adrenal enzyme dysfunction in PCOS involves only one key branch point enzyme that accelerates the conversion of progestogens to androgens.

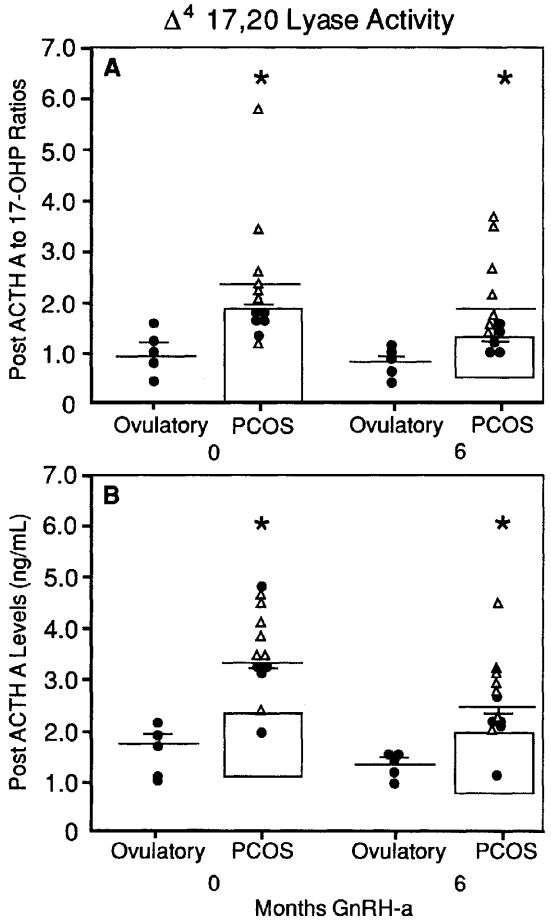

Figure 7. The ratios of androstenedione (A) to 17 hydroxyprogesterone (17-OHP) and the maximum absolute values of A following administration of a pharmacological dose of ACTH representing 17,20 lyase activity in women with PCOS who have androgen excess and in ovulatory controls before and after GnRH agonist treatment for 6 months. Fifty-six percent of this subgroup of women with PCOS (Δ) exhibited pre- and post-treatment values that were greater than 2 SD above the mean of ovulatory controls consistent with intrinsic 17,20 lyase hyperactivity. Asterisks indicate a significant difference ($P < 0.05$) between women with PCOS and ovulatory controls. (Reproduced with permission from Gonzalez et al.[17] © The American Society for Reproductive Medicine.)

Adrenal Androgen Hyperresponsiveness to ACTH

Studies using a variety of adrenal dynamic testing modalities have demonstrated adrenal androgen hyper-responsiveness to ACTH in PCOS. Increases in the adrenal capacity and/or sensitivity for DHEA-S, 11βA, DHEA, A and T are apparent in women with PCOS whether or not basal levels of DHEA-S or 11βA are elevated.[15,17,20,74,85,86] In fact, the presence of increased adrenal androgen sensitivity does not correlate with basal DHEA-S levels.[86] However, the degree of increase in either dynamic response is still greatest in women with PCOS who have adrenal involvement.[20] Adrenal androgen capacity reflects the adrenal response to pharmacological ACTH administration while adrenal androgen sensitivity is a measure of the adrenal response to physiological ACTH stimulation the latter of which is depicted in Figure 8.[24,74] Because circulating levels of ACTH are normal in PCOS,[87] adrenal androgen sensitivity may be more reflective of the in vivo circumstance.

Proposed Etiologies

The etiology of the adrenal abnormalities leading to adrenal androgen excess in PCOS has not been fully elucidated. The mechanisms involved are either intrinsic to the adrenal or must involve extrinsic factors that impact directly or indirectly on the adrenal and will be discussed separately.

