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2,4,6 세 소아에서 부신 안드로겐과 스테로이드 합성 효소 활성도의 변화: 전향적 코호트에서 스테로이드 호르몬 프로파일 분석

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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ABSTRACT

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 17α-hydroxylase, 17,20-lyase, 3β-hydroxysteroid dehydrogenase (HSD), 17β-HSD, and DHEA sulfotransferase. Steroid levels and enzyme activities were compared according to age and sex. 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 17α-hydroxylase and 17,20-lyase increased, although 3β-HSD and 17β-HSD activities decreased. In girls, 17,20-lyase activity was higher and 3β-HSD and 17β-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.

Conclusions: 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 3β-HSD activity. A longitudinal study with samples at the age of 8 years would be needed.

Keywords: Adrenarche, Adrenal androgen, Steroid profiling, Children, Cohort

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACTH, adrenocorticotropic hormone

BA, bone age

BMI, body mass index

DHEA, dehydroepiandrosterone

DHEA-S, dehydroepiandrosterone sulfate

EDC, Environment and Development of Children

IGF-1, insulin-like growth factor-1

LC-MS/MS, liquid chromatography-tandem mass spectrometry

LOQ, lower limit of quantification

SNP, Single nucleotide polymorphism

17-OHP, 17-hydroxyprogesterone

17-OHPreg, 17-hydroxypregnenolone

17β-HSD, 17β-hydroxysteroid dehydrogenase

3β-HSD, 3β-hydroxysteroid dehydrogenase

INTRODUCTION

The adrenal cortex synthesizes three different types of steroid hormones that play an important role in physiologic regulation during the human life span (1). Steroid hormones produced in adrenal glands have distinct characteristics: mineralocorticoids in zona glomerulosa, glucocorticoids in zona fasciculata, and adrenal androgens in zona reticularis (2, 3). These steroid hormones secreted from the adrenal cortex shows widespread effects in homeostasis of the human body.

Adrenal glands exhibit different histologic features between during fetal life and after birth. The fetal adrenal cortex is composed of an outer definitive zone, and a much larger inner fetal zone. In the definitive zone glucocorticoids and mineralocorticoids are synthesized, whereas in the fetal zone, androgenic precursors such as dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) are produced. A transitional zone exists between these two regions in the later phase of fetal development, although its function has not yet been elucidated (4, 5).

Adrenarche occurs through gonadotropin-independent adrenal activation before the onset of puberty, which is caused by gonadotropin-dependent hypothalamus-pituitary-gonadal axis activation (6-9). Adrenarche exists only

in human beings and some primates, although its clinical significance remains to be determined (7). Adrenal androgens, such as DHEA-S are known to affect neuroprotection, growth, bone density and erythropoiesis (10).

Adrenarche refers to the increase in adrenal androgens in childhood (11, 12). Biochemical adrenarche refers to the increased levels of adrenal androgen in blood, whereas clinical adrenarche refers to the status with clinical symptoms involving adrenal androgens, such as adult-type body odor, greasy hair, acne and/or comedone, axillary hair and/or pubic hair (6).

In the adrenal glands, various hormones are synthesized though the action of many steroidogenic enzymes (1, 13). DHEA and DHEA-S are adrenal androgens, which are very weak agonists of androgen receptors. The level of adrenal androgen does not appear to have any direct correlation with clinical symptoms (14, 15). Several enzymes, such as 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase 5 (17β-HSD), and 5α-reductase exhibit critical roles in adrenal androgen action. Moreover, dihydrotestosterone can be synthesized through the back-door pathway, without androstenedione or testosterone (16).

Adrenarche is a physiologic process during the normal growth and development of children. It commences before the onset of puberty, although longitudinal studies have not yet been performed to investigate changes in steroid hormone profiles and steroidogenic enzyme activities in early

childhood. Most research involving adrenarche has been cross-sectional. A study performed in a longitudinally followed cohort of children aged 2 years or more was lacking (17). Moreover, there is only one study involving Korean girls aged around 8 years, with a cross-sectional design (18). Factors affecting the onset of adrenarche remains to be elucidated.

For the measurement of steroid hormones, immunoassays have been employed. Although there are many advantages of immunoassay, critical limitations have been highlighted such as antibody selectivity, relatively large sample volumes, higher cost per sample, and a wide range of reproducibility. To the overcome the disadvantages of immunoassays, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been alternatively used to measure accurate levels of steroid hormones (19). LC-MS/MS has several strengths including high sensitivity, relatively small sample volumes, relatively low cost per sample, high reproducibility and quantification of multiple analytes in a single measurement (20, 21).

In the present study, we aimed to measure steroid hormone profiles and to evaluate steroidogenic enzyme activities related to adrenarche in a prospective children's cohort longitudinally followed-up from 2, 4 and 6 years of age. Factors affecting changes in DHEA-S concentration from 2 to 6 years of age and DHEA-S concentration at 6 years of age were investigated.

MATERIALS AND METHODS

Study participants

This study was approved by the Institutional Review Board of Seoul National University Hospital. Among children who participated in the Environment and Development of Children (EDC) cohort study (IRB No. C-1201–010-392), those who visited from 2, 4, and 6 years of age were included in this substudy.

The EDC cohort study is a prospective community-based cohort study that aims to investigate the effects of early-life environmental exposures from the prenatal period to early childhood, in terms of physical and neurobehavioral development (22). A total of 645 children (425 children aged 2 years and 220 children aged 4 years) were enrolled during 2012-2013, and 52 and 29 aged 4 years were additionally enrolled during 2014-2015. Enrolled subjects in the EDC cohort were followed up in the 2-year interval. Among 425 children who first participated in the EDC cohort at 2 years of age, 229 children who visited from 2, 4, and 6 years of age agreed to participate in the substudy (IRB No 1102-097-357 and 1806-072-949).

At each visit, responses to a structured questionnaire including details of birth history (gestational age and birth weight), sociodemographic and life

style factors, family history, and exposure to environmental factors, were obtained. Height (cm) and weight (kg) of participants were measured to one decimal place using a Harpenden Stadiometer (Holtain Ltd., Crymych, UK) and a digital weighing scale, respectively.

Height, weight, and body mass index (BMI) of study participants were transformed to z-scores using the 2017 Korean National Growth Charts (23). Additionally, bone age (BA) of the participants, which reflects skeletal maturation, was also assessed using Greulich-Pyle method. Body composition measurements were conducted via bioelectrical impedance analysis using an InBody 770 instrument (Biospace Co., Seoul, Republic of Korea). Blood samples were obtained in the morning after overnight fasting.

Small for gestational age (SGA) was defined as children born below the 3rd percentile from the mean weight for gestational age based on Korean reference (24). Biochemical adrenarche was defined as a DHEA-S concentration of 188.1 ng/mL using LC-MS/MS.

Steroid hormone profiling

(1) Reagents for steroid profiling and Calibration

Reagents for steroid profiling were used as follows: DHEA, DHEA-S, 17-hydroxypregnenolone (17-OHPreg), pregnenolone, and testosterone from Cerilliant Corporation (Round Rock, TX, USA); androstenedione,

pregnenolone sulfate, 17-hydroxyprogesterone (17-OHP), and d7-androstenedione from Steraloids Inc. (Newport, RI, USA); cholesterol sulfate, progesterone d5-DHEA, d5-DHEA-S, d9-progesterone, formic acid, zinc sulfate, hydroxylamine, bovine serum albumin, and charcoal from Sigma-Aldrich Inc. (St. Louis, MO, USA); d3-17-hydroxypregnenolone, d4-pregnenolone, d4-pregnenolone-S, d7-cholesterol-S, d2-testosterone, and d8-17-hydroxyprogesterone from C/D/N Isotopes Inc. (Pointe-Claire, Quebec, Canada). Other agents and organic solvents used were appropriate for HPLC.

Calibration stock solutions were prepared using 100% methanol, with a concentration of 1 mg/mL for DHEA-S and cholesterol sulfate, 100 μg/mL for pregnenolone sulfate, 10 μg/mL for DHEA, 17-OHP, 17-OHPreg, and pregnenolone, and 1 μg/mL for androstenedione, testosterone, and progesterone. A total of 8 calibration stock solutions were prepared after dilution with 1% bovine serum albumin in phosphate buffered saline (Table 1). Internal standards were constructed using 80% methanol, as described in Table 1.

