RESEARCH

Female-dominant estrogen production in healthy children before adrenarche

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Abstract

Objective: Ultra-sensitive hormone assays have detected slight sex differences in blood estradiol (E_2) levels in young children before adrenarche. However, the origin of circulating E_2 in these individuals remains unknown. This study aimed to clarify how E_2 is produced in young girls before adrenarche.
Design: This is a satellite project of the Japan Environment and Children's Study organized by the National Institute for Environmental Studies.
Methods: We collected blood samples from healthy 6-year-old Japanese children (79 boys and 71 girls). Hormone measurements and data analysis were performed in the National Institute for Environmental Studies and the Medical Support Center of the Japan Environment and Children's Study, respectively.
Results: E_2 and follicle stimulating hormone (FSH) levels were significantly higher in girls than in boys, while dehydroepiandrosterone sulfate (DHEA-S) and testosterone levels were comparable between the two groups. Girls showed significantly higher E_2/testosterone ratios than boys. In children of both sexes, a correlation was observed between E_2 and testosterone levels and between testosterone and DHEA-S levels. Moreover, E_2 levels were correlated with FSH levels only in girls.
Conclusions: The results indicate that in 6-year-old girls, circulating E_2 is produced primarily in the ovary from adrenal steroids through FSH-induced aromatase upregulation. This study provides evidence that female-dominant E_2 production starts several months or years before adrenarche. The biological significance of E_2 biosynthesis in these young children needs to be clarified in future studies.

Key Words
- puberty
- paediatric endocrinology

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Introduction

The first physical signs of puberty in boys and girls are testicular enlargement and breast budding, respectively (1). Typically, these signs appear at 11–12 years of age in boys and at 9–10 years of age in girls (1). Several years before developing physical signs, children undergo conspicuous changes in the blood levels of sex hormones. Usually, an increase in circulating adrenal steroids ('adrenarche') occurs at 7 or 8 years of age in children of both sexes (1, 2, 3). Subsequently, an increase in circulating gonadal steroids ('gonadarche') takes place in a sex-specific manner. In girls, blood levels of estradiol (E_2) start to increase around 8 years of age, while in boys, testosterone levels typically increase at 9 years of age or later (1, 4, 5, 6). Thus, sexual dimorphism in blood sex hormone levels is evident in children above 8 years of age. Furthermore, ultra-sensitive hormone assays using liquid chromatography-tandem mass spectrometry (LC-MS/MS) have revealed slight sex differences in circulating E_2 levels in children below 7 years of age (6). These results indicate that sex-specific hormone production begins before adrenarche. However, given the limited number of previous reports, these data require further validation. Moreover, this notion raises a fundamental question of how E_2 is produced in young girls before adrenarche. To clarify the origin of circulating E_2 in young girls, we measured serum levels of sex hormones and gonadotropins in 150 healthy children at 6 years of age.

Materials and methods

Subjects

This project was conducted in the framework of a pilot study of the Japan Environment and Children's Study (JECS). JECS is a large-scale birth cohort study of the Japan Ministry of the Environment, which is organized by the National Institute for Environmental Studies. The participants of the present study were recruited during a JECS pilot study targeting 6-year-old children. The present study was approved by the ethics committee of the National Institute for Environmental Studies and that of four JECS pilot study-centers. This study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from the participants' parents or guardians.

The participants of this study consisted of 79 boys and 71 girls aged at 5.75–6.58 years (Supplementary Table 1, see section on supplementary materials given at the end of this article). These children were recruited from four JECS pilot study-centers. All children were apparently healthy and exhibited no physical signs of puberty. The age, height, and body weight were comparable between the boy and girl groups (Table 1). Blood samples were obtained either in the morning (42 boys and 36 girls) or afternoon (30 boys and 30 girls), while information about the sampling time was unavailable for seven boys and five girls.

