國立臺灣大學公共衛生學院職業醫學與工業衛生研究所

碩士論文

Institute of Occupational Medicine and Industrial Hygiene
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Master thesis

一、環境中鉛與錳的產前暴露對兒童早期氣質表現的可能影響

Prenatal exposure to lead and manganese on temperament performance in early childhood

二、台灣年輕人高血壓世代研究族群雙酚 A 與頸動脈內膜中層厚度之相關性

Association between bisphenol A and carotid intima-media thickness in a young hypertension cohort of Taiwan

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致謝

回想起兩年前第一次參加保中老師Group meeting時，老師說：「想好要作什麼題目了嗎？接下來很快就要畢業囉！」，那時的我心想：「畢業？！還要很久吧！」。兩年的時間中，修課、收案、做實驗、跑統計、寫論文……，一項項的填滿我的生活，忙著學習與適應各種學過與沒學過的事物，一切快的讓我沒注意到時間已悄悄流逝，現在的我心裡想的是：「我畢業了，好快！」。短短的兩年，經歷了好多，也學習了好多，一路以來感謝身旁的你們，因為有你們才讓我有今天微甜的成果，謝謝你們！

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Part I

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Prenatal exposure to lead and manganese on temperament performance in early childhood

中文摘要

研究背景與目的：鉛與錳金屬是環境中普遍知道的神經毒物，目前已知共同暴露這兩種金屬時，可能會造成兒童行為問題與神經發展上的負面影響。另外，嬰兒時期的氣質表現可以用來預測未來兒童的行為問題，視為未來行為問題的早期表現，然而，現在仍然不清楚當媽媽懷孕期間暴露鉛與錳金屬是否會對於兒童早期的氣質有影響。因此本篇的研究目的在於釐清母親懷孕期的鉛錳共同暴露與兒童早期氣質表現的相關性。

方法：本篇研究的族群來自於台灣出生世代追蹤研究(Taiwan Birth Panel Study, TBPS)，共 275 對母親與孩童參與，在母親生產時立即收集孩童的臍帶血，利用 Agilent 7500C ICP-MS 進行鉛與錳的濃度量測，在兒童氣質表現的量測選用中文版幼兒氣質量表進行評估，由主要照顧者進行填答評估。我們利用 linear regression 與 mixed-effect model 來分析母親鉛錳暴露與兒童氣質的相關性。

結果：我們發現在高錳暴露組，隨著鉛濃度上升，兒童氣質適應度分數會有下降的趨勢[β: -0.385; *p*-value: 0.058]，且我們也發現反應閾分數趨勢亦同[β: -0. 404; *p*-value: 0.015]。

結論：媽媽懷孕期間鉛錳共同暴露對於兒童早期氣質表現可能具有交互作用的趨勢，但機制仍需要後續研究進一步釐清。

關鍵字：氣質、鉛、錳、幼兒期

Abstract

Background: The lead and manganese are the common neurotoxic metals in the environment. Co-exposure to lead and manganese could injure child neurodevelopment and cause behavior problems. Additionally, temperament performance in infant period may be a predictor of behavior problems in childhood. However, it is not clear that association between prenatal lead and manganese co-exposure and temperament performance in early childhood.

Aims: The purpose of this study is to understand the effect of prenatal exposure to lead and manganese on child temperament.

Methods: A total of 275 newborns from the Taiwan Birth Panel Study (TBPS) were followed up in northern Taiwan. We collected their cord blood for measuring lead and manganese levels by an Agilent 7500C ICP-MS. We used the Chinese Toddler Temperament Scale which was collected from parental reports for measuring temperament at infants and toddlers. We examined the association between lead and manganese co-exposure and child temperament by linear regression and mixed-effect models.

Results: We found that under the higher manganese level, lead level in cord blood was associated with the adaptability ($\beta = -0.385$, p-value = 0.058). We also found that the co-exposure of lead and manganese was associated with threshold of responsiveness ($\beta = -0.404$, p-value = 0.015).

Conclusions: Lead and manganese prenatal exposure may have an effect on early child temperament performance. Mechanistic studies are needed to elucidate the causal relationship.

Key words: temperament, lead, manganese, early childhood

Introduction

Manganese (Mn) and lead (Pb) are the common environmental metal neurotoxin, and even low doses may lead to neurotoxicity, particularly for children (Grandjean & Landrigan, 2006; Wright et al., 2006).

Manganese is an essential element for human, but if concentration exceeds the homeostatic range, Mn can be neurotoxin. The neurotoxicity of Mn has been associated with dopaminergic dysregulation, inhibition of metabolism (Wei et al., 1998), and oxidative injury (MA, 1999) at the synaptic level. High Mn exposure was found have an association with hyperactive behavior, mood dysregulation, and intellectual performance (Bouchard et al., 2006; Ericson et al., 2007). Additionally, children even exposure to low level Mn may cause negative effect on cognition, motor function and behavior (Bouchard et al., 2006; Claus Henn et al., 2012; Menezes-Filho et al., 2011; Wright et al., 2006).

Lead store in blood, soft tissues, and bone, individually, the half-life of Pb in these tissues is about 40 days for blood, months for soft tissues, and years for bone (Barbosa et al., 2005; Surya et al., 2008). A paper showed that 94% of absorbed Pb is deposited in bone and teeth in adults, but that is only 70% in children. Additionally, a part of the 70% absorbed Pb will release from bone to bloodstream in children, because their bones are undergoing remodeling. Therefore, Pb may cause serious health effects on children (Barbosa et al., 2005). The main target for Pb toxicity is central nervous system, and brain is the organ which is often studied in lead toxicity. Within the brain, Pb may lead to damages in the prefrontal cerebral cortex, hippocampus, and cerebellum (Liu et al., 2013). A study found that lead exposure not only inhibits glutamate release, which is an important neurotransmitter, but also decrease the number of the gene for the receptor in part of the brain (Hwang, 2007). In 2007, the Centers for Disease Control

(CDC) in USA published a review paper, and it summarized studies which were published since 1991 to 2006, many strong evidence about children may have physical and development problems at blood lead level <10 μg/dL (Binns et al., 2007). Cognitive deficits were the most concerned adverse effect of Pb exposure (Lanphear et al., 2005). Co-exposure to lead and manganese could injure child intelligence and neurodevelopment (Kim et al., 2009; Lin et al., 2013).

Temperament had an advantage in predicting childhood behavior problems, and it can be readily assessed in infancy (Prior et al., 1992; Kuo et al., 2008). The definition of temperament was the unique behavior patterns of response to internal or external stimuli (Tsou et al., 1987). Temperament didn't affect intelligence and future achievement, but it would affect the personality development of infants and children. Individual temperament characteristics of parents and child may involve with fitness for parent-child relationship and individualized education. In the beginning, the temperament was used to assess and resolve parent-child problems, but some studies found temperament may be a good predictor of future behavior problems, like Attention deficit hyperactivity disorder (ADHD) (Nigg et al., 2004; Martel and Nigg, 2006).

However, only a study found Pb have effects on child intelligence (Lucchini et al., 2012). The effects of Pb-Mn interaction are still not clear. Additionally, the association between prenatal lead and manganese co-exposure and temperament performance in early childhood is also not clear. Therefore, we conducted this study to understanding the effect of lead and manganese exposure in pregnancy of child temperament.

Material and Methods

Study design and population

This study was a part of the Taiwan Birth Panel Study (TBPS) which was conducted at a medical center in Taipei from May 2004 to January 2005 (Hsieh et al., 2011). The trained researcher interviewed mothers after delivery by structured questionnaire and specialized medical staffs collected the specimen of umbilical cord blood at birth. The structured questionnaire included information about parents' age, education level, annual household income and infant's primary caregiver. Maternal characteristics such as occupation, nationality, gestational age, breastfeeding, tobacco smoke, drinking behavior, and environmental tobacco smoke exposure (ETS) during pregnancy were also collected. We collected the neonatal information included infant sex, parity, gestational age and birth weight from medical records. The well-trained researchers visited participants' home to measure the home environment when children were 6 months and 2 years of age by the Home Observation for Measurement of the Environment-Infant/Toddler version (HOME) (Caldwell & Bradley, 2003).

A total of 335 newborns and their parents were participated in this study. We excluded 3 subjects whose mother were active smoke during pregnancy, after eliminated missing metal data (N = 55), very preterm case (gestation less than 32 weeks, N = 2), there were 275 mother-infant pairs in this study. We measured infant' temperament status by the Chinese Toddler Temperament Scale at the age of 4, 12 and 24 months, and the case number were 212, 138 and 170, respectively. At the beginning of the study and before each follow-up, all the mothers gave informed consent and the study protocol was approved by the Ethics Committee of the National Taiwan University Hospital.

Measurement of metals

We collected their umbilical cord blood for measuring lead and manganese levels by an Agilent 7500C Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Following the standard protocol, umbilical cord blood were collected at birth by ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA) tubes, and stored at -80°C until processed for metal analysis. Before analyzing, those samples would be diluted with solution containing 5 g/L of 25% ammonia, 0.5 g/L Triton X-100 and 0.5 g/L EDTA in double de-water with a sample to solution volume ratio of 1:9. Then we used filters to remove crud. In this study, the detection limits for manganese and lead were 1.50 and 0.35 μg/L. All samples were above detection limits.

Measurement of temperament

We used the Chinese Toddler Temperament Scale (CTTS) for assessing the toddlers' temperament situation. The child's temperament was different on sex and culture (Campbell & Eaton, 1999; Fullard et al., 1984; Weiss et al., 2004). The CTTS was modified from the Toddler Temperament Scale (Fullard et al., 1984) to adapt to Chinese culture. There were 95 items with a 6-point Likert scale with options ranging from "almost never" to "almost always" in the CTTS, and that information was collected from main caregivers' reports to rate their children's behavior in the past month. The CTTS would have scores on nine dimensions of temperament: activity level (higher scores mean much more activity), rhythmicity (higher scores mean worse rhythmic), approach/ withdrawal (higher scores mean more withdrawal), adaptability (higher scores mean lower adaptability), intensity of reaction (higher scores mean intense), quality of mood (higher scores mean negative), persistence (higher scores mean lower persistence), distractibility (higher scores mean concentration) and

threshold of responsiveness (higher scores mean lower threshold). The CTTS was standardized on 349 infants whose age were less than 12 months and 308 toddlers who were 12 to 36 months of age in Taiwan with acceptable internal consistency (Cronbach $\alpha = 0.55–0.82$) (Tsou et al., 1987).

Other co-variables

We collected the socioeconomic, characteristics information and other factors via questionnaires and medical records to check which ones could confound the relationship between manganese, lead and temperament: parents' age, education level, occupation, nationality, annual household income, gestational age, breastfeeding, tobacco smoke, drinking behavior, environmental tobacco smoke exposure (ETS) during pregnancy, and infant's sex, parity, birth weight and primary caregiver. We also use the Home Observation for Measurement of the Environment-Infant/Toddler version (HOME) to measure the quality and quantity of home environment about caregiving support when children were 6 months of age and 2 years old by the well-trained researchers visited participants' home.

Statistical analysis

The main effect of manganese was showed an inverted U-shaped association between manganese concentration and neurodevelopment performance (Claus Henn et al. 2010). Therefore we categorized the manganese concentration in to two groups ($<75^{th}$ percentile and $\geq 75^{th}$ percentile, representing the low and high exposure group, respectively). We fit separate adjusted linear regression model of lead level in higher or lower manganese category and each CTTS dimension for each follow-up time point. We also fit one linear mixed-effect model of the CTTS and lead level in higher or lower

manganese category over time.

We adjusted all models for the same variables as the confounders, maternal age, education, occupation, environmental tobacco smoke during pregnancy, family annual household income and home score (6 months home score adjusted for 4 months temperament, 2 years old home score adjusted for 12 and 24 months temperament), infant preterm, breastfeed period (4months), birth weight, primary caregiver at night. Those confounders were included because those could change the manganese and lead effect estimates more than 10% when we added one at a time and could affect more than five temperament dimensions. We would choose only one from the two correlated variable (correlation coefficients ≥ 0.4). We conducted statistical analyses using SAS (version 9.2; SAS Institute, Inc., Cary, NC, USA) and SPSS (version 16; SPSS Inc., Chicago, IL, USA). We considered results statistically significant at p-value < 0.05.

Results

In the Table 1, we presented the manganese and lead concentration in each characteristics of the study population, and try to compare any significant different in subgroups. We found both manganese and lead concentration were significant higher in the lower maternal education level subgroup (*p*-value = 0.021, *p*-value = 0.020). The children' cord blood lead concentrations were higher if their mother were exposure to environmental tobacco smoke during pregnancy (*p*-value = 0.015). We also found primiparous infants had significant higher lead concentration in cord blood (*p*-value = 0.007), and the infants who were cared by their mothers at night would have lower cord blood manganese concentrations (*p*-value = 0.049).

At the 4,12,24 months of age, the cord blood manganese arithmetic mean (±SD) concentration were 52.2±17.4 μg/L, 51.2±1.2 μg/L, and 51.4±1.3 μg/L (range, 18-123), and lead arithmetic mean (±SD) concentration were 1.2±0.7 μg/dL, 1.2±0.7 μg/dL, and 1.3±0.7 μg/dL (range, 0-4). The Pearson correlation coefficient of manganese and lead was 0.36 (data not shown).

Table 1. Manganese and lead concentration in each characteristics of the study population

			Cord blood Mn (μg/L)			Cord blood Pb (μg/dL)		
	N	%	Mean	SD	*p*-value	Mean	SD	*p*-value
Total	275		50.65	17.39		1.22	0.72	
Maternal characteristics								
Age (years)					0.459			0.248
<25	10	3.64	44.45	13.66		0.87	0.52	
26-34	192	69.81	51.21	16.54		1.23	0.73	
>35	73	26.55	50.03	19.87		1.27	0.71	
Education					**0.021**			**0.020**
High school and below	112	40.73	53.58	18.64		1.35	0.75	
University and above	163	59.27	48.64	16.23		1.14	0.68	
Occupation					0.453			0.059
employed	215	78.18	51.07	17.82		1.27	0.76	
housewife	60	21.82	49.16	15.82		1.07	0.50	
Drinking during pregnancy					0.229			0.855
yes	10	3.64	44.15	15.13		1.18	0.80	
no	265	96.36	50.90	17.45		1.23	0.72	
ETS* during pregnancy					0.100			**0.015**
yes	51	18.55	54.27	19.12		1.44	0.73	
no	224	81.45	49.83	16.91		1.17	0.71	
Parity					0.145			**0.007**
primipara	123	44.73	52.35	18.54		1.35	0.81	
multipara	152	55.27	49.28	16.34		1.12	0.62	
Breastfeeding					0.876			0.887
no	6	2.18	51.75	25.23		1.27	0.53	
yes	269	97.82	50.63	17.24		1.22	0.72	
Breastfeeding period					0.650			0.096
≤4monthes	212	77.09	50.39	17.02		1.18	0.73	
＞4monthes	63	22.91	51.53	18.69		1.36	0.68	
Family annual household income(NT)					0.135			0.996
< $1,000,000	126	45.82	48.95	17.14		1.22	0.81	
≥ $1,000,000	149	54.18	52.09	17.53		1.22	0.63	
Infant characteristics								
Gender					0.108			0.899
Male	153	55.64	52.16	18.64		1.23	0.72	
Female	122	44.36	48.76	15.55		1.22	0.72	
Preterm(<37weeks)					0.917			0.139
yes	17	6.18	51.08	16.59		0.97	0.49	
no	258	93.82	50.62	17.47		1.24	0.73	
1-6month primary caregivers								
day					0.896			0.534
mother	76	27.64	50.43	15.84		1.27	0.65	
others	199	72.36	50.74	17.98		1.21	0.75	
night					**0.049**			0.274
mother	236	85.82	49.81	16.80		1.20	0.72	
others	39	14.18	55.73	20.11		1.34	0.70	

	Mean	SD
Gestational age (weeks)	38.78	1.46
Birth weight(kg)	3.22	0.45
HOME score		
6 month	38.79	2.29
24 month	40.81	2.56

*ETS: environmental tobacco smoke

In the Table 2, we could find the adaptability scores were less with the arisen lead concentration in the higher manganese group at the 24 month time point (β = -0.603, *p*-value = 0.032) and over time (β= -0.385, *p*-value = 0.058), which mean they had positively over the normal range of adaptability. Figure 1 shows a regression line for the association of lead with adaptability score among children in higher manganese group (≥75th percentile) versus lower manganese group (< 75th percentile). The threshold of responsiveness scores were less with the arisen lead concentration in higher manganese group at over time (β = -0.404, *p*-value = 0.015) and 24 month time point (β = -0.451, *p*-value = 0.055), which mean those children' threshold of responsiveness were higher. Figure 2 shown a regression line for the association of lead with threshold of responsiveness score among children in higher manganese group (≥75th percentile) versus lower manganese group (< 75th percentile).We also found that the higher manganese group would tend to more withdrawal with arisen lead concentration in cord blood at 12 months time point (β = -0.648, *p*-value = 0.041).

