The steroid metabolome of adrenarche

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Abstract
Adrenarche is an endocrine developmental process whereby humans and select nonhuman primates increase adrenal output of a series of steroids, especially DHEA and DHEAS. The timing of adrenarche varies among primates, but in humans serum levels of DHEAS are seen to increase at around 6 years of age. This phenomenon corresponds with the development and expansion of the zona reticularis of the adrenal gland. The physiological phenomena that trigger the onset of adrenarche are still unknown; however, the biochemical pathways leading to this event have been elucidated in detail. There are numerous reviews examining the process of adrenarche, most of which have focused on the changes within the adrenal as well as the phenotypic results of adrenarche. This article reviews the recent and past studies that show the breadth of changes in the circulating steroid metabolome that occur during the process of adrenarche.

Introduction
The human adrenal produces large quantities of the 19-carbon (C19) steroids DHEA and DHEAS during fetal development, but the production of these steroids falls rapidly after birth and remains low for the first few years of life. Adrenarche refers to the re-emergence of adrenal C19 steroid production at around 6 years of age that results from the expansion and differentiation of the adrenal zona reticularis (ZR; Cutler & Loriaux 1980, Miller 1988, 2009, Parker 1991, Auchus & Rainey 2004; Fig. 1). Neither DHEA nor DHEAS are bioactive androgens, but they act as precursors for production of more potent androgens including testosterone in peripheral tissues that include hair follicles, genital skin, and prostate (Kaufman et al. 1990, Rosenfield 2005, Pelletier 2008; Fig. 2). The initiation of androgen-dependent growth of axillary and pubic hair (pubarche) is the phenotypic hallmark of adrenarche (Ducharme et al. 1976, Auchus & Rainey 2004, Havelock et al. 2004). The peripherally converted bioactive androgens also stimulate the development of apocrine glands in the skin to produce characteristic adult-type body odor and can act on the sebaceous glands leading to signs of acne (Rosenfield & Lucky 1993, Zouboulis et al. 2007). There are numerous reviews examining the process of adrenarche, most of which have focused on the adrenarche-associated changes in adrenal steroidogenic enzymes and differentiation (Cutler & Loriaux 1980, Parker 1991, Auchus & Rainey 2004, Havelock et al. 2004, Miller 2009, Nakamura et al. 2009a). Herein, we have reviewed the recent and past studies that show the breadth of changes in the circulating steroid metabolome that occur during the process of adrenarche.

C19 steroids and adrenal morphology
While most of our focus will be placed on the adrenarchal rise in adrenal production of C19 steroids, it should be noted that during development the fetal adrenal produces large amounts of DHEAS. During this period, C19 steroids arise from a developmental specific fetal zone that comprises 80% of the fetal adrenal. Till birth, the fetal zone continues to secrete dramatic quantities of the C19 steroid DHEAS, which is used by the placenta for production of remarkably large amounts of estrogens during pregnancy (Siiteri & MacDonald 1963, Frandsen & Stakemann 1964, Bolte et al. 1966). After birth, there is a dramatic involution of the fetal zone, which accounts for the sharp decline in DHEAS synthesis in the first few months of life (Fig. 1). The levels of DHEAS remain low until adrenarche commences as a result of the expansion of the ZR (Dhom 1973, Suzuki et al. 2000, Hui et al. 2009). This phenomenon was first described and given
the name adrenarche by Albright et al. (1942) when they observed the growth of axillary and pubic hair in the absence of gonadal androgens due to congenital absence or malformation of ovaries. Dhom (1973) characterized the morphological changes that occurred in the prepubertal and pubertal adrenal. According to this study, the adrenal cortex of the infant shows distinct zona glomerulosa (ZG) and zona fasciculata (ZF) with little ZR. However, in the adrenals of children around age 3 years, focal islands of ZR appear, which expand at age 4–5 years (Dhom 1973, Hui et al. 2009). A continuous layer of ‘functional’ ZR is first seen at age 6, which continues to expand till age 12–13 years (Dhom 1973, Hui et al. 2009). The emergence of the developed ZR corresponds with the rise in circulating DHEAS (Dhom 1973). It has also been demonstrated that the thickness of the ZR is directly proportional to the production of DHEAS (Dhom 1973, Reiter et al. 1977; Fig. 3).

