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Urinary Markers of Adrenarche: Reference Values in Healthy Subjects, Aged 3–18 Years

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Information on the urinary excretion of dehydroepiandrosterone (DHEA) and its direct metabolites is scarce for healthy subjects during growth. We used gas chromatography-mass spectrometry urinary steroid profiling to noninvasively study adrenarchal metabolome in 400 healthy subjects, aged 3–18 yr. Urinary 24-h excretion rates of DHEA did not increase significantly before age 7–8 yr. However, DHEA together with its 16α-hydroxylated downstream metabolites, 16α-hydroxy-DHEA and 3β,16α,17β-androstenetriol (DHEA&M), as well as the DHEA metabolite, 5-androstene-3β,17β-diol (ADIOL), and the sum of major urinary androgen metabolites (C19) rose consistently from the youngest to the oldest age group. The significant increases ($P < 0.01$) observed for 24-h excretion rates of C19, ADIOL, and DHEA&M were 2- to 4-fold in boys and girls between age 3 and 8 yr. DHEA&M, for example, rose from about 20 to 80 μg/d ($P < 0.0001$) during this period. Until the age of 16 yr, DHEA&M excretion also increased to nearly 1000 μg/d. Patterns of steroidogenic enzyme activities were assessed (from definite ratios of urinary steroid metabolites) for 21-hydroxylase, 3β-hydroxysteroid dehydrogenase, 17β-hydroxysteroid dehydrogenase, and 5α-reductase. Our results indicate for healthy boys and girls that adrenarche is a gradual process starting much earlier than hitherto believed. Efficient metabolism of DHEA, especially to 16-hydroxylated steroids, may explain the almost constant levels seen for this steroid until age 7–8 yr. The established reference values for DHEA, DHEA&M, ADIOL, C19 (including androsterone and etiocholanolone), and urinary parameters of steroidogenic enzyme activities could be useful to identify nutritional, environmental, and pathophysiological interrelations with the progressive maturational process of adrenarche. Our data may also be used as reference data for the diagnosis of steroid-related disorders. (*J Clin Endocrinol Metab* 90: 2015–2021, 2005)

DURING CHILDHOOD, THE adrenal cortex changes in size, cell distribution, and function. This is impressively reflected by adrenarche, which is defined as the increase in adrenal secretion of C19 steroids occurring several years before the onset of gonadarche. Adrenal androgen secretion, principally dehydroepiandrosterone (DHEA) and its sulfate ester, continues to rise until age 20–30 yr. The factors that regulate adrenarche remain unknown (1, 2). Most researchers believe that adrenarche begins in midchildhood at about age 6–8 yr (1, 3). In contrast, two longitudinal studies, one performed in healthy children collecting 24-h urine samples at yearly intervals (4) and the other in girls (with idiopathic central precocious puberty during long-term pituitary-gonadal suppression) providing blood samples at 3- to 6-month intervals (2), suggest that adrenarche is a gradual process that might begin earlier in childhood. However, until now, this has not been shown in any cross-sectional study. We performed the present study to examine whether an early increase in adrenal androgen secretion is also apparent cross-sectionally in healthy children and to establish reference values for urinary markers of adrenarche analyzed by gas chromatography-mass spectrometry (GC-MS) urinary steroid profiling. Additionally, to gain more insight into potential steroidogenic enzyme-related mechanisms that may be involved in the regulation of adrenarche (5), activities of 3β-hydroxysteroid dehydrogenase (3β-HSD), 21-hydroxylase (21-OH), 17β-hydroxysteroid dehydrogenase (17β-HSD), and 5α-reductase (5α-Red) were also determined from defined ratios of urinary steroid metabolites.

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Abbreviations: ADIOL, 5-Androstene-3β,17β-diol; C19, sum of major urinary androgen metabolites; DHEA, dehydroepiandrosterone; DHEA&M, sum of dehydroepiandrosterone, 16α-hydroxydehydroepiandrosterone, and 3β,16α,17β-androstenetriol; DONALD study, Dortmund Nutritional and Anthropometric Longitudinally Designed study; GC-MS, gas chromatography-mass spectrometry; HSD, hydroxysteroid dehydrogenase; 21-OH, 21-hydroxylase; 5α-Red, 5α-reductase.

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Subjects and Methods

Subjects

The study group comprised 400 healthy children and adolescents (200 boys and 200 girls, aged 3–18 yr) participating in the DONALD (Dortmund Nutritional and Anthropometric Longitudinally Designed) study, an ongoing observational study, investigating the interrelations among nutrition, growth, and metabolic and endocrine changes during childhood and adolescence (6, 7). Fifty 24-h urine samples (25 from boys and 25 from girls) were randomly selected for each of the eight equally wide age groups with dates of urine collection starting at 3–4 yr and ending at 17–18 yr. The study was approved by the institutional review board of the Research Institute for Child Nutrition Dortmund, and parental consent and child's assent were obtained before entry into the study.

