Sex hormone levels in young children: a pilot study of the Japan Environment and Children's Study (JECS)

LB-P6

Tadayuki Ayabe,[1] Maki Fukami,[1] Kiwako Yamamoto-Hanada,[1] Hidetoshi Mezawa,[1] Mizuho Konishi,[1] Kazue Ishitsuka,[1] Mayako Saito,[1] Hatoko Sasaki,[1] Miori Sato,[1] Minaho Nishizato,[1] Shin Yamazaki,[2] Takehiro Michikawa,[2] Yukifumi Monden,[3] Nathan Mise,[3] Fujio Kayama,[3] Masayuki Shimono,[4] Koichi Kusuhara,[4] Toshihiro Kawamoto,[2,4] Masafumi Sanefuji,[5] Kiyoko Kato,[5] Masako Oda,[6] Hiroshi Mitsubuchi,[6] Takahiko Katoh,[6] Hirohisa Saito,[1] and Yukihiro Ohya[1]

[1] Medical Support Center for Japan Environment and Children's Study, National Center for Child Health and Development, Tokyo, Japan
[2] Programme Office for Japan Environment and Children's Study, National Institute for Environmental Studies, Tsukuba, Japan
[3] Regional Center for Pilot Study of Japan Environment and Children's Study, Jichi Medical University, Shimotsuke, Japan
[4] Regional Center for Pilot Study of Japan Environment and Children's Study, University of Occupational and Environmental Health, Kitakyushu, Japan
[5] Regional Center for Pilot Study of Japan Environment and Children's Study, Kyushu University, Fukuoka, Japan
[6] Regional Center for Pilot Study of Japan Environment and Children's Study, Kumamoto University, Kumamoto, Japan

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Background

Gender-related differences in sex hormone levels in preschool children remain unclear.
Measurements are difficult because levels are generally below the lower limit of quantitation (LOQ) of conventional sex-hormone immunoassay methods, while current immunoassay methods are also limited by cross-reactivity.
However, liquid chromatography-tandem mass spectrometry (LC-MS/MS) demonstrates excellent sensitivity and specificity for measuring sex hormone levels in children.

Objective

To measure sex hormone levels in preschool children using LC-MS/MS.
To compare the levels of sex hormones between preschool boys and girls in relation to upstream hormones and background factors.

Participants and Methods

The present study was a cross-sectional study and comprised part of the JECS study.
Our participants are **151** preschool children (80 boys and 71 girls) who participated in the detailed survey at the age of 6 of the JECS pilot study.
None of the participating children demonstrated precocious puberty.

Blood samples were collected from the participating children.
Serum levels of E2, testosterone (T), and DHEA-S were assayed using LC-MS/MS. Serum levels of LH and FSH were assayed CLEIA kits.

The gender-stratified distributions of hormone levels were compared by Mann-Whitney U-test (equality of variance) or Welch's t-test (unequal variance).
We generated a multiple linear regression model with downstream hormone levels (DHEA-S, T and E2) as the outcome variable and upstream hormone levels, anthropometric data, and specimen-collection time as explanatory variables.
The significance of the regression coefficients was tested by the Wald test.

Results

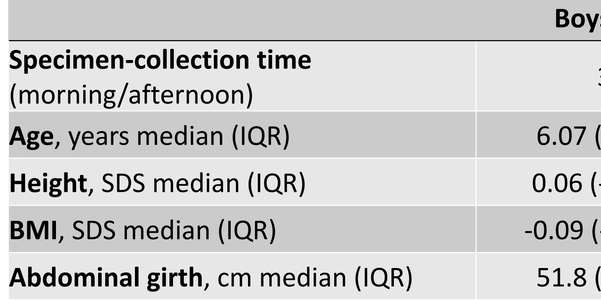
Table 1. Gender-stratified profiles and hormone levels in participating children

	Boys (N = 80)	**Girls** (N = 71)	*p*-value†,‡,§
Specimen-collection time (morning/afternoon)	34/46	33/38	0.76†
Age, years median (IQR)	6.07 (5.99; 6.14)	6.06 (5.95; 6.14)	0.61‡
Height, SDS median (IQR)	0.06 (-0.67; 0.67)	0.16 (-0.56; 0.89)	0.61‡
BMI, SDS median (IQR)	-0.09 (-0.59; 0.51)	-0.32 (-0.85; 0.21)	0.06‡
Abdominal girth, cm median (IQR)	51.8 (49.1; 54.4)	51.2 (48.5; 53.6)	0.21‡
LH, mIU/mL median (IQR)	<0.1 (<0.1; 0.1) 55 below lower LOQ	<0.1 (<0.1; <0.1) 68 below lower LOQ	-
FSH, mIU/mL median (IQR)	1.0 (0.8; 1.5)	2.0 (1.4; 2.4)	4.35e-08‡
DHEA-S, ng/mL median (IQR)	75.15 (41.21; 139.06)	73.18 (43.62; 124.10)	0.14 §
Testosterone, pg/mL median (IQR)	18.3 (11.4; 24.4) 4 below lower LOQ	16.4 (11.9; 23.7) 1 below lower LOQ	0.70‡
Estradiol, pg/mL median (IQR)	0.102 (0.060; 0.163) 1 below lower LOQ	1.020 (0.640; 1.606)	3.19e-15§

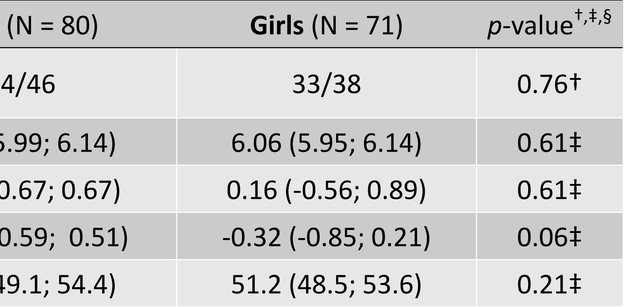
†Fisher's exact test; ‡Mann-Whitney U-test; §Welch's t-test.

