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Cord blood bisphenol A levels and reproductive and thyroid hormone levels of neonates: The Hokkaido Study on Environment and Children's Health

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Running Head

Cord blood BPA and reproductive and thyroid hormone levels of neonates

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Abstract

Background: Bisphenol A (BPA) is widely used and BPA exposure is nearly ubiquitous in developed countries. While animal studies have indicated adverse health effects of prenatal BPA exposure including reproductive dysfunction and thyroid function disruption possibly in sex-specific manner, findings from epidemiological studies have not been enough to proof these adverse effects. Given very limited research on human, the aim of this study was to investigate associations between cord blood BPA levels and reproductive and thyroid hormone levels of neonates and whether associations differed by neonate sex.

Methods: Among 514 participants of the Hokkaido study who were recruited from 2002 to 2005 at one hospital in Sapporo, Japan. BPA level in cord blood was determined by ID-LC/MS/MS and the limit of quantification was 0.040 ng/ml. 9 types of reproductive hormone levels in cord blood were measured and thyroid hormone levels were obtained from neonate mass screening test data. 283 subjects those who had both BPA and hormone levels measurements were included for the final analyses.

Results: Geometric mean of cord blood BPA was 0.051 ng/ml. After adjustment, BPA level was negatively associated with prolactin (PRL) ($\beta=-0.38$). There was an

interaction between infant sex and BPA levels on PRL, weak negative association was found in boys ($\beta=-0.12$) whereas weak positive association was found in girls ($\beta=0.14$). BPA level showed weak positive association with testosterone, estradiol and progesterone levels in boys. No association was found between BPA and thyroid hormone levels.

Conclusions: Our findings suggested that fetal BPA levels might be associated with certain reproductive hormone levels of neonate with sex-specific manner, though further investigations are necessary.

Introduction

Bisphenol A (BPA) is widely used in plastics such as food and drink containers, and as an additive in thermal paper, dental sealant, medical equipment and flame retardant.

BPA exposure is nearly ubiquitous in developed countries as it has been detected from most of adults and children¹⁻³ as well as amniotic fluid⁴, cord blood and placental tissue⁵, which indicate in utero BPA exposure. A large number of publications linked BPA exposure to adverse health effects in animal studies. Although evidences from animal studies suggest that exposure to BPA may cause detrimental effects on human health, there are relatively limited number of researches have carried out in humans. Especially, BPA exposure to fetus and its potential influences on their health outcomes are yet largely unknown.

Hormones are some of the most important chemicals and are essential in the process of reproductive, growth and development in human. Among claimed adverse health effects of BPA exposure in epidemiology, studies have found changes in circulating reproductive hormone levels in relation to BPA exposure⁶⁻⁹. BPA has a weak estrogenic property. Experimentally, BPA has shown to interact with estrogen signaling pathways through binding to the estrogen receptors¹⁰. Further, BPA can act as an antiestrogen¹¹ and also can directly bind to androgen receptors¹². BPA also

act as thyroid hormone agonist and antagonist¹³. There has been only one study focused on associations between cord blood BPA levels and reproductive hormones and their study only focused on boys with and without cryptorchidism¹⁴. To our knowledge, there was no published study of prenatal BPA exposure in association with reproductive hormone levels of neonate in general population including both sex.

In addition to adverse influences of BPA exposure on reproductive hormones, effects on thyroid hormones are also reported. Thyroid hormones particularly play an essential role in brain development. *In vitro* studies indicated that BPA might change thyroid homeostasis by modifying thyroid hormone signal pathways¹⁵. Considering that the structure of BPA is similar to thyroid hormones, it has been tested its thyroid disrupting activity *in vivo* and some published studies have observed increased serum thyroxine (T4) levels in rat pups with maternal BPA exposure^{13,16}. Several prospective cohort studies have investigated the association between BPA levels and thyroid function and showed suggestive inverse associations with thyroid stimulating hormone (TSH) and T4 and positive associations with triiodothyronine (T3)^{17, 18}.

From a few prospective birth cohort studies, it has been suggested that prenatal BPA

exposure may modify reproductive and/or thyroid hormone homeostasis and may be in sex-specific manner, yet research on reproductive and thyroid hormone levels in association with prenatal exposure to BPA in human is very limited. Thus, the aim of this study was to investigate the association between cord blood BPA levels and reproductive and thyroid hormone levels of neonates in prospective birth cohort study.