Measurements and urine collection

Body weight was measured with an electronic scale (Seca 753E, Seca Weighing and Measuring Systems, Hamburg, Germany) to the nearest 0.1 kg and standing height to the nearest 0.1 cm using a digital telescopic wall-mounted stadiometer (Harpenden, Crymych, UK).

Subjects and parents received instruction and written guidance to ensure compliance in the 24-h urine collection, which was performed at home by each participant. All micturitions were stored immediately in

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TABLE 1. Characteristics of the 400 children and adolescents

Age (yr)	Boys (n = 200)				Girls (n = 200)			
	Height (cm)	Weight (kg)	BMI (kg/m^2)	Creatinine (mmol/d)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Creatinine (mmol/d)
3–4	104.4 ± 5.1	16.9 ± 2.0	15.5 ± 1.1	2.4 ± 0.4	102.2 ± 5.7	16.7 ± 2.6	15.9 ± 1.6	2.2 ± 0.5
5–6	117.7 ± 5.4	21.2 ± 2.7	15.2 ± 1.1	3.4 ± 0.8	114.2 ± 5.0	20.3 ± 2.7	15.5 ± 1.4	2.9 ± 0.6
7–8	127.5 ± 6.9	26.8 ± 5.4	16.3 ± 1.8	4.4 ± 1.2	126.9 ± 5.3	25.6 ± 5.1	15.8 ± 1.9	3.7 ± 0.9
9–10	142.1 ± 7.9	35.2 ± 6.3	17.3 ± 2.0	6.0 ± 1.3	139.3 ± 7.6	33.1 ± 6.6	16.9 ± 2.3	5.3 ± 1.4
11–12	154.1 ± 8.4	45.1 ± 9.9	18.9 ± 3.2	7.1 ± 1.8	151.9 ± 7.0	43.6 ± 10.6	18.7 ± 3.3	6.4 ± 1.1
13–14	163.4 ± 8.4	53.1 ± 8.7	19.8 ± 1.8	9.7 ± 3.4	161.9 ± 6.2	51.5 ± 9.3	19.6 ± 3.4	7.9 ± 1.8
15–16	174.7 ± 8.3	62.3 ± 8.7	20.4 ± 2.2	12.8 ± 2.3	167.3 ± 7.0	59.8 ± 11.3	21.3 ± 2.8	9.8 ± 2.0
17–18	180.2 ± 6.3	73.1 ± 10.3	22.5 ± 3.0	14.8 ± 4.1	170.3 ± 7.6	62.4 ± 10.1	21.4 ± 2.8	9.9 ± 2.2

Data are presented as the mean ± SD. Each age group includes 25 boys and 25 girls. BMI, Body mass index.

preservative-free, Extran-cleaned (Extran, MA03, Merck, Darmstadt, Germany), 1-liter plastic containers at temperatures between −12 and −18 C before transfer to the research institute organized by a dietitian visiting the families and discussing collection completeness in detail (7, 8).

Creatinine was measured in all 24-h urine samples by the Jaffé method using a Beckman-2 creatinine analyzer (Beckman Coulter, Fullerton, CA). Urinary steroid profiles were determined using quantitative data produced by GC-MS analysis according to the method described previously (8, 9). Free and conjugated urinary steroids were extracted by solid phase extraction (Sep-Pak C18 cartridge, Waters Associates, Milford, MA), and the conjugates were enzymatically hydrolyzed (type I powdered *Helix pomatia*, Sigma-Aldrich Corp., St. Louis, MO). The hydrolyzed steroids were recovered by Sep-Pak extraction. Known amounts of three internal standards (5α-androstane-3α,17α-diol, stigmasterol, and cholesteryl butyrate) were added to a portion of each extract before formation of methyloxime-trimethylsilyl ethers.

GC was performed using an Optima-1 fused silica column (Macherey-Nagel, Dueren, Germany). Helium was used as carrier gas at a flow rate of 1 ml/min. The gas chromatograph (Agilent 6890 series GC, Agilent 7683 Series Injector, Agilent Technologies, Waldbronn, Germany) was directly interfaced to a mass selective detector (Agilent 5973N MSD, Agilent Technologies) operated in selected ion monitoring mode. Calibration of the GC-MS was achieved by analysis of a reference mixture containing known amounts of all separation compounds. The injections took place with an 80 C (2 min) GC oven; the temperature was then increased by 20 C/min to 190 C (1 min). For separation of steroids, it was increased by 2.5 C/min to 272 C. After calibration, values for the excretion of individual steroids were determined by measuring the selected ion peak areas against the internal standards.