2. Sample preparation for standards and quality control

In glass tubes, aliquots of 550 μL of serum samples or quality control materials and calibrators were added to combined internal standards (50 μL). The samples were extracted with 2 mL of methyl t-butyl ether (MTBE).

One mL of the organic phase was transferred into a glass vial and evaporated under nitrogen at 50 ℃. The dried residues were reconstituted in 100 μL of 70:30 methanol/water. For the analysis pregnenolone and 17-OHPreg, 400 μL of the organic phase was transferred into another glass vial and evaporated under nitrogen at 50 ℃. The dried residues were derivatized in 75 μL of hydroxylamine (0.7 mol/L, in 70:30 methanol/water) at 70 ℃ for 15min and 75 μL of water was added.

3. UPLC-MS/MS

An ACQUITY UPLC system (Waters, Milford, MA, USA) with Unison UK-C8 column (2 × 50 mm, 3 μm; Imtakt, Portland, OR, USA) was used for the analysis. After injection of 20 μL of reconstituted sample, analysis was performed for 10 minutes with 0.2% formic acid for mobile phase A and with 0.2% formic acid and 100% methanol for mobile phase B. Flow velocities and mobile phase A by time were as follows: 0.3 mL/min for 0-1 minutes, 90%; 0.3 mL/min for 1-6 minutes, from 90% to 1%; 0.3-0.8 mL/min for 6-6.1 minutes, 1%; 0.8 mL/min for 6.1-8.5 minutes, 1%; 0.8-0.3 mL/min for 8.5-9 minutes, from 1% to 90%; 0.3 mL/min for 9-10 minutes, 90%.

A Waters Xevo TQ-S tandem mass spectrometer (Waters) was used with a condition of electrospray ionization and multiple reaction monitoring (MRM). Sulfate steroids were measured in negative ion mode, and others were measure in positive ion mode. Detailed condition for analysis was descried in Table 2, including MRM transition (*m/z*), cone voltage, collision energy and dwell time. Measured steroids were analyzed using Target Lynx 4.0 software.

4. Evaluation of performance

In the evaluation of LC-MS/MS performance, within-run and between-run accuracy, linearity and lower limit of quantification (LOQ) are described in Table 3. Values below LOQ were calculated as LOQ divided by $\sqrt{2}$.

Assessment of steroidogenic enzyme activities

Steroidogenic enzyme activity was calculated using product/precursor ratio.

Pregnenolone sulfotransferase: Pregnenolone sulfate/pregnenolone

DHEA sulfotransferase: DHEA-S/DHEA

17α-hydroxylase: (17-OHPreg+DHEA)/pregnenolone

17,20-lyase: (DHEA+androstenedione)/17-OHPreg

3β-HSD: (Testosterone+androstenedione)/DHEA

17β-HSD: Testosterone/androstenedione

Statistical analysis

Statistical analysis was performed using Stata 14.2 software (StataCorp LP, College Station, TX, USA) and R software version 3.5.1 (www.r-project.org). Steroid hormone profiles and steroidogenic enzyme activities were log-transformed for analysis and were expressed as geometric mean ± standard error. Other data were expressed as mean ± SD. Student's *t*-test was used to compare means of adrenal androgens and steroidogenic enzyme activities according to sex at each visit. Repeated measure ANOVA was used to compare adrenal androgens and steroidogenic enzyme activities at the ages of 2, 4, and 6 years. Latent class mixed model analysis was used to classify groups according to trend of change of adrenal androgen (DHEA-S concentrations from 2, 4, and 6 years). Generalized additive mixed model analysis was used to evaluate factors affecting DHEA-S concentration from 2, 4, and 6 years. Univariate and multivariate linear regression analysis were performed to investigate factors affecting DHEA-S concentrations at 6 years of age. Univariate and multivariate logistic regression analyses were performed to investigate factors affecting biochemical adrenarche at 6 years of age. A P value < 0.05 was considered as statistically significant.

Table 1. Concentration of internal standards (ng/mL), standard stock solutions (μg/mL) and eight calibrators (ng/mL) for steroid compounds

Steroid compound	Internal Standards	Standard stock solution	Serially diluted concentration							
			0	1	2	3	4	5	6	7
DHEA-S	1000	1000	0	10	50	100	500	1000	5000	10000
Cholesterol sulfate	1000	1000	0	5	25	50	250	500	2500	5000
Pregnenolone sulfate	100	100	0	1	5	10	50	100	500	1000
DHEA	10	10	0	0.1	0.5	1	5	10	50	100
17-OHP	10	10	0	0.05	0.25	0.5	2.5	5	25	50
17-OHPreg	10	10	0	0.05	0.25	0.5	2.5	5	25	50
Pregnenolone	10	10	0	0.1	0.5	1	5	10	50	100
Androstenedione	1	1	0	0.01	0.05	0.1	0.5	1	5	10
Testosterone	1	1	0	0.01	0.05	0.1	0.5	1	5	10
Progesterone	1	1	0	0.01	0.05	0.1	0.5	1	5	10

Abbreviations: DHEA-S, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone; 17-OHP, 17-hydroxyprogesterone; 17-OHPreg, 17-hydroxypregnenolone.

Table 2. Tandem mass spectrometry conditions for steroid compounds

Steroid compounds	Ion mode	Parent Ion (*m/z*)	Daughter Ion (*m/z*)	Cone voltage (v)	Collision energy (v)	Dwell (sec)
DHEA, Quantifying	Positive	271.2	253.2	30	13	0.05
DHEA, Qualifying	Positive	271.2	213.2	30	15	0.05
d5-DHEA	Positive	276.3	258.3	25	13	0.05
Testosterone, Quantifying	Positive	289.1	97	30	20	0.003
Testosterone, Qualifying	Positive	289.1	109.1	30	25	0.003
d2-testosterone	Positive	291.2	99	40	25	0.003
Androstenedione, Quantifying	Positive	287.1	97.1	30	20	0.003
Androstenedione, Qualifying	Positive	287.1	109.1	30	23	0.003
d7- Androstenedione	Positive	294.2	100.1	30	20	0.003
Progesterone, Quantifying	Positive	315.2	97.1	25	25	0.003
Progesterone, Qualifying	Positive	315.2	109.2	25	25	0.003
d9-progesterone	Positive	324.2	100.1	25	23	0.003
17-OHP, Quantifying	Positive	331.2	97.1	25	25	0.003
17-OHP, Qualifying	Positive	331.2	109.2	25	25	0.003
d8-17-OHP	Positive	339.2	100.2	25	25	0.003
DHEA-S	Negative	367.3	97	25	30	0.05
d6-DHEA-S	Negative	373.3	98	25	30	0.05
Pregnenolone-S	Negative	395.2	97	25	32	0.05

d4-pregnenolone-S	Negative	399.2	97	25	30	0.05
Cholesterol-S	Negative	465.5	97	30	35	0.035
d7-cholesterol-S	Negative	472.5	97	30	35	0.035
Pregnenolone Quantifying	Positive (Oxime derv.)	332.3	86.2	35	26	0.02
Pregnenolone Qualifying	Positive (Oxime derv.)	332.3	300.3	35	22	0.02
d4-pregnenolone	Positive (Oxime derv.)	336.3	90.2	35	26	0.02
17-OHPreg, Quantifying	Positive (Oxime derv.)	348.3	330.3	25	10	0.05
17-OHPreg, Qualifying	Positive (Oxime derv.)	348.3	312.3	25	10	0.05
d3-17-OHPreg	Positive (Oxime derv.)	351.2	333.3	25	10	0.05

Abbreviations: DHEA-S, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone; 17-OHP, 17-hydroxyprogesterone; 17-OHPreg, 17-hydroxypregnenolone.