Hormone measurements

Serum levels of E_2, testosterone, and dehydroepiandrosterone sulfate (DHEA-S) were measured using LC-MS/MS (LSI Medience, Tokyo, Japan). The methods were described previously (7). The lower detection limits of E_2, testosterone, and DHEA-S were 0.092 pmol/L (0.025 pg/mL), 0.017 nmol/L (5 pg/mL), and 0.14 μmol/L (50 pg/mL), respectively. Serum levels of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured using standard chemiluminescent enzyme immune assays (LSI Medience). The lower detection limits of LH and FSH were both 0.1 IU/L. We calculated blood E_2/testosterone ratios, which reflect the enzymatic activity of aromatase (8).

Statistical analysis

Sex differences in the hormone values and E_2/testosterone ratios were analyzed by the Wilcoxon rank sum test. Differences in hormone levels between samples obtained in the morning and afternoon were analyzed using the Wilcoxon signed rank test. Correlations between two hormone values were analyzed by the Spearman rank correlation test and the relationship between E2 and other sex hormones were examined by linear regression analysis. All statistical analyses were performed using R (version 4.0.0, R Core Team, Vienna, Austria). *P*-values of less than 0.05 were considered significant.

Table 1 Clinical data of the participants. Data are presented as the median (range).

	Boys	Girls	*P*-value
Number	79	71	
Age (years)	6.00 (5.83–6.58)	6.00 (5.75–6.50)	0.63
Height (cm)	114.2 (101.8–124.7)	113.5 (101.6–127.9)	0.73
Body weight (kg)	20.4 (14.4–35.1)	19.0 (13.9–27.7)	0.12

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Results

Blood hormone levels

The results of the 150 children are shown in Fig. 1 and Supplementary Table 1. In most children, serum levels of LH were undetectable, while levels of testosterone, E_2, DHEA-S, and FSH were low but above the detection limit. Thus, LH was excluded from statistical analyses.

Sex differences in blood hormone levels and E_2/testosterone ratios

Blood levels of E_2 and FSH were significantly higher in the girl group than in the boy group, whereas levels of DHEA-S and testosterone were comparable between the two groups (Fig. 1). Consequently, E_2/testosterone ratios were significantly higher in the girl group than in the boy group.

Blood samples obtained in the morning and afternoon contained almost equal levels of hormones, except for testosterone and E_2 (Supplementary Table 2). Testosterone levels in the boy and girl groups and E_2 levels in the boy group were relatively high in the samples obtained in the morning. However, the differences between samples obtained in the morning and afternoon were small.

In children of both sexes, serum E_2 levels were positively correlated with testosterone levels, and testosterone levels were correlated with DHEA-S levels (Table 2). Furthermore, E_2 levels were positively correlated with FSH levels in girls, but not in boys. Likewise, linear regression analysis revealed that E_2 levels was associated with testosterone and FSH levels only in girls (Table 3).

Discussion

We measured serum levels of sex hormones and gonadotropins in 150 healthy children at 6 years of age. In most children, levels of E_2, testosterone, DHEA-S, and FSH were above the detection limit, while LH levels remained extremely low. Blood levels of DHEA-S and testosterone in these children were lower than the levels previously documented for children during adrenarche (9, 10), indicating that our participants had not yet entered adrenarche. These results are consistent with the report by Tung *et al.* that the average age of adrenarche in the East