Steroidogenic enzymes and cofactors involved in C19 steroid production

Although some steroidogenic enzymes and cofactor proteins are common to all zones of the cortex, the zone-specific production of steroids results in part due to differential expression of key steroidogenic enzymes. The pathway leading to the synthesis of DHEAS is quite simple and requires only three steroidogenic enzymes. However, across the period of adrenarche there are changes in the expression pattern of the steroidogenic enzymes and cofactors that facilitates C19 steroid production (Fig. 4A). Immunohistochemistry studies have demonstrated the varied expression of key steroidogenic enzymes within the cortical zones (Gell et al. 1996, 1998, Suzuki et al. 2000, Hui et al. 2009).

The initial conversion of cholesterol to pregnenolone is an essential step in all steroidogenic organs and is accomplished by the enzyme cytochrome P450 cholesterol side-chain cleavage (CYP11A1). This enzyme is expressed in all zones of the adrenal and the expression pattern within the ZR does not change during adrenarche (Nakamura et al. 2009a). CYP17A1, a single steroidogenic enzyme localized in the endoplasmic reticulum, is necessary for production of both cortisol and DHEA as it catalyzes two biosynthetic activities: 17β-hydroxylase and 17,20-lyase (Yanase et al. 1991, Imai et al. 1993, Brock & Waterman 1999). Thus, this enzyme is needed for both ZF and ZR functions. However, Suzuki et al. (2000) reported changes in expression of CYP17A1 in the adrenal ZF and ZR of pre-adrenarche vs post-adrenarche children. Their semiquantitative immunohistochemical analysis showed that after age 5 years, the CYP17A1 protein appeared to increase in both zones and reached a plateau level after 13 years; suggesting that increased CYP17A1 could impact the capacity for DHEA synthesis.

The expression of the flavoprotein mediating the dual activity of CYP17A1, namely cytochrome P450 oxidoreductase (CPR) has also been studied across adrenarche and was found to increase in all the three adrenal zones, especially the ZR (Suzuki et al. 2000). The increased conversion of 17OHpreg to DHEA due to the elevated 17,20-lyase activity of CYP17A1 (Katagiri et al. 1995) is central to understanding the mechanisms underlying adrenarche. The lyase activity of the enzyme is enhanced by the hemoprotein cytochrome b5 (CYB5A; Katagiri et al. 1995, Auchus et al. 1998, Brock & Waterman 1999, Dharia et al. 2004). CYB5A expression was detected in the DHEAS-synthesizing fetal zone throughout

Figure 1 Serum DHEA and DHEAS levels before and after the onset of human adrenarche. Based on steroid levels from de Peretti & Forest (1976, 1978).

Figure 2 Adrenal-derived C19 steroids act as precursors for the production of more potent androgens in peripheral tissues including hair follicles, genital skin, and prostate. The classical pathway for bioactive androgen synthesis as well as a proposed alternative pathway using 11β-hydroxysteroid dehydrogenases (type 5 or type 3).
of the poor androgen DHEA into more potent androgens. Numerous studies have demonstrated that the transitional and fetal zones of the human fetal adrenal abundantly express SULT2A1 (Korte et al. 1982, Barker et al. 1994, Parker et al. 1994). Suzuki et al. (2000) carried out immunohistochemical studies in the postnatal period from infancy to old age and established that SULT2A1 became highly discernible in the ZR at ages 5–13 years and reached a plateau thereafter; thus confirming it as marker for ZR development.

The sulfonation of steroids by SULT2A1 requires a sulfate donor, namely 3’-phosphoadenosine 5’-phosphosulfate (PAPS; Weinstilboum et al. 1997, Strott 2002). In humans, PAPS synthesis requires two isoforms of the enzyme PAPS synthase, namely PAPSS1 and PAPSS2. Noordam et al. (2009) examined an 8-year-old girl with early pubic and axillary hair, and concluded that the cause of hyperandrogenism in this subject was a set of compound heterozygous mutations in PAPSS2. Mutated PAPSS2 generated a decreased amount of PAPS, which is a prerequisite for steroid sulfonation. This was consistent with impaired DHEA sulfonation; thereby making the unconjugated DHEA pool available for conversion into bioactive androgens such as testosterone.