Table 3. Performance evaluation data for the developed methods including precision, linearity and lower limit of quantification (LOQ)

Compounds	Within-run (%)			Between-run (%)			Linearity		LOQ
	Low	Medium	High	Low	Medium	High	Range	R^2	(ng/ml)
DHEA	8.1	3.3	7.7	8.5	7.8	7.2	0~100	0.9925	0.1
DHEA-S	10.9	7.8	6.9	8.9	15.1	17.8	0~10000	0.9970	10
Androstenedione	5.3	4.2	3.6	5.1	4.1	12.7	0~10	0.9993	0.025
Pregnenolone sulfate	15.2	13.9	15.0	14.4	9.4	10.8	0~1000	0.9995	2.5
Cholesterol sulfate	4.1	4.4	3.6	17.1	6.3	2.7	0~50000	0.9996	10
Testosterone	5.3	3.8	2.1	6.6	7.5	10.8	0~10	0.9936	0.025
17-OHP	9.2	5.5	3.8	5.2	7.0	7.5	0~50	0.9964	0.1
Progesterone	12.7	8.2	9.2	17.6	7.4	10.2	0~10	0.9998	0.05
17-hydroxy-pregnenolone	4.6	3.9	2.9	9.0	9.5	5.9	0~50		0.1
Pregnenolone	3.7	3.0	5.5	11.7	6.7	5.2	0~100		0.1

Abbreviations: DHEA-S, dehydroepiandrosterone sulfate; DHEA, dehydroepiandrosterone; 17-OHP, 17-hydroxyprogesterone; 17-OHPreg, 17-hydroxypregnenolone.

RESULTS

Clinical characteristics of the study subjects

Study participants visited 3 times. At each visit, mean age was 1.96 ± 0.06, 3.90 ± 0.08, and 5.93 ± 0.10 years in boys and 2.00 ± 0.06, 3.93 ± 0.09, and 5.97 ± 0.12 years in girls, respectively (Table 4). There were no significant differences in age, height z-score, weight z-score and BMI z-score at each visit between boys and girls (Table 5). Gestational age, birth weight, and number of participants born small for gestational age were not significantly different by sex. There were no data regarding clinical symptoms and/or signs of adrenarche.

Changes in steroid hormone profiles from 2, 4, and 6 years of age by sex

Analyses were performed for 200 participants (boys 114, girls 86) with available serum samples from 3 consecutive visits. Measured steroid hormones levels are shown in Tables 6 and 7 and in Figures 1-10.

DHEA and DHEA-S showed increasing tendency with age in both sexes. Pregnenolone, 17-OHP, pregnenolone sulfate and androstenedione increased between 4 and 6 years in both sexes. Except for testosterone and cholesterol sulfate, most androgens increased between 4 and 6 years (Table 6).

DHEA, androstenedione and cholesterol sulfate were significantly higher in girls at each visit. DHEA sulfate and 17-OHP at 4 years of age was higher in girls. Pregnenolone, 17-OHPreg, testosterone, progesterone, pregnenolone sulfate showed no significant differences between sexes at each visit (Table 7).

Changes in steroidogenic enzyme activities from 2, 4, to 6 years of age by sex

Values of steroidogenic enzyme activities are shown in Tables 8 and 9 and in Figures 11-16.

Activities of 17α-hydroxylase and 17,20-lyase increased between 4 to 6 years in both sexes. Activities of 3β-HSD and 17β-HSD decreased between 4 and 6 years of age in both sexes. DHEA sulfotransferase activity increased between 2 and 4 years in both sexes. However, DHEA sulfotransferase activity plateaued in boys and decreased in girls (Table 8). Activity of 17,20-lyase was higher in girls at each visit, although 17α-hydroxylase activity was not different between sexex. Activities of 3β-HSD and 17β-HSD were lower in girls at each visit. DHEA sulfotransferase activity was higher in boys at the age of 6 years (Table 9).

Factors affecting changes in DHEA-S concentrations from 2, 4, 6 years of age

Participants were divided into 2 Classes by the trend of DHEA-S levels after latent class mixed model analysis. Class 1 (88 boys and 70 girls) refers to a group with steady DHEA-S concentrations over visits. Class 2 (26 boys and 16 girls) refers to a group with increasing DHEA-S concentrations over visits. DHEA-S concentration among subjects in Class 1 were higher in girls than in boys at the ages of 4 and 6 years (Table 10 and Figure 17). Gestational age and birth weight between Class 1 and 2 were not different at each visit for both sexes. BA at 6 years of age in girls in Class 2 were significantly higher than those in Class 1, although BA of boys was not different (Table 10). Gestational age and birth weight were not significantly different in both sexes. Generalized additive mixed model analysis revealed that older age and higher BMI were significantly associated with increasing levels of DHEA-S. However, other variables, such as lower gestational age and birth weight, and female sex, were not consistently related to DHEA-S concentration according to the model employed (Table 11).

Factors affecting biochemical adrenarche and DHEA-S concentrations at 6 years of age

By regression analysis, factors affecting DHEA-S concentration at the age of 6 years were: those born small for gestational age ($P = 0.022$), and with increased BA at the age of 6 years ($P < 0.001$) (Table 12).

At 6 years of age, 27 (13.5%) children (15 boys, 12 girls) showed biochemical adrenarche. There was no sex difference. Enzyme activities were higher in the biochemical adrenarche group, such as 17,20-lyase, 3β-HSD, 17β-HSD, DHEA sulfotransferase and pregnenolone sulfotransferase (Table 13). However, in girls only DHEA sulfotransferase activity was significantly higher. By univariate logistic regression analysis, birth weight (OR 0.45, 95% CI 0.22-0.94) and BA at 6 years of age (OR 1.64, 95% CI 1.01-2.65) were significantly associated with the presence of biochemical adrenarche (Table 14).

Table 4. Baseline characteristics of study participants by visit

	Boys (n = 124, 54.2%)				Girls (n = 105, 45.8%)			
	Visit 1	Visit 2	Visit 3	*P* value	Visit 1	Visit 2	Visit 3	*P* value
Age (yr)	1.98±0.06	3.93±0.12	5.93±0.11	<0.001[abc]	1.98±0.06	3.94±0.11	5.94±0.13	<0.001[abc]
Height (cm)	86.8±3.9	102.3±3.6	116.1±4.4	<0.001[abc]	85.6±2.7	101.5±3.4	115.5±3.9	<0.001[abc]
Height z-score	-0.10±1.27	-0.20±0.89	0.03±0.95	0.229	-0.03±0.85	-0.11±0.82	0.15±0.87	0.079
Weight (kg)	12.6±1.3	16.6±1.9	21.6±3.5	<0.001[abc]	12.0±1.2	16.2±1.7	21.1±3.0	<0.001[abc]
Weight z-score	0.30±0.91	-0.20±0.89	0.03±1.10	<0.001[ac]	0.30±0.81	-0.13±0.93	0.06±0.92	0.003[a]
Body mass index (kg/m^2)	16.8±1.7	15.8±1.3	15.9±1.9	<0.001[ac]	16.3±1.4	15.7±1.2	15.8±1.7	0.002 [ac]
Body mass index z-score	0.50±1.28	-0.13±1.08	-0.18±1.21	<0.001[ac]	0.42±0.98	-0.09±1.02	-0.13±1.10	0.015[ac]
Gestational age (week)	39.1±1.6	-	-	-	39.0±1.6	-	-	-
Birth weight (kg)	3.2±0.5	-	-	-	3.1±0.5	-	-	-
Small for gestational age,	7 (5.7%)	-	-	-	9 (8.6%)	-	-	-

n (%)								

Data are expressed as mean±SD.

Superscripts mean $P < 0.05$ from post-hoc analysis using Bonferroni's method: a between Visits 1 and 2; b between Visits 2 and 3; and c between Visits 1 and 3.