Table 2. Linear regression models and linear mixed-effect model of lead concentration (μg/dL) and temperament in high (≥59.3μg/L) or low (<59.3μg/L) manganese exposure group

	crude															
	Mn<59.3μg/L		Mn≥59.3μg/L		Mn<59.3μg/L		Mn≥59.3μg/L		Mn<59.3μg/L		Mn≥59.3μg/L		Mn<59.3μg/L		Mn≥59.3μg/L	
	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value
	4 months[1]				12 months[1]				24 months[1]				0-24 months[2]			
Activity level	0.156	0.117	-0.209	0.321	-0.038	0.768	-0.091	0.655	0.020	0.868	0.173	0.524	0.015	0.863	-0.106	0.350
Rhythmicity	-0.163	0.151	0.142	0.562	0.142	0.241	-0.233	0.424	-0.051	0.626	0.166	0.672	-0.050	0.578	0.074	0.618
Approach/Withdrawal	0.030	0.749	-0.254	0.208	0.006	0.955	**-0.534**	**0.004**	-0.069	0.457	-0.063	0.797	0.014	0.852	**-0.220**	**0.035**
Adaptability	-0.011	0.920	-0.388	0.122	-0.027	0.778	-0.397	0.080	-0.078	0.420	**-0.598**	**0.017**	-0.056	0.487	**-0.377**	**0.002**
Intensity of reaction	**0.188**	**0.039**	0.021	0.925	-0.095	0.406	-0.252	0.319	-0.078	0.499	0.072	0.799	0.008	0.914	0.006	0.962
Quality of mood	0.156	0.094	-0.071	0.766	-0.132	0.296	-0.015	0.958	-0.051	0.637	0.243	0.355	-0.003	0.972	-0.027	0.835
Persistence	0.158	0.124	-0.187	0.444	0.168	0.209	-0.236	0.401	-0.075	0.584	-0.003	0.989	0.052	0.571	-0.141	0.309
Distractibility	0.083	0.414	-0.169	0.477	-0.044	0.667	-0.318	0.158	-0.051	0.644	0.062	0.784	0.013	0.877	-0.154	0.188
Threshold of responsiveness	0.084	0.405	-0.394	0.105	-0.017	0.883	-0.169	0.434	0.143	0.167	-0.443	0.056	0.028	0.737	**-0.305**	**0.007**
	adjusted*															
	4 months[1]				12 months[1]				24 months[1]				0-24 months[2]			
Activity level	0.078	0.528	-0.150	0.580	-0.028	0.853	-0.045	0.873	0.047	0.712	0.231	0.452	0.086	0.362	-0.085	0.627
Rhythmicity	-0.214	0.132	0.252	0.413	0.080	0.584	0.150	0.687	-0.028	0.806	0.187	0.674	-0.055	0.565	0.108	0.640
Approach/Withdrawal	-0.120	0.332	-0.289	0.296	-0.082	0.493	**-0.648**	**0.041**	-0.024	0.812	-0.057	0.834	0.059	0.452	-0.280	0.100
Adaptability	-0.180	0.163	-0.308	0.375	-0.122	0.233	-0.465	0.142	-0.093	0.381	**-0.603**	**0.032**	-0.079	0.344	-0.385	0.058
Intensity of reaction	0.110	0.335	0.008	0.976	-0.164	0.237	-0.490	0.164	-0.171	0.176	0.072	0.819	0.015	0.852	0.025	0.891
Quality of mood	0.083	0.499	-0.040	0.906	-0.154	0.287	-0.019	0.963	-0.075	0.521	0.258	0.354	0.050	0.581	-0.063	0.767
Persistence	0.118	0.400	-0.237	0.417	0.066	0.646	0.037	0.931	-0.066	0.650	0.042	0.875	0.111	0.249	-0.116	0.598
Distractibility	-0.009	0.944	-0.332	0.281	-0.067	0.540	-0.528	0.145	-0.035	0.768	0.082	0.725	-0.016	0.863	-0.052	0.778
Threshold of responsiveness	-0.016	0.905	-0.141	0.643	-0.113	0.393	-0.142	0.706	0.181	0.106	-0.451	0.055	0.074	0.407	**-0.404**	**0.015**

*adjusted for maternal nationality, age, education, environmental tobacco smoke during pregnancy, parity, gestational age, family annual household income and home score(6 months home score adjusted for 4 months temperament, 2 years old home score adjusted for 12, 24, and 0-24 months temperament), infant breastfeed period(4 month), primary caregiver at day time.

1: Linear regression model

2: Linear mixed-effect model

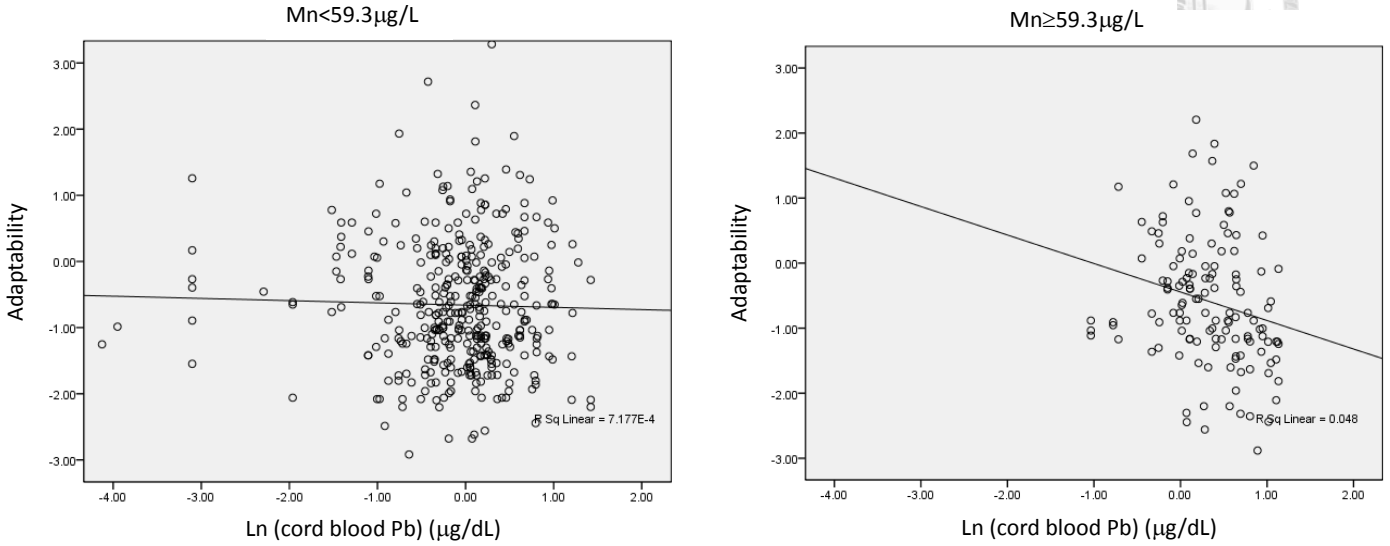

Figure 1. Scatter plots and regression lines of cord blood lead and adaptability temperament among children with cord blood manganese in percentile less than 75th (Mn1, interaction *p*-value =0.76) and more than 75th (Mn2, interaction *p*-value =0.07).

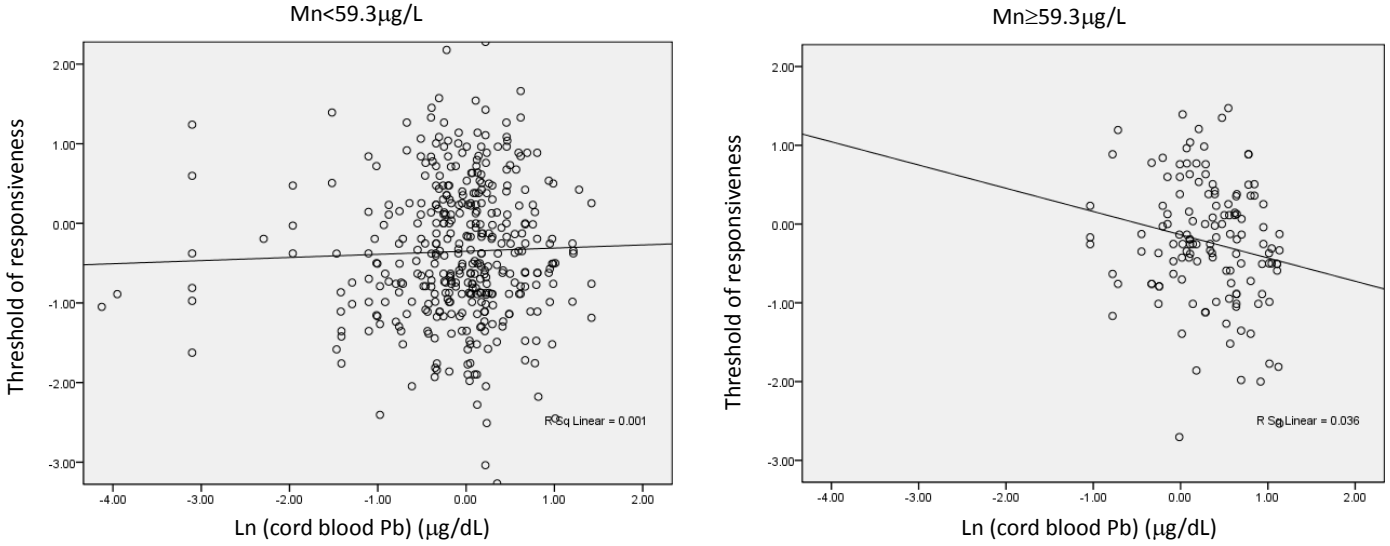

Figure 2. Scatter plots and regression lines of cord blood lead and threshold of responsiveness temperament among children with cord blood manganese in percentile less than 75th (Mn1, interaction *p*-value =0.13) and more than 75th (Mn2, interaction *p*-value =0.08).

Discussion

Our findings indicate that in utero co-exposure to environmental manganese and lead may have an interaction for temperament, especially with the adaptability and threshold of responsiveness.

In our result shows that in the higher manganese group the adaptability scores were less, which mean the adaptability were better, with the arisen lead concentration. However, we do not think it a protective situation. The Chinese Toddler Temperament Scale was used to assessing the temperament and trying to intervene between parents and child when they have upbringing problem. The CTTS have to be standardized, and we consider the score over one standard deviation were un-normal status. So, it was considered with more un-normal that the higher manganese group has fewer score with the arisen lead concentration. It is the same concept on threshold of responsiveness and approach-withdrawal (Tsou et al., 1987).

The mechanisms of lead and manganese interactions on temperament are still unclear. We conjecture the interaction of manganese and lead may be according to same target organ, and potentially similar biological mechanisms. The neurotoxicity of lead can affect the protein kinase C and the molecules involved in signal transduction, inhibit Ca^{2+} dependent acetylcholine and dopamine release (Devoto et al., 2001; Long et al., 1994). The manganese has been shown associated with

neurotransmitters (Finkelstein et al., 2007), inhibit protein transports (Lockman et al., 2001) and involved in cellular signal transduction (Spranger et al., 1998). Therefore, we believe that lead and manganese may have interaction.

In epidemiological study findings, the elevated blood lead level would have a temperament alteration of children, especially in activity level, approach-withdrawal and adaptability dimensions (Liu et al., 2011), and the manganese exposure would cause the subtle negative associated with motor speed and coordination which were kind of neurodevelopment (Hernandez-Bonilla et al., 2011). We can understand the lead has effect on temperament, but there are still no direct evidence about the relationship between single manganese exposure and temperament. However, the lead and manganese are really could cause the effect on neurodevelopment, and in utero co-exposure to manganese and lead had an effect on intelligence, cognitive and neurodevelopment (Claus Henn et al., 2012; Lin et al., 2013; Lin et al., 2011), so we think there may have an interaction on temperament.

The Chinese toddler temperament scale (CTTS) was reported by main caregiver, but the main caregiver's pressure status may influence the results of CTTS. However, we didn't have the pressure status information about main caregiver when child was 4, 12, and 24 month of age. We only have the mother pressure status data during pregnancy and the work status after delivery, which were alternative factors.

Therefore, we used the linear mixed-effect model to analysis the mother pressure status during pregnancy and children temperament performance (appendix 2). We didn't found significant effects on most temperament dimensions, except the rhythmicity. The main findings in this study may not confound with mother pressure during pregnancy. We also compared children over time temperament performance between occupational woman and housewife by ANOVA analysis. We found there were no significant different between two work status categories in all temperament dimensions (the *p*-value were 0.460-0.874).

The manganese concentration in our study is higher than the concentration in other studies (Abdelouahab et al., 2010; Jones et al., 2010; Kim et al., 2009; Zota et al., 2009). The situation may cause from the fuel in Taiwan. A Taiwan studies suggested that the cord blood manganese level would elevate that exposure to manganese-containing fuel from vehicles (Lin et al., 2011). In this study no any case is the very low manganese (<15μg/L), but all cases' concentration in higher manganese group are over the 56μg/L, if use the 15-56μg/L as the standard (Su et al., 2007). It may be the reason for explaining why we can find the interaction in higher manganese group in crude and adjusted models. Additionally, the mechanisms and target organ of manganese and lead are similar that may another reason for interaction. The lead concentration level in our study is lower than other studies (Claus Henn et al.,

2012; Liu et al., 2011). After 2000 year, Taiwan ban to use tetraethyl lead in gasoline, and a study found the average lead concentrations in cord blood had significantly decreased (Hwang et al., 2004).

The strength of our study is that we have comprehensive information about social-economical and home environment factors, which can use to adjust the confounder of children' temperament. However, our study still has limitations. The CTTS only could adapt to similar races and cultures, so our result could not compare with another country, but we may still conjecture other countries which have similar races and cultures, may have similar trends. Furthermore, we don't know the manganese and lead exposure after delivery, so we can't understand the environment metal postnatal exposure will lead to how much effect on temperament.