During adrenarche, as the ZR expands, this zone was found to have substantially lower levels of HSD3B2 compared with the adjacent ZF (Fig. 4B). The relative lack of HSD3B2 expression/activity facilitates increased DHEAS synthesis because HSD3B2 competes with CYP17A1 for pregnenolone and 17OHPreg (Conley & Bird 1997, Rainey et al. 2002). Thus, HSD3B2 expression in the expanding ZR coincides with elevated adrenal DHEAS synthesis through the postnatal period to adult life and is one of the driving forces of adrenarche (Gell et al. 1996, 1998, Suzuki et al. 2000, Hui et al. 2009). The phenomenon of increased androgen production as a result of decreased HSD3B2 activity was also demonstrated by McCartin et al. (2000) when they reported the presence of premature adrenarche (PA) in an 11-year-old subject with a profound loss of HSD3B2 activity owing to a compound heterozygous mutation in the HSD3B2 gene (McCartin et al. 2000). However, it should be noted that there are cortical cells located at the border between the ZF and ZR in normal adult adrenal glands that co-express HSD3B2 and CYB5A, suggesting that these cells have the potential for direct production of androstenedione (Nakamura et al. 2011). Human ovarian theca cells also co-express HSD3B2, CYP17A1, and CYB5A and similarly have potential for androstenedione biosynthesis (Dharia et al. 2004, Simard et al. 2005). Nevertheless, these results await further investigations to clarify the regulatory mechanisms of co-expression of the two enzymes in this population of cortical cells.

**Control of adrenarche**

The precise mechanisms that control adrenal androgen biosynthesis have not been clearly defined. ACTH has been considered as the accepted primary mediator of adrenarche.

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**Figure 3** DHEAS and expansion of the adrenal reticularis. (A) The relationship between the development of the ZR and plasma DHEAS levels (Dhom 1973, Reiter et al. 1977). (B) The morphological development of the adrenal zona reticularis depicting the appearance of focal islands of reticular tissue and a continuous ‘functional’ ZR (Dhom 1973).

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**References**

Rainey et al. 2002, Dharia et al. 2004, Nguyen et al. 2008. Immunohistochemical studies indicated that CYB5A is weakly synthesized in all the adrenal zones of pre-adrenarchal children (Suzuki et al. 2000). However, CYB5A expression markedly rises in the ZR after age 5 years and plateaus at age 13 years, whereas the levels remain extremely low in the ZG and ZF at all ages (Suzuki et al. 2000). A reduction in the proportion of the adrenal cortex that expresses CYB5A and DHEA levels was also detected with aging (Parker 1999, Parker et al. 2000, Dharia et al. 2005). These finding shed light on the significance of CYB5A in adrenarche.

The sulfonation of steroids occurs extensively in the adrenal cortex and is primarily carried out by the enzyme SULT2A1, of the human hydroxysteroid sulfotransferase family (Luu-The et al. 1996, Strott 2002, Shi et al. 2009). SULT2A1 has a broad substrate specificity, which includes DHEA, pregnenolone, and 17OHPreg. Sulfonation of pregnenolone and 17OHPreg precludes metabolism by HSD3B2 and CYP17A1 and thereby their utilization as precursors for the mineralocorticoid, glucocorticoid, and androgen biosynthetic pathways. Also, conversion of DHEA into its sulfate ester, i.e. DHEAS, prevents the transformation of the poor androgen DHEA into more potent androgens.
for the past few decades (Rosenfeld et al. 1971a, b, Reiter et al. 1977). Clearly, dexamethasone suppression of adrenal androgens suggests a regulatory role for ACTH (Abraham 1974, Kim et al. 1974, Rich et al. 1981). Moreover, children with an ACTH receptor defect fail to experience adrenarche (Weber et al. 1997), thereby supporting the postulate that ACTH plays an essential role in this phenomenon. However, several studies have demonstrated that ACTH and cortisol levels both remain constant even during adrenarche’s rise in adrenal androgens (Mellon et al. 1991, Parker 1991, Penhoat et al. 1991, Auchus & Rainey 2004). This is partly due to the tight regulatory feedback system between ACTH and cortisol, which keeps their levels within the physiologic range throughout life (Reader et al. 1982, 1983) as opposed to
DHEA and other C_{19} steroids that have no clear feedback system. Thus, although ACTH affects both cortisol and adrenal androgen production at adrenarche. A related hypothesis proposing that the proximal 18-amino acid hinge region (amino acids 79–96) of pro-opiomelanocortin (POMC) was a stimulator for adrenal androgen synthesis, was not supported by in vitro studies (Parker et al. 1989, Mellon et al. 1991, Penhoat et al. 1991). However, there have been numerous studies indicating that plasma levels of POMC-related peptides like β-lipotropin and β-endorphin correlate to the increasing levels of DHEAS during adrenarche (Genazzani et al. 1983a,b, O’Connell et al. 1996). Several studies also indicated no positive correlation between other potential endocrine regulatory candidates including prolactin, insulin, and insulin-like growth factor 1 with the DHEAS levels seen during adrenarche (Aubert et al. 1974, Parker et al. 1978, Smith et al. 1989, Guercio et al. 2002). Thus, determination of the controlling factor of adrenarche still needs more investigation.

