**11-Oxyandrogens from the viewpoint of pediatric endocrinology**

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**Highlights**

- 11-Oxyandrogens are newly specified human androgens.
- 11-Oxyandrogens are frequently overproduced in patients with 21-OHD and PCOS.
- Maternal-fetal transfer of 11-oxyandrogens is a novel cause of 46,XX DSD.

**Abstract.** 11-Oxyandrogens, such as 11-ketotestosterone (11-KT), 11-ketodihydrotestosterone (11-KDHT), 11β-hydroxytestosterone (11-OHT), 11β-hydroxyandrostenedione (11-OHA4), and 11-KA4, are newly specified human androgens. These 11-oxyandrogens are present in the cord blood and placenta, as well as in the blood of men and women of various ages, and are produced primarily in the adrenal gland. Accumulating evidence suggests that these steroids contribute to androgen excess in patients with 21-hydroxylase deficiency or polycystic ovary syndrome. More importantly, unlike classic androgens, 11-oxyandrogens produced in maternal tumors can pass through the placenta without being converted into estrogens, and cause severe virilization of female fetuses. Thus, overproduction of 11-oxyandrogens represents a new mechanism of 46,XX disorders of sex development. On the other hand, the physiological roles of 11-oxyandrogens remain to be clarified. This mini-review introduces the current understanding of 11-oxyandrogens, from the perspective of pediatric endocrinology.

**Key words:** 11-oxygenated androgen, 11-oxygenated C19 steroid; 11-ketotestosterone, fetus, disorders of sex development (DSD)
Introduction

Testosterone (T), dihydrotestosterone (DHT), and androstenedione (A4) are major human androgens (1, 2). These classic androgens promote male-specific phenotypes by binding to the androgen receptor (AR) (1, 2). Recent studies have identified 11-oxyandrogens, such as 11-ketotestosterone (11-KT), 11-ketodihydrotestosterone (11-KDHT), 11β-hydroxytestosterone (11-OHT), 11β-hydroxyDHT (11-OHDHT), 11β-hydroxyandrostenedione (11-OHA4), and 11-ketoandrostenedione (11-KA4), as novel human androgens (3, 4). These 11-oxyandrogens are produced primarily in the adrenal gland and exert AR binding activity of various degrees. This mini-review introduces the current understanding of 11-oxyandrogens from the viewpoint of pediatric endocrinology.

11-Oxyandrogens from the viewpoint of pediatric endocrinology

Identification of 11-oxyandrogens in Humans

11-KT has long been known as the major androgen in fish (5). Although the presence of 11-KT in the blood of humans and other mammals was suggested several years ago (5), the clinical significance of 11-oxyandrogens in these species has poorly been investigated until recently. In 2016, Imamichi et al. documented that human plasma contains large amounts of 11-KT and that the plasma levels of 11-KT in reproductive aged women are almost as high as those of T (6). The authors proposed that 11-KT represents one of the major androgens in humans. Other researchers also reported high levels of 11-oxyandrogens in the blood (3, 4). Currently, 11-KT is considered as the major circulating androgen in humans, particularly in prepubertal children and women of all ages. Rege et al. found that the blood levels of 11-oxyandrogens are variable among species and relatively high in primates, pigs, guinea pigs, and fish (7).