Materials and methods

Study population

This prospective birth cohort study was based on the Sapporo Cohort, Hokkaido Study on Environment and Children's Health¹⁹. Briefly we recruited pregnant women at 23-35 weeks of gestation between July 2002 and October 2005 from the Sapporo Toho Hospital in Hokkaido, Japan. All subjects were resident in Sapporo City or surrounding areas. The participants completed the self-administered questionnaire after the second trimester during their pregnancy. The questionnaire contained baseline information including their dietary habits, smoking history, alcohol consumption, caffeine intake, family income, educational levels of themselves and partners. The perinatal information of the mothers and their children was collected

from their medical records. This study was conducted with the informed consent of all participants in written forms. The protocol used in this study was approved by the Institutional ethical board for epidemiological studies at the Hokkaido University Graduate School of Medicine and Hokkaido University Center for Environment and Health Sciences.

Measurement of Bisphenol A

Cord blood was obtained at delivery. All samples were stored at -80°C until analysis. The concentration of BPA in cord blood was measured by using isotope dilution liquid chromatography-tandem mass spectrometry (ID-LC/MS/MS) at IDEA Consultants, Inc. (Shizuoka, Japan). The details can be found elsewhere²⁰. The limit of quantification (LOQ) of BPA was 0.040 ng/ml.

Reproductive hormone levels

9 kinds of reproductive hormone levels were measured in cord blood at Aska Pharmaceutical Co., Ltd (Tokyo, Japan). Estradiol (E2), testosterone (T) and progesterone (P4) were measured using LC/MS/MS. Luteinizing hormone (LH), follicle stimulating hormone (FSH), sex-hormone binding globulin (SHBG) and prolactin (PRL) were measured by immunoradiometric assay (IRMA). Inhibin B was measured using Enzyme-Linked Immuno Sorbent Assay (ELISA). Insulin-like factor

3 (INSL3) was measured using Enzyme Immunoassay (EIA). The details of reproductive hormone measurement were found in our previous study²¹.

Thyroid hormone levels

We obtained data of TSH, free T4 (FT4) from mass screening test for endocrine disorders conducted by Sapporo City Institute of Public Health (Hokkaido, Japan). A heel-prick blood sample of newborns was obtained as spots on a filter paper for the Guthrie test. The blood samples were obtained from infants between 3 and 7 days of age after birth. Blood samples were applied to 0.3 cm filter disks and TSH and FT4 levels were measured using ELISA (TSH: Enzaplate N-TSH, Bayer Co., Tokyo, Japan; FT4: Enzaplate N-FT4, Bayer Co.). The FT4 values of all samples were detected, and for samples with TSH levels below the detection limit (0.50 μ U/ml), we used a value of half the detection limit.

Data Analysis

We used the following eligibility for criteria for analyses of subjects; no serious illness or complications during pregnancy and delivery, no intake of medication which may modify maternal hormone levels, singleton babies born at term (37 to 42 weeks of gestation), and neonates without congenital anomalies or diseases. Among all 514 participants of Sapporo Cohort Study, 285 cord blood samples for BPA

measurements were available. Among 285 samples, 278 subjects had available reproductive hormone levels, 283 subjects had available thyroid hormone levels.

Since the distributions of cord blood BPA concentrations were right skewed, these variables were transformed by logarithms (log) 10 to improve their linear relation with hormone levels. Reproductive and thyroid hormone levels were also log 10 transformed. BPA concentration below the LOQ was assigned the value of one-half of the LOQ, 0.020 ng/ml. Mann-Whitney test was used for comparisons between boys and girls. To examine the association between cord blood BPA levels and various hormone levels, linear regression models were used. Then models were stratified by infant sex. Estimated marginal means (EMMs) were determined for selected hormone levels in association with tertile BPA levels. Tertiles were 0.041; 0.042-0.067; > 0.068 ng/ml, respectively. To select covariates to include in the final models, factors known or suspected of being associated with the BPA concentrations and/or hormone levels were reviewed in the literatures. The covariates used in this study were child sex and days of mass screening test. All analyses were conducted using SPSS (Version 22.0J; SPSS, Chicago, IL, USA).

Results

Basic characteristics of mothers and infants included in this study were shown in Table 1. The average maternal age at delivery was 30.4 ± 5.0 (mean $\pm$ SD). About half of the participants were nulliparas (51.6%). More than half of mothers had education longer than 12 years (54.4%), 70.9% had annual income below 5 million yen. 16.1 % of mothers were smoking during pregnancy. The mean birth weight was 3128 ± 33 g and birth length was 48.5 ± 1.6 cm. The geometric mean of cord blood BPA was 0.051 ng/ml and interquartile range (IQR) was <LOQ to 0.076 ng/ml in this study. The detection rate of cord blood BPA was 83.2 %.