Anderson (1980) postulated that adrenarche is triggered through an unknown mechanism involving the increased intra-adrenal levels of cortisol associated with the growth of the gland. Recent in vitro studies by Topor et al. (2011) also established that cortisol inhibits HSD3B2 and stimulates the biosynthesis of DHEA at concentrations above 50 μM. Dickerman et al. (1984) and Byrne et al. (1985, 1986) confirmed that there were age-dependent increases in intra-adrenal concentrations of several C_{19} and C_{21} steroids and proposed that these concentrations were in the range of the Michaelis–Menten constant (K_m) for HSD3B2 and might therefore inhibit the enzyme and promote 17OHPreg metabolism to DHEA at adrenarche. However, the same study demonstrated that 1 μM cortisol did not inhibit HSD3B2 (Byrne et al. 1986). Also, DHEAS levels appear to be high in adrenarchal children with untreated classic congenital adrenal hyperplasia where intra-adrenal cortisol levels should be low (Brunelli et al. 1995). Thus, the exact role for the modulation of HSD3B2 enzymatic activity by intra-adrenal steroid inhibitors remains unclear, while decreased expression of HSD3B2 in the post-adrenarche ZR appears clear.

**Steroid production in adrenarche**

The difference in expression and activity of the various adrenal steroidogenic enzymes from infancy to adulthood suggest that there should also be age-related changes in serum concentrations of several Δ5- and Δ4-steroids that would underlie the physiologic processes of adrenarche.

**Serum C_{21} steroids in adrenarche**

As described earlier, it has been established that adrenarche is associated with the expansion of the ZR having deficient expression of HSD3B2 along with increasing expression of CYP17A1. Also, 17OHPreg and 17OHPreg are the key intermediates in cortisol and androgen metabolism, respectively, thereby making them the most analyzed C_{21} steroids in adrenarche. Abraham et al. (1973) demonstrated by RIA that the concentration of 17OHPreg was higher in neonates than in preadolescents, adult males, and nonpregnant women, presumably due to the low activity of HSD3B2 in neonatal adrenal cortex as compared with the adult cortex. Hughes & Winter (1976) also observed age-related changes in serum concentrations of 17OHPreg. However, a detailed age-dependent analysis of 17OHPreg and 17OHPreg was described by Shimozawa et al. (1988). They examined the levels of 17OHPreg and 17OHPreg by RIA in 11 umbilical cord blood specimens and sera from 82 normal children of various ages and 20 normal adults (Shimozawa et al. 1988). According to this study, the levels of these steroids are the highest in the cord blood owing to their metabolism by the feto-placental unit; and after birth they start declining to reach a nadir at 1–2 years age, followed by a gradual increase from ages 3 to 6 years till adulthood. This is in agreement with the observation that CYP17A1 expression in the ZF and ZR increases during adrenarche (Suzuki et al. 2000).

The pattern of some of the unconjugated C_{21} steroids has been traced in humans from birth to adulthood. Toscano et al. (1989) examined the levels of serum pregnenolone (50±7 ng/dl), cortisol (13±1.8 μg/dl), and 11-deoxy-cortisol (52±3.6 μg/dl) in 20 children between ages 5-9 and 9 years. Several studies carried out in large cohorts of children between 2 and 12 years of age have established that serum cortisol levels do not increase in adrenarche (Parker et al. 1978, Franckson et al. 1980). Thus, it is clear that the serum levels of most of the adrenal unconjugated C_{21} steroids are not grossly affected during the course of adrenarche.