Intrinsic Mechanisms

Hyperfunction of P450 17 or of the 17,20 lyase arm in particular could be caused by genetic or regulatory abnormalities intrinsic to the adrenal. It is well documented that defects in the gene encoding P450 17 can cause 17,20 lyase deficiency in children[88,89] and combined 17αhydroxylase-17,20 lyase deficiencies in adults.[90–91] However, evidence to support a genetic basis for hyperfunction of P450 17 is limited to a recent investigation of women with PCOS.[92] This report identified a point mutation in the P450 17 gene that may upregulate its transcription (Fig. 9). Because this mutation was also apparent in 20% of normal control subjects, it is believed to only promote the phenotypic expression of PCOS caused by another currently unknown primary genetic defect. From a regulatory standpoint, microsomal phospholipids are considered to coordinate the association of P450 reductase with the various P450 enzymes.[40] Because

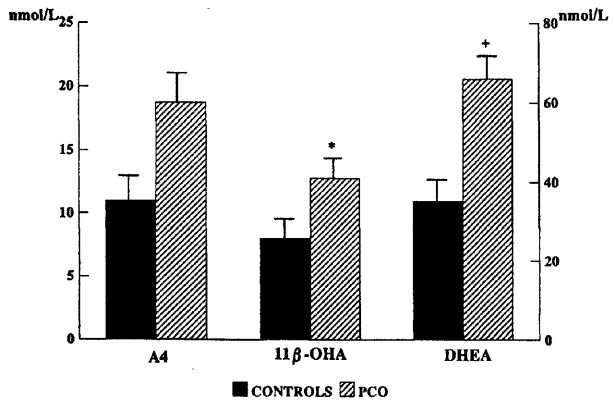

Figure 8. The responses of androstenedione (A4), 11β hydroxyandrostenedione (11β-OHA) and DHEA following CRH administration in ovulatory controls and in women with PCOS who have adrenal androgen excess. These data demonstrate a significant increase in the adrenal androgen response to endogenous physiological ACTH release in this subgroup of women with PCOS compared to ovulatory controls as indicated by the asterisk and cross ($P < 0.03$ and $P < 0.02$, respectively). (Reproduced with permission from Ditkoff et al[24] © The Endocrine Society.)

higher P450 reductase availability to P450 17 results in enhanced 17,20 lyase activity, the unusual presence of a phospholipid capable of increasing P450 reductase availability to P450 17 could culminate in 17,20 lyase hyperactivity. However, more data is required to determine how genetically induced hyperfunction of P450 17 becomes clinically evident and a specific phospholipid favoring P450 reductase association with P450 17 remains to be identified.

Adrenal androgen hyper-responsiveness to the normal circulating levels of ACTH found in PCOS may be promoted by insulin-like growth factors (IGF-I and IGF-II) locally produced within the adrenal. IGF-I receptors have been demonstrated within the adrenal cortex primarily within the zona reticularis.[93,94] The presence of IGF-I and IGF-II mRNAs and the respective peptides have been demonstrated in adrenocortical cells and have been shown to increase in response to ACTH.[95,96] Just recently, promotion of adrenal steroidogenesis by IGF-II has been shown to be mediated through interaction with the IGF-I receptor.[94] Thus, it is possible that excess bioavailability of either of these IGFs amplifies the direct and indirect actions of ACTH to promote hypertrophy and hyperplasia of the adrenocortical cells responsible for adrenal androgen synthesis.[40]

Extrinsic Factors

CASH and insulin have been proposed as extrinsic factors affecting adrenal androgen secretion in PCOS. With the controversy surrounding the potency of the POMC fragment presumed to be CASH notwithstanding, oversecretion of this pituitary substance could theoretically lead to adrenal androgen excess.[97] Unfortunately, data for or against this mechanism is completely lacking. Early studies of the effects of insulin on adrenal androgen secretion reported opposite results. While acute infusion of insulin was shown to lower DHEA-S levels in normal women,[98] hyperinsulinemia was shown to enhance adrenal androgen hyper-responsiveness to ACTH in women with PCOS.[99] Subsequent investigations have also failed to achieve a consensus reporting that insulin either stimulates or has no effect on adrenal androgen secretion. A correlation between hyperinsulinemia and increased 17,20 lyase activity in women with PCOS[100] as well as evidence of increased 17α hydroxylase activity and decreased 17,20 lyase activity in hyperandrogenic women following ACTH stimulation during acute insulin infusion[101] are considered to indicate insulin induced P450 17 dysregulation by proponents of this abnormality. On the other hand, adrenal androgen hyper-responsiveness to CRH was blunted,[102] while elevated serum DHEA-S concentrations remained unaltered[103,104] following acute pharmacological suppression of insulin in women with PCOS. In addition, circulating DHEA-S levels declined in