Conclusions

Lead and manganese co-exposure during pregnancy may have interaction to affect on early child temperament performance. Mechanistic studies are needed to elucidate the causal relationship.

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Appendix 1

Appendix 1. One way ANOVA of temperament Z score in eliminate and include group

	Metal missing data group* (N=55)		Include group (N=275)		
	Mean	SD	Mean	SD	*p*-value
Activity level					
4 month	-0.35	1.04	-0.47	0.86	0.42
12 month	0.74	0.97	0.62	0.86	0.50
24 month	0.02	0.98	-0.15	0.88	0.31
Rhythmicity					
4 month	0.45	1.04	0.39	0.98	0.72
12 month	-0.11	0.74	-0.12	0.87	0.97
24 month	0.18	0.80	-0.15	0.95	0.05
Approach/Withdrawal					
4 month	-0.64	0.90	-0.54	0.82	0.47
12 month	-0.22	0.69	-0.04	0.69	0.20
24 month	0.03	0.93	0.02	0.71	0.95
Adaptability					
4 month	-0.30	0.96	-0.05	0.95	0.13
12 month	-1.14	0.65	-0.95	0.70	0.16
24 month	-1.25	0.74	-1.11	0.79	0.36
Intensity of reaction					
4 month	-0.40	0.88	-0.43	0.81	0.86
12 month	-0.43	0.96	-0.53	0.82	0.53
24 month	-0.84	0.93	-0.72	0.82	0.43
Quality of mood					
4 month	-0.38	1.07	-0.24	0.85	0.36
12 month	1.21	1.02	1.11	0.88	0.56
24 month	0.81	1.25	0.61	0.82	0.21
Persistence					
4 month	-0.11	0.99	0.01	0.90	0.44
12 month	0.11	0.96	-0.19	0.94	0.11
24 month	-0.69	0.99	-0.90	1.06	0.25
Distractibility					
4 month	-0.08	0.92	0.09	0.90	0.30
12 month	-1.86	0.87	-1.97	0.73	0.46
24 month	-2.21	0.78	-2.27	0.81	0.69
Threshold of responsiveness					
4 month	-0.14	0.97	-0.35	0.90	0.18
12 month	-0.54	0.82	-0.45	0.78	0.59
24 month	-0.02	0.96	-0.19	0.83	0.29

*Mn and/ or Pb were missing

Appendix 2

Appendix 2. Linear mixed-effect model of the over time temperament performance and the mother pressure

	crude																			
	Vitality		Nervous		Depression		Peaceful		Vigorous		Sullen		Exhausted		Happy		Tired		Working Pressure	
	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value
Activity level	-0.041	0.371	0.090	0.095	0.137	*0.044*	-0.021	0.693	-0.010	0.828	0.083	0.232	-0.037	0.416	-0.052	0.303	0.109	*0.047*	0.012	0.773
Rhythmicity	0.084	0.092	-0.012	0.845	-0.206	**0.006**	0.071	0.213	0.070	0.152	-0.053	0.487	0.051	0.290	0.109	*0.048*	-0.038	0.523	0.030	0.491
Approach/Withdrawal	-0.026	0.525	0.068	0.149	0.120	*0.045*	-0.065	0.159	-0.089	**0.021**	0.107	0.078	-0.011	0.784	-0.090	*0.042*	-0.016	0.741	0.059	0.097
Adaptability	-0.051	0.251	0.033	0.522	0.067	0.306	-0.029	0.563	-0.059	0.170	0.101	0.133	0.000	0.994	-0.091	0.063	0.013	0.801	0.073	0.060
Intensity of reaction	0.002	0.954	0.001	0.978	-0.037	0.555	0.025	0.604	0.023	0.573	0.573	0.305	0.011	0.780	-0.027	0.564	0.564	0.861	0.025	0.498
Quality of mood	-0.067	0.155	0.057	0.305	0.091	0.188	-0.054	0.320	-0.042	0.356	0.049	0.497	0.016	0.737	-0.068	0.185	0.024	0.663	0.028	0.508
Persistence	0.003	0.948	0.044	0.443	-0.010	0.896	0.009	0.869	0.009	0.853	0.045	0.550	-0.032	0.512	0.064	0.233	0.022	0.704	0.015	0.722
Distractibility	-0.002	0.959	0.046	0.392	-0.024	0.708	0.065	0.196	-0.008	0.851	0.025	0.719	0.002	0.972	0.012	0.802	0.081	0.125	0.057	0.153
Threshold of responsiveness	-0.006	0.887	-0.020	0.711	0.035	0.609	0.023	0.657	-0.060	0.173	-0.003	0.964	0.006	0.896	-0.039	0.433	-0.073	0.172	-0.039	0.330
	adjusted*																			
	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value
Activity level	-0.030	0.533	0.073	0.182	0.131	0.060	-0.021	0.697	-0.006	0.891	0.061	0.389	-0.041	0.377	-0.046	0.377	0.100	0.070	0.007	0.860
Rhythmicity	0.100	*0.054*	-0.013	0.825	-0.203	**0.008**	0.072	0.210	0.098	*0.052*	-0.071	0.356	0.044	0.374	0.124	**0.027**	-0.033	0.580	0.016	0.723
Approach/Withdrawal	-0.028	0.496	0.054	0.261	0.117	*0.056*	-0.048	0.297	-0.082	*0.041*	0.104	0.092	-0.017	0.662	-0.084	0.062	-0.020	0.681	0.048	0.186
Adaptability	-0.029	0.514	0.024	0.641	0.076	0.245	-0.025	0.620	-0.049	0.258	0.068	0.307	0.021	0.692	-0.084	0.084	0.018	0.738	0.056	0.152
Intensity of reaction	0.001	0.976	0.003	0.953	-0.017	0.794	0.020	0.679	0.019	0.646	0.068	0.296	0.025	0.622	-0.036	0.455	0.008	0.882	0.020	0.593
Quality of mood	-0.044	0.354	0.053	0.350	0.096	0.176	-0.059	0.278	-0.049	0.298	0.021	0.774	0.102	0.075	-0.070	0.186	0.019	0.730	0.030	0.482
Persistence	0.021	0.671	0.051	0.386	-0.011	0.884	-0.012	0.825	0.006	0.895	0.032	0.670	-0.042	0.482	0.071	0.196	0.043	0.462	0.028	0.520
Distractibility	0.005	0.907	0.045	0.415	-0.017	0.801	0.059	0.256	0.016	0.714	-0.014	0.842	-0.030	0.576	0.030	0.548	0.109	0.043	0.058	0.159
Threshold of responsiveness	-0.018	0.689	-0.024	0.663	0.054	0.435	0.037	0.478	-0.051	0.263	-0.002	0.980	0.032	0.569	-0.038	0.465	-0.078	0.156	-0.050	0.224

*adjusted: maternal nationality, age, education, environmental tobacco smoke during pregnancy, parity, gestational age, family annual household income and home score(2 years of age), infant breastfeed period(4month), primary caregiver at day time.

Appendix 3

嬰兒氣質評估問卷

(4-8 個月嬰兒用，1977 年修訂版)

編　　號：_____

寶寶姓名：_____　出生日期：_____年_____月_____日

填寫日期：_____年_____月_____日

這份問卷的目的是要了解您寶寶對其內在刺激(如肚子餓)和外在刺激(如替他剪指甲)的反應模式(或稱為氣質)。這份問卷裡一共有 95 個具體的行為描述，每一題都和您寶寶的日常行為表現有關。請您按照您寶寶實際的情形，將每題中最符合您寶寶的情形的數字圈起來。雖然有些題目看起來相似，但他們並不一樣，請您每題都填答。假如有些題目您無法回答，或某一題目對您寶寶來說不適用，請在該題後面寫上不適用或無此經驗。

例如：

14. 第一次與其他小孩見面時會顯得害羞臉轉開或抱住母親

如果您寶寶從來沒有這經驗，請在該題後面註明: 無此經驗。

假如您寶寶自出生到現在，在某一題目所問的行為表現模式有所改變，請圈選符合他最近所建立反應模式的代表數字。

這份問卷的回答沒有好壞或對錯的區別。我們是想了解您寶寶的氣質特徵，然後與您討論如何根據此特徵來因材施教，以促進寶寶身心的健全發展。

問卷的後面另有幾個問題，是想了解您對寶寶氣質的綜合看法，請您按照您的主觀感覺回答。

謝謝您的合作！

當您填寫以下的量表時，請依照您寶寶最近的行為表現，以您主觀的判斷，在每題中圈選出最恰當的代表數字，假如題目中有多項事件而您寶寶反應不相同時，則請您將其多項行為總合起來作主觀判斷；假如有些題目不適合您寶寶的行為表現，將註明原因而不加圈選。

	從不	很少	偶爾	有時	常常	總是
1. 除牛奶或母奶外，每天大約吃等量的麥片、稀飯、麵條等食物。	1	2	3	4	5	6
2. 在正要入睡及醒來時，顯得煩躁不安(例如吵鬧、哭泣、或皺緊眉頭)。	1	2	3	4	5	6
3. 每玩一種玩具的時間不到一分鐘，就想玩其他的東西，或做其他的動作。	1	2	3	4	5	6
4. 當在看電視或注意近處的事物時，能安靜地坐著。	1	2	3	4	5	6
5. 餵奶時，對於餵奶姿勢與地點的改變或換人餵奶時，均能立刻接受。	1	2	3	4	5	6
6. 清醒時，可乖乖的讓你剪指甲。	1	2	3	4	5	6
	從不	很少	偶爾	有時	常常	總是

7.	當肚子餓而哭泣時，用奶嘴或圍圍兜、抱他，可使其停止哭泣一分鐘以上。	1	2	3	4	5	6
8.	當玩一種喜歡的玩具時，可連續玩 10 分鐘以上。	1	2	3	4	5	6
9.	在一天中的任何時間洗澡，都能接受。	1	2	3	4	5	6
10.	餵食時，不論對喜歡與不喜歡的食物，表現的反應都是輕微的。	1	2	3	4	5	6
11.	當尿布被大便弄髒時，會有不舒服的表現(例如：煩躁不安、扭動身體等)。	1	2	3	4	5	6
12.	洗澡時，能安靜地讓您洗而不亂動。	1	2	3	4	5	6
13.	每天在相同的時間想要吃奶(時間相差在 1 小時以內)。	1	2	3	4	5	6
14.	第一次見到陌生的其他小孩時，會顯得害羞(例如：把頭轉開或抱緊母親)。	1	2	3	4	5	6
15.	換尿布時，雖然利用種種方法分散其注意力(例如：唱歌，以玩具逗他等），仍顯得煩躁不安。	1	2	3	4	5	6
16.	可以自己一個人玩半個小時以上(例如：玩身邊的玩具或看周圍的東西等)。	1	2	3	4	5	6
17.	穿衣服或換尿布時動得很厲害(手舞足蹈、身體扭動)。	1	2	3	4	5	6
18.	吃飽或不想再吃時，會強烈地抗拒再給的食物(例如：大聲哭鬧、嘴巴緊閉、吐出食物、或推開奶瓶、湯匙)。	1	2	3	4	5	6
19.	試圖改變餵食的時間(差別在 1 小時以上），經兩次嘗試以後，仍然拒絕。	1	2	3	4	5	6
20.	每天大便不一定（時間相差在 1 小時以上)。	1	2	3	4	5	6
21.	當有人從身邊走過時，會停止原來的活動而注意觀看。	1	2	3	4	5	6
22.	正在玩一個喜歡的玩具時，會忽略談話聲或熟悉的聲音而不加以注意。	1	2	3	4	5	6
23.	在換尿布或換衣服時，會發出愉快的聲音或愉悅的表情。	1	2	3	4	5	6
24.	能馬上接受新食物，並迅速嚥下。	1	2	3	4	5	6
25.	觀看其他小孩遊戲時，不到一分鐘就轉移視線看別的地方。	1	2	3	4	5	6
26.	由陰暗處迅即轉至強光下，嬰兒只表現出輕微的反應(或眨眼睛或短暫的驚嚇)。	1	2	3	4	5	6
27.	第一次到陌生的環境時(例如：到朋友家玩或到百貨公司），會有愉快的表情。	1	2	3	4	5	6
28.	每天晚上在一定的時間入睡（時間相差在半小時之內)。	1	2	3	4	5	6
29.	對於日常生活例行的事件（例如：梳髮、洗臉）易接受而不反抗。	1	2	3	4	5	6
30.	在行進的汽車中可安靜的被抱著，或在行進的嬰兒車，可安靜地坐著。	1	2	3	4	5	6
		從不	很少	偶爾	有時	常常	總是

31.	對新保姆的最初反應是拒絕的(表現哭泣或抱緊母親)。	1	2	3	4	5	6
32.	練習一種新的動作時(例如：翻身或拿東西)可持續進行數分鐘。	1	2	3	4	5	6
33.	醒來躺在床上時，會動得很厲害(例如：手舞足蹈、全身扭動)。	1	2	3	4	5	6
34.	對於洗澡地點的改變或換人洗澡，經過 2、3 次的嘗試之後，仍表現出抗拒的樣子。	1	2	3	4	5	6
35.	每次吃牛奶的食量相差很大（差別在 50c.c 以上)。	1	2	3	4	5	6
36.	置身陌生地方，或新的環境時，最初的幾分鐘會表現煩躁不安。	1	2	3	4	5	6
37.	當母親的外表或服飾有明顯的改變時(換髮型或新衣著)寶寶會凝神觀看。	1	2	3	4	5	6
38.	對食物好壞的反應程度差別很大，不論是喜好(大叫、大笑)或厭惡(哭鬧、拒絕)的表現均很強烈。	1	2	3	4	5	6
39.	對於日常生活例行的事件，例如：梳頭、洗臉時會表現出愉快的神情。	1	2	3	4	5	6
40.	在哭泣時，雖由通常照顧者給予數分鐘的安撫，仍無法安靜下來。	1	2	3	4	5	6
41.	對於喜歡的玩具，雖然伸手拿不到但會嘗試用各種方法去獲得(包括喊叫），而且時間持續兩分鐘以上。	1	2	3	4	5	6
42.	對於會發出聲響的玩具，會有強烈的情緒表現(不論是喜歡或討厭的表情均可)。	1	2	3	4	5	6
43.	跟照顧者玩耍時，手腳及身體都會舞動得很厲害。	1	2	3	4	5	6
44.	雖然手中已握有一個玩具，但仍會注視再給他的另一個玩具。	1	2	3	4	5	6
45.	在家裡與陌生的客人接觸時，初次反應是接受的。	1	2	3	4	5	6
46.	在白天，小睡的入睡時間是不定的(相差 1 小時以上)。	1	2	3	4	5	6
47.	改變副食品的味道或濃度時，可繼續進食而沒有反應。	1	2	3	4	5	6
48.	自己一個人玩耍時會哭泣。	1	2	3	4	5	6
49.	大約在 10 分鐘之內，就能適應新的環境(例如：改變家庭擺設、進入商店或遊戲場所等)。	1	2	3	4	5	6
50.	每天白天小睡的睡眠時間長度大約相同(相差在半小時內)。	1	2	3	4	5	6
51.	在餵食時，會動得很厲害(用手抓東西、身體扭動、手腳亂踢)。	1	2	3	4	5	6
52.	當光線突然改變時(如閃光燈、開燈)會有反應(凝視光線或嚇一跳)。	1	2	3	4	5	6
53.	想睡覺時，可用兒語或遊戲的方式使其安靜。	1	2	3	4	5	6
		從不	很少	偶爾	有時	常常	總是
54.	換尿布或換衣服時，表現出強烈的情緒(大聲哭鬧	1	2	3	4	5	6

