E2, Testosterone, and Thyroid Related Function and serum PCDD/Fs levels in general populations

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Introduction

Exposures to environmental PCDD/Fs have long been suggested to result in many adverse health effects, such as immune deficiency and liver damage. PCDD/Fs were also known as endocrine disruptors, a group of chemicals that disrupt the normal endocrine system of animal and human. They were found to alter thyroid hormone and insulin levels in serum, and inhibit 17 beta-estradiol (E2)-induced responses from *in vivo* and *in vitro* study [1]. The hormones described are known to play critical roles on several aspects of neural and reproductive developments. Yet, inconsistent findings have been drawn for different populations regarding the association between the performance of thyroid function and exposure to PCDD/Fs. Study with workers from factory producing 2,4,5-trichorophenol identified a significantly higher adjusted mean free thyroxine index compared to those from the residential neighborhood of each study worker. In the studies conducted in US with general populations, decreasing levels of total triiodothyronine (T3), thyroid-stimulating hormone (TSH), and testosterone were associated with rising TCDD levels [1,2]. These previous investigations are intriguing in a sense that dose-response relationship between hormone functions and effects resulting from exposure to dioxin-like compounds may be highly dependent on the range of exposures. Meanwhile, synthetic environmental estrogens, such as polychlorinated biphenyls (PCBs) and PCDD/Fs, are even more stable in the body than natural estrogens. It was shown in vivo study that the observed estrogen receptor-beta mRNA increased by exposing to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) [3]. Yet, limited epidemiological data has been available in this regard.

As the global populations in general are increasingly exposed to all types of dioxin-like compounds in our daily life, our study was therefore aimed to examine the relationship between serum PCDD/Fs levels and the corresponding thyroid and E2 functions of the study subjects.

Materials and methods

Four hundred and forty-two subjects were representatively selected and invited to participate in this study from 3 counties/cities in Taiwan. All subjects were between 18-65 years old, were asked to contribute a blood sample of at least 60 mL for PCDD/Fs and endocrine analysis. Each serum sample was spiked with a mixture containing fifteen $^{13}C_{12}$-labeled PCDD and PCDF standards as defined in USEPA Method 1613. Serum samples were enriched and fractionated by C18, SCX, silica, and Florisil cartridges before analysis. Each analytical run consisted of a method blank, a quality control, and seven unknown samples. The quantification of PCDD/Fs was performed with high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) with a Rtx-5MS column [4]. All PCDD/Fs were adjusted to the lipid content analyzed from the corresponding samples. Serum PCDD/Fs concentrations were successfully obtained for 436 subjects.

Results and discussion

Table 1 showed higher abnormal rate, defined as percentage of subjects whose levels were not in the range of average values for that specific gender, of E2 in the younger subjects (18-25 years old), and subjects older than 45 years old. We further analyzed the female subjects older than 45 years of age, n=23, but found 3 of them still had E2 levels above the normal levels. In addition, higher PCDD/Fs level (33.3 pg WHO-TEQ/g lipid) was observed in subjects with high E2 levels than those with levels under the normal range (27.0 pg WHO-TEQ/g lipid), though with no statistical significance (p=0.471). Although it is thought that the release of E2 was reduced after menopause in women [5], we observed 3 of the 120 women older than 45 years of age presented the E2 levels that were significantly above the normal range. These 3 subjects were also measured with a higher average serum concentration of PCDD/Fs than those with levels under the normal range. It could be tentatively hypothesized that high PCDD/Fs burden is considered a risk factor for E2 dysfunction in women.

Number distribution between subjects within or over the normal range measured levels for 3 hormones, TSH, T3, and T4, were summarized in table 2. No particular statistical association for the factors of age, sex, Lipid content values, and smoking habit, was confirmed in this current analysis. In table 3, comparison of average PCDD/Fs concentrations was made between the groups with hormone measurements above or under than the normal range for 5 different hormones. The only significant difference were found for E2 and TSH, p=0.003 and p=0.092 respectively. Multiple logistic regression was performed, controlling for age, to examine the association between serum PCDD/Fs levels, and E2 or TSH. No significant association was found between serum PCDD/Fs levels and hormone levels of either E2 or TSH in male subjects. However, a statistical relationship, $p<0.05$, was shown for E2 levels in women between the subjects of PCDD/Fs level higher than 32 pg WHO-TEQ/g lipid and those lower than 16 pg WHO-TEQ/g lipid, OR=4.65, 95% CI=1.12-19.43, a finding consistent with the previous animal study where the increase of Estrodiol Receptor mRNA after exposure to TCDD for 48 hours [3]. In addition, several cohort studies have also demonstrated that a neurodevelopmental delay was associated with prenatal or early postnatal PCB-exposure at environmental levels.

Conclusion

Higher serum PCDD/Fs level is suggested to be a risk factor involved in hormone dysfunction, especially for estrogen in women. No similar relationship was observed for thyroid dysfunction in the current study. Further research is imperative to characterize the exact effects and mechanisms regarding the effect of exposure to low levels of PCDD/Fs on specific hormone dysfunction and related health performance.