Daily urinary excretion rates were determined for DHEA, 5-androstene-3β,17β-diol (ADIOL), and the sum of DHEA and its 16-hydroxylated downstream metabolites (8, 10), 16α-hydroxy-DHEA and 3β,16α,17β-androstenetriol (DHEA&M), reflecting major adrenarchal secretion products. Overall androgen metabolite excretion (C19) was determined as the sum of androsterone, etiocholanolone, 5-androstene-3β,17α-diol, ADIOL, DHEA, 16α-hydroxy-DHEA, and 3β,16α,17β-androstenetriol. Additional steroid metabolites profiled for the assessment of enzyme activities were 11-keto-pregnanetriol (5β-pregnane-

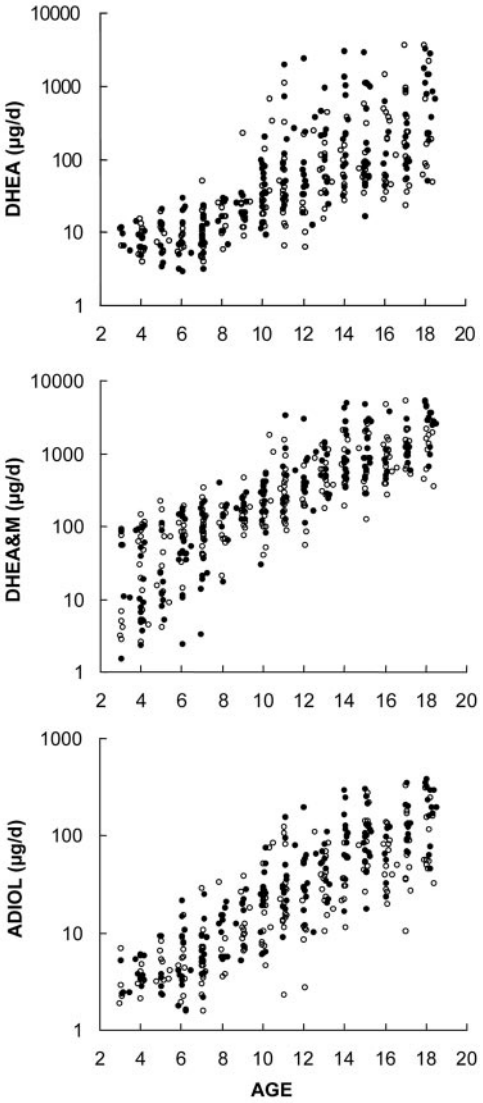

FIG. 1. Age-dependent increases in urinary 24-h excretion rates of DHEA, DHEA&M, and ADIOL in 400 healthy children (●, boys; ○, girls).

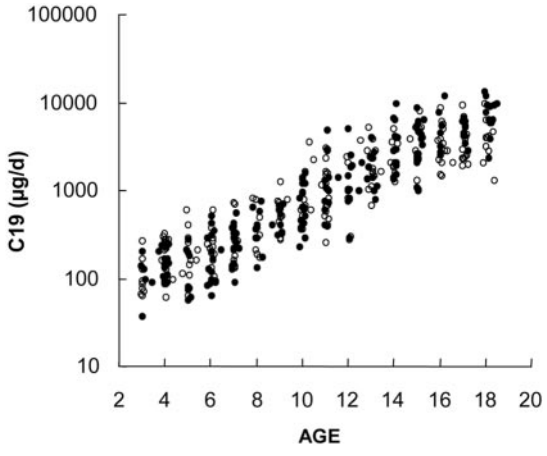

FIG. 2. Age-dependent increase in urinary 24-h excretion rate of C19 (sum of major urinary androgen metabolites: androsterone + etiocholanolone + 5-androstene-3β,17α-diol + ADIOL + DHEA + 16α-hydroxy-DHEA + 3β,16α,17β-androstenetriol) in 400 healthy children (●, boys; ○, girls).

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TABLE 2. Urinary 24-h excretion rates of DHEA according to age and sex (μg/d)

Age (yr)	Boys							Girls						
	P5	P25	P50	P75	P95	Mean[a]	SD[a]	P5	P25	P50	P75	P95	Mean[a]	SD[a]
3–4	4.77	6.09	8.23	10.4	14.5	0.91	±0.15	3.98	5.60	7.59	11.3	15.5	0.90	±0.19
5–6	3.07	5.07	7.13	13.4	26.0	0.90	±0.30	5.48	6.90	9.80	13.2	20.3	0.99	±0.18
7–8	5.15	8.18	11.9	17.5	28.0	1.08	±0.26	4.56	6.62	10.1	16.4	25.6	1.02	±0.28
9–10	11.2	18.5	32.9	60.7	98.5	1.53	±0.33	11.6	15.5	22.0	28.1	338	1.47	±0.48
11–12	18.5	32.8	51.7	120	1957	1.90	±0.59	6.60	18.9	29.8	51.0	328	1.57	±0.52
13–14	32.1	81.7	209	380	1335	2.27	±0.55	20.9	41.6	61.8	117	383	1.86	±0.37
15–16	43.4	59.3	86.4	165	1111	2.09	±0.53	34.7	52.8	91.0	378	1115	2.16	±0.52
17–18	55.0	151	308	880	2780	2.55	±0.54	36.7	82.2	165	378	3708	2.31	±0.61

Data are presented as percentiles and median (P50). Number of subjects per age group: 25 boys and 25 girls, with the exception of the three youngest age groups, in which DHEA was not measurable in a limited number of children. Data represent 11 boys and 12 girls in group 3–4 yr, 20 boys and 20 girls in group 5–6 yr, and 22 boys and 24 girls in group 7–8 yr.