	或笑出聲音來)。						
55.	入睡到醒來時,身體躺的位置相同,並沒有變換。	1	2	3	4	5	6
56.	當睡覺的地方或睡眠的時間改變,在1、2天之內就能適應,而且睡得很好。	1	2	3	4	5	6
57.	當牛奶的種類或溫度改變時,或以果汁代替時,通常會注意到而且有所反應。	1	2	3	4	5	6
58.	看電視時,每次都可持續5分鐘以上。	1	2	3	4	5	6
59.	當尿布濕而煩躁不安時,可用下列的方法使其安靜(例如:將他/她抱起、跟他/她玩或讓他/她看電視等)。	1	2	3	4	5	6
60.	每天大約在同一時間想吃麥片、稀飯、麵條等副食品(相差在1小時以內)。	1	2	3	4	5	6
61.	在餵食牛奶或副食品時,若中斷餵食,仍表現愉快的樣子(微笑或發出愉悅的聲音)。	1	2	3	4	5	6
62.	換人或換地方洗澡時,在數分鐘內就可接受。	1	2	3	4	5	6
63.	打針後,在1分鐘內便停止哭泣。	1	2	3	4	5	6
64.	哭泣時,身體動得很厲害(手舞足蹈)。	1	2	3	4	5	6
65.	在同一天內,對於同一種吵鬧聲會持續作多次的反應(例如:打鐘聲、狗叫聲)。	1	2	3	4	5	6
66.	當副食品的濃度、味道或溫度改變時,最初的反應是退縮或拒絕(將頭轉開或將食物吐出)。	1	2	3	4	5	6
67.	每天早晨醒來的時間差異很大(相差在1小時以上)。	1	2	3	4	5	6
68.	當寶寶拒絕不喜歡的食物或藥品時,不管努力用什麼方法逗他/她玩,都無法分散其注意力。	1	2	3	4	5	6
69.	清醒時即使輕輕地摸一下身體,也會有所反應(驚嚇、不安的扭動、哭或笑)。	1	2	3	4	5	6
70.	對陌生人的反應是強烈的(大笑或大哭)。	1	2	3	4	5	6
71.	在嬰兒可達到的範圍內的東西,會主動去抓或摸(例如:頭髮、湯匙、眼鏡等)。	1	2	3	4	5	6
72.	願意吃下各種餵他/她的東西,而沒有注意到它們之間的差別。	1	2	3	4	5	6
73.	每天差不多在同一時間,身體的活動量達到最高峰。	1	2	3	4	5	6
74.	當寶寶第一次被放在不同的地方睡覺時,會顯得煩躁(哭泣或不安)。	1	2	3	4	5	6
75.	遇到熟人時的反應並不熱烈(只是安靜微笑或沒反應)。	1	2	3	4	5	6
76.	當身體不舒服時(例如感冒或腸胃不好)會表現煩躁不安的情緒。	1	2	3	4	5	6
		從不	很少	偶爾	有時	常常	總是
77.	每天在不同的時間,要求給予額外的食物(時間相差1小時以上)。	1	2	3	4	5	6
78.	與陌生人在一起時,雖經過15分鐘仍表現出警覺或害怕的樣子。	1	2	3	4	5	6

79.	可安靜地躺者或坐著玩玩具，且動作輕微。	1	2	3	4	5	6
80.	遇到某些令人煩躁不安或坐臥不定的日常生活時(例如：剪指甲、梳頭等)可以用遊戲、唱歌或電視等，分散其注意力。	1	2	3	4	5	6
81.	在輕微的傷害下(例如：擦傷、撞傷)仍保持愉快或安靜。	1	2	3	4	5	6
82.	看醫生時，最初的反應是接受的。	1	2	3	4	5	6
83.	將嬰兒不喜歡的食物與喜歡食物混合後，仍會感覺出來而有所反應。	1	2	3	4	5	6
84.	玩玩具時，大都安靜地玩，很少發出聲音或兒語。	1	2	3	4	5	6
85.	每天大約在同一時間煩躁不安(例如：早上、中午或晚上)。	1	2	3	4	5	6
86.	在接受日常生活例行的活動時(例如：剪指甲或梳頭)可安靜地躺著或坐著而不亂動。	1	2	3	4	5	6
87.	在喝牛奶時，聽到不尋常的聲音(例如：電話鈴聲或門鈴聲)會停止吸食而注視聲音來源。	1	2	3	4	5	6
88.	與父母玩耍時，集中注意力的時間不超過1分鐘。	1	2	3	4	5	6
89.	洗澡時很安靜，喜歡或不喜歡的表情很輕微(只是微笑或皺眉頭)。	1	2	3	4	5	6
90.	在接受(嚥下)一種新的食物之前，約需要3次以上的嘗試。	1	2	3	4	5	6
91.	對任何新的日常生活事件(第1次剪頭髮、吃藥等），第一次的反應是拒絕的。	1	2	3	4	5	6
92.	當尿布濕了以後，沒有什麼反應。	1	2	3	4	5	6
93.	在給醫生作健康檢查時，會煩躁不安或哭泣。	1	2	3	4	5	6
94.	改變餵食副食品時(種類、數量或時間），在1、2次之內就可接受。	1	2	3	4	5	6
95.	獨自一個人玩時，動作很多(舞動雙手、踢腳等)而且可持續數分鐘。	1	2	3	4	5	6

母親對嬰兒氣質的一般印象

甲. 與您知道的同年齡的嬰兒比較，請以下列的標準來評估您寶寶(圈選一個)

1. ***活動量***—在睡眠、餵食、玩耍、穿衣等時的身體活動量，您的寶寶是 □(1)高， □(2)中等， □(3)低。
2. ***規律性***—在睡眠、飢餓、大便等反覆性的生理功能 □(1)很有規律，□(2)不一定， □(3)沒有規律。
3. ***對新刺激的反應***—對新刺激，如食物、人物、地方、玩具或日常生活事件的改變，最初的反應 □(1)接受，□(2)不一定， □(3)退縮。
4. ***對日常生活改變的適應力***—不管最初反應是接受與否，而著重在是否趨向社會大眾期望的方式 □(1)通常適應快，□(2)不一定，□(3)通常適應慢。
5. ***反應強度***—一但有了反應，不管寶寶的反應是正向的或負向的，寶寶所作的反應程度 □(1)通常很強烈，□(2)不一定，□(3)通常很微弱。
6. ***情緒正負***—每天所表現於外的正向情緒(愉快、友善、高興)和負向情緒(不愉快、不友善、不高興)兩者間的比較是 □(1)大部分是正向的，□(2)不一定， □(3)大部分是負向的。
7. ***堅持度和注意力廣度***—不論是否有外在干擾，寶寶持續作某些行為的程度 □(1)堅持度強， □(2)不一定， □(3)不堅持。
8. ***注意力分散度***—寶寶在進行某些活動時，外來的刺激容易或不容易使寶寶分心 □(1)容易分心， □(2)不一定， □(3)不容易分心。
9. ***感覺閾***—要引起寶寶產生反應所需的外在刺激的量，像聲音、食物或人的改變 □(1)反應閾高，□(2)中度，□(3)反應閾低(只需較小的刺激便有反應)。

乙. (1) 您在寶寶的養育上是否有一些困難？ □ (1)是，□ (2)否。

(2) 如果是的話，請您回顧氣質量表上哪一些題目造成您養育上的困難請將題號列出

Appendix 4

幼兒氣質評估表(1-2 歲)

一般而言，您覺得這個孩子 □ 1.好帶 □ 2.普通 □ 3.難帶

這份問卷的目的是要瞭解您孩子對其內在刺激(如肚子餓)和外在刺激(如替他剪指甲)的反應模式(或稱氣質)。這份問卷一共有 95 個具體的行為描述，每一題都和您寶寶的日常行為表現有關。您按照您寶寶實際的行為表現，以您主觀的判斷將每題中最恰當的數字圈出。雖然有些題目看起來相似，但它們並不一樣，請您盡量每題都填答。假如有些題目您實在無法回答，或某一項目對您寶寶來說不適用，則不必圈選，並請在該題選項後面寫上不適用或無此經驗。

假如您寶寶自出生到現在，在某一題目所問的行為表現模式有所改變，請圈選符合他最近所建立反應模式的代表數字：假如題目中提到多項事件，而您寶寶反應不相同時，則請您將其多項行為總和起來作主觀判斷。

這份問卷的回答沒有好壞或對錯的區別。我們是想瞭解您寶寶的氣質特徵，以追蹤寶寶身心的健全發展。

謝謝您的合作！

	從不	很少	偶爾	有時	常常	總是
1.除牛奶或母奶外，每天大約吃等量的麥片、稀飯、麵條等食物。	1	2	3	4	5	6
2.在正要入睡及醒來時,顯得煩躁不安(例如吵鬧、哭泣或皺緊眉頭)	1	2	3	4	5	6
3.每玩一種玩具的時間不到一分鐘,就想玩其他的東西，或做其他的動作。	1	2	3	4	5	6
4.當在看電視或注意近處的事物時，能安靜地坐著。	1	2	3	4	5	6
5.餵奶時,對於餵奶姿勢與地點的改變或換人餵奶時，均能立刻接受。	1	2	3	4	5	6
6.清醒時，可乖乖的讓你剪指甲。	1	2	3	4	5	6
7.當肚子餓而哭泣時，用奶嘴或圍圍兜、抱他，可使其停止哭泣一分鐘以上。	1	2	3	4	5	6
8.當玩一種喜歡的玩具時,可連續玩 10 分鐘以上。	1	2	3	4	5	6
9.在一天中的任何時間洗澡，都能接受。	1	2	3	4	5	6
10.餵食時,不論對於喜歡與不喜歡的食物,表現的反應都是輕微的。	1	2	3	4	5	6
11.當尿布被大便弄髒時，會有不舒服的表現（例	1	2	3	4	5	6

如：煩躁不安、扭動身體)。						
12.洗澡時，能安靜地讓您洗而不亂動。	1	2	3	4	5	6
13.每天在相同的時間想要吃奶(時間相差在 1 小時以內)	1	2	3	4	5	6
14.每一次見到陌生的其他小孩時，會顯得害羞(例如:把頭轉開或抱緊母親)。	1	2	3	4	5	6
15.換尿布時，雖然利用種種方法分散其注意力(例如:唱歌,以玩具逗他等)仍顯得煩躁不安。	1	2	3	4	5	6
16.可以自己一個人玩半個小時以上(例如：玩身邊的玩具或看周圍的東西等)	1	2	3	4	5	6
17.穿衣服或換尿布時動得很厲害(手舞足蹈,身體扭動)。	1	2	3	4	5	6
18.吃飽或不想再吃時，會強烈地抗拒再給的食物(例如：大聲哭鬧、嘴巴緊閉、吐出食物、或推開奶瓶、湯匙)	1	2	3	4	5	6
19.試圖改變餵食的時間(差別在 1 小時以上)，經兩次的嘗試以後，仍然拒絕。	1	2	3	4	5	6
20.每天大便時間不一定(時間相差在 1 小時以上)	1	2	3	4	5	6
21.當有人從身邊走過時，會停止原來的活動而注意觀看。	1	2	3	4	5	6
22.正在玩一個喜歡的玩具時，會忽略談話聲或熟悉的聲音而不加以注意。	1	2	3	4	5	6
	從不	很少	偶爾	有時	常常	總是
23.在換尿布或換衣服時,會發出愉快的聲音或愉悅的表情。	1	2	3	4	5	6
24.能馬上接受新食物,並迅速嚥下。	1	2	3	4	5	6
25.觀看其他小孩遊戲時，不到一分鐘就轉移視線看別的地方。	1	2	3	4	5	6
26.由陰暗處迅即轉至強光下，嬰兒只表現出輕微的反應(如眨眼或短暫的驚嚇)	1	2	3	4	5	6
27.每一次到陌生的環境時(例如:到朋友家玩或到百貨公司)，會有愉快的表情。	1	2	3	4	5	6
28.每天晚上在一定的時間入睡(時間相差在半小時以內)。	1	2	3	4	5	6
29.對於日常生活的例行事件(例如：梳髮、洗臉)易接受而不反抗。	1	2	3	4	5	6
30.在行進的汽車中可安靜的被抱著，或在行進中的嬰兒車，可安靜地坐著。	1	2	3	4	5	6
31.對新保姆的最初反應是拒絕的(表現哭泣或緊抱母親)	1	2	3	4	5	6
32.練習一種新的動作時(例如：翻身，或拿東西)可持續進行數分鐘。	1	2	3	4	5	6

33.醒來躺在床上時，會動得很厲害(例如：手足舞蹈,全身扭動)。	1	2	3	4	5	6
34.對於洗澡地點的改變或換人洗澡，經過2、3次的嘗試之後,仍表現出抗拒的樣子。	1	2	3	4	5	6
35.每次吃牛奶的食量相差很大(差別在50c.c以上)	1	2	3	4	5	6
36.置身於陌生的地方，或新的環境時，最初的幾分鐘會表現煩躁不安。	1	2	3	4	5	6
37.當母親的外表或服飾有明顯的改變時(換新髮型或新衣著)寶寶會注意觀看。	1	2	3	4	5	6
38.對食物好壞的反應程度差別很大，不論是喜好(大叫大笑)或厭惡(哭鬧、拒絕)的表現均很強烈。	1	2	3	4	5	6
39.對於日常生活例行的事件，例如：梳頭、洗臉時會表現出愉快的表情。	1	2	3	4	5	6
40.在哭泣時，雖由通常照顧者給予數分鍾的安撫，仍無法安靜下來。	1	2	3	4	5	6
41.對於喜歡的玩具，雖然伸手拿不到但會嘗試用各種方法獲得(包括喊叫),而且時間持續兩分鐘以上。	1	2	3	4	5	6
42.對於會發出聲響的玩具，含有強烈的情緒表現(不論是喜歡或討厭的表情均可)	1	2	3	4	5	6
	從不	很少	偶爾	有時	常常	總是
43.跟照顧者玩耍時,手腳及身體會舞動得很厲害。	1	2	3	4	5	6
44.雖然手中已握有一個玩具，但仍會注視再給他的另一個玩具。	1	2	3	4	5	6
45 在家程與陌生的客人接觸時，初次反應是接受的。	1	2	3	4	5	6
46.在白天,小睡的入睡時間是不定的(相差1小時以上)。	1	2	3	4	5	6
47.改變副食品的味道或濃度時，可繼續進食而沒有反應。	1	2	3	4	5	6
48.自己一個人玩耍時會哭泣。	1	2	3	4	5	6
49.大約在10分鐘之內，就能適應新的環境(例如：改變家庭擺設, 進入商店或遊戲場所等)。	1	2	3	4	5	6
50.每天白天小睡的睡眠時間長度大約相同(相差在半小時以內)。	1	2	3	4	5	6
51.在餵食時，會動得很厲害(用手抓東西、身體扭動、手腳亂踢)。	1	2	3	4	5	6
52.當光線突然改變時(如閃光燈、開燈)會有反應(凝視光線或嚇一跳)	1	2	3	4	5	6
53.想睡覺時，可用兒語或遊戲的方式使其安靜。	1	2	3	4	5	6
54.換尿布或換衣服時,表現出強烈的情緒(大聲哭	1	2	3	4	5	6

