

Changes in adrenal androgens and steroidogenic enzyme activities in children aged 2, 4, and 6 years: Steroid hormone profiling from the prospective cohort study

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Introduction

- Adrenarche refers to the increase in adrenal androgen synthesis. However, process of adrenal androgen production in early childhood remains to be elucidated.
- The aim of this study was to evaluate changes in adrenal androgen levels and steroidogenic enzyme activities associated with adrenarche using a prospective cohort.

Methods

- A total of 229 children (124 boys, 52.4%), who had participated in the Environment and Development of Children (EDC) cohort at age 2, 4, and 6 years old were enrolled. Anthropometric data at each visit and birth data were collected.
- Steroid profiles were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). A total of 10 adrenal hormones were measured including dehydroepiandrosterone (DHEA), DHEA sulfate (DHEA-S), 17-hydroxyprogesterone, androstenedione, testosterone, pregnenolone sulfate, cholesterol sulfate, testosterone, progesterone, 17-hydroxypregnenolone, and pregnenolone.
- Steroidogenic enzyme activities were calculated using precursor/product ratios, such as 17 α -hydroxylase, 17,20-lyase, 3 β -hydroxysteroid dehydrogenase (HSD), 17 β -HSD, and DHEA sulfotransferase.
- Steroid levels and enzyme activities were compared according to age and sex. Biochemical adrenarche was defined as a DHEA-S concentration of 188.1 ng/mL using LC-MS/MS. Factors affecting increasing levels of DHEA-S were analyzed.

Results

- Data for 200 subjects (114 boys, 57.0%) with all steroid profiling results at 2, 4, and 6 years were analyzed.
- DHEA, DHEA-S, and androstenedione increased between 2 and 4 years in both sexes. DHEA and androstenedione were higher in girls than in boys at the age of 6 years. DHEA sulfotransferase activity increased between 2 and 4 years in both sexes. Between 4 and 6 years, activities of 17 α -hydroxylase and 17,20-lyase increased, although 3 β -HSD and 17 β -HSD activities decreased. In girls, 17,20-lyase activity was higher and 3 β -HSD and 17 β -HSD activities were lower than in boys.
- Factors associated with increasing DHEA-S concentration over visits were age and body mass index. DHEA-S levels at the age of 6 years were significantly associated with being born small for gestational age and bone age on the third visit.
- Biochemical adrenarche was observed in 27 children (13.5%) with no sex difference.

Table 1. Steroid profiles by sex and visit

	Boys (n=114)				Girls (n=86)			
	Visit 1	Visit 2	Visit 3	P value	Visit 1	Visit 2	Visit 3	P value
DHEA	0.083±0.002	0.084±0.003	0.236±0.019	<0.001 ^{bc}	0.098±0.006	0.120±0.009	0.362±0.037	<0.001 ^{bc}
DHEA-S	14.0±1.1	19.0±1.5	52.1±5.3	<0.001 ^{abc}	15.9±1.3	24.9±2.6	58.8±6.0	<0.001 ^{abc}
Androstenedione	0.049±0.003	0.046±0.002	0.080±0.004	<0.001 ^{bc}	0.058±0.003	0.067±0.003	0.103±0.006	<0.001 ^{bc}
Pregnenolone-S	4.45±0.33	4.15±0.28	6.42±0.54	<0.001 ^{bc}	3.78±0.27	4.02±0.29	5.44±0.45	<0.001 ^{bc}
Cholesterol-S	1032.0±39.4	993.1±35.9	934.3±32.8	0.027 ^c	807.5±34.0	779.0±27.5	764.5±26.1	0.344
Testosterone	0.130±0.004	0.138±0.007	0.110±0.005	<0.001 ^{bc}	0.122±0.005	0.149±0.007	0.102±0.007	<0.001 ^{ab}
17-OH Progesterone	0.088±0.004	0.095±0.005	0.148±0.012	<0.001 ^{bc}	0.091±0.005	0.117±0.010	0.153±0.013	<0.001 ^{abc}
Progesterone	0.067±0.004	0.079±0.005	0.084±0.005	0.007 ^c	0.060±0.004	0.090±0.005	0.078±0.005	0.001 ^{bc}
17-OH Pregnenolone	1.224±0.099	0.919±0.087	1.391±0.119	<0.001 ^{ac}	1.000±0.087	0.949±0.093	1.177±0.125	0.135
Pregnenolone	0.171±0.008	0.216±0.010	0.294±0.016	<0.001 ^{abc}	0.180±0.011	0.244±0.016	0.291±0.020	<0.001 ^{abc}