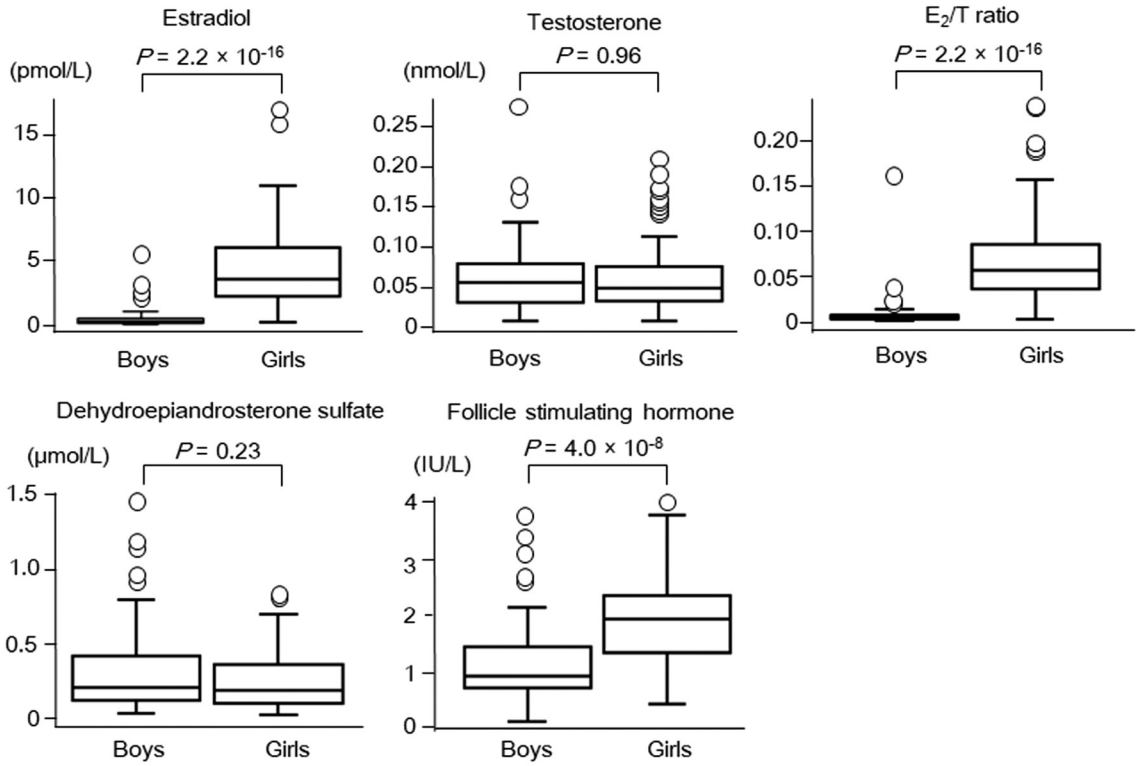

Figure 1
Blood hormone levels and estradiol/testosterone (E_2/T) ratios in boys and girls. The upper and lower borders of each box indicate the 75th and 25th percentiles, respectively. Thick horizontal lines represent the median; upper whisker ends, 75th percentile plus 1.5 interquartile range (IQR); and lower whisker ends, 25th percentile minus 1.5 IQR. The values that exceed indicated ranges are plotted as individual circles.

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Table 2 Correlation between two hormone values.

		P-value			
		E_2	DHEA-S	Testosterone	FSH
Boys					
r	E_2		3.12×10^{-15}	1.59×10^{-11}	0.81
	DHEA-S	0.75		1.04×10^{-13}	0.70
	Testosterone	0.67	0.72		0.14
	FSH	0.03	0.04	0.17	
Girls					
r	E_2		0.19	6.12×10^{-4}	3.09×10^{-3}
	DHEA-S	0.16		5.57×10^{-10}	0.40
	Testosterone	0.40	0.66		0.88
	FSH	0.35	−0.10	0.02	

E_2, estradiol; DHEA-S, dehydroepiandrosterone sulfate; FSH, follicle stimulating hormone.

Asian population is 7.7 years (3). Nevertheless, we found a significant difference in serum E_2 levels between boys and girls. This sexual dimorphism in circulating E_2 levels cannot be ascribed to the female-dominant E_2 production in the adrenal gland, because serum levels of DHEA-S, a marker for adrenal steroidogenesis (2, 11), were comparable between the boy and girl groups. Instead, a significant sex difference was observed in the E_2/testosterone ratios. These results indicate that in healthy children, female-dominant aromatase activation precedes adrenarche. This notion provides an answer to the previously raised question of why serum testosterone levels of a girl with congenital aromatase deficiency remained within the normal range until 5 years of age and became abnormally high thereafter (12). Notably, we found a slight difference in testosterone levels between samples obtained in the morning and afternoon. These results are consistent with the previous findings that there is a diurnal rhythm of testosterone secretion in both prepubertal and pubertal children (10, 13).