鬧或笑出聲音來)。						
55.入睡到醒來時，身體躺的位置相同，並沒有變換。	1	2	3	4	5	6
56.當睡覺的地方或睡眠的時間改變時在1、2天之內就能適應，而且睡得很好。	1	2	3	4	5	6
57.當牛奶的種類或溫度改變,或以果汁代替時,通常會注意到而且有所反應。	1	2	3	4	5	6
58.看電視時，每次都可持續5分鐘以上。	1	2	3	4	5	6
59.當尿布濕而煩躁不安峙,可用下列的方法使其安靜(例如：將他/她抱起，跟他/她玩，或讓他/她看電視等)	1	2	3	4	5	6
60.每天大約在同一時間想吃麥片、稀飯、麵條等副食品(相差在1小時以內)	1	2	3	4	5	6
61.在餵食牛奶或副食品時，若中斷餵食，仍表現愉快的樣子(微笑或發出愉悅的聲音)。	1	2	3	4	5	6
62.換人或換地方洗澡時，在數分鐘內就可接受。	1	2	3	4	5	6
63.打針後,在1分鐘內便停止哭泣。	1	2	3	4	5	6
64.哭泣時，身體動得很厲害(手舞腳踢〉	1	2	3	4	5	6
65.在同一天內，對於同一種吵鬧聲持續作多次的反應(例如：打鐘聲、或狗叫聲)。	1	2	3	4	5	6
	從不	很少	偶爾	有時	常常	總是
66.當副食品的濃度、味道或溫度改變時，最初的反應是退縮或拒絕(將頭轉開或將食物吐出)。	1	2	3	4	5	6
67.每天早晨醒來的時間差異很大(相差在1小時以上)	1	2	3	4	5	6
68.當寶寶拒絕不喜歡的食物或藥品時,不管努力用什麼方法逗他/她玩,都無法分散其注意力。	1	2	3	4	5	6
69.清醒時即使輕輕地摸一下身體, 也會有反應(驚嚇、不安的扭動、哭或笑)	1	2	3	4	5	6
70.對陌生人的反應是強烈的(大笑或大哭)	1	2	3	4	5	6
71.在幼兒可達到的範圍內的東西，會主動去抓或摸(例如:頭髮、湯匙、眼鏡)	1	2	3	4	5	6
72.願意吃下各種餵他/她東西,而沒有注意到它們之間的差別。	1	2	3	4	5	6
73.每天差不多在同一時間，身體的活動量達到最高峰。	1	2	3	4	5	6
74.當寶寶第一次被放在不同的地方睡覺時，會顯得煩躁(哭泣或不安)	1	2	3	4	5	6
75.遇到熟人時的反應並不熱烈(只是安靜微笑或沒反應)。	1	2	3	4	5	6
76.當身體不舒服時(例如感冒或腸胃不好)會表現煩躁不安的情緒。	1	2	3	4	5	6

77.每天在不同的時間,要求給予額外的食物(時間1小時以上)	1	2	3	4	5	6
78.與陌生人在一起時,雖經過15分鐘仍表現出警覺或害怕的樣子。	1	2	3	4	5	6
79.可安靜地躺著或坐著玩玩具,且動作輕微。	1	2	3	4	5	6
80.遇到某些令人煩躁不安或坐臥不定的日常活動時(例如：剪指甲、梳頭等)可以用遊戲、唱歌或電視等，分散其注意力。	1	2	3	4	5	6
81 在輕微的傷害下(例如：擦傷、撞傷)仍保持愉快或安靜。	1	2	3	4	5	6
82.看醫生時，最初的反應是接受的。	1	2	3	4	5	6
83.將幼兒不喜歡的食物與喜歡的食物混合後,仍會感覺出來而有所反應。	1	2	3	4	5	6
84.玩玩具時,大都安靜地玩,很少出聲音或兒語。	1	2	3	4	5	6
85.每天大約在同一時間煩躁不安(例如：早上、中午或晚上)	1	2	3	4	5	6
86.在接受日常生活例行的活動時(例如:剪指甲或梳頭)可安靜地躺著或坐著而不亂動。	1	2	3	4	5	6
	從不	很少	偶爾	有時	常常	總是
87.在喝牛奶時，聽到不尋常的聲音(例如：電話鈴聲或門鈴聲)會停止吸食而注視聲音來源。	1	2	3	4	5	6
88.與父母玩耍時，集中注意力的時間不超過1分鐘。	1	2	3	4	5	6
89.洗澡時很安靜,喜歡不喜歡的表情很輕微(只是微笑或皺眉頭)。	1	2	3	4	5	6
90.在接受(嚥下)一種新的食物之前，約需要3次以上的嘗試。	1	2	3	4	5	6
91.在任何新的日常生活事件(第1次剪頭髮、吃藥等),第一次的反應是拒絕的。	1	2	3	4	5	6
92.當尿布濕了之後,沒有什麼反應。	1	2	3	4	5	6
93.在給醫生作健康檢查時，會煩躁不安或哭泣。	1	2	3	4	5	6
94.改變餵副食品時(種類、數量或時間)，在1、2次之內就可接受。	1	2	3	4	5	6
95.獨自一個人玩時,動作很多(舞動雙手、踢腳等)而且持續數分鐘。	1	2	3	4	5	6

Part II

二、 台灣年輕人高血壓世代研究族群雙酚A與頸動脈內膜中層厚度之相關性

Association between bisphenol A and carotid intima-media thickness in a young hypertension cohort of Taiwan

中文摘要

研究背景與目的：雙酚 A 普遍存在於聚碳酸酯塑膠、環氧樹脂製品及金屬罐頭襯裡...等食品包裝中，經接觸食品後釋出而造成暴露。雙酚 A 是一種內分泌干擾物，影響體內荷爾蒙與代謝機制，造成肥胖、動脈硬化、心血管疾病...等疾病，現已知頸動脈內膜中層厚度為上述疾病的危險因子與早期的表現，然而目前探討雙酚 A 與頸動脈內膜中層厚度的研究有限，因此本篇研究目的在於探討在青少年與年輕成人族群中，雙酚 A 的暴露與頸動脈內膜中層厚度的相關性。

方法：本研究使用極致液相層析串聯質譜儀(UPLC/MS/MS)分析 887 位青少年與年輕成人血中雙酚 A 濃度，並使用超因波儀量測頸動脈內膜中層厚度，代謝相關生化值則經由血液及健康檢查取得。我們利用線性迴歸探討雙酚 A 暴露與頸動脈內膜中層厚度的相關性。

結果：我們發現相較於最低暴露組，在較高雙酚 A 暴露組中，雙酚 A 與頸動脈內膜中層增厚有關(p-value <0.0001, p for trends < 0.0001)，亦發現與低密度膽固醇(p-value =0.001, p for trends = 0.001)及總膽固醇(p-value = 0.001, p for trends = 0.003)增加有關。

結論：在青少年和年輕成年人族群中，我們發現雙酚 A 暴露可能與頸動脈內膜中層厚度增加有關，亦可能直接傷害血管，加劇對頸動脈內膜中層厚度的影響，且對於女性或健康族群影響更為顯著，但機制仍需要後續研究進一步釐清。

關鍵字：雙酚 A、頸動脈內膜中層厚度、青少年

Abstract

Background: The main exposure route of bisphenol-A (BPA) is dietary ingestion. BPA can release from foods containers, and pass to the food or beverage under various conditions. BPA is one of endocrine disrupting chemicals which will interfere with human hormone system, and cause adverse health effects. Low level of BPA exposure may have an association with obesity, cardiovascular disease, and arteriosclerosis. Carotid intima-media thickness (CIMT) is a risk factor and early indicator of those diseases in adult population. However, there is still limited information of BPA and CIMT in adolescent population.

Aims: This study wants to examine the exposure of BPA level in the teenagers and young adults in Taiwan, and try to understand the association between BPA and CIMT.

Methods: We used the ultra-performance liquid chromatography – tandem mass spectrometry (UPLC/MS/MS) to measure the concentration of BPA in 887 blood samples of adolescent and young adult. A high-resolution B-mode ultrasonograph was used to measure CIMT. Other information was collected by blood exam, health-check, and interview. We used liner regression model to investigate the association between BPA and CIMT, and between BPA and biochemical levels.

Results: In the highest BPA exposure group, BPA exposure was significant associated with an increase of averaged carotid intima-media thickness (p-value < 0.0001, p for trends < 0.0001), compared with the lowest exposure group when BPA levels were categorized into four groups. We also found BPA exposure was significant associated with an increase of LDL (p-value = 0.001, p for trends = 0.001), Cholesterol (TCHO) (p-value = 0.001, p for trends = 0.003) in the highest exposure group when BPA levels were categorized into three groups.

Conclusions: In adolescents and young adult populations, we found BPA exposure level may have a positive association with carotid intima-media thickness. In addition, BPA exposure may also hurt blood vessel directly, and exacerbated the effects on carotid intima-media thickness. The association was more significant in female or healthier population.

Key words: bisphenol A, carotid intima-media thickness, adolescents

Introduction

The main exposure route of bisphenol-A (BPA) is dietary ingestion via potential exposure sources for general population (Morgan et al., 2011). BPA are extensively in environment, the BPA exposure source for human include aquatic environment, air and soil, but the food is believed to be the major source of exposure (Kang et al., 2006). BPA can release from foods containers and pass to the food or beverage under various conditions. Many kind of daily necessities are produced including BPA. BPA had been found to release from polycarbonate plastics (PC) product and epoxy resins, for example, the lining of beverages and food cans, food and water containers, and so on (Cao et al., 2009; Greens et al., 2011; Maragou et al., 2008). In daily life, BPA exposure through ingestion is common. After BPA enter human body, it mainly reacts with glucuronic acid, and then discharge through urine. A part of BPA store in lipid tissues, which release from blood slowly (Fernandez et al., 2007). BPA is one of endocrine disrupting chemicals (EDCs) which will interfere with human hormone system, and cause adverse effects on the natural hormones in body responsible for the maintenance of homeostasis and the regulation of developmental processes (RJ, 1999).

Some previous studies found low level of BPA exposure may have an association with coronary heart disease (Melzer et al., 2010), and also found an association between coronary heart disease and arteriosclerosis (Hulthe et al., 1997). Carotid intima-media thickness is a risk factor and early indicators of arteriosclerosis and heart disease in adult population (Daniel et al., 1999). There is still limited information of BPA and carotid intima-media thickness in adolescent population. Therefore, this study wants to examine the exposure of bisphenol A level in the teenagers and young adults in Taiwan, and try to understand the association between this compound and carotid intima-media thickness.

Materials and methods

Study design and population

This study population was school age children in Taiwan. They were collected from an annual urine screening of approximately 2,615,000 to 2,932,000 school age children in grades 1-12 which was conducted by the Chinese Foundation of Health in Taipei, Taiwan from 1992 to 2000. A urine strip was used for the screening. If school-age children with two positive results on proteinuria, glucosuria or hematuria, and they would undergo the third urine screening test and a general health check-up. The check-up included anthropometric measures, fasting blood tests and blood pressure (BP). Totally, there were 103,756 school children received the third urine screen and health check-up, and they also had complete data profiles after detailed data checking. According to the American Heart Association criteria (2004), among these children, 9,227 had elevated blood pressure (EBP) and 94,529 had normal BP (Wei et al., 2003).

Base on the 1992-2000 urine screening population, we established a cohort study, the Young Hypertension Cohort Study, from 2006-2008. This cohort study population included childhood EBP students and without childhood EBP students. In the follow-up, we invited those childhood EBP students in Taipei area to join study by telephone after mailed invitation letters, there were 707 students. Those normal BP students were 6390 students in Taipei area, and we only via mail to invited they joined. Totally, we had 347 childhood EBP students (response rate: 49%) and 641 normal BP students (response rate: 10%) joined our study. The detailed information is available in a recent report (Tsai et al., 2011). In this study, we selected 887 subjects who live in Taipei area whose serum samples were available for further analysis (figure 1). The protocol of this study was approved by the ethics committee of the National Taiwan University Hospital (Research Ethics Committee, NTUH). When participants joined this cohort follow-up study, they

and their parents (for children and adolescents participants) signed informed consent.

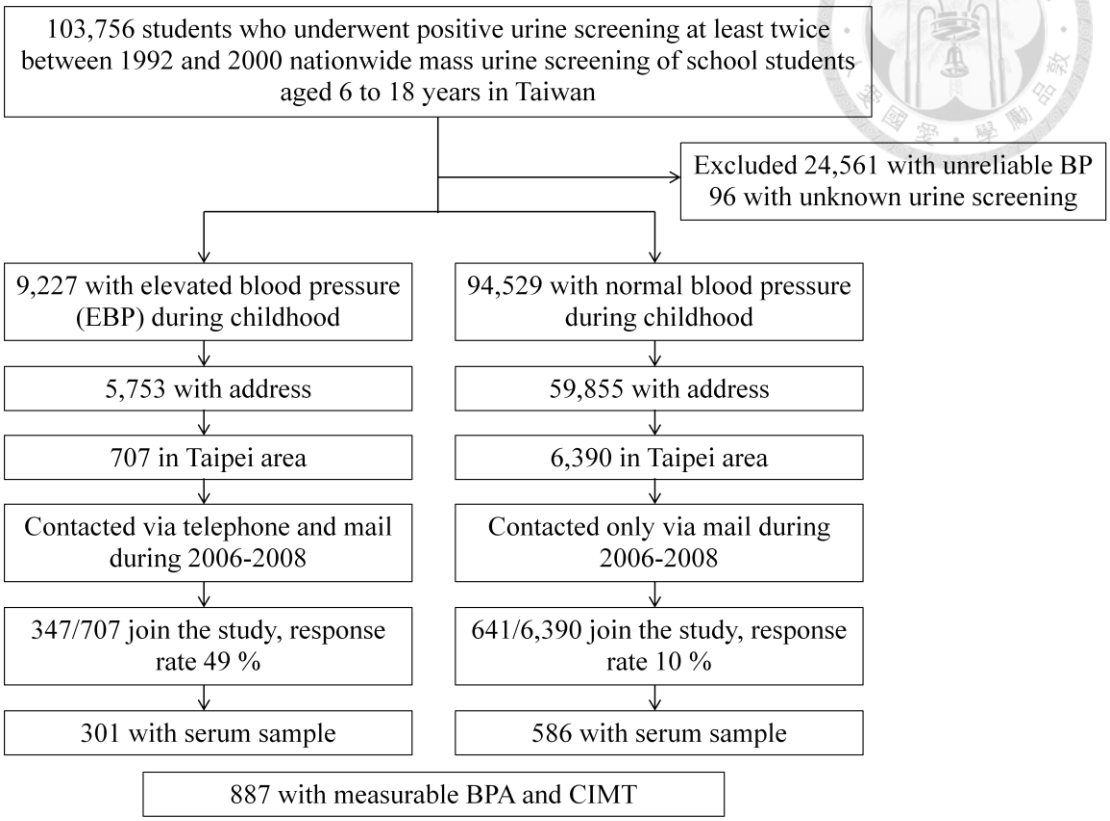

Figure 1. The flow chart of study population

Anthropometric and biochemical data

We collected socio-demographic information such as age, gender, history of medication and household income by interview. We also used questionnaire to collect the degree of alcohol intake information, and we categorized into current alcohol consumption and no alcohol drinking. Smoking status was subdivided into active smoker, passive smoker or has never smoked. We distinguished the household income into higher or lower categorization by 70,000 New Taiwan Dollars (NTD) per month. Weight and height were measured by standard methods, and BMI was calculated. Two seated blood pressure and heart rate measurements were made at least 1 min apart after 3 min of rest by using a mercury manometer and the appropriate cuff size. The

definition of hypertension was an average BP greater than 120/80 mmHg or basing on their self-reported current use of anti-hypertensive medication. Childhood EBP definition was according to the American Heart Association criteria (2004) that systolic blood pressure, diastolic blood pressure, or both greater than or equal to the modified sex- and age-specific criteria for blood pressure values. If their fasting glucose was greater than 126 mg/dl or they were current using oral hypoglycemic agents or insulin, we defined them were diabetes.