Table 5. Baseline characteristics of study participants by sex

	Visit 1 (2 years old)			Visit 2 (4 years old)			Visit 3 (6 years old)		
	Boys (n = 124, 54.2%)	Girls (n = 105, 45.8%)	*P* value	Boys (n = 124, 54.2%)	Girls (n = 105, 45.8%)	*P* value	Boys (n = 124, 54.2%)	Girls (n = 105, 45.8%)	*P* value
Age (yr)	1.98±0.06	1.98±0.06	0.996	3.93±0.12	3.94±0.11	0.856	5.93±0.11	5.94±0.13	0.534
Height (cm)	86.8±3.9	85.6±2.7	0.008	102.3±3.6	101.5±3.4	0.081	116.1±4.4	115.5±3.9	0.237
Height z-score	-0.10±1.27	-0.03±0.85	0.652	-0.20±0.89	-0.11±0.82	0.410	0.03±0.95	0.15±0.87	0.310
Weight (kg)	12.6±1.3	12.0±1.2	<0.001	16.6±1.9	16.2±1.7	0.094	21.6±3.5	21.1±3.0	0.267
Weight z-score	0.30±0.91	0.30±0.81	0.989	-0.20±0.89	-0.13±0.93	0.414	0.03±1.10	0.06±0.92	0.442
BMI (kg/m^2)	16.8±1.7	16.3±1.4	0.032	15.8±1.3	15.7±1.2	0.430	15.9±1.9	15.8±1.7	0.525
BMI z-score	0.50±1.28	0.42±0.98	0.616	-0.13±1.08	-0.09±1.02	0.768	-0.18±1.21	-0.13±1.10	0.739
Gestational age (week)	39.1±1.6	39.0±1.6	0.671	-	-	-	-	-	-
Birth weight (kg)	3.2±0.5	3.1±0.5	0.077	-	-	-	-	-	-

Small for gestational age, n (%)	7 (5.7%)	9 (8.5%)	0.387	-	-	-	-	-	-

Data are expressed as mean±SD.

Abbreviations: BMI, body mass index.

Table 6. Steroid profiles by 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	0.062±0.003	0.103±0.006	<0.001[bc]
Pregnenolone sulfate	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 sulfate	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-OHP	0.088±0.004	0.095±0.005	0.148±0.012	<0.001[bc]	0.091±0.005	0.117±0.010	0.152±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[ac]
17-OHPreg	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]

All steroid hormone values were log-transformed for the analysis and described in terms of ng/mL.

Data are expressed as geometric mean±SE.

Superscripts mean $P < 0.05$ from post-hoc analysis using Bonferroni's method: a between Visits 1 and 2; b between Visits 2 and 3; and c between Visits 1 and 3.

Abbreviations: DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; 17-OHP, 17-hydroxyprogesterone; 17-OHPreg, 17-hydroxypregnenolone.

Table 7. Steroid profiles by sex

	Visit 1 (2 years old)			Visit 2 (4 years old)			Visit 3 (6 years old)		
	Boys	Girls	*P* value	Boys	Girls	P value	Boys	Girls	*P* value
DHEA	0.083±0.002	0.098±0.006	0.007	0.084±0.003	0.120±0.009	<0.001	0.236±0.019	0.362±0.037	0.001
DHEA-S	14.0±1.1	15.9±1.3	0.250	19.0±1.5	24.9±2.6	0.040	52.1±5.3	58.8±6.0	0.410
Androstenedione	0.049±0.003	0.058±0.003	0.023	0.046±0.002	0.062±0.003	<0.001	0.080±0.004	0.103±0.006	0.002
Pregnenolone sulfate	4.45±0.33	3.78±0.27	0.124	4.15±0.28	4.02±0.29	0.761	6.42±0.54	5.44±0.45	0.172
Cholesterol sulfate	1032.0±39.4	807.5±34.0	<0.001	993.1±35.9	779.0±27.5	<0.001	934.3±32.8	764.5±26.1	<0.001
Testosterone	0.130±0.004	0.122±0.005	0.175	0.138±0.007	0.149±0.007	0.261	0.110±0.005	0.102±0.007	0.414
17-OHP	0.088±0.004	0.091±0.005	0.641	0.095±0.005	0.117±0.010	0.022	0.148±0.012	0.152±0.013	0.801
Progesterone	0.067±0.004	0.060±0.004	0.183	0.079±0.005	0.090±0.005	0.156	0.084±0.005	0.078±0.005	0.362
17-OHPreg	1.224±0.099	1.000±0.087	0.093	0.919±0.087	0.949±0.093	0.816	1.391±0.119	1.177±0.125	0.218
Pregnenolone	0.171±0.008	0.180±0.011	0.476	0.216±0.010	0.244±0.016	0.116	0.294±0.016	0.291±0.020	0.923

All steroid hormone values were log-transformed for the analysis and described in terms of ng/mL.

Data are expressed as geometric mean±SE.

Abbreviations: DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; 17-OHP, 17-hydroxyprogesterone; 17-OHPreg, 17-hydroxypregnenolone.

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Table 8. Steroidogenic enzyme activity by visit

	Boys (n = 114)				Girls (n = 86)			
	Visit 1	Visit 2	Visit 3	*P* value	Visit 1	Visit 2	Visit 3	*P* value
17α-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]
3β-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]
17β-HSD	2.66±0.15	3.00±0.20	1.36±0.09	<0.001[bc]	2.08±0.12	2.40±0.15	0.99±0.08	<0.001[bc]
DHEA Sulfotransferase	168.2±11.5	225.3±16.3	220.2±18.8	<0.001[ac]	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[ac]	20.9±1.7	16.5±1.2	18.7±1.7	<0.001[a]

Data are expressed as geometric mean±SE.

Superscripts mean $P < 0.05$ from post-hoc analysis using Bonferroni's method: a between Visits 1 and 2; b between Visits 2

and 3; and c between Visits 1 and 3.

Abbreviations: HSD, hydroxysteroid dehydrogenase; DHEA, dehydroepiandrosterone.

Table 9. Steroidogenic enzyme activity by sex

	Visit 1 (2 years old)			Visit 2 (4 years old)			Visit 3 (6 years old)		
	Boys	Girls	*P* value	Boys	Girls	*P* value	Boys	Girls	*P* value
17α-hydroxylase	7.85±0.62	6.27±0.58	0.066	4.85±0.41	4.67±0.40	0.758	6.04±0.41	5.86±0.53	0.778
17,20-lyase	0.112±0.009	0.162±0.014	0.002	0.148±0.013	0.201±0.020	0.025	0.240±0.023	0.429±0.044	<0.001
3β-HSD	2.21±0.08	1.90±0.10	0.016	2.28±0.10	1.82±0.13	0.005	0.84±0.06	0.60±0.05	0.003
17β-HSD	2.66±0.15	2.08±0.12	0.003	3.00±0.20	2.40±0.15	0.018	1.36±0.09	0.99±0.08	0.002
DHEA Sulfotransferase	168.2±11.5	162.8±11.9	0.746	225.3±16.3	207.9±16.6	0.458	220.2±18.8	162.2±15.4	0.018
Pregnenolone Sulfotransferase	26.0±2.1	20.9±1.7	0.062	19.2±1.2	16.5±1.2	0.118	21.9±1.7	18.7±1.7	0.191

Data are expressed as geometric mean±SE.

Abbreviations: HSD, hydroxysteroid dehydrogenase; DHEA, dehydroepiandrosterone.

Table 10. Comparison of factors affecting changes in DHEA-S concentrations from 2, 4, and 6 years of age by sex and class

Age	Variables	Boys (n = 114)			Girls (n = 86)		
		Class 1 (n = 88)	Class 2 (n = 26)	*P* value	Class 1 (n = 70)	Class 2 (n = 16)	*P* value
All	Gestational age (week)	39.3±1.5	38.6±1.7	0.052	39.1±1.6	38.5±2.0	0.206
	Birth weight (kg)	3.3±0.5	3.1±0.6	0.079	3.1±0.5	3.0±0.8	0.411
2	DHEA-S (ng/mL)	9.8±0.5	47.0±5.6	<0.001	13.7±1.0	30.7±8.1	<0.001
	Body mass index (kg/m^2)	16.8±1.7	16.8±2.0	0.862	16.3±1.3	16.1±1.3	0.578
	Body mass index z-score	0.5±1.2	0.4±1.7	0.697	0.4±1.0	0.3±1.0	0.600
4	DHEA-S (ng/mL)	13.7±0.9	57.3±6.5	<0.001	18.1±1.6	100.4±13.5	<0.001
	Body mass index (kg/m^2)	15.7±1.3	16.0±1.4	0.394	15.6±1.2	15.3±1.2	0.325
	Body mass index z-score	-0.2±1.1	0.0±1.1	0.394	-0.1±1.0	-0.4±0.9	0.330
6	DHEA-S (ng/mL)	38.4±4.1	146.7±20.1	<0.001	41.8±3.7	262.5±21.3	<0.001

	Body mass index (kg/m^2)	15.9±1.9	15.9±2.0	0.953	15.7±1.7	15.5±1.9	0.592
	Body mass index z-score	-0.2±1.3	-0.2±1.2	0.948	-0.2±1.1	-0.4±1.2	0.498
	Bone age	5.8±0.6	6.0±0.6	0.304	6.5±0.8	7.3±0.5	0.001

*Class was divided after latent class mixed model analysis: Class 1, steady DHEA-S concentration over visits; Class 2, increasing DHEA-S concentration over visits.