In children of both sexes, serum levels of E_2 were correlated with testosterone levels, which inturn were correlated with DHEA-S levels. These results imply that circulating E_2 in these children was synthesized primarily from adrenal steroids through the intermediacy of testosterone. We speculate that the conversion of testosterone to E_2 in girls occurs mostly in the ovary, because (i) *CYP19A1*, the gene encoding aromatase, is strongly expressed in the ovary (8, 14), (ii) blood E_2 levels in our female participants were higher than those in previously reported girls and young adults with pure ovarian dysgenesis (2.41 ± 1.67 pmol/L) (15), and (iii) Wilson *et al.* observed an increase in blood E_2 levels in healthy prepubertal girls at 5–12 years of age but not in age-matched girls with ovarian dysfunction due to Turner syndrome (16). However, it is possible that other *CYP19A1*-expressing tissues, such as the skin, adipose tissues, and bone (8) also contribute to the aromatization of circulating testosterone in young girls.

FSH levels were slightly higher in girls than in boys. Female-dominant FSH secretion in prepubertal children has been documented in previous reports (4, 17) and is likely to reflect sex-specific hypothalamic function that governs gonadotropin secretion (1). Notably, serum FSH levels were positively correlated with E_2 levels only in girls. These results indicate that in girls, FSH upregulates *CYP19A1* in the ovary to facilitate E_2 production. Indeed, FSH is known to stimulate *CYP19A1* expression in the ovary (18). Moreover, Francois *et al.* have shown that in normal infant mice, FSH induces E_2 production in the ovary without causing follicular growth (19). Interestingly, the authors proposed that E_2 production in the immature murine ovary is indispensable for the programming of future reproductive function. Thus, the relatively small increase in serum E_2 levels observed in our female participants may also be essential for ovarian function in later life. In contrast, LH is unlikely to contribute to the female-dominant E_2 production in young children, because serum levels of LH remained undetectable in most of our participants.

Table 3 Multiple linear regression model with estradiol as the outcome variable.

	Explanatory variable	Coefficient	Standard error	*T*-value	*P*-value
Boys	(intercept)	0.09	0.05	1.74	0.09
	DHEA-S	7.01×10^{-4}	2.68×10^{-4}	2.62	0.01
	Testosterone	6.51×10^{-5}	2.39×10^{-3}	0.03	0.98
	FSH	−0.02	0.03	−0.60	0.55
	Model summary; adjusted R-squared = 0.11, F-statistic *P*-value = 7.07×10^{-3}				
Girls	(intercept)	0.06	0.30	0.21	0.84
	DHEA-S	-1.28×10^{-3}	1.84×10^{-3}	−0.70	0.49
	Testosterone	0.038	0.01	3.75	3.68×10^{-4}
	FSH	0.27	0.12	2.33	0.02
	Model summary; adjusted R-squared = 0.26, F-statistic *P*-value = 4.01×10^{-5}				

DHEA-S, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone.

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This study has several limitations. First, this is a cross-sectional study. Thus, we could not determine the onset age of female-dominant E_2 production. Since previous studies have shown that blood FSH levels are constantly higher in girls than in boys from infancy through adolescence (4), there may be a sex difference in aromatase activity even in children under 6 years of age. Second, we did not measure levels of sex hormone binding globulin. Blood levels of total fractions of sex hormones may not be parallel with the levels of unbound (free and bioavailable) hormones (20). Third, our conclusions are based solely on the results of blood hormone measurement. Venous sampling is useful to determine the tissue origin of circulating sex hormones in children, although this test is generally not feasible in humans. Lastly, we did not address the functional importance of E_2 production in young girls. Further studies are necessary to clarify whether sex hormone production in childhood is associated with sexual maturation during puberty or reproductive function in adulthood.

In conclusion, the results show that sexual dimorphism in sex hormone production is already apparent in 6-year-old children. Excessive circulating E_2 in these girls is most likely synthesized in the ovary from adrenal steroids through FSH-induced aromatase upregulation. The biological significance of E_2 production in such young children needs to be clarified in future studies.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-21-0134.

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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