Measurement of carotid intima-media thickness (CIMT)

CIMT was defined as the distance from the front edge of the first echogenic line (lumen-intima interface which was interface between lumen and vascular intima) to the front edge of the second echogenic line (media-adventitia interface which was interface between vascular media and adventitia) in the far wall of the vessel (Su et al., 2006).

We used a high-resolution B-mode ultrasonograph (GE Vivid ultrasound system, Horten, Norway) equipped with a 3.5-10 MHz real-time B-mode scanner to exam CIMT at extracranial carotid arteries (ECCAs) by an experienced technician, and then applied a software package for vascular ultrasound to offline automatic calculation.

The maximum and mean CIMTs at the common carotid artery (CCA) proximal to the carotid bifurcation, bulb, and internal carotid artery were obtained bilaterally. CCA1 and CCA2 are points located at 0-1 cm and 1-2 cm, respectively, on the CCA distal from the carotid bifurcation. We averaged CCA1 and CCA2 to get a mean be representative of CCA. CIMT in this analysis was determined by averaging four measurements on bilateral CCA.

All scans were recorded on a digitalized memory system in DICOM format for subsequent off-line analysis. A moving-image clip of the carotid bulb and CCA with

duration of 5 s was acquired and stored. The digitized M-mode was later analyzed off-line using a computer program, in which each image was recalled with magnification and the CIMT between 2 successive R waves was measured by automated analyzing software provided by the manufacturer. Comparing with manual measurement, the mean value of 150 measurements on a 10-mm segment of the CCA, automated measurement is efficient, reliable and less time-consuming. To determine the reliability of repeated measurements, the technician conducted a second reading for the same participant of random selected 30 subjects after 2 weeks later. The reliability of CIMT measurement at bilateral CCA (mean of right and left CCA) had excellent intraobserver coefficient of correlation reliability (ICCR) around 98.8% and 98.5%, respectively.

Measurement of BPA concentration

Chemicals and reagents

Bisphenol A was supplied by AccuStandard (New Haven, Connecticut, USA). Bisphenol A-D16 was obtained from Sigma/Aldrich (Saint Louis, MO, USA). We prepared stock solutions of those compounds at a concentration of 500 μg/mL in methanol and stored at -20℃. Milli-Q water was obtained from a Millipore water purification system (Milford, MA, USA). N-methylmorpholine (purity > 99.5%) were provided by J.T. Baker (Phillipsburg, NJ, USA). Methanol and acetonitrile were used to be solvents which were all LC/MS grade (J.T. Baker). Bovine serum was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Sample preparation and calibration experiments

All glassware was rinsed with methanol before used for experiments. The concentrations of BPA in serum were quantified using modified analytical methods previously described (Anari et al., 2001).The underivatization method was used in this study. 50 μL serum was diluted with 0.5 mL of water and added in 50μL of internal standard (Bisphenol A-D16; 200ng/ml in methanol), and then gentle mixing. After that, add 2mL of ethyl acetate in each test tube of samples, and then gentle mixing again by vortex-mixer. The organic phase (1.5mL) of the samples was transferred to another tube, then filtrated through 0.22μm PVDF syringe filters into a 2mL auto-sampler vial, and evaporated to dryness by SpeedVac concentrator (Thermo Savant SPD 1010, Holbrook, NY, USA). The residues were reconstituted with 50μL of methanol and transferred to 150-μL insert for UPLC/MS/MS analysis.

Matrix-matched standard calibration solution was prepared in bovine serum and through the same procedure of sample preparation. The linear range was 1-500 ng/ml and 1-1000 ng/ml for BPA, and spiked 200 ng/ml of internal standard (BPA-d16) in each solution.

Instrumental analysis

We use ultra-performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) to measure the concentration of bisphenol A (BPA) in adolescent serum. The UPLC/MS/MS was performed using a Waters Acquity UPLC system (Waters Corporation, Milford, MA, USA) and controlled by MassLynx V4.1 with QuanLynx Application Manager. We used an ACQUITY UPLC BEH C18 column (2.1 mm×50 mm, 1.7μm) for analysis. The temperature and the flow rate were maintained at 60℃ and 0.5mL/min, respectively. The mobile phase was composed of 10 mM

N-methylmorpholine (pH 9.6) and acetonitrile. The gradient of LC system was initiated at the composition of 40% acetonitrile for 0.5 min and continued by a linear gradient to 60% acetonitrile in 0.5 min, and then increased to 95% acetonitrile in 2 min and held at 95% acetonitrile for 1 min before being returned to the initial condition. At last, the column was re-equilibrated at 40% acetonitrile for 1 min. The total run time of gradient program was 5 minutes and the sample injection volume was 5μL.

To achieve maximal analyte signal intensities, the instrumental parameters were referenced to previously described (Lien et al., 2009). The mass spectrometer was performed in native electrospray ionization (ESI-) and the capillary voltage was maintained at 3.0 kV. The desolvation gas flow, cone gas flow, desolvation temperature, and source temperature were set at 1100 L/hr, 50 L/hr, 500℃ and 120℃, respectively. Extractor voltage was 5.0 V and RF lens voltage was 0 V. Collision gas was argon at 3.26×10^{-3} mbar. Ion energy 1 and 2 were set at 1 and 1, respectively. Both LM 1 and LM 2 resolution were set at 13. The multiplier voltage was set at 650 V. Those instrumental parameters of the mass spectrometer were shown in appendix 1. The dwell time for BPA was 0.1 second. Ions were monitored by selected reaction monitoring (SRM) as shown in appendix 2 as well as the individual collision energy and cone voltage.

Method validation and quantification

The sample preparation of serum with UPLC/MS/MS method was validated regarding the precision, accuracy, and detection limit. The calibration standards in bovine serum were analyzed in the same day (n=3) for intra-day precision and accuracy and were analyzed between different days for the inter-day precision and accuracy (n=3). The accuracy and precision of intra- and inter-day of matrix-matched calibration were shown in appendix 3. The RSD% ranges were 4.3 to 21. The bias% ranges were not over 12.5 disparities. The recovery of the method was determined by three duplicates of bovine serum spiked known amounts (10, 25 and 100 ng/ml) of BPA with fixed levels of internal standard, and was calculated by dividing the measured quantities with the theoretical (spiked) quantities. The recovery of analyte with different concentration in bovine serum and overall process efficiency with different concentration in human serum were shown in appendix 4. Recovery calculated formula as follow: recovery (%) = (measured value / theoretical value) X 100%. Using the quantitative method of matrix-matched calibration with one internal standard, the recovery of BPA were 93.7% - 108.4%. The overall process efficiency of the method was determined by area of pre-extraction spike of human serum without spiked and spiked known amounts (5, 25 and 100 ng/ml) of BPA with fixed levels of internal standard (n=3), and was calculated by dividing the area of standard. The overall process efficiency calculated formula as follow: overall process efficiency = (area of pre-extraction spike / area of standard) X 100%. Comparing the area of pre-extraction spike and area of standard to realize the BPA value in human serum were loss situation through extraction process. The RSD of overall process efficiency in 5, 25, and 100 ng/ml were 22.6, 13.4, and 9.1, respectively.

BPA was found in blanks, but the signal was low which not influence the

quantification was. Most the human serum were detected the peak but some signal intensities were below the background level, it is impractical to calculate their limits of detection (LODs), therefore we used the lowest concentration of calibration curve to define their LOQs. BPA LOQs was 1ng/ml which defined as the lowest concentration of calibration curve.

For quantification accuracy, the quality control (QC) samples were prepared from a serum pool obtained from multiple serums which provide from this study. The serum pool was divided into four subpools, one subpool was used to analyze the phenols levels of the samples (no spike), and the three different concentration (5, 25 and 100 ng/ml) were spiked into the other three subpools. Three different concentrations which were 5, 25, and 100 ng/ml were spiked into the human serum sample to evaluate the method accuracy and precision. In the appendix 5, we found that the measured concentration of those spiked sample were very close to the spike levels basing on the no spike sample concentration. A solvent blank sample spiked fixed level of internal standard with each batch of samples to check experimental contamination and background level of native analytes. Two duplicate samples were chose from every sixty sample for quality control (QC) samples were prepared from human serum, to check the stability of method, and every six hours we would used 200 ng/ml mixed standard to check the stability of instrument. The linear calibration ranges in serum with 1/ x weighted were 1-500 ng/ml for BPA. Only for few high level samples, we used the linear calibration range in serum with 1/ x weighted were 1-1000 ng/ml to get more accurate data. The square of the correlation coefficient (R^2) was equal to or greater than 0.997. The data acquired and processed using MassLynx V4.1 Software.

Statistical analysis

The CIMT and biochemical levels in serum were modeled as continuous variables. We used liner regression model to investigate the association between BPA and CIMT, and between BPA and biochemical levels. The potential confounders contained sex, age, smoking status, alcohol status and household monthly income, which were adjusted in model.

For exploring the dose-response relationship between BPA levels and the biochemical levels, and between BPA levels and CIMT, we categorized BPA levels into four groups (<50th percentile, 50-75th, 75-90th and ≥90th percentile) and set the lowest group as a reference group. The BPA levels were natural log transformation, and the results were presented as thickness of CIMT in different BPA levels. To confirm the LDL, SBP, and CRP, which were some important factors of cardiovascular disease whether affect the results of BPA and CIMT or not, we base on the main linear model, and add LDL, SBP, and CRP as the confounder, individually. The cut-point of LDL, SBP, and CRP were 75 percentile.

We considered BPA was a kind of EDCs, may have different effect on sex, and also concerned BMI, hypertension and diabetes, LDL, sugar, and CRP may affect the outcome which we try to explore. To clarify the contribution of those factors and fast food consumption frequency on the association between BPA and CIMT, we assessed the association between natural log-transformed BPA and CIMT by sensitivity analysis. We categorized BPA exposure level into three groups (<50th percentile, 50-75th, and ≥75th percentile), because concerned the sample size in each subgroup. We conducted statistical analyses using SAS (version 9.2; SAS Institute, Inc., Cary, NC, USA) and SPSS (version 16; SPSS Inc., Chicago, IL, USA). We considered results statistically significant at p-value < 0.05.

Results

BPA levels in human serum

In this study, a total of 887 subjects' serums were used. The BPA detection rates were 44.7%, and there were 55.3% samples BPA levels were under the LOQs. Therefore, we used half value of LOQs (0.5 ng/ml) to alternate those samples concentration of BPA, if those samples peak signal-to-noise ratios(S/N ratios) over 3. If the S/N ratios under 3, we would define those samples were none detect. The serum BPA arithmetic mean (±SD) concentration was 19.77±79.89ng/ml, median was 2.4 ng/ml, and the range were N.D to 1097.4 ng/ml.

In the Table 1, we presented the BPA concentration in each characteristics of the study population, and try to compare any significant different in subgroups. In this study population, female were about sixty percent, and most subjects' age were 18-24 years old. In different subgroup including sex, age, household monthly income, BMI, hypertension, and diabetes, the BPA levels were no significant. Other characteristic about life styles and diet habits which may have association with CIMT, we found there were no significant different in most characteristics, except the fast food consumption. BPA concentration in the consumption frequency over 3 times per week was significant higher than another subgroup, and there was a significant difference between subgroups (*p*-value = 0.001).

Table 1. BPA concentration in each characteristics of the study population

	N	%	Bisphenol A (ng/ml)		
			Mean	SD	*p*-value
Total	887		19.77	79.89	
Sex					
Female	537	60.10	20.29	77.12	0.83
Male	350	39.20	18.91	84.75	
Age (year)					
< 18	127	14.20	25.35	85.17	0.68
18 -24	595	66.60	19.46	83.13	
≥ 24	165	18.50	15.59	60.98	
Household income (NTD/month)					
< 50,000	311	34.80	17.27	71.55	0.47
50,000 <70,000	184	20.60	28.16	105.37	
70,000-100,000	155	17.40	23.01	97.37	
≥ 100,000	204	22.80	14.12	53.63	
BMI (kg/m^2)					
< 24	687	77.5	20.0	76.4	0.88
≥ 24	200	22.5	18.9	91.5	
Current Hypertension					
yes	151	1.80	28.49	120.30	0.16
no	638	97.40	16.15	67.59	
Current Diabetes					
yes	10	1.13	1.10	1.16	0.57
no	876	98.87	19.97	80.55	
Smoking status					
Non smokers	739	82.80	20.86	86.39	0.72
Past smokers	29	3.20	13.65	27.99	
Current smokers	110	12.30	13.36	29.45	
Current number of cigarettes (cigarettes/day)					
<10	33	30.84	14.73	37.45	0.89
10 - <20	40	37.38	14.80	31.55	
≥20	34	31.78	11.00	14.11	
Alcohol drinking status					
never	778	87.10	19.94	81.19	0.28
past	17	1.90	48.04	156.33	
current	79	8.80	9.46	14.12	
Exercise					
never	228	25.50	21.03	98.65	0.73
past	378	42.30	21.89	85.18	
current	266	29.80	15.97	53.82	
Coke consumption (times/week)					
0	279	35.40	12.50	44.12	0.33
1-2	273	34.60	19.25	78.25	
≥ 3	237	30.00	25.42	112.85	
Ham consumption (times/week)					
0	131	16.60	24.96	103.30	0.25
1-2	431	54.60	21.41	91.95	
≥ 3	227	28.80	9.60	21.80	
Fast food consumption (times/week)					
0	387	49.00	17.50	77.88	**0.001**
1-2	367	46.50	14.31	48.93	
≥ 3	35	4.40	79.57	238.26	
Sweet food consumption (times/week)					
0	98	11.10	8.52	20.62	0.66
1-2	380	42.90	21.93	87.60	
≥ 3	405	45.70	20.64	82.68	
Fatty meat consumption (times/week)					
0	466	59.10	14.19	58.67	0.16
1-2	260	33.00	28.03	115.67	
≥ 3	63	8.00	12.40	30.80	

BPA exposure and carotid intima-media thickness (CIMT)

In the Table 2, in the 75-90th and the highest (≥90th) BPA exposure group, BPA exposure was significant associated with an increase of averaged carotid intima-media thickness (CIMT), bilaterally common carotid artery (CCA), bulb, and internal carotid artery (ICA), compared with the lowest exposure group when BPA levels were categorized into four groups. We also found the right internal carotid artery (RICA) had an association with BPA in every level category, which compared with the lowest exposure group.

In the Table 3, we also found BPA exposure was significant associated with an increase of BMI in the 75-90th category (β = 1.019, *p*-value = 0.023, *p* for trends = 0.050), LDL in highest category (β = 15.647, *p*-value = 0.0003, *p* for trends = 0.006), cholesterol (TCHO) in highest category (β = 15.945, *p*-value = 0.0008, *p* for trends = 0.019), and associated with a decrease of sugar (*p* for trends = 0.03), which were compared with the lowest exposure group when BPA levels were categorized into four groups.

