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Growth and Thyroid Function in Children With *In Utero* Exposure to Dioxin: A 5-y Follow-Up Study

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ABSTRACT: Because placental polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs) levels are associated with decreased free thyroxine (FT_4) and thyroid stimulating hormone (TSH) in neonates, we assessed development by gender and maternal PCDD/Fs exposure at years 2 and 5 in 92 mother and newborn pairs. RIA quantified thyroid, sex, and growth hormones. Of 200 subjects followed up from November 2000, 136 and 149 were observed at year 2 and year 5, respectively. PCDD/Fs exposure levels were low ($n = 35$) or high ($n = 35$) in 70 subjects at year 2, low ($n = 21$) or high ($n = 20$) in 41 at year 5. Height, weight, BMI, and head circumference were significantly higher in males, chest girth significantly higher in females at year 2. Significantly, more girls had higher bone age (BA) and chronological age (CA) at both times. Height, weight, $FT_4 \times TSH$, and transthyretin (TTR) at year 2; and height, triiodothyronine, and IGF-1 at year 5 differed significantly by PCDD/Fs level. In females, height, weight, CA, BA, and thyroid hormones differed significantly at year 2. In males, $FT_4 \times TSH$ at year 2 and IGF-1 at year 5 were significantly higher in the high PCDD/Fs group. *In utero* exposure to PCDD/Fs differentially affects growth and hormone levels in male and female preschool children. (*Pediatr Res* 67: 1–2, 2010)

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Dioxins [polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs)] are highly toxic environmental contaminants, which are lipophilic and resistant to biodegradation. These compounds enter the human body through the food chain and finally accumulate in adipose tissue (1). A series of deleterious health effects are thought to be associated with organochlorine exposure. These include fetal and infant growth retardation and developmental abnormalities (2,3), altered reproductive capacity (4,5), carcinogenicity (6,7), cognitive deficits (8), and impaired thyroid function (9). It has been confirmed that *PCDD/Fs* can be transferred from mother to fetus via the placenta (10) and from mother to infant via breast milk (11). The effects of the endocrine disruption during these critical stages of early development might persist long after birth.

Similar to other industrialized and developing countries, Taiwan is confronted with the problem of environmental *PCDD/Fs* contamination. It has been reported that high concentrations of *PCDD/Fs* are more prevalent in central Taiwan than other areas of Taiwan (12). In a previous study, we examined the association between *in utero* exposure to *PCDD/Fs*/polychlorinated biphenyls (PCBs) and altered thyroid and growth hormone concentrations in newborns from central Taiwan (13), we found that after adjusting for other dioxins and PCB congeners in neonates from the general population, the multiplied concentration of free thyroxine (FT_4) × thyroid stimulating hormone (TSH) decreased with increasing levels of non-*ortho* PCBs in the placenta. In the same study, we also noted a significant and positive association between thyroxine (T_4) and thyroid-binding globulin concentrations with levels of PCDD/Fs (13).

Because thyroid hormones are crucial for growth and development, thyroid dysfunction in early infancy/childhood may adversely impair these processes and have serious long-term consequences. Hence, we conducted follow-up assessments at preschool ages for the cohort of neonates in our previous study (13). Because a previous report found that schoolchildren (aged 7.5–15 y) whose serum level of PCBs was two to four times higher than normal children had no significant impairment of thyroid function (14), the focus of this study was the relationship between *in utero* exposure to PCDD/Fs and thyroid function and growth hormones. The objectives of the follow-up study were to examine possible abnormalities in growth and development, bone age (BA) and growth, and hormones related to neurodevelopment in children aged between 2 and 3 y and 5 and 6 y who were exposed to differing levels of PCDD/Fs *in utero*. Specifically, we made comparisons between children who were exposed to low and high levels of PCDD/Fs.

MATERIALS AND METHODS

Subjects. A total of 92 mother and newborn pairs were recruited from the general population in central Taiwan. We consecutively followed up the subjects ($n = 200$) of our previous study (13) from November 2000 to February 2007. We studied the subjects at 2–3 y of age ($n = 188$, during 2003) and at 5–6 y ($n = 149$, during 2006–2007). These groups are referred to as year 2 and year 5, respectively. Children were also classified into low exposure and high exposure groups by the cutoff value 15 pg-TEQ/g lipid based on the mother's PCDD/Fs TEQ levels. This study was approved by the

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Abbreviations: BA, bone age; **CA,** chronological age; **E_2,** estradiol; **FT_4,** free thyroxine; **IGFBP-3,** insulin-like growth factor binding protein 3; **PCBs,** polychlorinated biphenyls; **PCDD/Fs,** polychlorinated dibenzo-p-dioxins, dibenzofurans; **T_3,** triiodothyronine; **T_4,** thyroxine; **Tg,** thyroglobulin; **TRAb,** TSH receptor autoantibody; **TSH,** thyroid stimulating hormone; **TTR,** transthyretin

ethics review committee of our university hospital, and written parental consent was obtained for all participants. The study abided by the ethical standards of the Declaration of Helsinki (15).