Distributions of reproductive hormone levels in cord blood and thyroid hormone levels from mass screening data were shown in Table 2. E2, T, P4 were detected from 100% of the subjects, and SHBG, PRL were detected from 99.6%. The detection rate of Inhibin B was 99.2% in boys, however, only 27.0% in girls. For girls, INSL3 was measured in only 24 participants. Thus consequently Inhibin B and INSL3 were eliminated for the further analyses in girls. LH, FSH were detected from 16.8% and 21.3% of the subjects, respectively and thus, we did not include these hormones for further analysis. TSH and FT4 were detected from all the subjects. Since reproductive hormone levels were known to have sex differences, sex stratification analysis was conducted. T, P4, SHBG level was higher in boys

compared to girls.

Adjusted linear regression coefficients (β) of reproductive and thyroid hormone levels in relation to cord blood BPA levels were shown in Table 3. Cord blood BPA levels was negatively associated with PRL overall ($\beta = -0.38$). There was an interaction between infant sex and BPA levels on PRL, weak negative association was found in boys ($\beta=-0.12$) whereas weak positive association was found in girls ($\beta=0.14$). BPA level showed weak positive association with T, E2 and P4 levels in boys. Dose-response relationship between tertiles of BPA and selected hormone levels which showed associations with BPA concentrations were shown in Figure 1. No clear dose-response relationships were found in any of the hormone levels investigated.

Discussion

We observed weak positive association between cord blood BPA and T, E2 and P4 among boys. However, after stratification of BPA levels into tertiles, dose-response relationships were not found. There was only one published study investigated the association between perinatal BPA exposure and sex hormone levels only among boys, which found a positive correlation between cord blood BPA and total T and

Inhibin B in boys without cryptorchidism¹⁴. However, we did not observe correlation between cord blood BPA and T and Inhibin B among boys. This could be due to much higher cord blood BPA level in their study (1.12 ± 0.86 ng/ml, mean $\pm$ SD) compared to ours (0.057 ± 0.036 ng/ml, mean $\pm$ SD). Cross-sectional studies of adult, especially among male population have also suggested changes of circulating reproductive hormone levels in relation to BPA exposure⁶⁻⁹. These cross-sectional studies suggested possible interference with aromatase activity by BPA⁶, and direct stimulation by estrogenic activity of BPA⁷.

In animal studies, pre- and postnatal exposure to BPA caused decrease in T of male offspring²², on the other hand, prenatal BPA exposure did not change T levels of male offspring²³. Human and rodent have vastly different sensitivity levels to estrogen during gestation, and the species difference in free fraction of the steroid in the plasma may magnify the difference in adverse effects of BPA exposure²⁴. Thus what observed in animal studies cannot be simply applicable in human and therefore epidemiological studies are necessary to elucidate effects of exposure to BPA on reproductive function among human.

Recently, Qiu et al.²⁵ observed increase in testicular T, whereas decrease in aromatase after 8 weeks of BPA exposure ranged from 0.0005 to 5 mg/kg/bw to

adult rats. The main functions of aromatase are to convert T into E2, and longtime exposure to BPA resulted in the inhibition of aromatase activity in an in vitro-based study²⁶, which suggested that BPA might increase serum T partially by down-regulating aromatase and further accumulating it via a decrease in the transformation of T into E2²⁵.

There was no human study that found association between BPA levels and P4 levels in cord blood. *In vitro* studies observed decrease in P4 production following BPA treatments in swine ovarian granulosa cells²⁷. Results from animal studies also showed decrease in P4 in BPA-treated group²⁸. Those observations found in experimental studies did not support our observation.

We observed negative association between BPA and PRL levels. Yet studies of association between BPA and PRL are very limited. PRL plays a critical role in modulating immune and inflammatory responses through various immune signaling pathways²⁹. It has been reported *in vitro* and *in vivo* studies that BPA mimics E2 and induces hyperprolactinemia³⁰. Since the endocrine physiology of pregnancy of animals and human are quite different as alternations in the secretion of maternal PRL, placental lactogen and placental androgen, or in the activity of corpus luteum aromatase, thus it should be well debated that low-dose in utero effects which may

be suggested in rats are plausible in human as well. One case-control study suggested that female workers occupationally exposed to BPA showed increased PRL levels³¹. In their study BPA exposure was an independent risk factor for increased serum PRL levels³¹. Additionally, recent evidence has found that BPA has major stimulatory impacts on PRL release²⁹. Further epidemiological studies, especially prospective studies, are necessary to elucidate associations between BPA and PRL.

We found no association between cord blood BPA levels and thyroid hormone levels. Epidemiological studies have reported that negative association between maternal BPA exposure and neonatal THS levels^{17,18,32}. Discrepancy between our study and other prospective studies could be explained by the differences in ethnicities and socioeconomic background and also in BPA exposure levels. The cord blood BPA levels in this study were much lower compared to the previous reports^{3,17}. Sample size might be also one of the reasons to see the discrepancy between studies. Additionally, co-exposures assessment and method of thyroid hormone measurements, whether heel-prick blood or cord blood, or timing of thyroid hormone measurement could be a reason of discrepant results.