Data are expressed as mean±SD, except for DHEA-S (geometric mean±SE).

Abbreviations: DHEA-S, dehydroepiandrosterone sulfate.

Table 11. Factors associated with changes in DHEA-S concentrations from 2, 4, and 6 years of age

Variables	Univariate			Multivariate (Adjusted R^2 = 0.274, P <0.05)		
	Coef.	S.E.	*P* value	Coef.	S.E.	*P* value
Age (yr)	0.34	0.02	<0.001	0.35	0.02	<0.001
Sex	0.18	0.11	0.104	0.15	0.11	0.175
Body mass index z-score	-0.10	0.04	0.009	0.11	0.03	0.001
Gestational age (week)	-0.08	0.03	0.024	-0.03	0.04	0.492
Birth weight (kg)	-0.27	0.11	0.010	-0.19	0.14	0.160

* Generalized additive mixed model

* Dependent variable (Class 2 over Class 1)

Table 12. Factors associated with dehydroepiandrosterone 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
Body mass index 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 (yr)	0.35	0.09	<0.001	0.42	0.10	<0.001

* Multiple linear regression analysis

* Dependent variable (log transformed level of dehydroepiandrosterone sulfate)

Table 13. Comparison of steroidogenic enzyme activities by the presence of biochemical adrenarche at the age of 6 years

Biochemical adrenarche	Total (n = 200)			Boys (n = 114)			Girls (n = 86)		
	No (n = 173)	Yes (n = 27)	*P* value	No (n = 99)	Yes (n = 15)	*P* value	No (n = 74)	Yes (n = 12)	*P* value
17α-hydroxylase	5.90±0.36	6.39±0.84	0.626	6.04±0.41	6.10±0.91	0.962	5.72±0.57	6.77±1.60	0.527
17,20-lyase	0.284±0.022	0.478±0.014	0.016	0.220±0.022	0.431±0.136	0.018	0.402±0.044	0.543±0.171	0.322
3β-HSD	0.79±0.04	0.46±0.08	0.001	0.93±0.07	0.46±0.09	0.001	0.63±0.05	0.46±0.16	0.181
17β-HSD	1.25±0.07	0.86±0.11	0.011	1.48±0.10	0.81±0.15	0.001	1.00±0.09	0.93±0.14	0.738
DHEA Sulfotransferase	165.8±9.9	513.0±103.0	<0.001	189.1±16.0	602.4±113.4	<0.001	139.0±10.9	419.7±163.3	0.018
Pregnenolone Sulfotransferase	19.3±2.1	29.7±5.5	0.013	20.5±1.7	33.2±7.8	0.038	21.9±1.7	18.7±1.7	0.151

Data are expressed as geometric mean±SE.

Abbreviations: HSD, hydroxysteroid dehydrogenase; DHEA, dehydroepiandrosterone.

Table 14. Factors associated with the presence of biochemical adrenarche at the age of 6 years

Variables	Univariate		Multivariate (P = 0.224)	
	OR (95% CI)	P value	OR (95% CI)	P value
Sex	1.07 (0.47, 2.42)	0.871	0.53 (0.18, 1.53)	0.242
Body mass index z-score	0.91 (0.64, 1.30)	0.620	0.87 (0.45, 1.69)	0.678
Percent body fat (%)	0.99 (0.93, 1.06)	0.809	1.00 (0.87, 1.14)	0.983
Gestational age (week)	0.82 (0.66, 1.02)	0.079	0.96 (0.68, 1.35)	0.801
Birth weight (kg)	0.45 (0.22, 0.94)	0.035	0.67 (0.18, 2.49)	0.801
Small for gestational age	2.56 (0.75, 8.72)	0.132	1.68 (0.32, 8.90)	0.542
Bone age (yr)	1.64 (1.01, 2.65)	0.045	1.94 (1.05, 3.59)	0.036

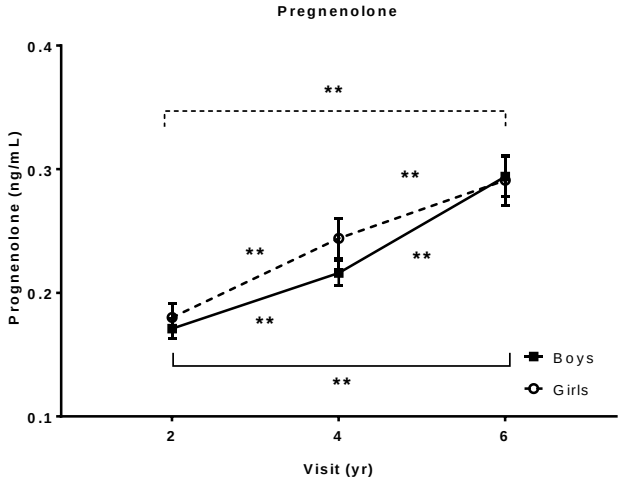

Figure 1. Plasma pregnenolone levels by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

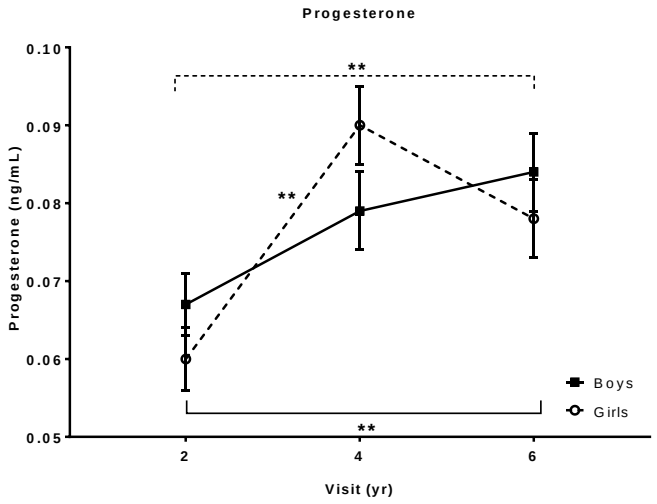

Figure 2. Plasma progesterone levels by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

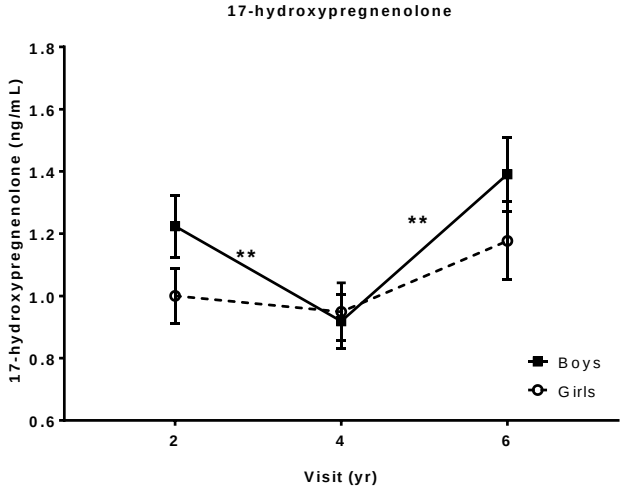

Figure 3. Plasma 17-hydroxypregnenolone levels by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

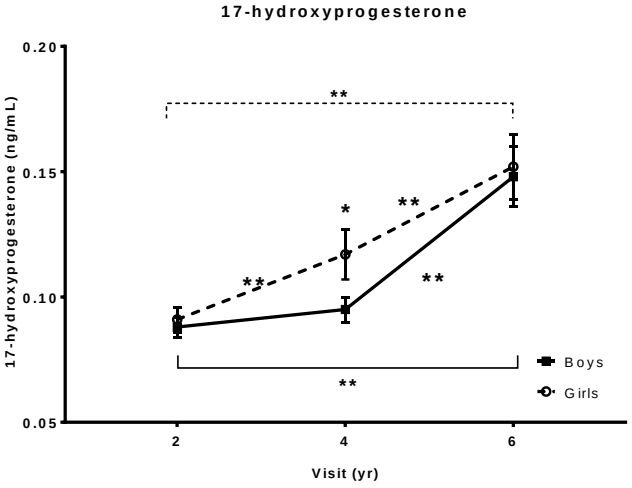

Figure 4. Plasma 17-hydroxyprogesterone levels by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