The robust expression of the adrenal-related sulfotransferase enzyme, namely SULT2A1 (Strott 2002), and its broad substrate specificity has also encouraged researchers to examine the sulfonated derivatives of various C_{21} steroids. de Peretti & Mappus (1983) traced the levels of pregnenolone sulfate (Preg-S) from birth to adulthood and observed that Preg-S levels were high in cord plasma as a consequence of residual fetal zone activity. The levels started declining during the first year after birth and remained low thereafter. Also, there was no detectable rise in Preg-S during the adrenarchal period as is seen for the classical markers of adrenarche, namely DHEA and DHEAS (de Peretti & Mappus 1983). Shimozawa et al. (1988) demonstrated that 17OHPreg sulfate (17OHPreg-S) exhibited an age-related change, wherein the 17OHPreg-S concentration was highest in cord blood and decreased to a minimum at 3–6 years, followed by a gradual increase from 7 years till adulthood as opposed to its nonsulfated form that showed a nadir at 1–2 years. These observations agree with what we now know regarding the adrenarchal expansion of the ZR that has high CYP17A1 and SULT2A1 and low HSD3B2 expression. This would explain the ability of 17OHPreg-S to act as an additional steroid marker of adrenarche.
Serum C₁₉ steroids in adrenarche

The weak C₁₉ steroids DHEA and DHEAS are the characteristic markers of adrenarche (Fig. 1). Extensive RIA studies tracing these steroids from birth to adolescence demonstrated that plasma DHEA levels rapidly declined in the first 2 years of life, stayed low for the next few years, and then dramatically increased after 6 years of age, corresponding to ‘adrenarche’ (Ducharme et al. 1976, Korth-Schutz et al. 1976, de Peretti & Forest 1976, 1978, Parker et al. 1978, de Peretti & Mappus 1983). DHEAS has been the most extensively studied steroid across adrenarche. A number of research groups traced the age-related changes in plasma DHEAS concentration and demonstrated that DHEAS decreased slowly during the first years of life, remained low till age 5 years and then abruptly started to rise (Korth-Schutz et al. 1976a, Parker et al. 1978, de Peretti & Forest 1978, Rich et al. 1981, Tung et al. 2004). Recent liquid chromatography–tandem mass spectrometry (LC–MS/MS) studies in a large cohort of children confirmed the same age-related pattern of serum DHEA levels (Kushnir et al. 2010). The patterns seen for both DHEA and DHEAS correlate well with intra-adrenal changes occurring during this period. Specifically, the high neonatal levels relate to the residual fetal zone that regresses over the first year leading to a nadir of DHEA/DHEAS, and their rise in the circulation correlates well with the expansion of the reticularis.

The age-related patterns of secretion of several other C₁₉ products have also been investigated but with varying results. While some immunoassay studies suggest no adrenarchal rise in androstenedione (Korth-Schutz et al. 1976, Parker et al. 1978), others show significant adrenarche-related increases in serum levels of androstenedione (Ducharme et al. 1976, Franckson et al. 1980, Likitmaskul et al. 1995, Tung et al. 2004). Recent LC–MS/MS analysis also showed a similar age-related rise in concentration of androstenedione (Kulke et al. 2010). Parker et al. (1978) did not find a relationship between serum 11β-hydroxyandrostenedione (11OHA) concentration and age, whereas Franckson et al. (1980) found that serum 11OHA increased with bone age throughout prepubertal childhood. The major difference between the studies was data analysis. Because of the circadian association of 11OHA with cortisol, Franckson et al. utilized a 11OHA/cortisol ratio to make steroid comparisons. Some immunoassay studies of testosterone in a large population of children established that this androgen did not rise demonstrably during the early stages of adrenarche (Ducharme et al. 1976, Korth-Schutz et al. 1976). Later studies using LC–MS/MS confirmed that the plasma levels of testosterone do not rise at adrenarche, but that its levels do rise as puberty approaches in boys (Kulke et al. 2010). The exact role of direct adrenal secretion of testosterone remains unclear. Previous studies in adult women exhibiting signs of androgen excess demonstrated that the adrenal could secrete testosterone under pathologic conditions (Stahl et al. 1973a,b, Greenblatt et al. 1976). In addition, the ZR expresses higher levels of the enzyme 17β-hydroxysteroid dehydrogenase type 5 (HSD17B5 or AKR1C3) than the adjacent ZF and its expression increases across adrenarche (Hui et al. 2009, Nakamura et al. 2009b). This enzyme has numerous activities, including the ability to convert androstenedione to testosterone (Fig. 4A). Thus, while it is clear that DHEA/DHEAS represent the most consistent markers of adrenarche, the production of more potent androgens needs further study and the analysis of these androgens may require consideration of adrenal circadian secretion as well.