Biochemical characteristics of 11-oxyandrogens

11-Oxyandrogens are synthesized from classic androgens (Fig. 1). The current understanding is that 11-OHA4 and 11-OHT are produced primarily in the adrenal gland from A4 and T, respectively (3, 4). CYP11B1, the enzyme that mediates these conversions, is strongly expressed in the adrenal gland. 11-OHA4 and 11-OHT are converted into 11-KA4 and 11-KT, respectively. This step is mediated by 11β-hydroxysteroid dehydrogenase type 2 (HSD11B2), probably in the peripheral tissues and adrenal glands. This notion is supported by the results of blood sampling from the adrenal vein and inferior vena cava (4). Finally, 11-OHT and 11-KT are converted into 11-OHDHT and 11-KDHT, respectively, through the enzymatic activity of 5α-reductase type 1 and 2 (SRD5A1 and 2) in the peripheral tissues. The major pathway from 11-OHA4 to 11-KDHT is predicted to be the route via 11-KA4 and 11-KT (4). Previous studies have shown that the blood levels of 11-KT are closely correlated with those of dehydroepiandrosterone-sulfate (DHEA-S), a steroid almost exclusively produced in the adrenal gland, supporting the notion that the adrenal gland is the major site of 11-oxyandrogen production. The minimal sex differences in the blood levels of 11-oxyandrogens argue for the non-gonadal origin of these steroids. However, Imamichi et al. speculated that gonads also contribute to the biosynthesis of 11-KT (6). Indeed, Leydig cells in the testis and theca cells in the ovary express CYP11B1 and HSD11B2.

11-Oxyandrogens have variable binding activities to AR. 11-KT and 11-KDHT are potent androgens, whereas 11-OHT and 11-OHDHT are less potent, and 11-A4 and 11-KA4 exert weak androgenic activity (8–10). Reportedly, the binding activities of 11-KT and 11-KDHT to AR are comparable to those of T and DHT, respectively (8, 9). In vitro assays have confirmed that 11-KT and 11-KDHT can alter the expression of AR-regulated genes (9). Given that AR is involved in all androgenic actions in the human body, the high binding activity of 11-KT to AR, together with its relatively high blood levels, indicates that this steroid is functionally important. Since 11-OHA4 is by far the most abundant 11-oxyandrogen in the blood (3), it may serve as an essential precursor of these androgens.

11-Oxyandrogens differ from classic androgens in terms of their sensitivity to aromatase (CYP19A1). Aromatase converts T and A4 into estrogens; however, it does not affect 11-oxyandrogens (1, 6, 11). Hence, 11-oxyandrogens, but not T or A4, can be transferred from maternal circulation to the fetus without being metabolized by placental aromatase (Fig. 2). Because of their non-aromatizable nature, 11-oxyandrogens can exert unique pathogenic effects on sex differentiation of female fetuses. This issue is described later in this review.

Classic androgens and 11-oxyandrogens in fetuses and placentas

O’Shaughnessy et al. measured the steroid levels in fetuses aborted during the first or second trimesters (12). The authors found that the classical and backdoor pathways are operating to produce classic androgens. The classical pathway refers to the conversion of A4 to DHT via T, whereas the backdoor pathway denotes a route from progesterone or 17-OH progesterone (17-OHP) to DHT without the intermediacy of T (1, 13, 14). In addition, O’Shaughnessy et al. showed that the blood levels of T and androsterone were significantly higher in male fetuses than in female fetuses, and proposed that these two androgens mediate the genital masculinization of genetic males (12). However, the levels of 11-oxyandrogens were not measured in that study.

11-Oxyandrogens are likely to be produced in
fetuses, because fetal adrenal glands clearly express several steroidogenic enzyme genes including CYP11B1 (5, 15, 16). Yoshida et al. measured multiple steroids in the placenta obtained from 10 healthy full-term neonates (17). The placentas invariably contained substantial amounts of 11-oxyandrogens, in addition to classic androgens and various androgen precursors. The levels of 11-KT and 11-KA4 were higher than those of T and A4, respectively. The high levels of 11-KT and 11-KA4 in the placenta were assumed to reflect high activity of HSD11B2 and relatively low activity of HSD11B1. Considering that CYP11B1 is barely expressed in the placenta (5, 15), 11-oxyandrogen production in the placenta should entail steroid transfer from other tissues. Presumably, 11-OHT and 11-OHA4 in the placenta are transferred from the fetal adrenal gland, where CYP11B1 is strongly expressed (5). Furthermore, 11-oxyandrogens in fetuses may also be derived from the maternal circulation. These data imply that the human placenta participates in a feto-maternal multi-organ network for 11-oxyandrogen production. Since Yoshida et al. found no sex differences in the tissue concentration of the tested steroids including 11-oxyandrogens (17), it remains unknown whether the placenta contributes to male-specific steroid metabolism.