Measurements and blood sampling. Demographic information included maternal age, gestational age, and infants' age, gender, height, weight, BMI, head circumference, chest girth, chronological age (CA), BA, and ratio of BA/CA. Body weight and height were compared with the population curve (data were obtained from the Taiwanese Department of Health) at each visit. Standing height was measured in triplicate using a wall-mounted stadiometer. BMI was calculated by dividing body weight (kg) by the square of height in meters (m^2). Left hand radiographs were taken, and BA was determined using the method of Greulich and Pyle (16).

In laboratory analysis, thyroid, sex hormone, and growth factor concentrations with respect to PCDD/F exposure level were collected for evaluation. Venous blood samples were obtained from children whose mothers' PCDD/F TEQ levels were available in our database. Blood samples were immediately centrifuged and the serum frozen at $-70°C$ for later analysis of triiodothyronine (T_3), T_4, FT_4, TSH, T_3 uptake, thyroglobulin (Tg), TSH receptor autoantibody (TRAb) uptake, transthyretin (TTR), IGF-1, IGF binding protein 3 (IGFBP-3), testosterone, and estradiol (E_2).

T_3, T_4, FT_4, TSH, and T_3 uptake analysis. Serum T_3, T_4, free T_4 and TSH concentrations, and T_3 uptake were measured using an automated chemiluminescent immunometric assay (Immulite 2000: Diagnostic Products Corp, Los Angeles, CA). Sensitivities were 19 ng/dL for T_3, 0.3 μg/dL for T_4, 0.002 μIU/mL for TSH, and 1.6 μg/mL for T_3 uptake. Intra- and interassay coefficients of variation (CV) were 4.4 and 5.3% for T_3; 4.6 and 5.6% for T_4; and 5.1 and 6.4% for TSH. $FT_4 \times$ TSH values were calculated based on the rationale that, for a normal hypothalamic-pituitary axis, a decrease in T_4 feedback inhibition to the hypothalamus resulted leads to TSH secretion by the anterior pituitary.

Tg analysis. Serum Tg levels were measured by immunoradiometric assay (RSR Ltd., Cardiff, UK). Sensitivity was 0.7 ng/mL, and the intra- and interassay CVs were 3.32 and 6.36%.

TRAb analysis. Serum TRAb levels were determined using a commercially available RIA kit (RSR limited, Cardiff, UK). Sensitivity of this assay was 5 units/L. Result was expressed as inhibition of TSH binding index, % = $100 \times (1-B/B_o)$. Intra- and interassay CVs were 4.15 and 6.02%.

TTR analysis. Serum TTR levels were measured using a kinetic nephelometric method by automated nephelometer (Beckman Coulter, Inc. Array System, Carlsbad, CA). The sensitivity of this assay was 1.17 mg/dL, and the intra- and interassay CV were 2.06 and 1.54%.

IGF-1 and IGFBP-3 analysis. Serum total IGF-I and IGFBP-3 levels were measured after acid extraction using commercial RIA kits (Diagnostic Systems Laboratories, Inc., Webster, TX). The sensitivities were 0.9 mg/L and 0.01 ng/mL for IGF-I and IGFBP-3, respectively. The intra- and interassay CV were 7.2 and 9.8% for IGF-I and 5.8 and 8.2% for IGFBP-3.

Testosterone analysis. Serum testosterone concentrations were measured by solid-phase, competitive chemiluminescent enzyme immunoassay (Immulite 2000 Advanced Immunoassay System, Siemens Medical Solutions Diagnostics, Deerfield, IL). The sensitivity of this assay was 15 ng/dL. Intra- and interassay CVs were 9.7 and 12.0%.

E_2 analysis. Serum E_2 levels were measured by RIA kit (Diagnostic Systems Laboratories). Sensitivity was 2.2 pg/mL, and intra- and interassay CV were 7.5 and 9.3%.