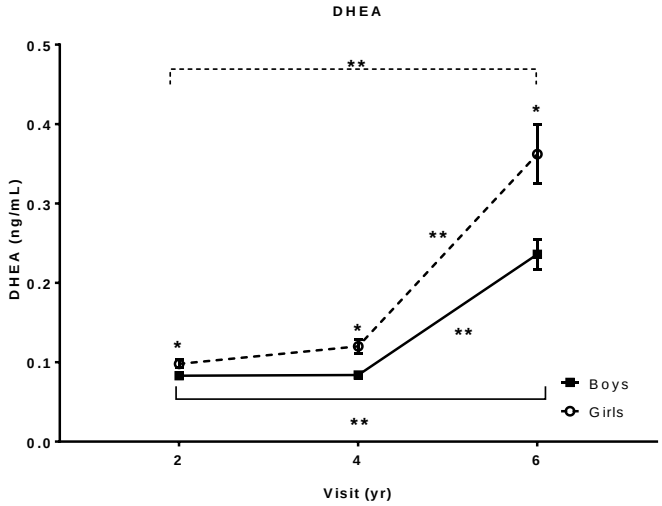

Figure 5. Plasma dehydroepiandrosterone (DHEA) levels by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

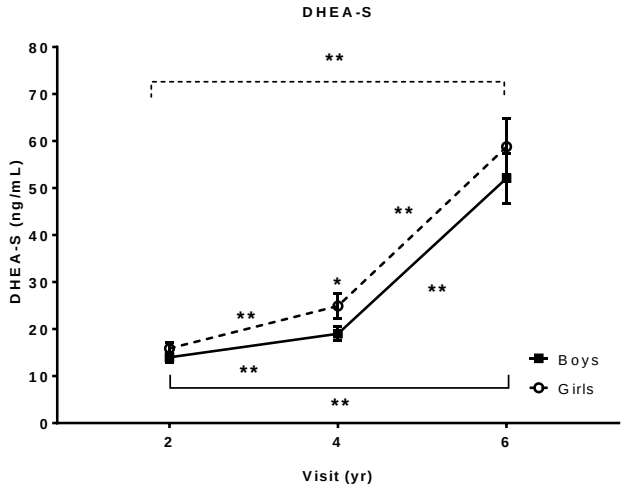

Figure 6. Plasma dehydroepiandrosterone sulfate (DHEA-S) levels by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

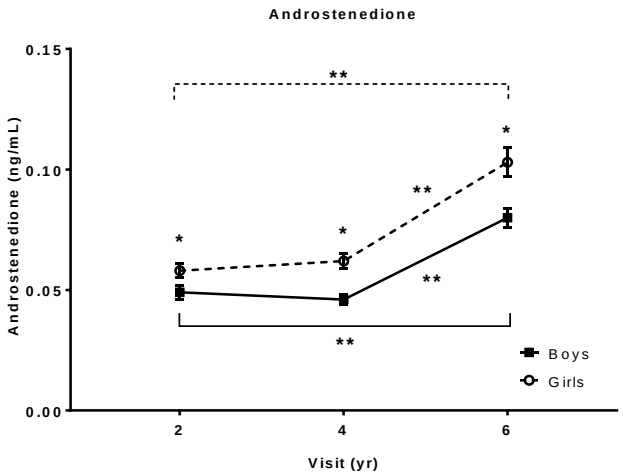

Figure 7. Plasma androstenedione levels by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

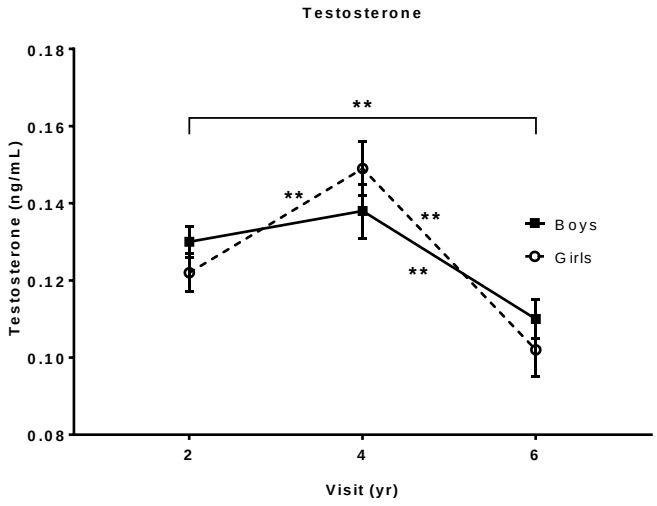

Figure 8. Plasma testosterone levels by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

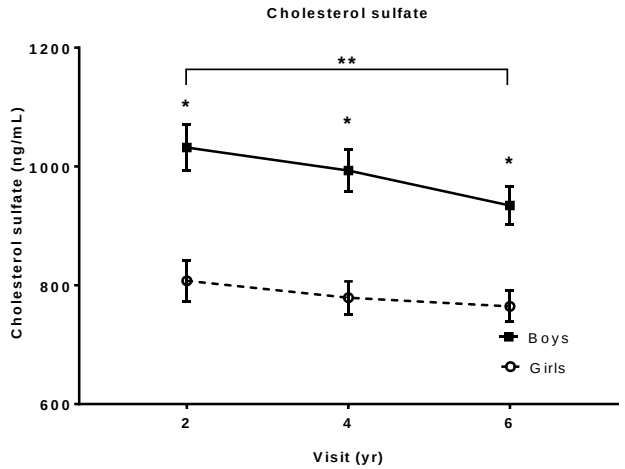

Figure 9. Plasma cholesterol sulfate levels by sex and visit. (*P < 0.05 between sex, ** P < 0.05 between visits)

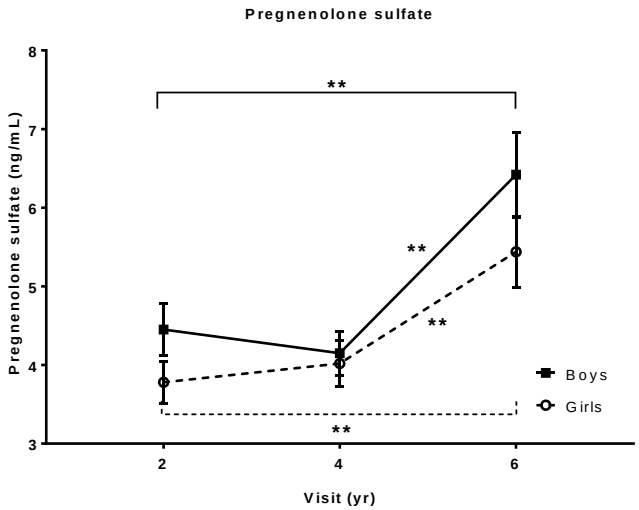

Figure 10. Plasma pregnenolone sulfate levels by sex and visit. (*P < 0.05 between sex, ** P < 0.05 between visits)

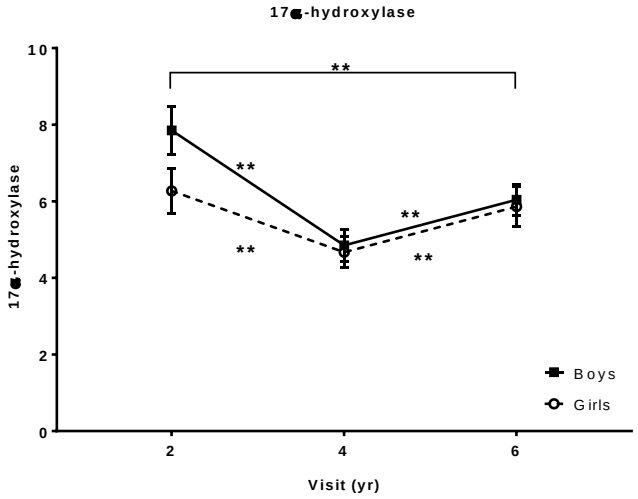

Figure 11. 17α-hydroxylase activity by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