*Unit: ng/mL

a: P <0.05 between Visit 1 and 2 from post-hoc analysis using Bonferroni method

b: P <0.05 between Visit 2 and 3 from post-hoc analysis using Bonferroni method

c: P <0.05 between Visit 1 and 3 from post-hoc analysis using Bonferroni method

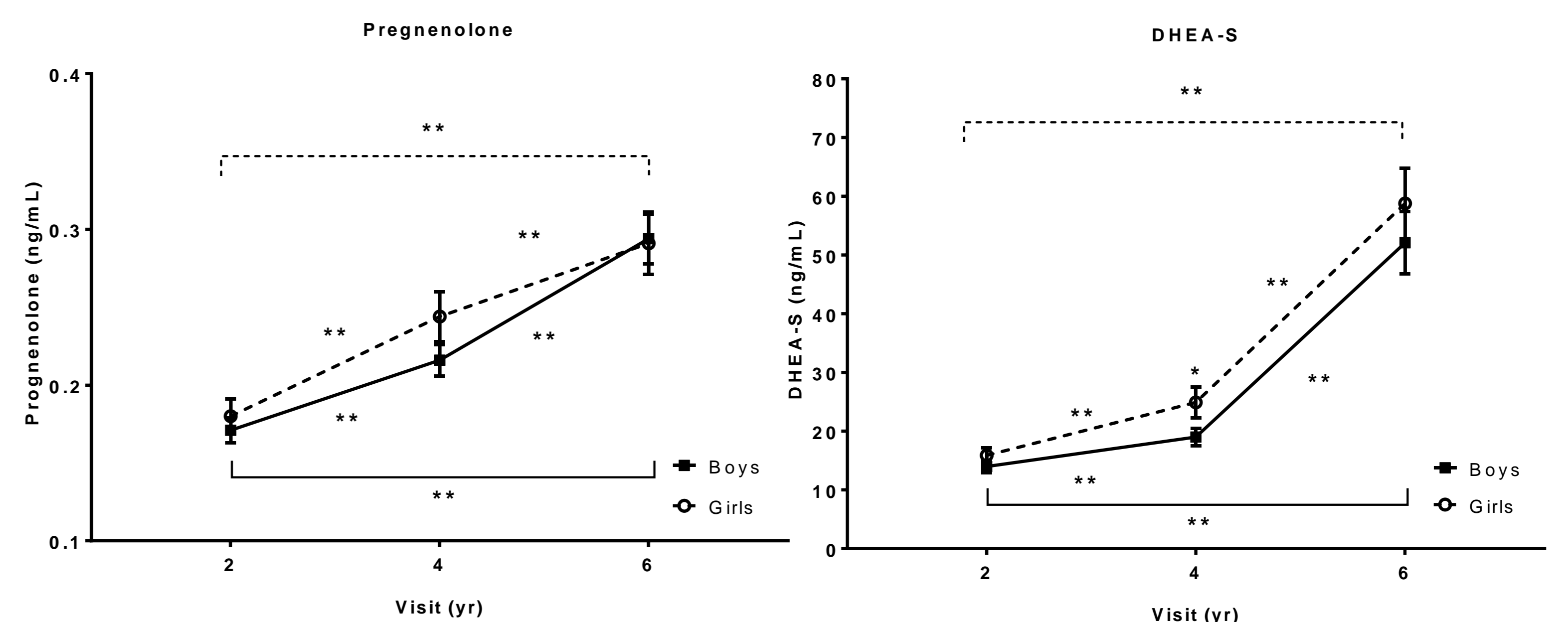


Figure 1. Plasma pregnenolone and DHEA-Sulfate levels by sex and visit. (*P < 0.05 between sex, ** P < 0.05 between visits)

Table 2. Steroidogenic enzyme activities by sex and age

	Boys (n=114)				Girls (n=86)			
	Visit 1	Visit 2	Visit 3	P value	Visit 1	Visit 2	Visit 3	P value
17 α -hydroxylase	7.85±0.62	4.85±0.41	6.04±0.41	<0.001 ^{abc}	6.27±0.58	4.67±0.40	5.86±0.53	<0.001 ^{ab}
17,20-lyase	0.112±0.009	0.148±0.013	0.240±0.023	<0.001 ^{abc}	0.162±0.014	0.201±0.020	0.429±0.044	<0.001 ^{bc}
3-beta HSD	2.21±0.08	2.28±0.10	0.84±0.06	<0.001 ^{bc}	1.90±0.10	1.82±0.13	0.60±0.05	<0.001 ^{bc}
17-beta HSD	2.66±0.15	3.00±0.20	1.36±0.09	<0.001 ^{bc}	2.08±0.12	2.40±0.15	0.98±0.08	<0.001 ^{bc}
DHEA Sulfotransferase	168.2±11.5	225.3±16.3	220.2±18.8	<0.001 ^{bc}	162.8±11.9	207.9±16.6	162.2±15.4	0.031 ^{ab}
Pregnenolone Sulfotransferase	26.0±2.1	19.2±1.2	21.9±1.7	<0.001 ^{bc}	20.9±1.7	16.5±1.2	18.7±1.7	<0.001 ^a