Figure 9. The sequence of the 5'region of the gene coding for P45017 on chromosome 10q 24.3 showing the -34 base pair mutation site where thymine (T) is replaced by cytosine (C) upstream from the proposed translation initiation site at the -60 base pair. This point mutation is believed to significantly modify the expression of PCOS but has been excluded as the primary genetic defect. (Adapted with permission from Picado-Leonard et al. DNA 6:442, 1987).

women with PCOS following chronic amelioration of insulin resistance and hyperinsulinemia in response to pharmacological therapy[105] but remained unaltered in obese hyperandrogenic women when the ameliorating effect was achieved through weight loss.[106] Therefore, postulates invoking the effects of CASH or insulin as part of the mechanism for adrenal androgen excess in PCOS should be viewed with caution at the present time.

Ovarian Induced Adrenal Hyperandrogenism

Substantial literature has been generated in efforts to determine if ovarian steroids are capable of inducing increases in adrenal androgen secretion in PCOS. A number of studies lend support to this contention.[20–24] Therefore, a comprehensive presentation is merited to document the evolution of this hypothesis and the various approaches taken to establish its validity.

The Anovulatory Ovary

The hormonal milieu created by the anovulatory ovary in PCOS may impact on adrenal steroidogenesis. Excess testosterone and androstenedione are secreted from the ovary in the circulation.[107] Elevations in testosterone lower sex hormone binding globulin (SHBG) production in the liver by as much as 50%.[108] Hyperinsulinemia, a phenomenon often present in PCOS further promotes the increases in ovarian androgen production and the decreases in SHBG.[104,109] While estradiol from the ovary is not secreted at high levels, a low SHBG level increases the active pool of circulating estrogens.[110] This pool is composed of estrone peripherally converted from androstenedione in addition to estradiol. Therefore, it has been postulated that the functional hyperestrogenic state of anovulation or the high levels of serum androgens of ovarian origin may influence the intra-adrenal microenvironment by altering key branch point enzyme activities culminating in increased adrenal androgen production.

The Fetal Adrenal Model

The hormonal milieu of the prenatal and immediate postnatal periods has often been cited to illustrate the impact of circulating steroids on adrenocortical function. In the past, it was believed that direct inhibition of the 3βHSD protein by the high levels of estrogen originating from the placenta caused low 3βHSD activity within the fetal zone of the adrenal to favor production of DHEA-S over cortisol.[111] However, recent studies have revealed an absence of 3βHSD protein and its mRNA within the fetal zone indicating a lack of 3βHSD gene expression.[112] Additional data has shown that estrogen is incapable of inhibiting 3βHSD expression in vitro[113] and that clearance of placental estrogens from the newborn circulation in vivo is not responsible for the postnatal rise in 3βHSD activity.[114] Therefore, the factor causing inhibition of fetal zone 3βHSD expression before birth and the mechanism by which 3βHSD expression is released from this inhibition after birth remains unknown.

Estrogen may still play a supporting role in conjunction with other factors to maintain the fetal adrenal microenvironment that favors DHEA-S production. Estrogen at high concentrations that are similar to what is present in fetal life has been shown to promote ACTH stimulated P450 17 expression in vitro.[113] However, estrogen receptors are absent in the fetal zone suggesting that estrogen does not apparently exert its effects in a receptor mediated fashion.[115] High P450 17 activity in conjunction with low 3βHSD activity promotes DHEA-S production while high placental cortisol clearance further increases ACTH secretion from an intact and functional fetal pituitary. The ACTH elevations may cause fetal adrenal hyperplasia for maintenance of fetal cortisol requirements. After birth, there is a decline in ACTH stimulation due to a reduction in the metabolic clearance of cortisol.[116] This phenomenon may explain the postnatal decline in the highly ACTH dependent activity of P450 17, which is concomitant with the involution of the fetal zone and reduction in gland size. Thus, the alterations in P450 17 activity observed in fetal life and soon after birth are primarily caused by the different degrees of ACTH stimulation that results from the changes in cortisol clearance. The chronic action of ACTH is subsequently potentiated by estrogen but through a mechanism that remains to be elucidated.

The mechanisms by which ovarian steroids could influence adrenocortical function in PCOS may not be entirely similar to the fetal model. First, 3βHSD deficiency is not prominent in women with PCOS.[17,70] Second, placental steroid levels in the fetal circulation are much higher than circulating steroid levels in women with PCOS.[111] Third, receptors for androgen and estrogen are present in the adult adrenal cortex including the zona reticularis.[115] Finally, efficiency of cortisol output following exogenous ACTH or CRH challenge is normal or even increased[20,69,117] and basal levels of endogenous ACTH are normal[87] in women with PCOS.