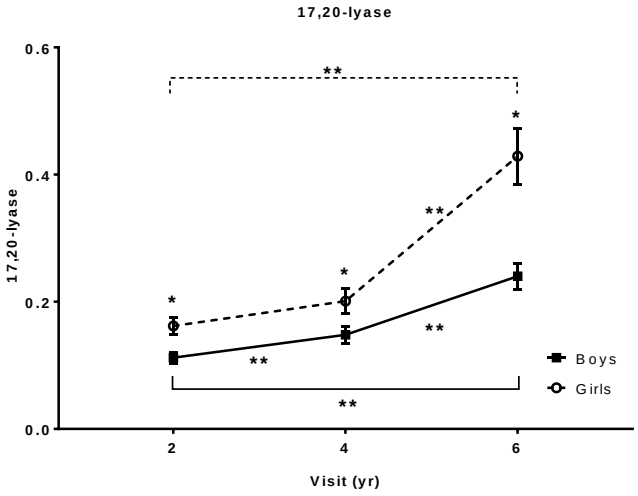

Figure 12. 17,20-lyase activity by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

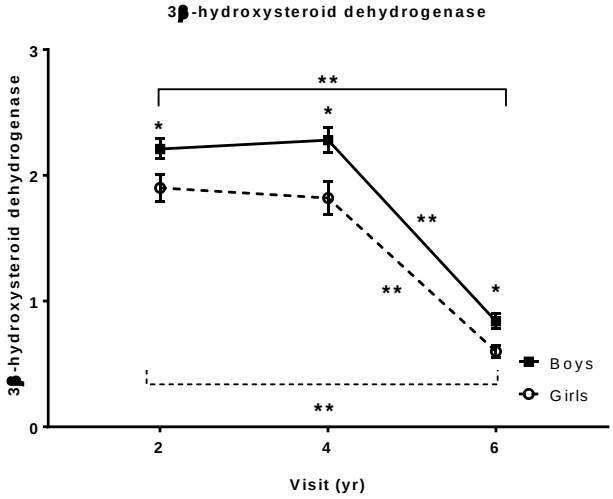

Figure 13. 3β-hydroxysteroid dehydrogenase activity by sex and visit. (*$P <$ 0.05 between sex, ** $P < 0.05$ between visits)

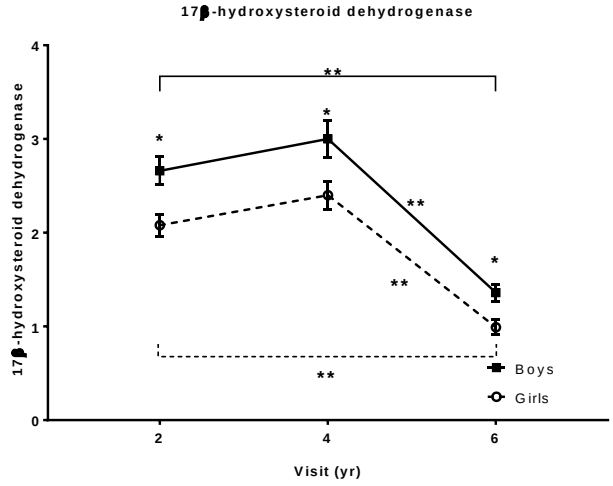

Figure 14. 17β-hydroxysteroid dehydrogenase activity by sex and visit. (*$P <$ 0.05 between sex, ** $P < 0.05$ between visits)

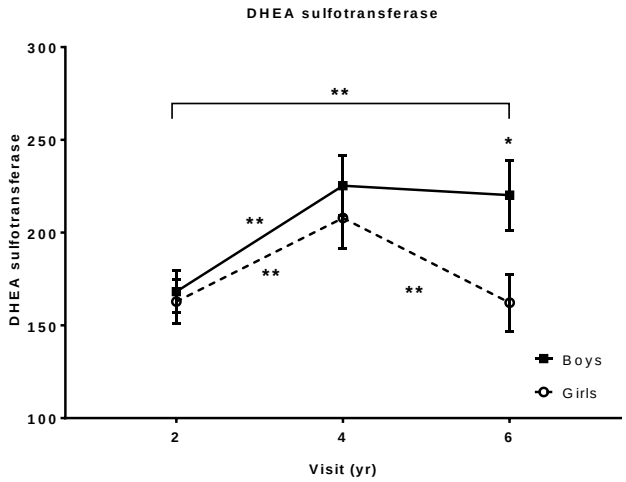

Figure 15. Dehydroepiandrosterone (DHEA) sulfotransferase activity by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

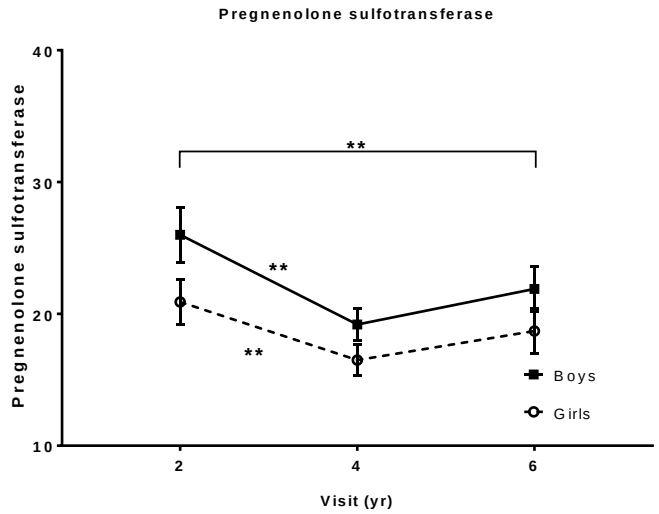

Figure 16. Pregnenolone sulfotransferase activity by sex and visit. (*$P < 0.05$ between sex, ** $P < 0.05$ between visits)

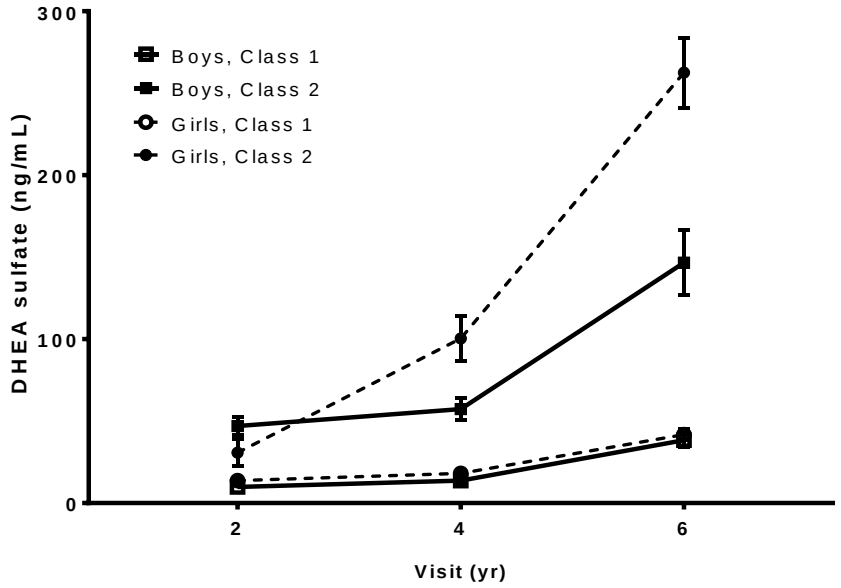

Figure 17. Dehydroepiandrosterone sulfate (DHEA-S) concentration by sex and Class. (Class 1, steady DHEA-S concentration over visits; Class 2, increasing DHEA-S concentration over visits.)

DISCUSSION

Adrenal glands have important roles in synthesizing and secreting steroid hormones. During the fetal period, adrenal cortex arises from the intermediate mesoderm. Approximately 33 days after conception, the adrenal blastema or the adrenal primordium becomes recognized as a distinct structure (25). By 50–52 days post-conception, fetal adrenal glands have two distinct zones: the inner fetal zone with large eosinophilic cells, and the outer definitive zone with small densely packed basophilic cells (26, 27). The morphology of the fetal adrenal cortex remains relatively constant during the fetal period. The transitional zone, which is located between definitive zone and fetal zone, has identified from the ultrastructural studies (4, 5). After birth, the fetal zone of the adrenal glands undergoes rapid involution, which results in a decreased levels of androgen (28, 29).