Statistical analysis. In general analysis, data were expressed as mean ± SD for continuous variables and frequency with percentage for categorical variables. For comparison of difference in means between groups, a *t* test was performed for normally distributed data, and the nonparametric Wilcoxon rank sum test was performed for data that were not normally distributed. A multiple regression analysis was performed with a stepwise selection for detecting the factors, which might affect the growth of hormone concentrations by adjusting the maternal age and sex of the infant. All comparisons were considered under a significance level $\alpha = 0.05$. All data were analyzed using SAS version 9.0 (SAS Institute Inc., Cary, NC).

RESULTS

Summary of anthropomorphic measurements by infant gender is presented in Table 1. Significant increases were apparent for each variable between genders at both ages. At year 2, height, weight, BMI, and head circumference were significantly higher in boys than girls. Chest girth was significantly higher in females at this visit. At year 5, there were no between-group differences in height, weight, BMI, head circumference, or chest girth.

Using BA as an indicator of abnormal growth, we compared BA with CA at both visits. At year 2, children (male and female combined) had a mean BA of 2.14 ± 0.45 y ($n = 136$; note: BA was not assessed for all subjects at year 2), whereas CA was 2.23 ± 0.58 y ($n = 194$). The difference between BA and CA was borderline significant ($p = 0.056$). At year 5, however, mean BA (4.39 ± 0.83 y) was significantly lower than mean CA (5.18 ± 0.19 y) ($p < 0.0001$) (data not shown). BA and CA comparisons with respect to gender were conducted as well; no difference was detected at year 2, but males had a significantly lower mean BA than females at year 5 ($p = 0.0058$). Significant differences were detected for the mean of ratio of BA and CA with respect to gender at both year 2 and year 5 ($p = 0.0160$ for year 2; $p < 0.0001$ for year 5) (Table 1).

In considering anthropomorphic data and PCDD/Fs exposure levels, high level ($\geq$15 pg-TEQ/g lipid) and low level (<15 pg-TEQ/g lipid) are summarized by age and gender. Overall, height and weight were significantly different between the PCDD/Fs exposure levels at year 2 ($p = 0.0029$ for height and 0.0076 for weight). However, only height remained significant at year 5. In subgroups (male and female), it was found that height, weight, CA, and BA were significantly different between the PCDD/Fs exposure levels at year 2 for female ($p = 0.0110$ for height, 0.0086 for weight, 0.0218 for CA, and 0.0461 for BA). In contrast, there were no such differences for males. Similarly, there were no significant differences between exposure levels by gender at year 5 (Table 2).

For children who were exposed PCDD/Fs, T_3, and IGF-1 concentrations at year 2 were significantly higher in females than males ($p = 0.0302$, 0.0434 for T_3 and IGF-1, respec-

Table 1. *Summary of anthropomorphic demographics at year 2 and year 5*

Demographics*	Year 2			Year 5		
	Male ($n = 69$)	Female ($n = 67$)	p	Male ($n = 70$)	Female ($n = 79$)	p
Height (cm)	88.96 ± 4.33	87.72 ± 3.56	0.0342‡	111.28 ± 4.16	110.78 ± 4.64	0.6000
Weight (kg)	13.45 ± 1.91	12.65 ± 1.66	0.0025†	20.33 ± 2.45	20.27 ± 3.87	0.9393
BMI (kg/m²)	16.96 ± 1.64	16.41 ± 1.57	0.0195†	18.24 ± 1.82	17.99 ± 2.81	0.6281
Head circumference (cm)	50.85 ± 2.72	48.18 ± 1.48	0.0362†	51.01 ± 1.50	50.81 ± 1.60	0.5534
Chest girth (cm)	48.64 ± 1.47	49.96 ± 2.83	0.0293†	56.95 ± 2.99	56.53 ± 4.06	0.5887
CA	2.20 ± 0.28	2.25 ± 0.27	0.1555	5.25 ± 0.32	5.32 ± 0.30	0.1815
BA	2.04 ± 0.34	2.25 ± 0.52	0.6419	3.99 ± 0.82	4.84 ± 0.59	0.0058
BA/CA	0.93 ± 0.17	1.00 ± 0.19	0.0160†	0.76 ± 0.16	0.91 ± 0.11	<0.0001†

* Demographics are presented as mean ± SD.

† $p < 0.05$ indicates statistically significant difference between genders.