Studies carried out in the 1970s and 80s (Mauvais-Jarvis et al. 1973, Moghissi et al. 1984) indicating that the conjugated androgen, androstane-3α,17β-diol glucuronide (Adiol-G) would be a good measure of testosterone transformation, led to further investigations that established the change in plasma steroid glucuronide levels at different ages (Belanger et al. 1986, Brochu & Belanger 1987). Brochu et al. determined the levels of androstosterone glucuronide and Adiol-G across various age groups in males and concluded that, since these 5α-reduced and conjugated steroids increase significantly before puberty, the adrenal C₁₉ steroids like androstenedione and androstane diol must be getting converted into androstosterone glucuronide and potentially into testosterone, which in turn is transformed into Adiol-G during prepubertal development (Brochu & Belanger 1987). Their findings support the premise that androgenic manifestations like appearance of pubic and axillary hair are induced by the conversion of adrenal C₁₉ steroids to active androgens that are further metabolized into their conjugated inactive products.

Urinary C₁₉ steroids in adrenarche

Nathanson et al. (1941) was one of the first groups of researchers to report the presence of androgen metabolites in the urine of a large cohort of children. The results of their study demonstrated that from ages 3 to 7 years, constant amounts of androgens are excreted; however, at about 7 years, the rate of excretion of these steroids begins to progressively increase. Several studies were carried out to confirm the age-related changes in androgen excretion. It was also demonstrated that urinary total DHEA and DHEAS levels are elevated from ages 8 to 12 years (Tanner & Gupta 1968, Gupta 1970, Kelnar & Brook 1983). In 1991, the normal ranges for urinary steroid excretion were determined for the first time by gas chromatography (GC), and it was demonstrated that urinary steroid excretion rates in childhood positively correlate with growth and activity of the adrenal cortex (Honour et al. 1991). With the advent of better techniques and availability of commercial assays, Remer et al. (1994a,b) demonstrated that urinary total 17-ketosteroid sulfate and DHEAS concentrations in 8-year-old normal children is significantly higher than preadrenal children (aged 4 years). Extensive studies by GC–MS to determine the urinary markers of adrenarche were carried out in a large population of healthy children and adolescents between 3 and
18 years (Remer et al. 2005), which established that DHEA and its metabolites, like 16α-hydroxy DHEA, 3β,16α,17α-androstenetriol, and androstenediol, show a continuous rise from ages 3–4 years to 17–18 years, thereby suggesting that adrenarche is a gradual process starting at an early age. The gradual nature of increasing steroids would appear to agree with Dhom’s histologic studies showing a gradual expansion of the adrenal ZR (Fig. 3). Recent studies by Shi et al. (2009) also confirmed the use of the C19 steroids as urinary markers of adrenarche.

**Future directions toward understanding the adrenarche steroid metabolome**

Hui et al. (2009) suggested that the process of adrenarche was associated with ZR increased expression of HSD17B5, an enzyme able to convert androstenedione to testosterone. They demonstrated the presence of HSD17B5 in the ZR of human adrenals around age 9 years, which tracks well with the onset of pubarche (Hui et al. 2009).

Testosterone derived from the testes as well as the adrenals is converted to a more potent androgen, 5α-dihydrotestosterone (DHT) by the enzyme 5α-reductases type I and II (SRD5A) in androgen target tissues like genital skin and prostate (Wilson et al. 1993, Chang et al. 2011). Recent studies suggest that there are likely several pathways leading to DHT biosynthesis (Wilson 1999, Wilson et al. 2003, Auchus 2004, Ghayee & Auchus 2007, Fluck et al. 2011). The peculiarity about these pathways is the synthesis of 5α-androstan-3α,17β-diol (androstenediol) involving 5α-pregnane-3α,17α-diol-20-one as a key intermediate. Androstenediol, in turn, acts as precursor for DHT, thereby bypassing the need for testosterone, which is the ‘classical’ precursor for DHT production. Recently, Fluck et al. (2011) confirmed that both the classical and alternative pathways of testicular androgen production are involved in the formation of normal human male sexual differentiation, whereas another group suggested that synthesis of DHT in castration-resistant prostate cancer requires 5α-androstenedione and not testosterone as an obligate precursor (Chang et al. 2011).

