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The Rise, Fall, and Resurrection of 11-Oxygenated Androgens in Human Physiology and Disease

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Abstract

The 11-oxyandrogens, particularly 11-ketotestosterone, have been recognized as a biologically important gonadal androgen in teleost (bony) fishes for decades, and their presence in human beings has been known but poorly understood. Today, we recognize that 11-oxyandrogens derive from the human adrenal glands and are major bioactive androgens, particularly in women and children. This article will review their biosynthesis and metabolism, abundance in normal and pathologic states, and potential as biomarkers of adrenal developmental changes and disease. Specifically, 11-oxyandrogens are the dominant active androgens in many patients with 21-hydroxylase deficiency.

Keywords

adrenal; androgen; adrenarche; 21-hydroxylase deficiency; 11-hydroxylase

I. Introduction

The archetypal circulating androgen in humans is testosterone (T). Dihydrotestosterone (DHT), which is the most potent recognized androgen, is synthesized within target cells, such as in the prostate or skin, by the 5 α -reduction of T, with little amounts in the circulation. In healthy men, the testes are the main source of T, but T also derives from the ovary and the adrenal glands, primarily via peripheral conversion of androstenedione (A4). Adrenal-derived androgen precursors, including dehydroepiandrosterone (DHEA), androstenediol (Adiol) and their respective sulfates (DHEAS and AdiolS) are much more abundant than T in women and children, yet the peripheral pathways for T formation from these steroids are inefficient. Several disorders are impacted by inappropriate androgen production, including congenital adrenal hyperplasia (CAH), polycystic ovary syndrome (PCOS), and premature adrenarche. Distinguishing the source of androgen synthesis in such conditions is particularly important to guiding therapy. While 11-oxygenated C₁₉ steroids (11-oxyandrogens) have been long identified and recognized as major androgens in teleost fishes, until recently their role(s) in human beings has been largely neglected. This review

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provides a historical perspective of 11-oxyandrogens and underlines their contributions, as currently understood, in disorders of androgen excess relevant to children and adolescents: CAH, PCOS and premature adrenarche.

II. Enzymatic machinery of 11-oxyandrogens synthesis in humans

The synthesis of all 11-oxyandrogens relies on the adrenal enzyme cytochrome P450 11 β -hydroxylase (CYP11B1), and patients with CYP11B1 deficiency or adrenal insufficiency have negligible amounts of 11-oxyandrogens [1–3]. CYP11B1 is expressed in the zonae fasciculata (ZF) and reticularis (ZR) of the adrenal gland [4] and its main function is to catalyze the last step in cortisol synthesis, under the regulation of adrenocorticotropin (ACTH). CYP11B1 can also use A4 and T as substrates, yielding 11 β -hydroxyandrostenedione (11OHA4) and 11 β -hydroxytestosterone (11OHT) (Figure 1). Alternatively, 11OHA4 synthesis can occur from cortisol, although this pathway is relatively minor [5]. 11OHA4 and 11OHT can be oxidized by the enzyme 11 β -hydroxysteroid dehydrogenase type 2 (11 β HSD2) to 11-ketoandrostenedione (11KA4) and 11-ketotestosterone (11KT), both in the adrenal gland and in periphery. In addition, 11KT can derive from reduction of 11KA4, via the enzyme aldo-keto reductase 1C3 (AKR1C3, or 17 β -hydroxysteroid dehydrogenase type 5, 17 β HSD5), which is expressed in the ZR and in many peripheral tissues [4]. Furthermore, Storbeck performed a series of *in vitro* studies demonstrating that 11OHA4 and 11OHT, as well as their cognate 11-ketosteroids, can serve as substrates for steroid 5 α -reductase (SRD5A) [6]. In addition, 11-ketodihydrotestosterone (11KDHT) can be generated either from 11 β -hydroxydihydrotestosterone (11OHDHT), via 11 β HSD2, or from 11-keto-5 α -androstenedione (11KDHA4), via AKR1C3 (Figure 1).