More recently, He et al. measured 11-oxyandrogen levels in maternal blood samples during pregnancy and at term, as well as in neonatal cord blood samples (18). The authors detected high levels of 11-OHA4 and 11-KA4 in these samples. Of these, 11-KA4 levels in the
maternal blood rose during pregnancy. In the cord blood, the levels of 11-KA4 exceeded those of 11-OHA4 and 11-KT. This is in contrast to the data of non-pregnant adult blood samples, in which 11-KA4 represents only a minor fraction of 11-oxyandrogens (19). The accumulation of 11-KA4 in the placenta can be explained by the high enzymatic activities of HSD11B2 and HSD17B2 (Fig. 1) (5, 15, 18). He et al. proposed that placental HSD17B2 protects fetuses from the androgenic action of excessive 11-KT (18).

**Classic androgens and 11-oxyandrogens in the body after birth**

Classic androgens are produced in the testis during the fetal and neonatal periods, and also after puberty (1, 2). Sexual differences in the blood T levels of fetuses are apparent from the first trimester. During reproductive ages, the average blood T levels are more than 10-fold higher in men than in women (19). These classic androgens are essential for genital masculinization, pubertal sex development, and reproduction (2).

11-Oxyandrogens are also produced from the fetal period. du Toit et al. documented that the blood samples of neonates contain more 11-oxyandrogens than classic androgens (5, 20). After the onset of adrenarche, the blood levels of 11-KT and other 11-oxyandrogens gradually increase (21, 22). In girls, the blood levels of 11-KT during adrenarche exceed those of T (22). This increase in 11-oxyandrogen levels during adrenarche is accompanied by an increase in the blood levels of DHEA, DHEA-S, and T, and can be ascribed to the activation of adrenal steroidogenesis (22, 23). Rege et al. reported that the blood levels of 11-OHT and 11-KT were significantly higher in girls with premature adrenarche than in age-matched unaffected girls (22), indicating that 11-oxyandrogens may play a role in the phenotype of premature adrenarche. In adulthood, the blood levels of 11-KT and 11-KA4 remain stable in women throughout their life, while these steroids gradually decline in men (19). The levels of 11-OHA4 and 11-OHT modestly increase with age in women, but remain constant in men. Sustained blood levels of 11-oxyandrogens in adult women are in contrast to the age-dependent decrease in the levels of T, DHEA, and DHEA-S (24). Notably, the levels of 11-OHA4, 11-KA4, and 11-KT are only minimally higher in adult men than in adult women. These data imply that aging and sex exert relatively minor effects on 11-oxyandrogen production in the adrenal gland. On the other hand, Davio et al. demonstrated that 11-oxyandrogen production is affected by body weight (19). Specifically, high body mass index (BMI) was associated with low 11-KA4 levels and high 11-KT levels in women and men, respectively. This can be explained by the relatively high HSD17B2 activity in obese individuals.

**Pathophysiologial Roles of 11-Oxyandrogens from the Viewpoint of Pediatric Endocrinology**

**Physiological roles of 11-oxyandrogens**

To date, the physiological roles of 11-oxyandrogens are poorly understood. It remains unknown whether these steroids are essential for the normal masculinization of genetic males. Given that 11-oxyandrogens are produced primarily in non-gonadal tissues (3, 4), these steroids may not be involved in male sex development under physiological circumstances. Indeed, 11-oxyandrogen measurement of full-term placentas and postnatal blood samples demonstrated no sexual dimorphism (17). However, under pathological conditions, accumulation of 11-oxyandrogens underlies androgen excess in genetic females. This issue is discussed in the next section.