[a] Mean ± SD of the logarithmized DHEA values; $P < 0.0001$ for age and $P < 0.05$ for sex (by two-way ANOVA, log DHEA).

3α,17α,20α-triol-11-one), α-cortolone (5β-pregnan-3α,17α,20α,21-tetrol-11-one), 5β-pregnane-3α,17α-diol-20-on, 5α-pregnane-3α,17α-diol-20-on, 17-hydroxypregnanolone, pregnanetriol, 11-oxopregnanetriol, tetrahydrocortisol, tetrahydrocortisone, and 5α-tetrahydrocortisol. Details on the calculation of each enzyme activity (3β-HSD, 21-OH, 17β-HSD, and 5α-Red) are given in the respective tables.

Statistical analysis

Data are presented as median, percentiles, and mean ± SD. For all adrenarchal hormone metabolites, the mean ± SD were obtained from logarithmized 24-h excretion values. Log transformation was performed to normalize the distribution before analysis. Gender and overall age group effects were tested by two-way ANOVA. Subsequent analyses of the influence of age on hormonal and enzymatic variables during specified periods of approximately 2- to 9-yr duration were performed (with age as a continuous predictor) using regression analysis. $P < 0.05$ was considered statistically significant. All tests were two-tailed. Calculations and analyses were performed using SAS for Windows (version 6.12, SAS Institute, Inc., Cary, NC).

Results

The mean ± SD for height, weight, body mass index, and daily creatinine excretion of the subjects according to age and gender are shown in Table 1. These values correspond closely to the means and normal ranges of other studies in the DONALD population (11). Scatter plots of age-dependent 24-h excretion rates of urinary markers of adrenarche and sum of major urinary androgen metabolites are shown in Figs. 1 and 2, respectively. For DHEA, DHEA&M, and ADIOL, the sex- and age-stratified median and 5th, 10th, 25th, 75th, and 95th percentiles are presented together with the mean ± SD of the logarithms of these steroids (Tables 2–4). Significant overall effects of age and gender were observed by two-way ANOVA for DHEA and ADIOL (Tables 2 and 4). For DHEA&M (Table 3), C19 (Table 5), and the quantitatively most important androgen metabolites in urine, androsterone and etiocholanolone (Table 5), primarily a highly significant influence of age ($P < 0.0001$) was seen; the overall influence of gender was not significant ($P \geq 0.3$). However, in the oldest age group, C19 and its major constituents, androsterone and etiocholanolone, differed between the sexes ($P < 0.01$; Table 5).

DHEA&M, ADIOL, and C19 (including androsterone and etiocholanolone) rose consistently during childhood and adolescence, but a less consistent increase was seen for DHEA during childhood (Fig. 1). Based on the retransformed (geometric) means of the logarithms of each adrenarche marker (Tables 2–5), average daily excretion rates rose about 4-fold for DHEA&M, 2-fold for ADIOL and C19, and only 1.4-fold for DHEA in boys and girls between 3–4 and 7–8 yr of age. Accordingly, regression analysis (with sex entered as a dummy variable) performed in all children under 7.5 yr of age (range, 3–7.2 yr; n = 133) yielded a strong age dependency for DHEA&M ($P < 0.0001$), ADIOL ($P = 0.004$), androsterone ($P = 0.0002$), etiocholanolone ($P = 0.0004$), and C19 ($P < 0.0001$), but not for DHEA ($P = 0.33$). In the subsequent approximately 2-yr period, ranging from age 7.8–10.2 yr (n = 64), age was a significant predictor ($P < 0.01$) for all androgen variables (DHEA, DHEA&M, ADIOL, androsterone, etiocholanolone, and C19).

Global enzyme activities of 3β-HSD and 21-OH were sig-

TABLE 3. Urinary 24-h excretion rates of the sum of DHEA and its direct 16-hydroxylated downstream metabolites (DHEA&M) according to age and sex (μg/d)

Age (yr)	Boys							Girls						
	P5	P25	P50	P75	P95	Mean[a]	SD[a]	P5	P25	P50	P75	P95	Mean[a]	SD[a]
3–4	2.38	5.26	10.8	86.4	97.5	1.26	±0.60	2.88	5.10	29.6	87.8	118	1.34	±0.62
5–6	5.29	11.8	42.3	90.2	167	1.53	±0.52	9.06	28.7	69.4	103	197	1.71	±0.44
7–8	13.8	36.1	132	151	230	1.89	±0.49	41.4	66.9	79.5	121	254	1.94	±0.27
9–10	81.8	159	226	299	426	2.32	±0.26	51.5	103	164	227	1070	2.25	±0.37
11–12	128	249	361	584	3004	2.61	±0.37	84.2	168	223	399	931	2.40	±0.34
13–14	270	501	811	1204	4228	2.93	±0.35	193	376	493	865	1544	2.76	±0.28
15–16	451	592	857	1679	3866	3.00	±0.33	278	412	985	1626	2630	2.93	±0.38
17–18	677	1000	1324	3069	4597	3.24	±0.29	456	644	1383	2145	5323	3.11	±0.31

Data are percentiles and median (P50). Number of subjects per age group: 25 boys and 25 girls, with the exception of age group 3–4 yr, in which two boys showed undetectable concentrations of DHEA and DHEA metabolites.