III. Androgenic potency of 11-oxyandrogens

Considerable evidence has now demonstrated that 11KT and its 5 α -reduced metabolite, 11KDHT are potent and clinically relevant agonists of the human androgen receptor (AR, *NR3C4*). Using an MDA-kb2 cell model expressing the human AR, Rege et al found that the maximum androgenic activity of 11KT was similar to that of T, and that the EC₅₀ of 11KT was only 5-fold higher than T [7]. The maximal androgenic activity of 11OHT was below that of 11KT and T. In a subsequent study, using an optimized *in vitro* reporter system of human AR activation, without the co-expression of glucocorticoid, mineralocorticoid or progesterone receptors, 11KT demonstrated reproducible androgen potency, while 11OHT was inferior [8]. Storbeck and colleagues obtained similar results in a COS-1 cell system expressing human AR [6]. In a separate study, 11KT and 11KDHT were shown to bind to the human AR with affinities similar to that of T and DHT, and all four steroids promoted AR-regulated gene expression and cell growth in two androgen-dependent prostate cancer cell lines [9]. In contrast, as demonstrated decades ago [10, 11], 11OHA4 and 11KA4 showed minimal androgenic activity in all of the more recent models.

IV. 11-oxyandrogens across species

In the 1960s, 11KT and 11OHT were isolated from salmon plasma [12, 13]. Since then, 11KT has been extensively studied in teleost fishes, where it was shown to act as the major

androgen, contributing to sexual maturation [14, 15] and to various functions, including male-type sexual behavior [16], induction of spermatogenesis [17], sperm motility [18], epidermal and dermal thickening [19].

The synthesis of 11KT in fish occurs by 11 β -hydroxylation of A4 to 11OHA4, which is then oxidized to 11KA4 by an 11 β HSD, prior to reduction by a 17 β -hydroxysteroid dehydrogenase [20, 21]. 11KT can also be generated from T, via 11OHT [21].

In fish, 11KT is much higher in males than females and can induce female-to-male sex reversal in some teleost species [16]. In contrast, 11KT was found to be similar in both sexes in mice, despite significantly larger amounts of T in males [22]. Murine Cyp11b1 and Hsd11b2 were detected in both testicular Leydig and ovarian theca cells, although the latter is more highly expressed in the ovary [22, 23]. The mechanisms of this sexual dimorphism remains unclear, but it has been postulated that Hsd11b2 might regulate ovarian steroidogenesis by limiting the local access of active glucocorticoids.

11OHA4 was identified as a major adrenal product in primates over 40 years ago [5, 24, 25], but its contributions to physiology were poorly understood. *In vitro* studies have shown that 11OHA4 is abundantly produced by the human adrenal cells, both under basal and cosyntropin-stimulated conditions [26]. Similarly, steroid studies with serum obtained from human adrenal veins have demonstrated that 11OHA4 is produced in higher amounts than A4, both at baseline and after cosyntropin stimulation [3, 7]. The adrenal gland also produces 11OHT, 11KA4, and 11KT in an ACTH-dependent manner, although less robustly than 11OHA4.

While it has been proposed that the gonads might contribute to the synthesis of 11-oxyandrogens in humans, gonadal CYP11B1 expression was found to be minimal compared to that in the adrenal cortex [27]. Moreover, like in mice, 11KT and 11OHT have been found to circulate in comparable concentrations between sexes in human beings, despite dramatically larger amounts of T in men [3, 27], suggesting that the gonadal synthesis of 11-oxyandrogens is trivial relative to the adrenal component.

V. Clinical applications of 11-oxyandrogens

V.1. Congenital adrenal hyperplasia

CAH encompass a heterogeneous group of autosomal recessive disorders in which one or more enzymes required for cortisol synthesis are defective. These inherited defects range from severe (also called classic) to mild (non-classic, with normal cortisol production but elevated precursor/product ratios), and from a single to multiple enzymatic steps. The negative feedback to the hypothalamus and the pituitary gland normally executed by cortisol becomes disrupted, leading to an over-activation of the hypothalamic-pituitary-adrenal axis. While this mechanism is meant to compensate for cortisol insufficiency, it leads to excessive production of upstream and off-target steroids.