**11-Oxyandrogens in patients with 21-hydroxylase deficiency**

21-Hydroxylase deficiency (21-OHD) is the most common form of congenital adrenal hyperplasia (25). Androgen excess and glucocorticoid deficiency are characteristic features of 21-OHD (25). In patients with 21-OHD, accumulated 17-OHP in the adrenal gland is converted into A4, which serves as a substrate for both T and 11-oxyandrogens. Indeed, the blood levels of classic androgens and 11-oxyandrogens are frequently increased in patients with 21-OHD. For example, Turcu et al. showed that the blood levels of 11-OHA4, 11-KA4, 11-OHT, and 11-KT in patients with 21-OHD under standard glucocorticoid supplementation therapy were three- to four-fold higher than those in age- and sex-matched unaffected individuals (3, 26).

11-Oxyandrogens may be used as biomarkers for disease control in patients with 21-OHD. Reportedly, the blood levels of 11-OHT and 11-KT were higher in patients with menstrual irregularity and hirsutism than in those without these features (3). Likewise, blood levels of 11-KT and other 11-oxyandrogens were increased in patients with 21-OHD and testicular adrenal rest tumors (TART) (27). Steroid measurement of spermatic vein blood samples suggested that TART can produce various 11-oxyandrogens (28). Particularly the high levels of 11-OHT in the spermatic vein blood samples of patients with TART (28) are indicative of high CYP11B1 activity in these tumors.

**11-Oxyandrogens in patients with polycystic ovary syndrome**

Polycystic ovary syndrome (PCOS) is a common multifactorial disorder affecting 5–15% of women of reproductive age (29, 30). Women with PCOS typically present with hyperandrogenic features, in addition to ovarian dysfunction and metabolic abnormalities (29, 30). Excessive androgens in PCOS patients are
assumed to be produced in both the ovary and adrenal gland (3). Both the classical and backdoor pathways for DHT production are activated in women with PCOS (31). Several studies have demonstrated that not only classic androgens, but also 11-oxyandrogens are often increased in the blood of PCOS patients (32). For example, Yoshida et al. compared the steroid levels in PCOS patients to those in eumenorrheic women, and found that eight of 28 patients had high levels of both 11-oxyandrogens and classic androgens, whereas four and 12 had an increase in only 11-oxyandrogen levels and classic androgen levels, respectively (33). These data indicate that PCOS is an etiologically heterogeneous condition and that 11-oxyandrogens are overproduced in certain subgroups of PCOS. On the other hand, Yoshida et al. found no evidence that accumulation of 11-oxyandrogens contributes to the high frequency of anxiety and depression in PCOS patients (34). Since the blood levels of 11-OHT and 11-KT in the patients were correlated with BMI, it is likely that overweight/obesity enhances 11-oxyandrogen overproduction in PCOS. In this regard, Torchen et al. reported that 11-KT levels were similarly elevated in peripubertal girls with obesity and premenarchial daughters of women with PCOS (35). These data suggest significant activation of androgen production in the adrenal gland. The A4:11-OHA4 ratio of the patients may be useful to assess the tissue origin of excessive androgens (the adrenal or the ovary) (4).

Maternal-fetal transfer of 11-oxyandrogens leading to 46,XX disorders of sex development

In 2020, Nagasaki et al. reported a case of a maternal adrenal tumor, in which the overproduction of 11-oxyandrogens caused severe virilization of a female fetus (36). The proband presented with virilized external genitalia of Prader grade 3. The 31-yr-old mother of the proband manifested hirsutism for several years. Eight months after the birth of the proband, the mother was diagnosed with adrenal adenoma and underwent comprehensive steroid measurements. The levels of T, A4, and DHT in the maternal blood remained within the normal range; however, the levels of 11-OHA4, 11-OHT, and 11-KT were markedly elevated. These findings provide the first evidence that 11-oxyandrogens can be synthesized in adrenal adenomas and pass through the placenta to cause severe virilization of female fetuses. Such a maternal-fetal transfer is unlikely to occur in classic androgens, because they are subjected to enzymatic conversion by placental aromatase (1, 2). The findings by Nagasaki et al. indicate that 11-oxyandrogen overproduction in maternal tumors represents a novel cause of 46,XX disorders of sex development (DSD).