Table 2. Linear regression models of BPA levels in blood and carotid intima-media thickness

	crude[1]							
	0.85 < ln BPA (N=312)	0.85 ≤ ln BPA < 2.51(N=156)		2.51 ≤ ln BPA < 3.45 (N=94)		ln BPA ≥ 3.45 (N=62)		*p* for Trends
	Thickness (mm) ± SD	Thickness (mm) ± SD	*p*-value	Thickness (mm) ± SD	*p*-value	Thickness (mm) ± SD	*p*-value	
Carotid intima-media thickness (CIMT)[3]	0.443 ± 0.049	0.447 ± 0.051	0.375	0.466 ± 0.058	**0.0004**	0.483 ± 0.077	**<0.0001**	**<0.0001**
RCCA[4]	0.454 ± 0.061	0.455 ± 0.063	0.774	0.471 ± 0.064	**0.023**	0.487 ± 0.088	**0.0003**	**0.001**
RCCA_Max	0.584 ± 0.079	0.577 ± 0.074	0.359	0.594 ± 0.071	0.281	0.615 ± 0.105	**0.006**	**0.049**
RBULB[5]	0.459 ± 0.102	0.472 ± 0.093	0.217	0.492 ± 0.097	**0.007**	0.499 ± 0.122	**0.006**	**0.001**
RICA[6]	0.408 ± 0.070	0.423 ± 0.068	**0.045**	0.430 ± 0.075	**0.013**	0.475 ± 0.105	**<0.0001**	**<0.0001**
LCCA[4]	0.448 ± 0.058	0.453 ± 0.061	0.505	0.474 ± 0.071	**0.001**	0.488 ± 0.089	**<0.0001**	**<0.0001**
LCCA_Max	0.577 ± 0.072	0.577 ± 0.080	0.980	0.598 ± 0.091	**0.030**	0.618 ± 0.112	**0.0003**	**0.001**
LBULB[5]	0.465 ± 0.096	0.477 ± 0.111	0.252	0.489 ± 0.102	**0.045**	0.507 ± 0.102	**0.004**	**0.002**
LICA[6]	0.406 ± 0.066	0.403 ± 0.060	0.647	0.428 ± 0.062	**0.006**	0.447 ± 0.084	**<0.0001**	**<0.0001**
	adjusted[1,2]							
Carotid intima-media thickness (CIMT)[3]	0.443 ± 0.050	0.449 ± 0.051	0.243	0.465 ± 0.059	**0.0002**	0.478 ± 0.070	**<0.0001**	**<0.0001**
RCCA[4]	0.453 ± 0.062	0.457 ± 0.063	0.527	0.472 ± 0.064	**0.013**	0.481 ± 0.077	**0.0023**	**0.001**
RCCA_Max	0.584 ± 0.080	0.578 ± 0.074	0.568	0.594 ± 0.072	0.224	0.606 ± 0.095	**0.033**	*0.07*
RBULB[5]	0.460 ± 0.103	0.473 ± 0.094	0.223	0.490 ± 0.097	**0.015**	0.500 ± 0.120	**0.008**	**0.001**
RICA[6]	0.408 ± 0.070	0.425 ± 0.068	**0.030**	0.431 ± 0.076	**0.008**	0.470 ± 0.105	**<0.0001**	**<0.0001**
LCCA[4]	0.449 ± 0.058	0.453 ± 0.062	0.428	0.474 ± 0.072	**0.001**	0.483 ± 0.084	**<0.0001**	**<0.0001**
LCCA_Max	0.576 ± 0.072	0.577 ± 0.081	0.897	0.598 ± 0.092	**0.019**	0.611 ± 0.098	**0.0013**	**0.001**
LBULB[5]	0.466 ± 0.097	0.478 ± 0.113	0.195	0.487 ± 0.102	**0.060**	0.502 ± 0.097	**0.0065**	**0.004**
LICA[6]	0.406 ± 0.065	0.404 ± 0.061	0.769	0.426 ± 0.062	**0.006**	0.438 ± 0.078	**0.0002**	**0.0002**

1. We set the lowest level(ln BPA < 0.85, N=312) as a reference
2. Model adjusted for sex, age, smoking status, alcohol status and household monthly income
3. CIMT were the mean of CCA, BUlB, and ICA
4. RCCA, LCCA: right or left side common carotid artery
5. RBULB, LBULB: right or left side bulb
6. RICA, LICA: right or left side internal carotid artery

Table 3. Linear regression models of BPA levels in blood and biochemical data

	crude[1]						
	0.85 ≤ ln BPA < 2.51 (N=156)		2.51 ≤ ln BPA < 3.45 (N=94)		ln BPA ≥ 3.45 (N=62)		*p* for Trends
	β	*p*-value	β	*p*-value	β	*p*-value	
Physical exam							
Waist(cm)	-0.070	0.946	0.642	0.617	3.339	**0.034**	**0.037**
Hip(cm)	-0.143	0.842	1.010	0.256	2.020	0.064	*0.065*
BMI	0.084	0.821	1.010	**0.027**	1.125	**0.045**	**0.019**
SBP	-1.935	0.134	-0.590	0.712	1.458	0.457	0.883
DBP	-1.059	0.289	1.654	0.181	1.272	0.402	0.392
Lipid							
HDL	0.038	0.966	-0.582	0.598	-0.630	0.643	0.453
LDL	-2.169	0.433	4.422	0.196	14.722	**0.001**	**0.001**
Triglyceride (TG)	-1.047	0.875	-3.220	0.695	13.824	0.171	0.446
Cholesterol (TCHO)	-0.747	0.809	3.317	0.387	15.901	**0.001**	**0.003**
Glucose homeostasis							
Insulin	0.181	0.754	0.868	0.224	0.189	0.830	0.525
Sugar	-2.083	0.237	-3.536	0.105	-2.786	0.298	**0.049**
HOMA[3]	-0.041	0.806	0.108	0.599	-0.071	0.778	0.946
C-reactive protein (CRP)	-0.003	0.849	0.009	0.673	-0.018	0.493	0.838
	adjusted[1,2]						
Physical exam							
Waist(cm)	0.310	0.742	0.834	0.470	2.372	0.102	*0.068*
Hip(cm)	-0.039	0.955	0.986	0.252	1.470	0.174	0.160
BMI	0.077	0.833	1.019	**0.023**	0.764	0.175	*0.050*
SBP	-1.344	0.252	-0.339	0.814	1.063	0.557	0.601
DBP	-0.630	0.511	1.679	0.154	0.657	0.657	0.608
Lipid							
HDL	-0.643	0.445	-0.779	0.451	-0.291	0.823	0.344
LDL	-2.005	0.467	4.663	0.169	15.647	**0.0003**	**0.006**
Triglyceride (TG)	-0.360	0.956	-2.537	0.750	1.114	0.911	0.808
Cholesterol (TCHO)	-1.338	0.663	3.538	0.348	15.945	**0.0008**	**0.019**
Glucose homeostasis							
Insulin	0.074	0.901	0.713	0.329	-0.034	0.971	0.590
Sugar	-2.251	0.220	-3.564	0.114	-2.927	0.302	**0.027**
HOMA[3]	-0.066	0.702	0.080	0.705	-0.123	0.643	0.801
C-reactive protein (CRP)	-0.002	0.899	0.010	0.630	-0.024	0.362	0.830

1. We set the lowest level(ln BPA < 0.85, N=312) as a reference
2. Model adjusted for sex, age, smoking status, alcohol status and household monthly income
3. HOMA index=Sugar*Insulin*0.05551*6.945/22.5

In the Table 4, we compared the main linear regression model (adjusted for sex, age, smoking status, alcohol status and household monthly income) and the models which were added LDL, SBP, and CRP as the confounder, individually. We found the results and trends were similar with main model. There were an association between BPA exposure and CIMT, and also associated with CCA, bulb, and ICA in different models.

The Table 5 was the results of sensitivity analysis. We found the significant association between BPA exposure and CIMT in both subgroups, including sex, age, LDL, sugar, and consumption of fast food. Basing on sensitivity analysis, we also found the healthier subgroups had more significant association between BPA exposure and CIMT in BMI, hypertension or diabetes subgroups, and CRP.

Table 4. Mean and standard deviation of carotid intima-media thickness across different categories of serum BPA levels in linear regression models

	Main model[1,2]							
	0.85 < ln BPA (N=312)	0.85 ≤ ln BPA < 2.51 (N=156)		2.51 ≤ ln BPA < 3.45 (N=94)		ln BPA ≥ 3.45 (N=62)		*p* for Trends
	Thickness (mm) ± SD	Thickness (mm) ± SD	*p*-value	Thickness (mm) ± SD	*p*-value	Thickness (mm) ± SD	*p*-value	
Carotid intima-media thickness (CIMT)[3]	0.443 ± 0.050	0.449 ± 0.051	0.243	0.465 ± 0.059	**0.0002**	0.478 ± 0.070	**<0.0001**	**<0.0001**
CCA[4]	0.451 ± 0.055	0.455 ± 0.057	0.443	0.473 ± 0.063	**0.0012**	0.482 ± 0.075	**0.0002**	**<0.0001**
BULB[5]	0.463 ± 0.082	0.476 ± 0.082	0.130	0.489 ± 0.092	**0.0095**	0.501 ± 0.094	**0.0012**	**0.0001**
ICA[6]	0.407 ± 0.058	0.415 ± 0.052	0.214	0.429 ± 0.059	**0.0012**	0.454 ± 0.082	**<0.0001**	**<0.0001**
	Main model confounders + LDL[1]							
Carotid intima-media thickness (CIMT)[3]	0.443 ± 0.050	0.449 ± 0.051	0.197	0.465 ± 0.059	**0.0006**	0.478 ± 0.070	**<0.0001**	**<0.0001**
CCA[4]	0.451 ± 0.055	0.455 ± 0.057	0.384	0.473 ± 0.063	**0.0024**	0.482 ± 0.075	**0.0017**	**<0.0001**
BULB[5]	0.463 ± 0.082	0.476 ± 0.082	0.100	0.489 ± 0.092	**0.0189**	0.501 ± 0.094	**0.0109**	**0.0013**
ICA[6]	0.407 ± 0.058	0.415 ± 0.052	0.167	0.429 ± 0.059	**0.0027**	0.454 ± 0.082	**<0.0001**	**<0.0001**
	Main model confounders + SBP[1]							
Carotid intima-media thickness (CIMT)[3]	0.443 ± 0.050	0.449 ± 0.051	0.180	0.465 ± 0.059	**0.0002**	0.478 ± 0.070	**<0.0001**	**<0.0001**
CCA[4]	0.451 ± 0.055	0.455 ± 0.057	0.380	0.473 ± 0.063	**0.0011**	0.482 ± 0.075	**0.0002**	**<0.0001**
BULB[5]	0.463 ± 0.082	0.476 ± 0.082	0.088	0.489 ± 0.092	**0.0078**	0.501 ± 0.094	**0.0011**	**<0.0001**
ICA[6]	0.407 ± 0.058	0.415 ± 0.052	0.159	0.429 ± 0.059	**0.0010**	0.454 ± 0.082	**<0.0001**	**<0.0001**
	Main model confounders + CRP[1]							
Carotid intima-media thickness (CIMT)[3]	0.443 ± 0.050	0.449 ± 0.051	0.238	0.465 ± 0.059	**0.0003**	0.478 ± 0.070	**<0.0001**	**<0.0001**
CCA[4]	0.451 ± 0.055	0.455 ± 0.057	0.443	0.473 ± 0.063	**0.0013**	0.482 ± 0.075	**0.0001**	**<0.0001**
BULB[5]	0.463 ± 0.082	0.476 ± 0.082	0.124	0.489 ± 0.092	**0.0107**	0.501 ± 0.094	**0.0006**	**<0.0001**
ICA[6]	0.407 ± 0.058	0.415 ± 0.052	0.214	0.429 ± 0.059	**0.0013**	0.454 ± 0.082	**<0.0001**	**<0.0001**

1. We set the lowest level(ln BPA < 0.85, N=312) as a reference
2. Model adjusted for sex, age, smoking status, alcohol status and household monthly income
3. CIMT were the mean of CCA, BUlB, and ICA
4. CCA: mean of bilaterally common carotid artery
5. BULB: mean of bilaterally bulb
6. ICA: mean of bilaterally internal carotid artery

Table 5. Linear regression model of BPA levels group in blood and CIMT by subgroups

	N	0.85 < ln BPA (N=312) Thickness (mm) ± SD	0.85 ≤ ln BPA < 2.51 (N=156) Thickness (mm) ± SD	*p*-value	ln BPA ≥ 2.51 (N=156) Thickness (mm) ± SD	*p*-value	*p* for Trends
Sex							
Female	537	0.433 ± 0.046	0.439 ± 0.042	0.378	0.464 ± 0.062	**<0.0001**	**<0.0001**
Male	350	0.457 ± 0.052	0.466 ± 0.060	0.549	0.481 ± 0.066	**0.0039**	**0.0055**
Age							
12~19	275	0.438 ± 0.050	0.447 ± 0.047	0.281	0.472 ± 0.057	**0.0001**	**0.0001**
≥20	612	0.446 ± 0.050	0.449 ± 0.053	0.440	0.469 ± 0.067	**<0.0001**	**0.0002**
BMI							
<24	687	0.437 ± 0.046	0.446 ± 0.047	0.127	0.462 ± 0.060	**<0.0001**	**<0.0001**
≥24	200	0.463 ± 0.058	0.459 ± 0.062	0.711	0.492 ± 0.069	**0.0402**	0.0600
Current Hypertension or Current Diabetes							
Yes[$]	158	0.463 ± 0.054	0.470 ± 0.056	0.565	0.470 ± 0.072	0.6725	0.5896
No	631	0.429 ± 0.041	0.429 ± 0.036	0.957	0.455 ± 0.059	**<0.0001**	**<0.0001**
LDL*							
<119	664	0.437 ± 0.046	0.446 ± 0.048	0.097	0.460 ± 0.055	**<0.0001**	**<0.0001**
≥119	223	0.462 ± 0.056	0.461 ± 0.061	0.983	0.496 ± 0.077	**0.0071**	**0.0112**
Sugar*							
<89	654	0.438 ± 0.047	0.444 ± 0.046	0.227	0.461 ± 0.059	**<0.0001**	**<0.0001**
≥89	233	0.458 ± 0.056	0.460 ± 0.060	0.817	0.498 ± 0.070	**0.0005**	**0.0012**
HOMA*							
<1.49687	665	0.438 ± 0.045	0.444 ± 0.047	0.234	0.464 ± 0.060	**<0.0001**	**<0.0001**
≥1.49687	222	0.457 ± 0.061	0.462 ± 0.059	0.497	0.487 ± 0.071	**0.0139**	**0.0157**
CRP*							
<0.09	658	0.436 ± 0.045	0.448 ± 0.050	**0.026**	0.468 ± 0.059	**<0.0001**	**<0.0001**
≥0.09	229	0.460 ± 0.058	0.450 ± 0.055	0.130	0.478 ± 0.076	0.1707	0.3476
Fast food consumption at least one time (times/week)							
Yes	402	0.437 ± 0.043	0.434 ± 0.043	0.846	0.461 ± 0.058	**0.0007**	**0.0028**
No	387	0.437 ± 0.049	0.440 ± 0.044	0.627	0.454 ± 0.065	**0.0304**	**0.0354**

* cut-point were 75 percentile

$ sample size in three BPA level were 61, 22, and 21

Model adjusted for sex, age, smoking status, alcohol status and household monthly income, but in sex/age sensitivity analysis, we didn't adjust for sex/age

Discussion

In an adolescent and young adult population, with increased BPA exposure level, the trends of CIMT were increased. Higher BPA exposure may have an association with overweight, higher LDL, higher cholesterol, and thicker of CIMT, no matter which measured position.