In Vitro Data

Early enzyme kinetic studies formed the initial basis for the hypothesis that ovarian steroids influence the adrenal in PCOS. High concentrations of androgens and estrogens in vitro were shown to lower 3βHSD activity by direct enzyme inhibition.[118,119] However, circulating ovarian steroids in vivo are incapable of inhibiting 3βHSD activity in this fashion. The steroid gradient formed across the adrenal cortex is a result of intradrenal production and secretion of corticosteroids from concentric layers. On the other hand, a significant gradient is unlikely to form from circulating steroids merely passing through the centripetal vasculature of the adrenal cortex. Therefore, the concentrations of androgens and estrogens originating from the ovary in women with PCOS would be unchanged within the zona reticularis remaining 1000-fold lower than what is required to directly inhibit 3βHSD at the protein level.[55]

Circulating steroids may still be capable of altering adrenal enzyme activities through receptor-mediated mechanisms. Androgens and estrogens of ovarian origin may bind their respective nuclear receptors within zona reticularis cells to influence adrenal enzyme gene expression. In a recent study, estrogen at a concentration similar to circulating levels was shown to increase P450 17 mRNA within adrenocortical cells of normal individuals in vitro.[120] Interestingly enough, this same estrogen concentration also increased 3βHSD mRNA reflected by a decline in DHEA and DHEA-S within the culture media. Therefore, circulating estrogens in normal individuals may actually attenuate adrenal androgen production. However, an intrinsic adrenal abnormality may exist in PCOS, permitting enhancement of P450 17 expression to be favored over that of 3βHSD in response to estrogen. It is also possible that ovarian steroids participate in indirect mechanisms to exert extrinsic modulation of adrenal androgen production in PCOS.

Adrenal Response to Estrogen in Hypoestrogenic States

An association between changes in circulating levels of estrogen and changes in those of DHEA-S can be observed throughout life with the exception of the pubertal interval when these levels do not rise concordantly.[36] Moreover, DHEA-S levels are low in hypoestrogenic states such as childhood and the menopause.[119,121] While the association between estrogen and DHEA-S levels does not prove a cause-and-effect relationship, DHEA-S levels undergo an accelerated decline with premature ovarian failure or oophorectomy.[122] The activities of P450 17 and 3βHSD vary with age rising and falling respectively in relation to the adrenal production of DHEA-S.[44–46,54] Therefore, in vivo phenomena also suggest that these adrenal enzyme activities may be modulated by estrogen or estrogen-mediated effects.

Studies designed to determine the DHEA-S response to estrogen administration in hypoestrogenic women have been reviewed extensively in the past.[25] The collective data generated by these investigations indicate that in this population, estrogen does not influence adrenal androgen production to maintain DHEA-S levels in premenopausal range. In support of this impression is the observation that GnRH agonist induction of chronic hypoestrogenism in ovulatory women does not cause decreases in DHEA-S.[20,23,123] Thus, another ovarian factor is probably involved in stimulating adrenal androgen production. This factor would most likely be associated with the presence of a viable but not necessarily active follicular apparatus within the ovary.

Findings obtained from hypoestrogenic states should be applied with caution to PCOS, which is a functionally hyperestrogenic state. Moreover, impact from estrogen and estrogen-mediated events not demonstrable in hypoestrogenic individuals may be possible in PCOS.

GnRH Agonist Studies in PCOS

The advent of GnRH agonists has allowed direct investigation of any possible extrinsic influence of ovarian steroids in promoting excess adrenal androgen secretion in PCOS. Pituitary down regulation by GnRH agonist administration causes suppression of LH and FSH secretion and subsequent inhibition of ovarian steroidogenesis. Proven reductions in estrogen, testosterone, and androstenedione to the castrate range have been demonstrated.[107] However, early studies failed to show an impact of ovarian steroids on the adrenal in PCOS. Serum adrenal androgen levels remained unaltered following acute administration (4 weeks) of a GnRH agonist to women with PCOS who had moderately elevated DHEA-S levels (mean 350 μg/dL) and chronic administration (6 months) of this agent to women with PCOS who had normal DHEA-S levels (mean 230 μg/dL).[123,124] Whereas, acute GnRH agonist treatment may have been insufficient should adrenal morphological alteration be required to observe changes in circulating adrenal androgens, none of the women treated chronically with GnRH agonist had adrenal in-

volvement. Thus, the length of treatment and the population of women with PCOS selected may have accounted for the negative results.