[a] Mean ± SD of the logarithmized DHEA&M values; $P < 0.0001$ for age and $P = 0.3$ for sex (by two-way ANOVA, log DHEA&M).

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TABLE 4. Urinary 24-h excretion rates of ADIOL according to age and sex (μg/d)

Age (yr)	Boys							Girls						
	P5	P25	P50	P75	P95	Mean[a]	SD[a]	P5	P25	P50	P75	P95	Mean[a]	SD[a]
3–4	2.40	2.66	3.52	5.32	5.85	0.57	±0.15	1.87	2.26	3.10	4.84	7.04	0.53	±0.19
5–6	1.61	2.61	3.67	8.61	18.3	0.64	±0.32	2.12	3.37	5.25	7.38	12.3	0.71	±0.22
7–8	3.85	5.32	6.69	13.8	21.4	0.91	±0.27	2.35	3.66	5.71	8.88	29.4	0.78	±0.32
9–10	6.04	12.4	21.9	27.7	51.3	1.28	±0.30	6.18	8.20	11.3	19.5	76.6	1.16	±0.34
11–12	10.1	21.7	30.0	51.5	157	1.53	±0.34	2.73	11.8	20.0	28.0	106	1.29	±0.41
13–14	20.2	45.6	64.5	107	245	1.83	±0.31	11.6	22.2	36.3	53.7	85.0	1.53	±0.27
15–16	23.9	62.0	99.2	122	253	1.93	±0.29	26.3	39.8	70.3	89.6	222	1.81	±0.31
17–18	63.6	93.9	159	234	354	2.18	±0.26	27.6	46.6	59.9	158	311	1.89	±0.37

Data are percentiles and median (P50). Number of subjects per age group: 25 boys and 25 girls, with the exception of the three youngest age groups, in which ADIOL was not measurable in a limited number of children. Data represent 12 boys and 10 girls in group 3–4 yr, 20 boys and 20 girls in group 5–6 yr, and 21 boys and 23 girls in group 7–8 yr.

[a] Mean ± SD of the logarithmized ADIOL values; $P < 0.0001$ for age and $P < 0.0001$ for sex (by two-way ANOVA, log ADIOL).

nificantly influenced by age (Tables 6 and 7; by ANOVA, $P < 0.01$), but a gender effect was only seen for 3β-HSD. 3β-HSD activity significantly decreased (precursor/product ratio increased) between 3 and 12.4 yr in boys (n = 125; $P < 0.001$) and between 3 and 8.2 yr in girls (n = 75; $P < 0.05$). Thereafter, from 12.6–16.2 yr (n = 50) in boys and from 8.9–16.3 yr (n = 100) in girls, changes in 3β-HSD were no longer significant ($P > 0.1$). With respect to 21-OH, as reflected by the ratio of 21-deoxy-α-cortolone/α-cortolone (Table 7), steroidogenic enzyme activity significantly increased between 3 and 8 yr in boys (n = 75; $P < 0.05$) and between 3 and 10 yr in girls (n = 100; $P < 0.0001$) and was constant thereafter. However, indexes for 21-OH activity, used for monitoring treatment in 21-hydroxylase deficiency either by determining precursor metabolites or by the ratio of 17-hydroxyprogesterone metabolites to major cortisol metabolites (12), fell strongly with increasing age (Table 8). No significant age-related changes were seen for 17β-HSD and 5α-Red in boys and girls, but 5α-Red was higher in boys than in girls, especially with increasing age.

Discussion

This is, to our knowledge, the first report on 24-h urinary excretion rates of DHEA, its direct metabolites, and steroidogenic enzyme activities quantified in a large population of healthy children and adolescents using GC-MS. The clear increase in DHEA&M, *i.e.* the sum of DHEA and its 16-hydroxylated downstream metabolites, and the quantitatively most important C19 steroid metabolites, *i.e.* androsterone and etiocholanolone, from age 3 yr on provides strong evidence that adrenarche actually is a gradual process starting much earlier than generally believed. Palmert *et al.* (2) put forward the idea of adrenarche as a gradual process after longitudinally studying girls with idiopathic central precocious puberty. The present cross-sectional findings on urinary adrenarche markers confirm this for healthy boys and girls. However, if only DHEA, the primary C19 secretion product of the adrenal gland is considered, textbook knowledge would be confirmed, in that adrenarche appears to start around the age of 7 yr. The fact that in the youngest age group, only 11 boys and 12 girls had DHEA concentrations above the detection limit of (3 μg/liter), whereas almost all children (23 boys and 25 girls) showed a measurable 24-h excretion of 3β,16α,17β-androstenetriol, indicates that 16α-hydroxylation of DHEA or its 17β-reduced metabolite ADIOL is efficient in 3- to 4-yr-old children. The importance of 16α-hydroxylation, is strongly reflected in childhood and adolescence by the urinary excretion rate of DHEA&M being 4- to 11-fold higher than that of DHEA alone. Thus, efficient metabolism of DHEA, especially 16α-hydroxylation, can mask a substantial part of the adrenarchal increase in adrenal androgen secretion if the age-dependent pattern of only DHEA is examined. In contrast, longitudinal studies appear sensitive enough to detect the small individual increases in