The vast majority of CAH cases result from defects in 21-hydroxylase (CYP21A2). Classic 21-hydroxylase deficiency (21OHD) affects ~1:16,000 newborns [28]. Non-classic 21OHD

is one of the most common inherited disorders, occurring in approximately 1:1,000 Caucasians and more commonly in Ashkenazi Jews, Hispanics and Mediterraneans [29]. In addition to the active *CYP21A2* gene, human beings have a highly homologous pseudogene (*CYP21A1P*), which does not encode an active enzyme. The frequent occurrence of mutations in the *CYP21A* genes is favored by their close proximity to each other in a duplicated locus within the HLA major histocompatibility complex and which includes the genes for the fourth component of complement. [30–32] This locus undergoes frequent recombinations and/or gene conversion events, which disrupt the *CYP21A2* gene with sequences from the *CYP21A1P* pseudogene.

The main substrate of *CYP21A2*, 17-hydroxyprogesterone (17OHP), which accumulates in 21OHD, has traditionally been used for both diagnosis and monitoring. Additionally, the *CYP21A2* blockage and ACTH elevation promote the diversion of 17OHP towards formation of 19-carbon (C₁₉) steroids (androgens and precursors). Although the catalytic efficiency of the human 17,20-lyase is much higher when using 17-hydroxypregnenolone as substrate [33], the high concentrations of 17OHP in 21OHD allow some conversion to A4, but *CYP11B1* converts much of nascent 17OHP to 21-deoxycortisol in the adrenal. In parallel, intra-adrenal A4 and T can serve as substrates for *CYP11B1*, leading to 11OHA4 and 11OHT, respectively, which are then further metabolized to 11KA4 and 11KT, predominantly in the periphery.

While 17OHP has been the main steroid for the diagnosis of 21OHD, 17OHP is a poor indicator of disease control. Serum 17OHP fluctuates rapidly and prominently in relation to glucocorticoid dosing. For adults, a 17OHP in the normal range indicates over-treatment with glucocorticoids, which is associated with a multitude of side effects. Similarly, DHEA and DHEAS, the characteristic adrenal C₁₉ steroids, are often paradoxically low in patients with classic 21OHD, even when treated conservatively [34]. In a cross-sectional study of 38 patients (19 men) with classic 21OHD between 3 and 59 years of age, we found that DHEAS and DHEA were 6- to 7-fold lower than in sex- and age-matched controls [3]. Surprisingly, however, pregnenolone sulfate was 3-fold higher in 21OHD patients than in their unaffected counterparts. While the mechanisms of DHEAS deficiency in 21OHD remain unclear, the utility of both DHEAS and DHEA as biomarkers of androgen excess in 21OHD is limited.

The most widely used biomarkers of androgen excess in 21OHD have been A4 and, in women, T. Clinical studies have shown that both A4 and T correlate poorly with phenotypic evidence of hyperandrogenism; moreover, both steroids are also produced from the gonads. Thus, in postpubertal males, T also reflects testicular function. Due to its adrenal origin, 11OHA4 has been previously proposed as a biomarker of androgen excess in 21OHD, but studies have been small and results inconclusive. In 1992, Carmina and colleagues studied the response of a set of androgens to 7-day dexamethasone suppression and found that the ratio of A4/11OHA4 was lower in women with non-classic 21OHD and higher in women with chronic anovulation as compared to controls [35]. Huerta et al evaluated the response of C₁₉ steroids to ACTH stimulation in women with untreated non-classic 21OHD and normal age- and BMI-matched controls. While 11OHA4 was higher at baseline in 21OHD women,

it did not differ between the two groups after ACTH stimulation, questioning its clinical relevance in such patients [36].

More recently, we have shown that all four 11-oxyandrogens are 3 to 4-fold higher in treated patients with classic 21OHD of both sexes than in age- and sex-paired controls [3]. Notably, while 11KT correlated directly with T in females and prepupertal boys, 11KT and T correlate inversely in post-pubertal males [3, 37]. Furthermore, while T correlated positively with LH, 11OHT and 11KT displayed a negative correlation with LH in sexually mature males. These findings suggest that 11KT synthesis relies predominantly on adrenal precursors and that its high concentrations in poorly controlled males with 21OHD suppress the hypothalamic-pituitary-gonadal axis, and subsequently testicular-derived T. Another important caveat is that 11KT was approximately twice as high as T in women and children with 21OHD, making 11KT the dominant circulating androgen in these patients. Collectively, these reports support the utility of 11KT as an adrenal-specific androgen, with applicability in both sexes.