Chronic administration of GnRH agonist to women with PCOS exhibiting baseline elevations in adrenal androgens suggest an impact of ovarian steroids on adrenal androgen secretion in a subgroup of these patients (Fig. 10). Elevated serum levels of DHEA-S and 11βA normalized in some patients but remained unaltered in others after GnRH agonist treatment.[20,23] The percentage of women with PCOS demonstrating normalized levels in response to therapy was greater for DHEA-S (48%) compared to 11βA (25%) and reductions in DHEA-S and 11βA did not correlate providing further confirmation that these two adrenal androgens are under separate control.[23] Thus, the mechanisms responsible for adrenal dysfunction in PCOS must be heterogenous in etiology.

Defects in 17,20 lyase activity known to occur in women with PCOS who have adrenal involvement exhibit variable dependency on ovarian steroids. This is apparent in the effect of chronic GnRH agonist administration on 17,20 lyase activity as determined by various adrenal dynamic testing modalities. After GnRH agonist treatment, the increased 17,20 lyase activity shown to occur in response to CRH or physiological ACTH normalized[24,85] while 17,20 lyase hyperactivity uncovered in response to pharmacological ACTH persisted in affected individuals.[17,85] Continued elevation in serum DHEA-S levels following chronic GnRH agonist therapy is also characteristic of those women with PCOS who have 17,20 lyase hyperactivity suggesting that this enzyme defect is an intrinsic adrenal disorder.[17] Because ovarian steroid reduction appears to overcome the abnormal 17,20 lyase response to physiological ACTH but not to pharmacological ACTH, it is possible that physiological ACTH stimulation may only uncover an acquired adrenal 17,20 lyase defect while pharmacological ACTH stimulation may be capable of revealing an abnormality of this enzyme that is intrinsic to the adrenal.

There is a lack of consensus regarding the impact of ovarian steroids on the adrenal androgen response to ACTH in women with PCOS who have adrenal involvement. Increased adrenal androgen capacity has been shown to normalize in some studies[17,22] but remained unaltered in others during chronic GnRH agonist administration to affected individuals.[20] On the other hand, currently available data suggests that increased adrenal androgen sensitivity normalizes in this subgroup of women with PCOS following chronic GnRH agonist treatment (Fig. 11).[24,85] As alluded to earlier, adrenal androgen sensitivity reflects the adrenal response to physiological ACTH stimulation that is similar to what is elicited by the normal endogenous ACTH levels found in PCOS.[74,87] Therefore, the evidence suggesting the ability of ovarian steroids to induce increased adrenal androgen sen-

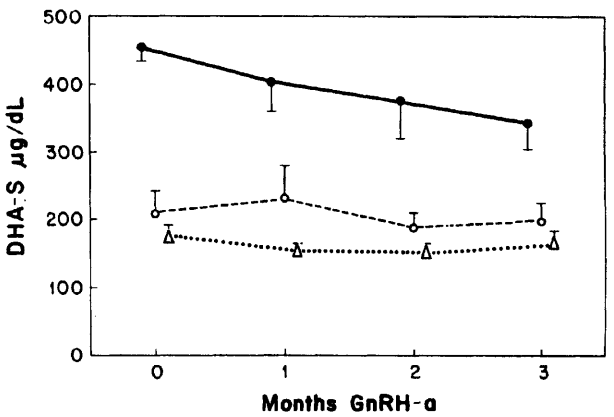

Figure 10. Basal serum DHEA-S concentrations (mean ± SE) in women with PCOS who have high (—•—) and normal (--o--) DHEA-S levels and in ovulatory controls (···Δ···) before and during GnRH agonist (GnRH-a) treatment for 3 months. Women with PCOS who have high DHEA-S levels exhibited a significant ($P < 0.02$) decline in DHEA-S by the end of GnRH-a treatment. These data demonstrate the ability of ovarian steroids to promote excess adrenal androgen secretion in some women with PCOS. (Reproduced with permission from Gonzalez et al.[20])