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Table 1. Abnormal rate of sexual hormone between different age, sex, body percentage and smoking status: number (%), Chi-Square test

	Testosterone†			E2†		
	Normal	Abnormal	P value	Normal	Abnormal	P value
Age(years old)						
18-25	29 (82.9)	6 (17.1)		30 (85.7)	5 (14.3)	
26-35	56 (96.5)	2 (3.5)	0.076	56 (96.5)	2 (3.5)	**0.007****
36-45	107 (94.7)	6 (5.3)		106 (93.8)	7 (6.2)	
46-55	132 (93.6)	9 (6.4)		119 (84.4)	22 (15.6)	
56-65	66 (95.6)	3 (4.4)		55 (79.7)	14 (20.3)	
Sex						
Male	185 (93.0)	14 (7.0)	0.550	175 (87.9)	24 (12.1)	0.550
Female	205 (94.5)	12 (5.5)		191 (88.0)	26 (12.0)	
Lipid content						
0-25%	100 (94.3)	6 (5.7)		91 (85.8)	15 (14.2)	
25-50%	96 (91.4)	9 (8.6)	0.234	96 (91.4)	9 (8.6)	0.620
50-75%	101 (95.3)	5 (4.7)		92 (86.8)	14 (13.2)	
75-100%	93 (93.9)	6 (6.1)		87 (76.0)	12 (24.0)	
Smoking habit						
Nonsmokers	157 (96.3)	6 (3.7)	0.180	145 (89.0)	18 (11.0)	0.826
Active smokers	101 (91.0)	10 (9.0)		96 (86.5)	15 (13.5)	
Passive smokers	132 (93.0)	10 (7.0)		125 (88.0)	17 (12.0)	

†: People have intake the hormone related medicines such as contraceptive drugs, the menopause drug, and other have been excluded in this analysis

**: $p<0.05$

Table 2. Number distribution between subjects within or over the normal range measured levels for 5 hormones, Testosterone, E2, TSH, T3, and T4: number (%), Chi-Square test

	TSH			T4			T3		
	Normal	Abnormal	P	Normal	Abnormal	P	Normal	Abnormal	P
Age									
18-25	38 (100)	0 (0)		38 (100)	0 (0)		38 (100)	0 (0)	
26-35	57 (98.3)	1 (1.7)	0.246	57 (98.3)	1 (1.7)	0.619	56 (96.5)	2 (3.5)	0.810
36-45	111 (94.9)	6 (5.1)		113 (96.6)	4 (3.4)		113 (96.6)	4 (3.4)	
46-55	140 (93.3)	10 (6.7)		143 (95.3)	7 (4.7)		144 (96.0)	6 (4.0)	
56-65	67 (91.8)	6 (8.2)		70 (95.9)	3 (4.1)		70 (96.6)	3 (3.4)	
Sex									
Male	190 (95.5)	9 (4.5)	0.668	191 (96.0)	8 (4.0)	604	191 (96.0)	8 (4.0)	604
Female	223 (94.1)	14 (5.9)		230 (97.0)	7 (3.0)		230 (97.0)	7 (3.0)	
Lipid content									
0-25%	100 (93.5)	7 (6.5)	0.288	102 (95.3)	5 (4.7)	0.578	104 (97.2)	3 (2.8)	0.156

25-50%	107 (98.2)	2 (1.8)		106 (97.2)	3 (2.8)		105 (96.3)	4 (3.7)	
50-75%	103 (94.5)	6 (5.5)		104 (95.4)	5 (4.6)		102 (93.6)	7 (6.4)	
75-100%	103 (92.8)	8 (7.2)		109 (98.2)	2 (1.8)		110 (99.1)	1 (0.9)	
Smoking habit									
Never	162 (94.7)	9 (5.3)	0.997	164 (95.9)	7 (4.1)	0.443	165(96.5)	6 (3.5)	0.313
Active	105 (94.6)	6 (5.4)		106 (95.5)	5 (4.5)		105 (94.6)	6 (5.4)	
Passive	146 (94.8)	8 (5.2)		151 (98.0)	3 (2.0)		151 (98.0)	3 (2.0)	

Table3. Difference of serum PCDD/Fs distribution between subjects above or under normal hormone range for specific gender

	PCDD/Fs levels (pg WHO-TEQ/g lipid)		P value
	Normal	Abnormal	
Testosterone	(410) 20.6±8.5	(26) 19.1±9.2	0.413
E2	(386) 20.0±8.1	(50) 23.8±10.7	**0.003****
TSH	(413) 20.3±8.5	(23) 23.4±9.1	**0.092**
T4	(421) 20.4±8.4	(15) 22.8±10.9	0.289
T3	(421) 20.6±8.6	(15) 18.1±5.7	0.270

**: p<0.05, (n)mean±standard deviation

Table 4. Logistic regression between serum PCDD/Fs levels, and E2 or TSH.

Hormone	Testosterone		E2		TSH	
	OR	95 % CI	OR	95 % CI	OR	95 % CI
Male						
Age	1.67	012-25.66	0.23	0.03-1.88	4.34	0.15-180.25
PCDD/Fs: <16	1.00	--	1.00	--	1.00	--
PCDD/Fs: 16-32	0.46	0.09-2.0	0.86	0.23-3.42	1.56	0.23-16.09
PCDD/Fs: >32	6.21	0.65-45.30	5.07	0.57-34.37	1.87	0.03-27.75
Nonsmokers	1.00	--	1.00	--	--	--
Smoker	1.18	0.25-6.13	1.91	0.59-6.51	--	--
Passive smokers	3.12	0.53-18.23	0.86	0.17-3.62	--	--
Female						
Age	0.01	0.0001-0.26	**94.9****	**8.08-1416**	2.13	0.10-44.64
PCDD/Fs: <16	1.00	--	1.00	--	1.00	-
PCDD/Fs: 16-32	0.49	0.07-4.71	0.34	0.10-1.21	2.22	0.45-19.37
PCDD/Fs: >32	5.50	0.08-131.03	**4.65****	**1.12-19.43**	3.41	0.31-43.78
Nonsmokers	1.00	--	--	--	--	--
Smoker	**16.28***	**1.51-147.63**	--	--	--	--
Passive smokers	0.32	0.05-1.81	--	--	--	--

**: p<0.05

: PCDD/Fs concentration, pg WHO-TEQ/g lipid