Two derivatives of testosterone, 11β-hydroxytestosterone (11OHT) and 11-ketotestosterone (11KT), may represent unique adrenal-derived androgens. While testosterone and DHT are the primary human androgens, 11OHT and 11KT are the major androgens found in a variety of fish (Rosenblum et al. 1985, Brantley et al. 1993, Yazawa et al. 2008). These C19 steroids are also produced in small quantities by mouse gonads (Yazawa et al. 2008). The physiologic role of these steroids in humans has not been studied, but circulating levels of these androgens have been examined in human blood (Kley et al. 1984, Schlaglecke et al. 1986). 11OHT could be speculated to be an adrenal-derived androgen as it might require the activity of 11β-hydroxylation via the adrenal-specific enzyme CYP11B1 can be derived by the 11β-hydroxylation testosterone by the adrenal-specific enzyme CYP11B1 in a reaction similar to that of the conversion of androstenedione to 11OHA (Schloms et al. 2012, Kley et al. 1984, Schlaglecke et al. 1986). Because the adrenal produces large amounts of DHEA, DHEAS, androstenedione, and 11OHA, it is likely that these androgen precursors will feed into both the classical and alternative peripheral metabolic pathways that can lead to active androgens.

**Premature adrenarche**

PA can be defined as the early rise in adrenal androgen production that usually results in the appearance of pubic or axillary hair before age 8 years in girls and 9 years in boys, without the appearance of other secondary sex characteristics (Talbot et al. 1943, Silverman et al. 1952). Girls with PA are susceptible to the development of polycystic ovary syndrome with hirsutism, irregular menses, and hyperandrogenism (Ibanez et al. 1993). PA is also an early onset predictor of hyperinsulinemia (Oppenheimer et al. 1995, Ibanez et al. 2000). Children with PA have been shown to exhibit higher serum levels of DHEA, DHEAS, androstenedione, and testosterone as well as their urinary metabolites (Doberne et al. 1975, Korth-Schutz et al. 1976b, Rosenfield et al. 1982, Voutilainen et al. 1983, Rosenfield & Lucky 1993, Likitmaskul et al. 1995, Ibanez et al. 2000). Recently studied LC–MS/MS steroid profiles of infants with fine genital hair showed a mild elevation of DHEAS as compared with healthy pre-adrenarchal children, thus indicating that pubic hair in infancy may represent a mild and early-onset variant of PA (Kaplowitz & Soldin 2007). Toscano et al. observed increases in plasma levels of C21 steroids like pregnenolone and 17OHPreg along with the C19 steroids like DHEA, DHEAS, and androstenedione but with no change in cortisol or 11-deoxycortisol in PA children as compared with controls. They interpreted these steroid changes to suggest that the PA adrenal had decreased HSD3B2 activity and increased CYP17A1 activity (Toscano et al. 1989). They also indicated that PA might not be exclusively dependent on ACTH regulation (Toscano et al. 1989). The levels of Adiol-G also increased in children with PA and showed a strong correlation with DHEA, DHEAS, and androstenedione (Montalto et al. 1990, Riddick et al. 1991, Balducci et al. 1992, 1993, Ibanez et al. 2000). Thus, it is clear that numerous adrenal-derived steroids can be used as markers in the diagnosis of PA.

**Conclusions**

Adrenarche is characterized by rising levels of C19 steroids like DHEA and DHEAS due to the expanding adrenal ZR, and in humans this occurs at ages 6–8 years. The phenotypic hallmark of adrenarche is the appearance of axillary and pubic
hair. This phenomenon is driven by the concerted actions of the enzymes CYP17A1, SULT2A1, and the cofactor CYB5A, which are highly expressed in the expanding population of ZR cells seen during adrenarche. The low expression of HSD3B2 in the ZR also enables the increased production of androgens by the adrenal cortex. Herein, we have reviewed recent and past studies that discuss the changes in the circulating steroid metabolome that occur during the process of adrenarche. It is clear that along with DHEA and DHEAS, serum levels of other C19 steroids like androstenedione, 11OHA, and Adiol-G are elevated during the process of adrenarche. It is still worth noting that although biochemical pathways leading to the formation of these steroids have been elucidated in detail, the primary signal(s) that drive adrenarche remains unknown. This makes the process of adrenarche one of the least understood of human endocrine developmental events.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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