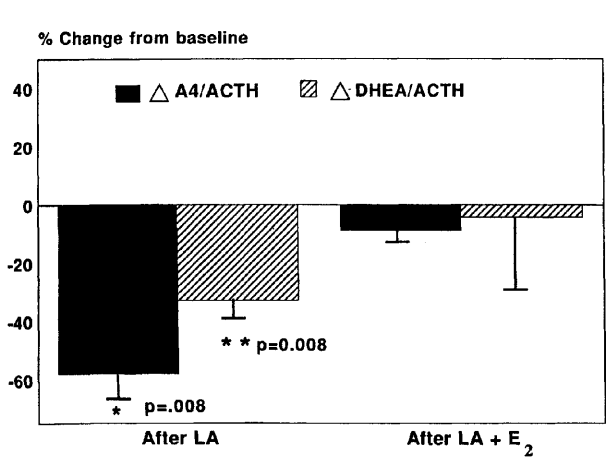

Figure 11. Percent change in the post-CRH ratios of the maximal incremental rises in androstenedione (A4) or DHEA to that of ACTH after treatment with the GnRH agonist, leuprolide acetate (LA) and after estrogen replacement following continued GnRH agonist suppression as compared to the pretreatment CRH responses in women with PCOS who have adrenal androgen excess. These data demonstrate the ability of estrogen to increase adrenal androgen sensitivity in this subgroup of women with PCOS. Asterisks indicate significant changes in CRH responses after LA administration compared to pretreatment values ($P < 0.01$). (Reproduced with permission from Ditkoff et al.[24] © The Endocrine Society.)

sitivity is the most compelling because it is most reflective of the in vivo circumstance.

The possibility has been raised that it is the decrease in pituitary luteinizing hormone (LH) secretion per se and not the subsequent inhibition of ovarian steroids, which accounts for the resolution of adrenal dysfunction observed during GnRH agonist administration in many women with PCOS who have elevated adrenal androgens. Elevated levels of circulating LH is often observed in PCOS[124,125] and considered a consequence of the functional hyperestrogenic state.[126] LH receptors have recently been identified in adult human reticularis and lower fasiculata cells, which are proven sites of adrenal androgen production.[127] Human chorionic gonadotropin (HCG), a structural and functional homolog of LH, has been shown to stimulate adrenal androgen secretion in guinea pig adrenocortical cells.[128] However, increases in 17,20 lyase activity and adrenal androgen sensitivity shown to normalize in response to GnRH agonist treatment in women with PCOS who have adrenal involvement once again become increased following estrogen administration to the degree evident before GnRH agonist therapy.[24] Therefore, ovarian steroids alone are indeed capable of causing the adrenal abnormalities observed in affected individuals with PCOS although the potentiation or separate induction of these abnormalities by LH cannot be excluded. Moreover, estrogen, in particular, may be the ovarian steroid responsible for altering adrenal steriodogenesis in PCOS. Whether estrogen can affect the adrenal in PCOS directly or through the indirect action of other substances within the ovarian or adrenal microenvironment such as insulin-like growth factors remains to be elucidated.

CONCLUSION

An attempt at understanding the factors that control normal adrenal function provides the basis for evaluating proposed mechanisms for excess adrenal androgen secretion in PCOS. ACTH promotes development growth and overall steroidogenic efficiency within the adrenal cortex.[43,55] While another recently isolated human pituitary factor has been proposed for separate regulation of adrenal androgen synthesis,[61] its role remains controversial and alterations in adrenal morphology may well serve this purpose.[62,63] It is well established that the unique centripetal circulation within the adrenal creates a steroid gradient across the cortex.[43,54] It is equally proven that sufficient adrenocortical mass and cortical thickness are achieved by adolescence under the influence of ACTH to allow maximum increases in corticosteroid concentrations within the deepest layers of the cortex.[43] What remains to be shown is whether or not a particular corticosteroid is required to achieve a high enough concentration to lower 3βHSD expression, increase P450 17 gene expression, or both via a receptor-mediated mechanism. Once the promotion of adrenal androgen production is accomplished, ACTH continues to initiate and maintain the steroidogenic process.[31,41,49] With the elusiveness of the exact mechanism for the induction of adrenarche notwithstanding, adrenal androgen excess in PCOS must involve mechanisms that alter androgen synthetic enzyme activities or elicit an unusual adrenal response to ACTH.