a: P <0.05 between Visit 1 and 2 from post-hoc analysis using Bonferroni method

b: P <0.05 between Visit 2 and 3 from post-hoc analysis using Bonferroni method

c: P <0.05 between Visit 1 and 3 from post-hoc analysis using Bonferroni method

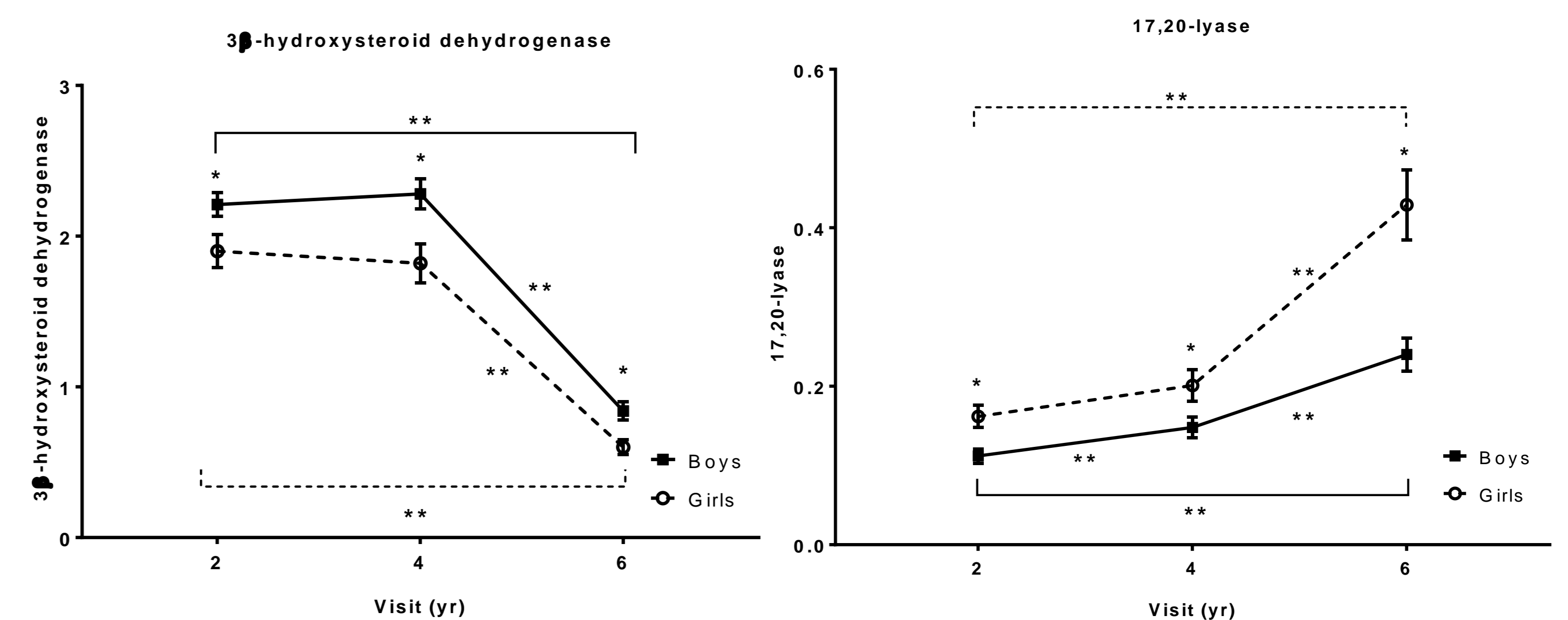


Figure 2. 3 β -hydroxysteroid dehydrogenase and 17,20-lyase activity by sex and visit. (*P < 0.05 between sex, ** P < 0.05 between visits)

Table 3. Factors associated with DHEA sulfate concentration at the age of 6 years

Variables	Univariate			Multivariate (Adjusted R ² = 0.106, P <0.001)		
	Coef.	S.E.	P-value	Coef.	S.E.	P-value
Sex	0.12	0.15	0.410	-0.26	0.16	0.111
BMI z-score	0.10	0.06	0.123	0.06	0.06	0.323
Small for gestational age	0.64	0.27	0.022	0.71	0.27	0.008
Bone age	0.35	0.09	<0.001	0.42	0.10	<0.001

* Multiple linear regression analysis

* Dependent variable (log-transformed DHEA sulfate)

Conclusion

- Adrenal androgens began to increase between the ages 2 to 4 years. Increased activity of DHEA sulfotransferase began between 2 and 4 years.
- Changes in steroidogenic enzyme activity to increase DHEA-S concentrations started between 4 and 6 years with increased 17,20-lyase and decreased 3 β -HSD activity.
- A longitudinal study with samples at the age of 8 years would be needed.