In this study, we found CIMT may be affected by BPA level. Though there are still limited information on association between BPA and CIMT. A Sweden study didn't find an association between BPA and CIMT, but it found there was an association between BPA and plaques in carotid (Lind & Lind, 2011). In addition, the CIMT was a risk factor of cardiovascular disease, coronary heart disease, and coronary artery disease (Daniel et al., 1999), and there are many studies shown that BPA exposure may have a positive association with those diseases (Melzer et al., 2012; Melzer et al., 2012; Melzer et al., 2010). Some potential mechanism of BPA would affect human health, including lipid metabolism, glucose homeostasis, and so on (Rochester, 2013). For the lipid metabolism, comparing our results with others studies results, it was consistent in lipid metabolism results, which were that higher BPA level may have an association with higher LDL and cholesterol level (Olsen et al., 2012). Moreover, the effect on HDL was null results in this study, and it is not clear in literature. Some studies found higher BPA level significant associated with lower HDL (Li et al., 2012; Teppala et al., 2012), but

some studies had opposite results (Olsen et al., 2012) or null results (Chou et al., 2011). The effects of waist and BMI which are risk factors of cardiovascular disease were also not consistent in literature (Maserejian et al., 2012; Zhao et al., 2012). For glucose homeostasis, we found that there was a negative association between BPA and sugar in this study. The result was inconsistent with previous studies (Rochester, 2013; Shankar & Teppala, 2011). However, we also found a study shown BPA may not have an association with blood sugar (Guang et al., 2011). Therefore, we check all subjects' sugar level. Most of their sugar levels were not less than 70 mg/dl and only two subjects' sugar level were 68 and 69 mg/dl. All subjects' sugar statuses were not too low to cause clinic effects. The other possible reason was that the negative association between BPA and sugar was just a temporary effect through long time fasting. Basing on those studies, we conjectured the major mechanism may be the lipid metabolism.

BPA may affect lipid metabolism, and then cause exaltation on BMI, LDL and cholesterol. Through affecting lipid metabolism, higher BPA exposure may cause thicker CIMT. According to the results of this study, there was a positive association between BPA exposure and CIMT, but a negative association between BPA and lipid metabolism in higher BPA level group. We conjecture the mechanism of BPA cause thicker CIMT was mainly through the negative effects on lipid metabolism. The LDL will be a precipitation on the vessel wall (Brain et al., 2012), and impact on the media

thickness of the carotid artery. In addition, BMI, LDL, and cholesterol are the important risk factors of cardiovascular disease (Eisenmann et al., 2005) which association with the CIMT and plaques (Hulthe et al., 1997).

A previous study showed that BPA was slowly released or metabolites from tissue sites may cause oxidative endothelial cell damage (Melzer et al., 2010). We conjectured BPA may not only have negative effect on lipid metabolism, but also hurt blood vessel directly. When endothelial cell of blood vessel were damage, LDL would through the damage endothelial cell and enter intima-media space easily. After LDL enter the intima-media space, LDL would have a reaction with free radical and then induce a series reaction of lipid peroxidation, and become a lipid peroxides in finally. Those lipid peroxides were swallowed by macrophages. However, macrophages would die, because macrophages can not break down lipid peroxidation. Thence, those dead macrophages would cause cell proliferation and fibrosis on vessel, and make vessel thicker and lack of flexibility (Hennig & Chow, 1988). Higher BPA exposure may lead to thicker CIMT through negative effects on lipid metabolism, and BPA may directly hurt vessel, which may exacerbate that LDL become a precipitation on vessel wall.

BPA levels in serum in this study were slight higher than other studies and countries (appendix 6). In this study, there were some extremes values may cause mean value higher. If we compare our mean with other studies, we would found the BPA

mean in this study was much higher than others (Luigi et al., 2009; Padmanabhan et al., 2008; Shen et al., 2013; Takeuchi & Tsutsumi, 2002). We think use median value to compare with other studies may more appropriate. Our median value was 2.4 ng/mL, and it was similar with United States study and higher than a Japan study (Bloom et al., 2011; Sugiura-Ogasawara et al., 2005). The BPA concentration range was ND – 1097.4ng/mL. The minimum was similar with other papers', but the maximum was much higher than other studies' (Choi et al., 2012; Chou et al., 2011). Those extremes in this study were found that most of them had higher consumption on coke, sweets, or fast food per week. The packages of those foods were a probably exposure source of BPA (Lopez-Espinosa et al., 2007; Noonan et al., 2011), which may cause the higher BPA level in serum.

BPA is a kind of estrogen disruptor chemicals (EDCs), and it structure is similar with natural estrogen. Those EDCs were concerned that had different effects on different sex. In this study results, we can find the association between BPA and CIMT appeared in both sex (Table 5). However, we still found the association was more significant in female. We already known estrogen play a protected role of cardiovascular disease in female (Subbiah, 2002). According to a previous study, low-level exposure of BPA may have an antagonist's function of estrogen (Li et al., 2012). We infer that the BPA exposure decreased the protected effect of natural

estrogen, so we found the association between BPA and CIMT was more significant in female.

Even in adolescent and young adult population, we found BPA exposure may have an association with CIMT. Age was concerned when discuss the risk factors of cardiovascular disease. In our study, we categorize age into two subgroups, 12-19 and ≥20 years old. In both subgroups, we found significant association between BPA and CIMT (Table 5). A Sweden study suggested that BPA played a role for plaques-associated chemicals in atherosclerosis in a 70 years old population (Lind & Lind, 2011). Basing on that Sweden study, our results suggest that not only elder people but also adolescent and young adult need to concern BPA exposure may exacerbate cardiovascular disease risks in the future.

In sensitivity analysis (Table 5), the association between serum BPA and CIMT was more evident in subjects with lower BMI, no hypertension or diabetes, and lower CRP. These findings deserve more attention, particularly in searching for novel risk factors of atherosclerosis in relatively healthy populations. However, the relationship between BPA and CIMT has not been elucidated at a mechanistic level. One possible explanation is that the effect of BPA on CIMT is much weaker than the effect of obesity trends, hypertension, diabetes, and cardiovascular diseases. When considering the atherosclerotic effect of BPA in the above populations, the trend is too small to be

statistically significant. Alternatively, it is also possible that the association between BPA and obesity trends, hypertension, diabetes, and cardiovascular diseases is due to an opposite synergistic effect (Lin et al., 2013). The sample sizes in 50-75th and ≥75th BPA exposure level which were less than 30 may be another reason in hypertension or diabetes subgroup.

The association between BPA and CIMT was not contributed from fast foods consumption. In the Table 1, we found the BPA level had significant different among subgroups. The packages of fast foods were a probably exposure source of BPA (Lopez-Espinosa et al., 2007; Noonan et al., 2011). For understanding whether the fast food consumption interfered the association or not, we did a sensitivity analysis. In the Table 5, the association between BPA and CIMT was found in both subgroups, at least one time fast food consumption every week or not. Therefore, we known the association didn't come from the fast food consumption contribution. However, we still found the association was more significant in the fast food consumption at least one time a week subgroup. A possible reason is that there are more fired foods in fast food, and the diet habits including too much fired foods may enhance the risk of cardiovascular disease. We didn't have more detail information about diet habits can explore the contribution.

There are several limitations to our study. First, we didn't have some information about the eating outside frequency, types of foods or more detail information of diet

habits for clarifying the contribution to BPA exposure level. Second, our study population is made up of adolescents and young adults with abnormal urinalysis in childhood and living in the Taipei area, and therefore we cannot infer that the same association might be similar in the general population. Third, we did not take into account all medications that may have impact on CIMT, which will be a confounding variable. However, more than 95% of participants self-reported no significant clinical diseases and no medication history. Finally, this study was a cross-sectional study, and it can not infer the causal. Besides, we used the cross sectional study design may make a slight impact of BPA on CIMT was overridden by greater impacts from other cardiovascular related diseases.

Conclusions

In adolescents and young adult populations, we found BPA exposure level may have a positive association with carotid intima-media thickness. In addition, BPA exposure may also hurt blood vessel directly, and exacerbated the effects on carotid intima-media thickness.

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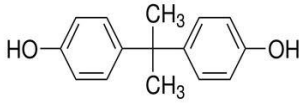
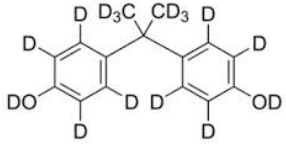
Appendix 1

Appendix 1. Instrumental parameters of the mass spectrometer

	Ionization Mode	ESI-
Voltages		
	Capillary (kV)	3.0
	Extractor (V)	5
	RF Lens (V)	0
Temperature		
	Source temperature (℃)	120
	Desolvation temperature (℃)	500
	Column Oven (℃)	60
Gas Flow		
	Desolvation gas flow (L/hr)	1100
	Cone gas flow (L/hr)	50
Analyzer		
	LM 1 resolution	13
	HM 1 resolution	13
	Ion Energy 1 (V)	1
	Entrance (V)	0
	Exit (V)	0
	LM 2 resolution	13
	HM 2 resolution	13
	Ion Energy 2 (V)	1
	Multiplier voltage (V)	650
	Gas Cell Pirani Pressure (mbar)	3.26 e^{-3}

Appendix 2

Appendix 2. Selected reaction monitoring(SRM) transitions, individual cone voltage and collision energy of the analytes

Analytes		MW	Precursor ion (m/Z)	Product ion (m/Z)	cone voltage (V)	collision energy (V)
BPA		228.3	227.2	212.1	40	20
				132.8	40	30
BPA-d16		244.4	241.0	222.6	40	30

BPA-d16 was used as internal standard of BPA.

Appendix 3

Appendix 3. Intra- and Inter-day and precision for BPA in bovine serum

concentration (ng/ml)		BPA	
		Intra-day	Inter-day
1	mean	1.1	1.0
	RSD%	18.3	10.0
	Bias%	12.5	0.0
2.5	mean	2.5	2.7
	RSD%	11.4	1.8
	Bias%	1.3	9.0
5	mean	4.7	4.9
	RSD%	12	16.9
	Bias%	-5.3	-2.0
10	mean	10.9	10.1
	RSD%	21	8.6
	Bias%	9.3	0.7
25	mean	23.9	23.0
	RSD%	11.7	9.6
	Bias%	-4.4	-7.9
50	mean	54.6	50.5
	RSD%	11.8	10.4
	Bias%	9.2	1.0
100	mean	111.6	101.0
	RSD%	16.1	10.7
	Bias%	11.6	0.9
200	mean	186.4	200.7
	RSD%	5.2	5.8
	Bias%	-6.8	0.3
500	mean	510.5	503.4
	RSD%	6.8	4.4
	Bias%	2.1	0.7
750	mean	738.0	699.6
	RSD%	4.3	10.6
	Bias%	-1.6	-6.7
1000	mean	1030.5	1010.3
	RSD%	5.5	4.7
	Bias%	3.1	1.0

RSD% = (standard deviation / mean) x 100%
Bias% = [(measured value – theoretical value) / theoretical value] x 100%

Appendix 4

Appendix 4. Recovery of analytes with different concentration in bovine serum and overall process efficiency with different concentration in human serum (n=3)

recovery	BPA		
	10 ng/ml	25 ng/ml	100 ng/ml
	101.7±7.1 (7.0)	93.7±6.4 (6.8)	108.4±8.0 (7.4)
overall process efficiency	BPA		
	5 ng/ml	25 ng/ml	100 ng/ml
	202.3±45.7 (22.6)	98.8±13.3 (13.4)	58.2±5.3 (9.1)

Values are mean ± SD (RSD%)

recovery(%)=(measured value / theoretical value) X 100%

overall process efficiency=(area of pre-extraction spike / area of standard) X 100%

Appendix 5

Appendix 5. Quantification of the spiked samples in human serum (n=3)

		no spike	low spike(5ng/ml)	medium spike(25ng/ml)	high spike(100ng/ml)
BPA	mean	17.83	22.77	43.57	127.23
	SD	4.20	4.04	10.93	17.65
	RSD%	23.57	17.75	25.09	13.87

Appendix 6

Appendix 6. Paper reviews of BPA concentration in blood

Country	Study year	No. subjects	Age	Concentration (ng/mL)			reference
				Mean (SD)	Median	Range	
Taiwan (Taipei)	2006-2008	887	21.3 (3.4)	19.8 (79.9)	2.4	ND - 1097.4	This study
Taiwan (Hsinchu)	2006-2007	Pregnant mothers: 97	28.8 (3.7)	5.4 (6.3)	--	0.3 - 29.4	Chou WC et. al., 2011
Korea	--	Mother: 40	--	9.0 (7.1)	--	0.0 - 32.4	Haemin Choi et. al., 2012
		Children: 80	--	2.6 (5.7)		0.0 - 5.0	
Japan	--	men: 11	29.4 (1.1)	1.5 (0.1)	--	--	Toru Takeuchi, et al., 2002
		women: 14	28.7 (0.7)	0.6 (0.1)			
Japan	2001-2002	32	32.0 (4.8)	0.8 (0.4)	0.7	0.2 - 1.6	Sugiura-Ogasawara M, et. al, 2005
China	2011-2013	124	30-50	7.5 (9.4)	--	1.0 - 62.8	Yang Shen, et al., 2013
Italy	--	11	18-44	2.9 (1.7)	--	0.8 - 7.1	Cobellis L, et al., 2009
USA	2006/8-11	Pregnant mothers: 40	28.9 (0.8)	5.9 (0.9)	--	0.5 - 22.3	Vasantha Padmanabhan, et al., 2008
USA	--	men: 27	38 (31-48)	--	0.5	0.0 - 22.7	Michael S. Bloom, et al., 2011
		women: 27	35 (31-44)	--	3.3	0.0 - 67.4	
USA	2007-2008	women: 44	35.8 (4.08)	7.22 (14.15)	2.5	0.0 - 67.4	Michael S. Bloom, et al., 2011

Appendix 7

Appendix 7. Characteristics and biochemical level of the study population

	Mean	SD
Characteristics and physical exam		
Age (year)	21.29	3.45
BMI (kg/m^2)	21.90	4.11
Waist (cm)	70.63	11.54
Female	66.40	9.50
Male	77.10	11.40
Hip (cm)	93.32	7.99
Female	91.44	7.16
Male	96.22	8.34
SBP (mmHg)	107.37	14.69
DBP (mmHg)	66.40	11.52
Lipid (mg/dl)		
HDL	50.18	9.91
LDL	101.75	30.94
Triglyceride	84.23	73.75
Cholesterol	175.09	34.59
Glucose homeostasis		
Insulin (μIU/mL)	5.58	6.41
Sugar (mg/dl)	87.13	19.58
HOMA	1.27	1.84
C-reactive protein (mg/dL)	0.10	0.19
Intima-media thickness (mm)	0.45	0.05
RCCA	0.46	0.06
RCCA_Max	0.58	0.08
RBULB	0.47	0.11
RICA	0.42	0.08
LCCA	0.45	0.07
LCCA_Max	0.58	0.08
LBULB	0.47	0.10
LICA	0.41	0.07