Table 2. *Summary of anthropomorphic variables with respect to PCDD/F exposure levels (low versus high) as determined at year 2 and year 5*

Demographics*	Male			Female			Overall		
	Low† (<15.1)	High† (≥15.1)	p	Low† (<15.1)	High† (≥15.1)	p	Low† (<15.1)	High† (≥15.1)	p
Year 2									
n	14	15		21	20		35	35	
Height (cm)	89.25 ± 3.24	91.63 ± 2.60	0.1531	85.77 ± 2.98	88.71 ± 2.47	0.0110‡	86.76 ± 3.38	89.88 ± 2.86	0.0029‡
Weight (kg)	13.08 ± 1.53	13.50 ± 1.20	0.5768	12.03 ± 1.06	13.71 ± 1.95	0.0086‡	12.33 ± 1.27	13.63 ± 1.65	0.0076‡
BMI (kg/m²)	16.36 ± 0.74	16.09 ± 1.35	0.6593	16.36 ± 1.18	17.38 ± 1.96	0.1073	16.36 ± 1.05	16.86 ± 1.82	0.2931
Head circumference (cm)	49.17 ± 1.75	49.75 ± 1.60	0.5291	47.83 ± 1.23	48.71 ± 1.47	0.1049	48.21 ± 1.49	49.13 ± 1.57	0.0641
Chest girth (cm)	50.50 ± 1.90	50.75 ± 1.65	0.7965	49.47 ± 2.90	51.04 ± 3.75	0.2295	49.76 ± 2.65	50.93 ± 3.03	0.1975
CA	2.20 ± 0.12	2.16 ± 0.17	0.6382	2.09 ± 0.18	2.24 ± 0.15	0.0218‡	2.12 ± 0.17	2.21 ± 0.16	0.0804
BA	2.24 ± 0.32	2.17 ± 0.41	0.7472	1.99 ± 0.35	2.40 ± 0.54	0.0461‡	2.07 ± 0.35	2.31 ± 0.49	0.1143
BA/CA	1.02 ± 0.14	1.02 ± 0.23	0.9797	0.96 ± 0.15	1.06 ± 0.21	0.2021	0.98 ± 0.14	1.04 ± 0.21	0.3022
Year 5									
n	6	8		15	12		21	20	
Height (cm)	110.77 ± 2.49	113.25 ± 3.18	0.1409	109.45 ± 4.99	112.75 ± 5.10	0.1030	109.82 ± 4.40	112.95 ± 4.34	0.0276‡
Weight (kg)	20.58 ± 3.26	20.54 ± 1.89	0.9740	19.57 ± 3.24	22.23 ± 5.19	0.1145	19.86 ± 3.20	21.56 ± 4.20	0.1520
BMI (kg/m²)	18.56 ± 2.70	18.13 ± 1.56	0.7165	17.80 ± 2.22	19.25 ± 3.47	0.1973	18.01 ± 2.32	18.81 ± 2.86	0.3362
Head circumference (cm)	51.30 ± 1.76	51.40 ± 1.68	0.9157	50.09 ± 1.74	51.41 ± 1.86	0.0685	50.43 ± 1.79	51.41 ± 1.74	0.0863
Chest girth (cm)	57.23 ± 4.08	56.81 ± 2.93	0.8254	55.95 ± 3.64	58.98 ± 4.68	0.0695	56.32 ± 3.71	58.12 ± 4.12	0.1503
CA	5.20 ± 0.12	5.15 ± 0.17	0.5504	5.12 ± 0.27	5.28 ± 0.14	0.0626	5.15 ± 0.24	5.23 ± 0.16	0.1891
BA	4.17 ± 0.75	3.81 ± 0.75	0.4008	4.63 ± 0.69	4.92 ± 0.56	0.2618	4.50 ± 0.72	4.48 ± 0.83	0.9188
BA/CA	0.80 ± 0.15	0.74 ± 0.16	0.4819	0.90 ± 0.13	0.93 ± 0.09	0.5828	0.88 ± 0.14	0.85 ± 0.15	0.6421

* Demographics are presented as mean ± SD.

† Low and high levels for PCDD/Fs levels were categorized as <15, and ≥15 pg-TEQ/g lipid.

‡ $p < 0.05$ indicates statistically significant difference between the high- and low-exposure levels for a given gender or overall.