Conclusions

It has become apparent that 11-oxyandrogens are the major circulating androgens in humans, particularly in children and women. 11-oxyandrogens underlie androgen excess in a certain percentage of patients with 21-OHD and PCOS. More importantly, because of their non-aromatizable nature, 11-oxyandrogens play a unique role in the development of DSD. Still, the physiological roles of 11-oxyandrogens need to be clarified in future studies.

Conflict of interests: The author declares no conflicts of interest.

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References

1. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endoc Rev 2011;32: 81–151. [Medline] [CrossRef]
2. Matsumoto AM, Anawalt BD. Testicular disorders. In: Melmed S, Auchus R, Goldfine AB, Koenig RJ, Rosen CJ, editors. Williams textbook of endocrinology. 14th ed. Elsevier 2020. p.668–755.
3. Turcu AF, Rege J, Auchus RJ, Rainey WE. 11-Oxygenated androgens in health and disease. Nat Rev Endocrinol 2020;16: 284–96. [Medline] [CrossRef]
4. Barnard L, du Toit T, Swart AC. Back where it belongs: 11β-hydroxyandrostenedione compels the re-assessment of C11-oxy androgens in steroidogenesis. Mol Cell Endocrinol 2021;525: 111189. [Medline] [CrossRef]
5. du Toit T, Swart AC. Turning the spotlight on the C11-oxy androgens in human fetal development. J Steroid Biochem Mol Biol 2021;212: 105946. [Medline] [CrossRef]
6. Imamichi Y, Yuki KI, Otsuka M, Kitano T, Mukai K, Ushikubi F, et al. 11-ketotestosterone is a major androgen produced in human gonads. J Clin Endocrinol Metab 2016;101: 3582–91. [Medline] [CrossRef]
7. Rege J, Garber S, Conley AJ, Elsey RM, Turcu AF, Auchus RJ, et al. Circulating 11-oxygenated androgens across species. J Steroid Biochem Mol Biol 2019;190: 242–9. [Medline] [CrossRef]
8. Storbeck KH, Bloem LM, Africander D, Schloms L, Swart P, Swart AC. 11β-Hydroxydihydrotestosterone and 11-ketodihydrotestosterone, novel C19 steroids with androgenic activity: a putative role in castration resistant prostate cancer? Mol Cell Endocrinol 2013;377: 135–46. [Medline] [CrossRef]
9. Pretorius E, Africander DJ, Vlok M, Perkins MS, Quansoo J, Storbeck KH. 11-Ketotestosterone and 11-ketodihydrotestosterone in castration resistant prostate cancer: potent androgens which can no longer be ignored. PLoS One 2016;11: e0159867. [Medline] [CrossRef]

10. Campana C, Rege J, Turcu AF, Pezzi V, Gomez-Sanchez CE, Robins DM, et al. Development of a novel cell based androgen screening model. J Steroid Biochem Mol Biol 2016;156: 17–22. [Medline] [CrossRef]

11. Stocco C. Tissue physiology and pathology of aromatase. Steroids 2012;77: 27–35. [Medline] [CrossRef]

12. O’Shaughnessy PJ, Antignac JP, Le Bizec B, Morvan ML, Svechnikov K, Söder O, et al. Alternative (backdoor) androgen production and masculinization in the human fetus. PLoS Biol 2019;17: e3000002. [Medline] [CrossRef]

13. Reisch N, Taylor AE, Nogueira EF, Ashby DJ, Dirh V, Berry A, et al. Alternative pathway androgen biosynthesis and human fetal female virilization. Proc Natl Acad Sci USA 2019;116: 22294–9. [Medline] [CrossRef]