TABLE 5. Mean values (± SD) of the logarithmized 24-h excretion rates of the quantitatively most important single androgen metabolites in urine, androsterone and etiocholanolone, and of the sum of major urinary androgen metabolites (C19) in boys and girls (μg/d)

Age (yr)	Boys			Girls		
	Androsterone[a]	Etiocholanolone[a]	C19[a]	Androsterone[a]	Etiocholanolone[a]	C19[a]
3–4	1.72 ± 0.16	1.64 ± 0.17	2.11 ± 0.19	1.78 ± 0.20	1.67 ± 0.20	2.16 ± 0.24
5–6	1.73 ± 0.25	1.62 ± 0.25	2.16 ± 0.28	1.87 ± 0.24	1.77 ± 0.22	2.31 ± 0.24
7–8	1.98 ± 0.21	1.90 ± 0.23	2.46 ± 0.24	1.98 ± 0.26	1.89 ± 0.27	2.45 ± 0.24
9–10	2.29 ± 0.23	2.19 ± 0.23	2.78 ± 0.22	2.37 ± 0.31	2.23 ± 0.26	2.80 ± 0.29
11–12	2.57 ± 0.27	2.42 ± 0.29	3.06 ± 0.30	2.53 ± 0.33	2.41 ± 0.32	2.96 ± 0.31
13–14	2.87 ± 0.29	2.73 ± 0.23	3.37 ± 0.27	2.88 ± 0.23	2.81 ± 0.23	3.33 ± 0.22
15–16	3.15 ± 0.28	2.96 ± 0.28	3.57 ± 0.25	3.05 ± 0.23	3.00 ± 0.26	3.51 ± 0.24
17–18	3.33 ± 0.18[b]	3.19 ± 0.23[b]	3.77 ± 0.19[b]	3.02 ± 0.20	2.98 ± 0.27	3.55 ± 0.22

Number of subjects per age group: 25 boys and 25 girls. C19 is the sum of androsterone, etiocholanolone, 5-androstene-3β,17α-diol, ADIOL, DHEA, 16α-hydroxy-DHEA, and 3β,16α,17β-androstenetriol.

[a] $P < 0.0001$ for age and $P > 0.3$ for sex (by two-way ANOVA).

[b] Due to significant sex by age interaction in each ANOVA ($P < 0.05$), gender differences were additionally tested by *t* test. Significant sex differences ($P < 0.01$) were observed only in the oldest age group for each androgen variable.

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TABLE 6. 3β-HSD activity [urinary ratio of (DHEA + 16α-hydroxy-DHEA + 3β,16α,17β-androstenetriol)/(androsterone + etiocholanolone)] according to age and sex

Age (yr)	Boys							Girls						
	P5	P25	P50	P75	P95	Mean[a]	SD[a]	P5	P25	P50	P75	P95	Mean[a]	SD[a]
3–4	0.03	0.05	0.14	0.75	0.83	0.37	±0.35	0.03	0.07	0.23	0.73	0.80	0.37	±0.34
5–6	0.10	0.15	0.54	0.76	1.00	0.49	±0.33	0.07	0.17	0.60	0.64	0.84	0.49	±0.26
7–8	0.12	0.38	0.61	0.75	1.45	0.61	±0.42	0.17	0.41	0.57	0.61	1.17	0.56	±0.26
9–10	0.40	0.48	0.62	0.76	0.92	0.63	±0.19	0.12	0.32	0.48	0.62	1.05	0.51	±0.27
11–12	0.29	0.48	0.59	0.78	1.65	0.76	±0.50	0.24	0.32	0.39	0.48	1.19	0.48	±0.31
13–14	0.28	0.41	0.59	1.07	1.88	0.77	±0.49	0.21	0.31	0.36	0.46	1.50	0.49	±0.36
15–16	0.12	0.25	0.37	0.65	1.66	0.57	±0.52	0.15	0.24	0.39	0.57	1.29	0.47	±0.31
17–18	0.22	0.28	0.44	0.67	1.65	0.56	±0.41	0.26	0.40	0.56	1.02	1.46	0.74	±0.44

Data representing the precursor/product ratio of 3β-HSD are given as percentiles, median (P50), and mean ± SD. Number of subjects per age group: 25 boys and 25 girls, with the exception of age group 3–4 yr, in which 3β-HSD could not be calculated in two boys due to undetectable concentrations of DHEA and DHEA metabolites.