Table 3. *Thyroid and sex hormone, and growth factor concentrations with respect to PCDD/F exposure level at year 2*

Variables*	Male			Female			Overall		
	Low† (<15.1)	High† (≥15.1)	p	Low† (<15.1)	High† (≥15.1)	p	Low† (<15.1)	High† (≥15.1)	p
n	14	15		21	20		35	35	
T_3 (ng/dL)	186.79 ± 41.05	188.06 ± 23.89	0.9357	154.77 ± 29.00	176.50 ± 18.29	0.0236‡	166.97 ± 36.72	181.12 ± 21.04	0.1281
T_4 (μg/dL)	9.83 ± 2.52	10.33 ± 2.21	0.6568	9.30 ± 2.79	9.36 ± 1.71	0.9469	9.50 ± 2.64	9.73 ± 1.93	0.7310
TSH (mIU/L)	2.77 ± 0.91	3.31 ± 0.86	0.2129	2.54 ± 1.05	3.19 ± 1.44	0.1857	2.63 ± 0.98	3.24 ± 1.22	0.0697
T_3 uptake (%)	26.14 ± 2.97	26.64 ± 2.98	0.7816	28.37 ± 4.51	27.97 ± 1.89	0.7996	27.63 ± 4.10	27.48 ± 2.36	0.9035
FT_4 (ng/dL)	1.12 ± 0.18	1.25 ± 0.15	0.1291	1.13 ± 0.23	1.20 ± 0.23	0.4689	1.13 ± 0.21	1.22 ± 0.20	0.1479
$FT_4 \times$ TSH	2.78 ± 0.54	4.11 ± 1.11	0.0122‡	2.83 ± 1.05	3.87 ± 2.00	0.1155	2.81 ± 0.88	3.97 ± 1.67	0.0079‡
Tg (ng/dL)	42.04 ± 19.69	35.82 ± 11.54	0.4409	38.05 ± 18.71	30.13 ± 11.16	0.2331	39.69 ± 18.61	32.57 ± 11.41	0.1791
TRAb (%)	1.61 ± 0.59	1.83 ± 0.81	0.5736	1.92 ± 0.84	1.75 ± 0.76	0.5928	1.81 ± 0.76	1.78 ± 0.76	0.8868
TTR (mg/dL)	12.42 ± 2.87	12.42 ± 3.14	0.9978	10.81 ± 2.67	13.87 ± 3.04	0.0045‡	11.50 ± 2.82	13.24 ± 3.12	0.0298‡
E_2 (ng/dL)	7.64 ± 1.60	8.44 ± 1.75	0.3539	8.48 ± 1.10	9.09 ± 2.31	0.3684	8.19 ± 1.32	8.83 ± 2.09	0.2132
Testosterone (ng/dL)	3.81 ± 1.52	3.42 ± 1.74	0.6224	3.62 ± 1.61	3.31 ± 1.24	0.5618	3.70 ± 1.54	3.35 ± 1.43	0.4371
IGF-1 (ng/mL)	94.73 ± 34.70	131.73 ± 39.55	0.0596	133.69 ± 80.20	168.89 ± 69.39	0.2515	118.11 ± 67.46	153.69 ± 60.80	0.0797
IGFBP-3 (ng/mL)	2.80 ± 0.54	3.18 ± 0.64	0.1921	3.79 ± 2.49	3.40 ± 0.68	0.5842	3.41 ± 2.02	3.31 ± 0.66	0.8230

* Variables including thyroid and sex hormone, and growth factor concentrations were presented as the mean ± SD.

† Low and high levels for PCDD/Fs levels were categorized as <15, and ≥15 pg-TEQ/g lipid.

‡ $p < 0.05$ indicates statistically significant difference between the high and low exposure levels for a given gender or overall.

tively). At year 5, FT_4 was significantly higher in males ($p = 0.0105$), whereas IGF-1 levels were significantly higher in females ($p = 0.0433$; data not shown).

The thyroid hormone, sex hormone, and growth factor concentrations with respect to PCDD/Fs exposure levels, high level (≥15 pg-TEQ/g lipid) and low level (<15 pg-TEQ/g lipid) were summarized by gender for year 2 and year 5, respectively (Table 3 and Table 4). Overall, $FT_4 \times$ TSH, TTR levels at year 2 and T_3, IGF-1 at year 5 were significantly higher in the group with high dioxin/PCB exposure level than the group with low exposure level ($p = 0.0079$, 0.0298 for $FT_4 \times$ TSH and TTR at year 2, respectively; $p = 0.0379$,

0.0243 for T_3 and IGF-1 at year 5, respectively). In females, only T_3 and TTR concentrations at year 2 were significantly higher in the high PCDD/Fs exposure level than the low level ($p = 0.0236$, 0.0045 for T_3 and TTR, respectively), whereas there was no significant difference detected in concentrations at year 5. In males, $FT_4 \times$ TSH at year 2 and IGF-1 at year 5 were significantly higher in the high PCDD/Fs exposure level than the low level ($p = 0.0122$ for $FT_4 \times$ TSH at year 2, and $p = 0.0076$ for IGF-1 at year 5).