14. Fukami M, Homma K, Hasegawa T, Ogata T. Backdoor pathway for dihydrotestosterone biosynthesis: implications for normal and abnormal human sex development. Dev Dyn 2013;242: 320–9. [Medline] [CrossRef]

15. Pezzi V, Mathis JM, Rainey WE, Carr BR. Profiling transcript levels for steroidogenic enzymes in fetal tissues. J Steroid Biochem Mol Biol 2003;87: 181–9. [Medline] [CrossRef]

16. Rainey WE, Rehman KS, Carr BR. The human fetal adrenal: making adrenal androgens for placental estrogens. Semin Reprod Med 2004;22: 327–36. [Medline] [CrossRef]

17. Yoshida T, Matsumoto K, Miyado M, Miyashiro Y, Sago H, Horikawa R, et al. Quantification of androgens and their precursors in full-term human placenta. Eur J Endocrinol 2021;185: K7–11. [Medline] [CrossRef]

18. He X, Banker M, Putthayatappa M, Padmanabhan V, Auchus RJ. Maternal 11-ketoandrostenedione rises through normal pregnancy and is the dominant 11-oxygenated androgen in cord blood. J Clin Endocrinol Metab 2022;107: 660–7. [Medline] [CrossRef]

19. Davio A, Woolcock H, Nanba AT, Rege J, O’Day P, Ren J, et al. Sex differences in 11-oxygenated androgen patterns across adulthood. J Clin Endocrinol Metab 2020;105: e2921–9. [Medline] [CrossRef]

20. du Toit T, Finken MJJ, Hamer HM, Heijboer AC, Swart AC. C11-oxy C21 steroids in neonates: UPC2-MS/MS quantification of plasma 11β-hydroxyandrostenedione, 11-ketotestosterone and 11-ketoprogesterone. Steroids 2018;138: 1–5. [Medline] [CrossRef]

21. Stárka L, Dušková M, Vítků J. 11-Keto-testosterone and other androgens of adrenal origin. Physiol Res 2020;69(Suppl 2): S187–92. [Medline] [CrossRef]

22. Rege J, Turcu AF, Kasa-Vubu JZ, Lerario AM, Auchus GC, Auchus RJ, et al. 11-ketotestosterone is the dominant circulating bioactive androgen during normal and premature adrenarche. J Clin Endocrinol Metab 2018;103: 4589–98. [Medline] [CrossRef]

23. Havelock JC, Auchus RJ, Rainey WE. The rise in adrenal androgen biosynthesis: adrenarche. Semin Reprod Med 2004;22: 337–47. [Medline] [CrossRef]

24. Nanba AT, Rege J, Ren J, Auchus RJ, Turcu AF. 11-oxygenated C19 steroids do not decline with age in women. J Clin Endocrinol Metab 2019;104: 2615–22. [Medline] [CrossRef]

25. Newell-Price JDC, Auchus RJ. The rise in adrenal androgen biosynthesis: adrenarche. Semin Reprod Med 2004;22: 327–36. [Medline] [CrossRef]

26. Van der Toit T, Finken MJJ, Hamer HM, Heijboer AC, Swart AC. C11-oxy C21 and C11-oxy C22 steroids in newborns: UPC2-MS/MS quantification of plasma 11β-hydroxyandrostenedione, 11-ketotestosterone and 11-ketoprogesterone. Steroids 2018;138: 1–5. [Medline] [CrossRef]

27. Yoshida T, Saito K, Kawamura T, Ishikawa T, Kato T, Matsubara K, et al. Circulating steroids and mood disorders in eumenorrheic women and patients with polycystic ovary syndrome. J Endocrinol 2013;227: 337–47. [Medline] [CrossRef]

28. Nagasaki K, Takase K, Numakura C, Homma K, Hasegawa T, Fukushima T. Foetal virilisation caused by overproduction of non-aromatisable 11-oxygenated C19 steroids in maternal adrenal tumour. Hum Reprod 2020;35: 2609–12. [Medline] [CrossRef]