The median E2 level was significantly higher in girls compared with boys.
The E2 levels in all the participating children were below the lower LOQ of conventional immunoassays (10 pg/mL).
T and DHEA-S levels were similar in boys and girls.
FSH levels were significantly higher in girls compared with boy.
LH levels in most children were below the lower LOQ of CLEIA (0.1 mIU/mL).

Histogram of boy's E2 level



Histogram of girl's E2 level



Results and Discussion

Table 2. Multiple linear regression model with DHEA-S level as outcome variable

Explanatory variable	Coefficient	S.E.	t	*p*-value†
Boys				
(intercept)	-190.2	149.3	-1.3	0.21
Height, SDS	4.0	13.0	0.3	0.76
Abdominal girth, cm	6.0	2.8	2.1	0.04
FSH, mIU/mL	3.6	16.4	0.2	0.83
Specimen-collection time, morning =1, afternoon = 0	25.3	23.5	1.1	0.29
Model summary; adjusted R-squared = 0.06, F-statistic *p*-value = 0.07				
Girls				
(intercept)	215.9	100.8	2.1	0.04
Height, SDS	31.9	8.0	4.0	0.0001
Abdominal girth, cm	-1.9	1.9	-1.0	0.34
FSH, mIU/mL	-12.3	8.6	-1.4	0.16
Specimen-collection time, morning =1, afternoon = 0	11.0	14.4	0.8	0.45
Model summary; adjusted R-squared = 0.31, F-statistic *p*-value = 2.16e-05				

†Wald test.

Associations between DHEA-S and different physical indexes in boys (abdominal girth) and girls (height) were found. We cannot exclude the possibility that a mid-growth spurt was only observed in some girls with adrenarche.

Table 3. Multiple linear regression model with testosterone level as outcome variable

Explanatory variable	Coefficient	S.E.	t	*p*-value†
Boys				
(intercept)	13.8	2.8	5.0	3.49E-06
Height, SDS	0.4	0.9	0.4	0.69
FSH, mIU/mL	-0.6	1.4	-0.4	0.69
DHEA-S, ng/mL	0.1	0.0	8.5	1.33E-12
Specimen-collection time, morning = 1, afternoon = 0	5.5	2.0	2.8	0.0074
Model summary; adjusted R-squared = 0.52, F-statistic *p*-value = 2.33e-12				
Girls				
(intercept)	-13.0	13.4	-1.0	0.34
Abdominal girth, cm	0.4	0.3	1.6	0.11
FSH, mIU/mL	2.0	1.3	1.5	0.14
DHEA-S, ng/mL	0.1	0.0	7.3	5.29E-10
Specimen-collection time, morning = 1, afternoon = 0	6.1	2.2	2.8	0.0060
Model summary; adjusted R-squared = 0.48, F-statistic *p*-value = 8.59e-10				

Abdominal girth in boys and height in girls were excluded from this model as per the previous model.

†Wald test.

In both boys and girls, a higher T level was associated with a higher DHEA-S level, and T levels were significantly higher in the morning than in the afternoon.
T levels are known to show diurnal variation and to be higher in the morning during pre-puberty and puberty, as confirmed by the current results.

Table 4. Multiple linear regression model with estradiol level as outcome variable

Explanatory variable	Coefficient	S.E.	t	*p*-value†
Boys				
(intercept)	0.1	0.1	0.8	0.42
Height, SDS	0.0	0.0	0.8	0.43
FSH, mIU/mL	-0.0	0.0	-0.4	0.71
Testosterone, pg/mL	0.005	0.002	2.4	0.02
Model summary; adjusted R-squared = 0.04, F-statistic *p*-value = 0.13				
Girls				
(intercept)	0.3	1.1	0.3	0.80
Abdominal girth, cm	-0.0	0.0	-0.3	0.75
FSH, mIU/mL	0.3	0.1	2.7	0.0095
Testosterone, pg/mL	0.04	0.01	4.9	6.67E-06
Model summary; adjusted R-squared = 0.30, F-statistic *p*-value = 6.75e-06				

Abdominal girth in boys, height in girls, DHEA-S and specimen-collection time in both were excluded from this model as per previous models.

†Wald test.

E2 level was only associated with both T and FSH levels in girls and not in boys. The present study demonstrated that there was no gender difference in T levels among 6-year-olds, while E2 and FSH levels were significantly higher in girls than in boys, and FSH levels were also positively correlated with E2 levels in girls. These results indicate that FSH is involved in E2 secretion even in preschool girls.

Conclusions

Obvious gender difference in E2 distribution exists even in young (preschool) children.
Furthermore, these results suggest that T is associated with DHEA-S in boys, while E2 is associated with both DHEA-S and FSH in girls.