Results of multiple linear regression analysis adjusting for maternal age indicated that, overall, only $FT_4 \times$ TSH at year 2 and IGF-1 at year 5 were significantly higher in the group

Table 4. *Thyroid hormone, sex hormone, and growth factor concentrations with respect to PCDD/F exposure level at year 5*

Variables*	Male			Female			Overall		
	Low† (<15.1)	High† (≥15.1)	p	Low† (<15.1)	High† (≥15.1)	p	Low† (<15.1)	High† (≥15.1)	p
n	6	8		15	12		21	20	
T_3 (ng/dL)	144.30 ± 11.74	151.88 ± 18.03	0.3897	135.50 ± 15.67	146.36 ± 15.80	0.0868	138.01 ± 14.93	148.57 ± 16.49	0.0379‡
T_4 (µg/dL)	7.98 ± 2.21	8.78 ± 2.01	0.4975	8.29 ± 2.02	8.33 ± 1.95	0.9641	8.20 ± 2.02	8.51 ± 1.94	0.6275
TSH (mIU/L)	2.60 ± 1.34	2.99 ± 0.95	0.5327	2.73 ± 1.72	2.86 ± 2.22	0.8700	2.69 ± 1.59	2.91 ± 1.78	0.6843
T_3 uptake (%)	26.72 ± 2.21	26.84 ± 2.34	0.9237	27.13 ± 3.37	26.09 ± 2.50	0.3824	27.01 ± 3.04	26.39 ± 2.40	0.4722
FT_4 (ng/dL)	1.29 ± 0.25	1.33 ± 0.22	0.7665	1.24 ± 0.28	1.11 ± 0.14	0.1166	1.26 ± 0.27	1.20 ± 0.21	0.4172
$FT_4 \times$ TSH	3.47 ± 2.32	4.12 ± 1.76	0.5643	3.38 ± 2.58	3.13 ± 2.24	0.7875	3.41 ± 2.45	3.52 ± 2.07	0.8735
Tg (ng/dL)	20.88 ± 7.62	24.41 ± 3.58	0.3129	24.32 ± 5.37	25.66 ± 4.19	0.5536	23.51 ± 5.90	25.08 ± 3.83	0.3852
E_2 (ng/dL)	6.74 ± 2.32	6.47 ± 1.72	0.8085	7.51 ± 2.30	5.93 ± 1.62	0.0563	7.29 ± 2.28	6.15 ± 1.64	0.0748
Testosterone (ng/dL)	6.60 ± 1.81	6.27 ± 1.53	0.7158	6.58 ± 1.59	6.72 ± 1.81	0.8319	6.59 ± 1.61	6.54 ± 1.68	0.9281
IGF-1 (ng/mL)	161.22 ± 57.79	263.85 ± 60.43	0.0076‡	252.96 ± 122.61	334.28 ± 114.97	0.0907	226.75 ± 114.73	306.11 ± 101.25	0.0243‡
IGFBP-3 (ng/mL)	4.79 ± 1.38	5.38 ± 0.98	0.3678	5.06 ± 0.98	5.46 ± 1.17	0.3412	4.98 ± 1.08	5.43 ± 1.07	0.1928

* Variables including thyroid and sex hormone, and growth factor concentrations were presented as the mean ± SD.
† Low and high levels for PCDD/Fs levels were categorized as <15 and ≥15 pg-TEQ/g lipid.
‡ $p < 0.05$ indicated a statistically significant difference between the high and low exposure levels for a given gender or overall.