[a] $P < 0.01$ for age and $P < 0.05$ for sex (by two-way ANOVA).

urinary DHEA (4), which is mainly excreted in the urinary steroid sulfate fraction (13), or circulating DHEA sulfate (2) early in childhood. The early increase in the urinary output of ADIOL, the direct conversion product of DHEA by 17β-hydroxysteroid dehydrogenase (14), also emphasizes that adrenarche actually starts at a preschool age. Our results of the adrenarchal metabolome in the youngest age groups are in accordance with the histomorphological findings of Dhom (15), who characterized the emergence of the zona reticularis in the adrenals of children; he observed that the focal island of adrenal reticularis appears in children from the age of 3 yr onward.

A deficiency in 21-OH expression is known to cause excessive production of adrenal C19 steroids (5, 16), suggesting that 21-OH could play a role in adrenarche. A decrease in the activity of this enzyme would allow more substrate to flow toward synthesis of DHEA. At first glance, our observation of a strong fall in urinary diagnostic parameters of 21-OH activity with increasing age seems to confirm that adrenarche could be driven by changing 21-OH expression. However, this is in contrast to published results of immunohistochemical examinations in adrenal glands from children and adults showing an overall constant activity of this enzyme not only with increasing age, but also between the fasciculata and the reticularis (5). In contrast, our alternatively suggested precursor/product ratio for the characterization of 21-OH, the 21-deoxy-α-cortolone/α-cortolone ratio, was at least partly in line with the immunohistochemical findings of Gell *et al.* (5); from 7–8 yr in boys and from 9–10 yr in girls this ratio was constant until age 17–18 yr. Whether the 21-deoxy-α-cortolone/α-cortolone ratio allows a more sensitive noninvasive monitoring of treatment effects in 21-hydroxylase deficiency than the usually determined urinary parameters (12) is speculative, but deserves additional study.

Rich *et al.* (17) examined changes in certain plasma precursor-product relationships in children with evidence of excess C19 steroid production and detected a decrease in 3β-HSD efficiency. Our 24-h urine-based hormone measurements in healthy children are in line with these earlier findings and correspond to the immunohistochemical examinations by Gell *et al.* (5) of sections of adrenal glands from children retrieved from autopsy files. Adrenal sections of children less than 5 yr of age demonstrated greater immunodetectable levels of 3β-HSD in the reticularis zone than that of children aged 8–13 yr (5). This loss of 3β-HSD would allow for steroid precursors to proceed toward the synthesis of DHEA and could thus explain part of the adrenarchal process (18). Decreased 3β-HSD expression in the adrenal zona reticularis is, according to Arlt *et al.* (1), one prerequisite for adrenarche. However, adrenal androgen production continues to rise throughout adolescence, although 3β-HSD activity no longer showed consistent decreases beyond age 11–12 yr. This might indicate that in older children, adrenarche is no longer primarily a result of adrenal loss of 3β-HSD

TABLE 7. 21-OH activity [urinary ratio of 21-deoxy-α-cortolone[a]/α-cortolone (5β-pregnane-3α, 17α,20α-triol-11-one/5β-pregnane-3α, 17α,20α,21-tetrol-11-one)] according to age and sex

Age (yr)	Boys							Girls						
	P5	P25	P50	P75	P95	Mean[b]	SD[b]	P5	P25	P50	P75	P95	Mean[b]	SD[b]
3–4	0.007	0.011	0.014	0.048	0.148	0.044	0.055	0.009	0.012	0.032	0.075	0.129	0.051	0.047
5–6	0.007	0.008	0.011	0.024	0.071	0.020	0.021	0.007	0.010	0.013	0.028	0.074	0.025	0.025
7–8	0.008	0.009	0.011	0.020	0.045	0.016	0.014	0.008	0.011	0.015	0.024	0.055	0.020	0.015
9–10	0.007	0.008	0.010	0.018	0.045	0.017	0.016	0.006	0.007	0.009	0.013	0.019	0.011	0.004
11–12	0.006	0.008	0.009	0.028	0.061	0.019	0.018	0.005	0.006	0.008	0.011	0.022	0.010	0.008
13–14	0.005	0.008	0.010	0.015	0.071	0.017	0.021	0.005	0.006	0.008	0.014	0.035	0.012	0.009
15–16	0.005	0.007	0.008	0.011	0.022	0.010	0.005	0.004	0.007	0.008	0.011	0.017	0.009	0.004
17–18	0.005	0.006	0.008	0.023	0.052	0.016	0.014	0.004	0.006	0.009	0.014	0.028	0.011	0.009

Data representing the precursor/product ratio of 21-OH are given as percentiles, median (P50), and mean ± SD. Number of subjects per age group: 25 boys and 25 girls.

[a] 21-deoxy-α-cortolone = 11-oxopregnanetriol.

[b] $P < 0.0001$ for age and $P = 0.57$ for sex (by two-way ANOVA).