Adrenarche is a phenomenon, which only occurs in humans and some primates. The exact mechanism of onset and progression of adrenarche remains to be determined (10). During adrenarche, DHEA and DHEA-S levels increase in the zona reticularis without changes in secretion of , adrenocorticotropic hormone (ACTH) and cortisol (30). DHEA and DHEA-S, which are produced in the fetal adrenal cortex, have been used as substrates

for estrogen synthesis in placenta (1). After birth, the concentrations of DHEA and DHEA-S decreased rapidly according to the involution of the fetal zone of adrenal glands. Histologically, the zona reticularis of adrenal glands appears at the age of 3 years in humans as a form of focal islands. This pattern progresses further by the ages of 4 to 5 years (31). A continuous form of the zona reticularis is observed at age 6, and grows further until 12 to 13 years of age.

In the previous studies, the onset of adrenarche was associated with decreased activity of 3β-HSD, and with increased activity of 17,20-lyase and DHEA sulfotransferase. Majzoub et al. speculated that the onset of adrenarche is caused by suppression of 3β-HSD activity and increased production of DHEA related to normal body growth and associated increase in cortisol secretion by the adrenal glands (32). Because childhood obesity promotes this process, adrenarche begins earlier in obese children. In a study performed in prepubertal Korean girls, obese girls exhibited increased adrenal androgen secretion, which was partly associated with elevated 17,20-lyase activity (18).

In the present study, DHEA and DHEA-S concentrations showed increasing tendency with age, especially between the ages 4 to 6 years. DHEA was higher in girls at the age of 4 to 6 years. Pregnenolone and 17-OHPreg, the precursor of DHEA and DHEA-S, increased between 4 to 6 years of age. DHEA and androstenedione was higher in girls. These results were similar to

the observations that adrenarche begins at the age of 5 to 8 years and that girls show earlier onset of adrenarche (33).

In this study, activity of 3β-HSD decreased and activity of 17α-hydroxylase and 17,20-lyase increased between ages 4 and 6 years. Interestingly, DHEA sulfotransferase activity increased between 2 and 4 years of age. DHEA sulfotransferase plays an important role in converting DHEA to DHEA-S, which is important to start adrenarche. The present study revealed that the change in DHEA sulfotransferase activity was an early sign of adrenal androgen production. The increased activity in DHEA sulfotransferase, followed by decreased activity in 3β-HSD and increased activities in 17α-hydroxylase and 17,20-lyase activities, led to DHEA-S production at 6 years of age. DHEA sulfotransferase activity plateaued in boys, although it decreased in girls between ages 4 to 6 years. This was caused by a more rapid increase in DHEA concentrations in girls, because the activity was calculated using a formula with DHEA-S concentration divided by DHEA concentration.

At age 6, biochemical adrenarche was observed in 13.5% of the study population. In this study, there was no sex difference in the presence of biochemical adrenarche. A study performed in prepubertal Finnish children showed female predominance of clinical signs of androgen action and male predominance of biochemical adrenarche at the median age of 7.6 years (33).

Previous studies have reported that onset of adrenarche is associated with sex, gestational age, and obesity (34). Body fat percentage is also associated with the clinical signs of adrenarche (33). Insulin, insulin-like growth factor-1 (IGF-1), and leptin are associated with the adrenarche (33, 35). Single nucleotide polymorphisms (SNPs) including *MC2R*, *CYP19*, and *IGF-1R* gene SNPs also affect the onset of adrenarche (36-38). The number of androgen receptor CAG repeats is an associated factor of adrenarche (39).

In a recently published study, low vitamin D was associated with PA, and insulin resistance may play a role in this association (40). In a Korean study performed in children aged around 8 years, BA was independently associated with adrenal androgen levels (41). In the present study, higher BMI, older age, small for gestational age, and advanced BA were significantly associated with increased levels of DHEA-S, which corresponded with the previous studies. However, sex, birth weight, and gestational age were not significantly associated with increased DHEA-S.

There are several limitations in the present study. First, clinical symptoms of signs of adrenarche were not available. Due to the lack of reference values for adrenal androgens and steroidogenic enzyme activities, comparison of adrenal androgen by sex and age was difficult. The relatively small number of subjects in each group made it difficult to analyze according to BMI category and DHEA-S concentration. However, the strength of this study is the

evaluation of steroid hormone profiles and enzyme activities longitudinally in a well-designed prospective cohort samples. To the best of the author's knowledge, this is the first study to evaluate longitudinal changes in adrenal androgens in children aged 2, 4, and 6 years.

CONCLUSIONS

Adrenal adrogens began to increase between ages 2 to 4 years, and showed marked increases between 4 and 6 years of age. Moreover, 13.5% of children reached biochemical adrenarche by the age of 6 years. Increased activity of DHEA sulfotransferase began bewteen 2 and 4 years. Changes in steroidogenic enzyme activities to increase DHEA-S concentration commenced between 4 and 6 years, with increased 17,20-lyase and decreased 3β-HSD activity. For the further study to elucidate the process of adrenal androgen synthesis and enzyme activity, additional analysis would be necessary, with samples at the age of 8 years. Moreover, reference values of adrenal androgen by sex and age will be generated for such comparison.

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국문 초록

서론: 성증발생(Adrenarche)은 부신에서 안드로겐이 생성되는 것을 나타내지만, 아직 소아시기에서의 정확한 시작 기전에 대해서는 알려져 있지 않다. 이번 연구에서는 전향적 코호트에서 수집된 자료로 한국 어린이에서 adrenarche 의 시작과 관련된 부신 스테로이드 호르몬 및 부신 스테로이드 합성효소의 활성도를 분석하였다.

방법: 어린이 환경발달 코호트에서 2, 4, 6 세 때 추적관찰을 실시한 229 명(남자 124 명, 52.4%)을 대상으로 하였다. 신체계측 기록 및 출생 정보가 수집되었다. LC-MS/MS 방법으로 스테로이드 프로파일 분석을 실시하였다. 측정된 부신 호르몬은 dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), 17-hydroxyprogesterone, androstenedione, testosterone, pregnenolone sulfate, cholesterol sulfate, testosterone, progesterone, 17-hydroxypregnenolone, pregnenolone 이다. 전구물질과 생성물질의 비를 통하여 17α-hydroxylase, 17,20-lyase, 3β-hydroxysteroid dehydrogenase (HSD), 17β-HSD, DHEA Sulfotransferase 의

활성도를 계산하였고 이를 성별, 연령별로 비교하였다. DHEA-S 농도 증가와 관련이 있는 요인을 분석하였다.

결과: 2,4,6세 때의 스테로이드 프로파일 결과가 모두 있는 200명 (남자 114명, 57%)를 대상으로 분석을 실시하였다. DHEA, DHEA-S, androstenedione는 남녀 모두에서 2-4세 사이에 증가하였다. DHEA와 androstenedione은 6세 때 여아에서 높았다. DHEA sulfotransferase 활성도는 남녀 모두 2-4세 사이에 증가하였다. 4-6세 사이에는 17α-hydroxylase, 17,20-lyase의 활성도는 증가하고, 3β-HSD와 17β-HSD 활성도는 감소하였다. 여아에서 남아보다 17,20-lyase의 활성도는 높았고, 3β-HSD와 17β-HSD의 활성도는 낮았다. DHEA-S 농도가 증가하는 추세를 보이는 것과 관련된 인자는 연령과 체질량지수였다. 6세 때의 DHEA-S 농도와 관련이 있는 인자는 부당경량아 여부와 골연령이었다. 생화학적 adrenarche는 6세 때 총 27명 (13.5%) 에서 발견되었으며, 남녀 차이는 없었다.

결론: 2-6세 한국 어린이에서 부신 안드로겐은 2-4세에 증가하기 시작하여 4-6세 사이에 많이 증가한다. 부신 스테로이드 합성효소는 2-4세에 DHEA sulfotransferase 활성도가 증가하고, 4-6세 사이에 17,20-lyase의 증가, 3β-HSD의 감소를 보인다. 추후 장기적인

추적관찰 연구가 필요하다.

주요어 : 성증발생, 부신 안드로겐, 스테로이드 프로파일, 어린이, 코호트

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