Table 5. *Stepwise multiple linear regression models for selection in thyroid hormone concentrations with respect to PCDD/F exposure level at year 2*

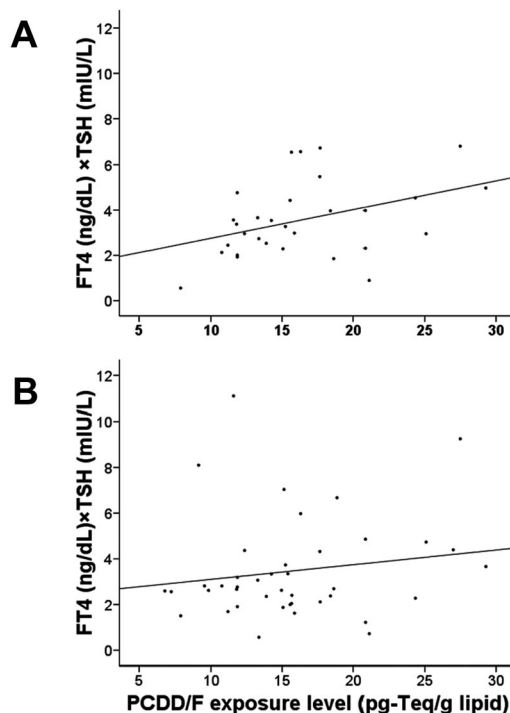| Model*	Estimate ± SE	p-Value
T_3, $R^2 = 0.3698$		
PCDD/F	12.59 ± 4.75	0.0133‡
(PCDD/F)^2	−0.32 ± 0.13	0.0201‡
TSH†, $R^2 = 0.2221$		
Maternal age	−0.05 ± 0.07	0.0238‡
PCDD/F	0.05 ± 0.02	0.0188‡
$FT_4 \times$ TSH†, $R^2 = 0.2880$		
Maternal age	−0.07 ± 0.03	0.0195‡
PCDD/F	0.06 ± 0.02	0.0089‡
TTR, $R^2 = 0.0619$		
PCDD/F	0.12 ± 0.08	0.1720

* Model as performed adjusting infants' gender.
† TSH and $FT_4 \times$ TSH were log transformed to meet regression assumptions.
‡ $p < 0.05$ indicates a statistically significant relationship.

Figure 1. PCDD/F exposure level in relation to $FT_4 \times$ TSH level. By simple linear model: (*A*) for 2 y preschool data, $FT_4 \times$ TSH $= 1.49 + 0.12 \times$ (PCDD/F), $R^2 = 0.1551$, $p = 0.0313$; (*B*) for 5-y follow-up, $FT_4 \times$ TSH $= 2.46 + 0.06 \times$ (PCDD/F), $R^2 = 0.0247$, $p = 0.3266$.

with high PCDD/Fs exposure level than the group with low level exposure ($p = 0.0002$ for $FT_4 \times$ TSH at year 2; $p = 0.0243$ for IGF-1 at year 5). In males, $FT_4 \times$ TSH at year 2 and IGF-1 at year 5 were still significantly higher in the group with high PCDD/Fs exposure level than the group with low exposure level ($p = 0.0072$ for $FT_4 \times$ TSH at year 2, and $p = 0.0228$ for IGF-1 at year 5). In females, TTR was still significantly higher in the high PCDD/Fs exposure level than the low level at year 2; $FT_4 \times$ TSH (instead of T_3) was significantly higher in the high PCDD/Fs exposure level. There was no significant difference in hormone levels at year 5 (data not shown).

Table 5 shows the results of the stepwise multiple linear regression analysis of selection of thyroid hormone concentrations at first follow-up (year 2) with respect to PCDD/Fs exposure level, considering mother's age, parity at first follow-up, and the quadratic form of PCDD/Fs. There were significantly positive correlations among T_3, TSH, $FT_4 \times$ TSH, and TTR concentrations and the level of PCDD/Fs exposure. Both TSH and $FT_4 \times$ TSH concentrations were significantly negatively correlated with maternal age. There was also a negative correlation between T_3 levels and the

quadratic form of PCDD/Fs. The significant relation between $FT_4 \times$ TSHlevels and PCDD/Fs exposure level at year 2 was further demonstrated by multiple linear model (Fig. 1).

DISCUSSION

PCDD/Fs are transferred from mother to the fetus through the placenta and to infants via breast milk (10–12). Therefore, maternal exposure to these endocrine disruptors may adversely impact fetal/infant development and functioning. In this study, we performed follow-up examinations of infants/children previously demonstrated to be exposed *in utero* to

varying levels of PCDD/F (13). Our findings indicate that children with higher levels of exposure to these environmental contaminants *in utero* were significantly taller and heavier and had higher serum level of several thyroid hormones (T_3, FT_4 × TSH, and TTR) and growth hormone (IGF-1) at the preschool ages. Whether there is a cause-effect relationship between growth and hormone remains to be studied.