In a more recent study of 114 children and adults with classic 21OHD ages 2 to 67 years (59% younger than 18 years), we explored the association of 23 steroids with surrogates of poor 21OHD control, such as adrenal volume, testicular and adrenal rest tumors (TART), bone age, menstrual irregularities and hirsutism [37]. We found that all four 11-oxyandrogens correlated tightly with adrenal volume (in adults) and with ACTH ($r \sim 0.7$, $p < 0.0001$ for all). In addition, all four 11-oxyandrogens, and in particular 11OHA4, were 3–7 fold higher in males with TART as compared to those without TART of similar ages. On the other hand, we and others [38] found no differences in T between males with and without TART. Neither the 11-oxyandrogens nor any of the other steroids measured demonstrated a correlation with TART volume, in agreement with a study from Reisch and colleagues [39]. Nonetheless, patients with TART had larger adrenal volume than those without TART, suggesting poorer long-term control. While poor disease control is thought to contribute to TART growth [40–42], their mere presence and ultimate size is influenced by embryological factors and remain incompletely understood [38, 39, 43–46].

In females, 11OHT and 11KT were found to be higher in the group with menstrual disturbances and hirsutism than in those without. It is worth mentioning that none of the steroids studied predicted bone age advancement. Because bone age is impacted not only by sex-hormone dysregulation, primarily estrogens, but glucocorticoid therapy as well, it is not surprising that single-point steroid biomarkers cannot provide clinical insight into bone age derangements. Nonetheless, taken together, our recent studies support 11-oxyandrogens as better indicators of poor 21OHD control than 17OHP and T.

V.2. Polycystic ovary syndrome

PCOS is the most common ovarian disorder, affecting 5–15% of reproductive age women [47–49]. PCOS is a complex heterogeneous condition, characterized by reproductive abnormalities, ovulatory dysfunction, polycystic ovarian morphology, and hyperandrogenism [50, 51], but its pathogenesis remains poorly understood. Importantly, patients with PCOS have an increased risk of metabolic abnormalities, such as obesity, insulin resistance, type 2 diabetes mellitus, cardiovascular disease and dyslipidemia.

Hyperandrogenism is the cardinal clinical feature of PCOS and can be defined by either hirsutism and/or excess of serum androgens [50]; however, not all patients with PCOS have hirsutism or elevated T [52]. The source(s) of androgen excess in PCOS has been debated. While the ovary was originally considered the primary source of androgen excess in PCOS women, elevated adrenal androgens, particularly DHEA and DHEAS, have been observed in many patients with PCOS [53, 54]. Thus the adrenal gland has also been recognized as a source of androgen excess. Nonetheless, the mechanism of adrenal androgen excess in PCOS remains unclear. Some investigators have observed an excessive response of adrenal androgens and cortisol after cosyntropin stimulation in patients with PCOS [55, 56], suggesting that the adrenal androgen excess in PCOS might result from generalized adrenocortical hyper-reactivity to ACTH.

Recently, A4 has been used as a more sensitive marker of hyperandrogenism in PCOS than T [57–59]. In addition, A4 was proposed as a predictor of metabolic risk in PCOS [57]. Studies from early 1990s demonstrated that 11OHA4 is also elevated in PCOS [35, 60]. In a recent study of 114 women with PCOS and 49 healthy controls, traditional androgens, precursor steroids and 11-oxyandrogens were measured in peripheral serum, as well as their metabolites in 24h urine collections using mass spectrometry [61]. Serum 11OHA4, 11KA4, 11OHT, and 11KT were significantly higher in patients with PCOS than in controls, as was the urinary 11-oxyandrogen metabolite 11 β -hydroxyandrosterone. Indeed, the relative contribution of the 11-oxyandrogens to the total circulating androgenic steroids was significantly greater in patients with PCOS than in controls. The authors also investigated the association of 11-oxyandrogens with markers of metabolic risk in patients with PCOS and found that serum 11OHA4 and 11KA4, but not the urinary 11-oxygenated T metabolites, correlated significantly with BMI, insulin and HOMA-IR.

While additional large scale studies in lean and obese women with PCOS are needed, these early findings suggest that the 11-oxyandrogens contribute significantly to the total androgen burden in PCOS and might explain clinical evidence of androgen excess in women with normal serum T. In addition, 11-oxyandrogen measurements might improve subtyping of PCOS and serve as biomarkers of metabolic risk.