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TABLE 8. Urinary diagnostic parameters (mean ± SD) of activities of 21-OH, 17β-HSD, and 5α-Red in boys and girls

Age (yr)	Boys				Girls			
	21-OH[a]	21-OH[b]	17β-HSD[c]	5α-Red[c]	21-OH[a]	21-OH[b]	17β-HSD[c]	5α-Red[c]
3–4	1.06 ± 0.28	0.046 ± 0.017	0.44 ± 0.13	1.20 ± 0.17	1.13 ± 0.26	0.057 ± 0.014	0.58 ± 0.22	1.30 ± 0.27
5–6	1.07 ± 0.30	0.046 ± 0.013	0.69 ± 0.28	1.32 ± 0.23	1.16 ± 0.26	0.056 ± 0.014	0.57 ± 0.19	1.29 ± 0.26
7–8	1.17 ± 0.21	0.060 ± 0.016	0.66 ± 0.17	1.26 ± 0.44	1.18 ± 0.26	0.066 ± 0.020	0.63 ± 0.22	1.25 ± 0.24
9–10	1.37 ± 0.20	0.064 ± 0.018	0.67 ± 0.40	1.27 ± 0.26	1.35 ± 0.27	0.066 ± 0.018	0.56 ± 0.28	1.45 ± 0.46
11–12	1.52 ± 0.20	0.074 ± 0.026	0.55 ± 0.36	1.42 ± 0.35	1.44 ± 0.19	0.075 ± 0.021	0.61 ± 0.33	1.34 ± 0.38
13–14	1.83 ± 0.29	0.099 ± 0.044	0.53 ± 0.55	1.50 ± 0.65	1.93 ± 0.32	0.118 ± 0.042	0.52 ± 0.24	1.23 ± 0.32
15–16	2.10 ± 0.31	0.133 ± 0.047	0.92 ± 0.61	1.66 ± 0.61	1.91 ± 0.31	0.134 ± 0.047	0.55 ± 0.35	1.30 ± 0.70
17–18	2.27 ± 0.28	0.163 ± 0.062	0.60 ± 0.48	1.51 ± 0.59	1.97 ± 0.28	0.151 ± 0.049	0.44 ± 0.25	1.21 ± 0.59

Number of subjects per age group: 25 boys and 25 girls. In the three youngest age groups, the metabolite ratio for 17β-HSD could not be calculated in all children due to partly undetectable concentrations of ADIOL or DHEA. 17β-HSD data represent 10 boys and six girls (3–4 yr), 17 boys and 17 girls (5–6 yr), and 20 boys and 23 girls (7–8 yr).

[a] Activity of 21-OH represented by logarithmized urinary 24-h excretion rates of the precursor metabolite 17-hydroxypregnanolone (sum of 5β-pregnane-3α,17α-diol-20-on + 5α-pregnane-3α, 17α-diol-20-on).

[b] Activity of 21-OH was calculated as precursor/product ratio from the urinary ratio of the sums of 17-hydroxyprogesterone metabolites to major cortisol metabolites, *i.e.* (17-hydroxypregnanolones + pregnanetriol + 11-oxopregnanetriol)/(tetrahydrocortisol + tetrahydrocortisone + 5α-tetrahydrocortisol).

[c] Activities of 17β-HSD and 5α-Red were calculated as product/precursor ratio from the urinary ratios of ADIOL to DHEA and androsterone to etiocholanolone, respectively.

[a,b,c] By two-way ANOVA: 21-OH[a], 21-OH[b], $P < 0.0001$ for age and $P > 0.1$ for sex; 17β-HSD, $P = 0.07$ for age and $P = 0.08$ for sex; 5α-Red, $P = 0.19$ for age and $P < 0.05$ for sex.

activity, but of the age-dependent growth of a 3β-HSD-deficient reticularis zone (5).

Although the enzymes 17β-HSD and 5α-Red, which are involved in sex steroid formation, do not appear to play a role in the developmental process of adrenarche itself, these enzyme activities and the patterns of the other variables of the urinary metabolome from childhood to young adulthood may be usable for the diagnosis of altered androgen metabolism, premature adrenarche, and/or polycystic ovary syndrome. In polycystic ovary syndrome, for example, enhanced peripheral 5α-Red activity seems to be a metabolic characteristic occurring together with an at least partly adrenal-induced hyperandrogenism (19, 20). Also, hypotheses, such as premature adrenarche as a potential forerunner of obesity and its sequelae (21, 22), might be studied in a greater detail using our adrenarchal reference values.

In summary, we have established reference values for urinary markers of adrenarche using GC-MS. DHEA together with its 16-hydroxylated downstream metabolites, ADIOL and C19, show a continuous rise from age 3–4 to 17–18 yr, which strongly suggests that adrenarche is a gradual process starting at an early preschool age. The urinary metabolome of healthy children confirms that decreasing 3β-HSD activity during childhood may contribute to the developmental increase in adrenal androgen secretion. The present reference values could be useful to identify nutritional, environmental, and pathophysiological influences on the progressive maturational process of adrenarche. Our data may also be used for the diagnosis of premature adrenarche and early metabolic signs of polycystic ovary syndrome.

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