Females seem to be more sensitive than males to PCDD/Fs exposure. Females exposed to PCDD/Fs *in utero* were significantly taller and heavier and had higher BA/CA ratio than male at both follow-up visits. We also noted the intriguing finding of significantly higher T3 and IGF-1 levels in females than males at year 2. IGF-1 remained higher at year 5 visit. Females exposed to high levels of PCDD/Fs were significantly taller and heavier and had higher BA and CA those with low exposure at year 2 follow-up visits. Similarly, we observed a significant correlation between exposure level of PCDD/Fs and thyroid hormones (T_3, TTR) in females at the year 2 follow-up visit. There was no significant correlation between PCDD/Fs exposure level and growth or hormone level in girls at the year 5 follow-up, suggesting that fetal exposure to PCDD/Fs can stimulate growth at early ages but it may become less apparent with increasing age. For males, exposure level did not significantly affect growth, but level of PCDD/Fs exposure *in utero* had a significant correlation with FT4 × TSH at both year 2 and year 5.

Correlation between PCDD/Fs exposure level *in utero* and hormone level during preschool ages was further confirmed using a stepwise multiple linear regression analysis. The results suggested that there were significantly positive correlations between T_3, TSH, FT_4 × TSH, and TTR concentrations and the level of PCDD/Fs exposure at year 2 follow-up. The finding of higher FT_4 × TSH values in the high exposure group suggested that T_4 feedback inhibition of TSH secretion was impaired in both males and females exposed to high levels of PCDD/Fs.

A number of previous studies have noted altered thyroid functioning in dioxin/PCB exposed infants. The major finding of this study was the demonstration of significant positive correlations between T_3, TSH, FT_4 × TSH, and TTR concentrations and the level of PCDD/Fs exposure at year 2 follow-up. Pluim *et al*. (9,17) also reported that term infants exposed to upper-median dioxin levels (as measured in breast milk) had increased plasma T_4 and TBG concentrations. Sauer *et al*. (18) investigated the growth and development of infants in highly industrialized western countries; they found a higher TSH at higher levels of dioxin exposure.

We have noted in a previous publication that FT_4 × TSH decreased with increasing concentrations of non-*ortho* PCB exposure levels in infants, indicating a lack of effective FT_4 feedback to stimulate the hypothalamus (13). It suggested that dioxin/PCB levels *in utero* may have differential effect in neonates and children 2–3 y of age. The reason for the difference between two developmental stages is unclear and warrants further investigation. Interestingly, there was no significant correlation between PCDD/Fs exposure level and hormone level at year 5 visit after stepwise multiple linear regression analysis. Mazhitova *et al.* (14) found that none of

the schoolchildren (aged 7.5–15 y), whose serum level of PCBs was two to four times higher than healthy Stockholm children, had any impairment of thyroid function, as revealed by the plasma levels of TSH and thyroxine; there was no relation between thyroid hormone status and the blood lipid levels of PCBs, hexachlorocyclohexane, and DDT. Findings from our previous study suggest that there is a negative correlation between thyroid hormone levels and maternal age (13). In accordance with that study, we also observed a significant negative correlation between TSH and FT_4 × TSH concentrations and maternal age, suggesting that children born to older mothers tend to have lower levels of thyroid hormone. It is apparent that further studies are needed to explore in more detail how changes in IGF-1, T_3, TTR, and FT_4 × TSH correlate with growth and development in infants/children following *in utero* exposure to PCDD/Fs. Exposure level did not affect E_2 and testosterone at the early stage of life. In the future, we plan to closely follow this cohort of children and investigate possible differences in the levels of hormones involved in regulating sexual development (*i.e.* E_2 and testosterone) with respect to exposure level.

The same cohort of children will be continuously followed up though school age and puberty. It will be particularly interesting to know if material exposure level and blood levels of PCDD/Fs have any effect on hormone status and development during puberty.

In conclusion, the results of this first complete follow-up study of preschool children exposed to PCDD/Fs *in utero* indicate that such exposure can significantly affect growth and development. Serum levels of several thyroid hormones showed positive correlation with the level of PCDD/Fs exposure *in utero*. Age-related changes were also observed. A number of gender-specific differences were detected with regard to growth, basal hormone concentrations, and PCDD/Fs exposure level. These findings emphasize that *in utero* exposure to PCDD/Fs can affect the growth and development of preschool age children.

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES 1

1—Kindly check whether the minor edit made to the title is OK.

2—The author names "Greulich and Pyle" do not match with the author names listed in Ref. 16. Kindly check.

3—Kindly indicate the significance of the symbol "‡" in Table 1 footnotes.