V.3. Premature adrenarche

Adrenarche is a developmental process clinically defined by the appearance of axillary and pubic hair around age 8 years [62]. This phenomenon is marked by an increase in adrenal C₁₉ steroid production, such as DHEA and DHEAS, which reflect the expansion of the ZR [62–64]. After regression of the fetal adrenal, infant adrenal glands display a distinct zona glomerulosa and ZF, but a small ZR [63]. Focal islands of ZR cells gradually appear in the adrenals of children around age 3 years and expand at age 4–5 years [63, 65]. A continuous layer of ZR cells is observed by age 6 years, and the ZR attains its maximum thickness around age 12–13 years [63, 65]. The expression of the steroidogenic enzymes and cofactors required for C₁₉ steroid production, such as 17 α -hydroxylase/17,20-lyase (CYP17A1) and cytochrome *b*₅ (CYB5A), increases along with the changes of adrenal morphology that occur during adrenarche [66–68].

Premature adrenarche is defined as the occurrence of pubic and axillary hair, adult type body odor, oily hair and skin, acne and accelerated growth before age 8 years in girls or 9 years in boys, accompanied by hormonal evidence of adrenal androgen production [69–72]. Importantly, girls with premature adrenarche are at increased risk for developing PCOS, infertility and features of metabolic syndrome. As a group, children with premature adrenarche exhibit elevated serum adrenal androgens for age, including DHEA, DHEAS, A4 and T; [73–75] however, some children with clinical signs typical of premature adrenarche have normal serum DHEAS concentrations [70, 73]. The secretion of 11-oxyandrogens during adrenarche has been explored as early as the 1970s, but initial results did not appear promising. Parker et al found no significant changes in 11OHA4 with age [76]. On the other hand, Holownia and colleagues observed a gradual rise in plasma concentrations of 11OHA4 from age 9 through 16 years, which was similar in both sexes [77]. In a recent study, Rege et al used liquid chromatography-tandem mass spectrometry to characterize the steroid metabolome in children with premature adrenarche and found that not only DHEAS, but also 11-oxyandrogens, including 11OHA4 and 11KT, were significantly higher in children with premature adrenarche as compared to controls [78]. Further studies are required to understand the role of 11-oxyandrogen in normal and premature adrenarche.

VI. Summary

Although the presence of 11-oxyandrogens in human beings has been documented for several decades, their functions in normal physiology and pathologic states remain poorly understood. Sufficient evidence has appeared to identify 11OHA4 as a major product of the adrenal gland and 11KT as the dominant active androgen metabolite of 11OHA4. The 11-oxyandrogens are the predominant androgens in most patients with classic 21OHD, except for men with good disease control and normal testicular function. Ironically, while T and DHT have been recognized as the main androgens in human beings for many years, their prominence might be limited to normal adult males. The contributions of 11-oxyandrogens to androgen excess disorders in women and children looms large and awaits further definition.

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Preg, pregnenolone; 17OHPreg, 17 α -hydroxypregnenolone; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; Adiol, 5-androstenediol; AdiolS, 5-androstenediol-3-sulfate; A4, androstenedione; T, testosterone; DHT, 5 α -dihydrotestosterone; 11OHA4, 11 β -hydroxyandrostenedione; 11KA4, 11-ketoandrostenedione; 11OHT, 11 β -hydroxytestosterone; 11KT, 11-ketotestosterone; 11OHDHA4, 11 β -hydroxy-5 α -androstenedione; 11KDHA4, 11-keto-5 α -androstenedione; 11OHDHT, 11 β -hydroxydihydrotestosterone; 11KDHT, 11-ketodihydrotestosterone; StAR, steroidogenic acute regulatory protein; CYP11A1, cytochrome P450 cholesterol side-chain cleavage; CYP17A1, cytochrome P450 17 α -hydroxylase/17,20-lyase; CYB5A, cytochrome b₅; SULT2A1, sulfotransferase family 2A member 1; 3 β HSD2, 3 β -hydroxysteroid dehydrogenase type 2; CYP11B1, cytochrome P450 11 β -hydroxylase; 11 β HSD2, 11 β -hydroxysteroid dehydrogenase type 2; 11 β HSD1, 11 β -hydroxysteroid dehydrogenase type 1; SRD5A, steroid 5 α -reductase; AKR1C3, aldo-keto reductase 1C3.