Adrenal dynamic testing has uncovered two types of adrenal abnormalities in the large population of women with PCOS who have adrenal involvement. Elevations in DHEA-S and 11βA observed in affected individuals[15,16] can be attributed to increased 17,20 lyase activity[17,24,86] or adrenal androgen hyper-responsiveness to ACTH.[15,20,72,74,85,86] Whereas increases in 17,20 lyase activity can be intrinsically or extrinsically induced, adrenal androgen hyper-responsiveness to ACTH may primarily be under the influence of an extrinsic factor.[17,24,86] The recent discovery of a point mutation thought to upregulate P450 17 transcription[92] coupled with the persistence of 17,20 lyase hyperactivity detected by pharmacological ACTH stimulation even after chronic ovarian steroid suppression in a subgroup of women with PCOS with elevated adrenal androgens[17] is highly suggestive of an intrinsic genetic defect. On the other hand, recent data also implicates the functional elevation in ovarian steroids as the extrinsic impetus for the increases in 17,20 lyase activity and the adrenal androgen hyper-responsiveness observed following physiological ACTH stimulation in yet another subgroup of women with PCOS who have adrenal androgen excess.[24,86] The ability of ovarian steroids to cause adrenal androgen hyper-responsiveness to pharmacological ACTH stimulation has been reported but remains controversial[17,20,22,23] and less reflective of the in vivo circumstances given the normal levels of ACTH in PCOS.[87] Thus, all the data generated to this point is sufficient to conclude that adrenal androgen excess in PCOS is heterogeneous in etiology.

Estrogen is the ovarian steroid recently shown to be the extrinsic factor responsible for altering adrenal steroidogenesis in some adrenal involved women with PCOS.[24] Interestingly enough, estrogen has long been suspected to play this type of role in the proposed ovarian–adrenal relationship believed to affect adrenal androgen synthesis.[55,118,119] Major attempts to demonstrate the influ-

ence of estrogen on adrenal androgen secretion in the hypoestrogenic population has yielded data that collectively speaks against any effect of estrogen on the adrenal in this regard.[25] On the other hand, none of these findings may be applicable to hyperestrogenic conditions such as PCOS. While the circulating estrogen concentrations present in PCOS would still be too low to directly alter enzyme activities at the protein level,[55] the latest in vitro data suggests that levels of estrogen similar to what is observed in PCOS may be capable of increasing 17,20 lyase activity through a receptor-mediated mechanism.[120] Of course, estrogen may also be capable of inducing both adrenal abnormalities described in PCOS through an indirect mechanism yet to be determined.

This review of the literature provides some indication as to the direction of future investigation. Characterization of the factors that could increase the potency of CASH and documentation of intra-adrenal steroid gradient-dependent receptor-mediated alterations of adrenal androgen steroidogenic enzyme activities could lead to immense breakthroughs in understanding the induction of adrenarche. Beyond the characterization of adrenal dysfunction in PCOS is the ellucidation of its heterogeneous etiologies. Research aimed at delineating genetic defects of P450 17 and possible phospholipid-induced P450 reductase regulatory abnormalities, which could cause intrinsic 17,20 lyase hyperactivity, is still in its early stages. The proposal that estrogen directly induces increases in 17,20 lyase activity and the adrenal androgen response to ACTH via a receptor-mediated mechanism is based on studies of fetal and normal adult adrenal tissue, which utilized the techniques of cell culture, northern blot analysis, and immunohistochemical staining.[113,115,120] However, direct examination of adrenal tissue obtained from women with PCOS who have adrenal involvement by using similar techniques would provide more definitive answers with the understanding that collection of these data will be dependent on limited tissue availability. Studies along these lines could also determine whether or not either of the IGFs are capable of potentiating the action of estrogen to elicit adrenal androgen hyper-responsiveness to ACTH. Finally, any possible role played by LH in stimulating excess adrenal androgen secretion in PCOS via intra-adrenal LH/HCG receptors could be investigated using basic and clinical approaches. In the former instance, adrenal androgen concentrations within cultured adrenocortical cells could be measured before and after HCG exposure. In the latter instance, the adrenal androgen response to HCG administration could be observed in women with PCOS who have adrenal androgen excess before and after GnRH agonist treatment. Given the rapid pace of research that has provided an explosion of information about adrenal involvement in the past 7 years, it may be possible to learn the answers to many of the questions detailed above within the very near future.

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