It is known that thyroid hormone is essential for fetal and child growth and brain

development, thus interference with thyroid hormone circulation by BPA exposure may have effects on development. Although results were not consistent among studies, animal studies have shown associations between prenatal BPA exposure and thyroid hormone levels of pups^{13,15}. Inconstancies from these studies could be due to different dosages of BPA, different timing and duration of BPA exposure, and different animals. Besides it is difficult to lead conclusions from these rodent studies as metabolic pathways of BPA were different between rodents and humans³³. For instance, orally dosed BPA in rodents are excreted through the bile with reabsorption by guts, whereas in humans, orally taken BPA is metabolized in the liver and then enters the blood stream. Additionally, humans are likely to be exposed lower levels of BPA but chronically, in animal models, they often exposed higher levels compared to humans.

In our study, BPA in cord blood was measured as prenatal exposure whereas maternal urine samples were used in the other epidemiological studies. BPA levels in cord blood were more directly represent fetal exposure rather than maternal exposure, on the other hand, our study was not able to assess early fetal exposure. BPA can be measured in serum as well as urine³⁴. Although urinary BPA testing is less invasive, it measures BPA excreted, which is not exactly reflecting the current *in*

vivo exposure. Thus, serum BPA may be a better for exposure assessment³⁵.

Besides studies used urinary BPA as exposure measurements, intra-individual variability of BPA concentrations was moderately correlated³⁶ and accurately characterizing exposure from a single measurement was difficult. Meanwhile using mean concentration of urinary BPA from several measurements would decrease the ability to identify time-sensitive window of development³⁷. We should note that measurement of BPA in blood samples have left space for further discussion as BPA blood concentration possibly overestimated due to external contamination. In our method of BPA measurements, we used glass cartridge instead of polypropylene one to reduce background levels and no free BPA was detected from samples, which was indication of null possible external contamination.

The limitations of this study need to be considered. First, there was limited statistical power with our sample size. Additionally, there have been concerns whether single measurement of cord blood sample represented the long-term prenatal BPA exposure due to short half-lives of BPA and there might be a possibility of accidental exposure near blood drawing period. However, in this study, only vaginal birth was included and chances were small that medical equipment such as syringe, tubes which possibly contained BPA was used during the process of delivery. Thus, we

consider accidental exposure did not occur. There might be a chance of selection bias in this study as we only included participants with available cord blood samples and reproductive and thyroid hormone levels. However, the comparison between original cohort profile and the present study profile did not show discrepancy. The strength of our study was that we used the BPA levels of cord blood, which accurately indicated the exposure of fetus. However, more studies are necessary to confirm adverse effect of prenatal BPA exposure as developmental exposure can result in changes in gene expression that may not produce observable effects at birth but may increase the risk of adverse health outcomes later in life.

Conclusion

In conclusion, this study provided some evidence for negative association between cord blood BPA levels and PRL levels overall. The association was sex-specific, negative association was found in boys whereas positive association was found in girls. We also found suggestive positive associations between BPA levels and T, E2 and P4 in boys. In ongoing cohort, effects on reproductive hormone levels needs to be investigated at pubertal stage in association with prenatal BPA exposure.

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Table 1 Basic characteristic of mothers and infants (n=285).

Characteristics		Mean $\pm$ SD or n (%) or Geometric mean (25 - 75%)
Mother		
Age at delivery (years)		30.4 $\pm$ 5.0
BMI (kg/m ²)		20.9 $\pm$ 2.9
Parity	0	147 (51.6)
	≥ 1	138 (48.4)
Education (years)	≤ 12	130 (45.6)
	≥ 13	155 (54.4)
Annual income (million yen) ^a	<5	202 (70.9)
	≥ 5	81 (28.4)
Smoking status ^b	No	239 (83.8)
	Yes	46 (16.1)
Caffeine intake (mg/day)		148.4 $\pm$ 121.6
Infant		
Sex	Male	127 (44.6)
	Female	158 (55.4)
Gestational age (days)		278 $\pm$ 7
Birth weight (g)		3128 $\pm$ 33
Birth length (cm)		48.5 $\pm$ 1.6
Cord blood BPA (ng/ml)		0.051 (<LOQ - 0.076)

^a n=283 because of missing values. ^b During pregnancy. LOQ: Limit of quantification.

Table 2 Median and inter quartile range of reproductive and thyroid hormone levels of infants.

Hormone	Boys		Girls	
	n	Med (IQR)	n	Med (IQR)
T (pg/ml)	124	98.3 (76.7-122.5)	154	69.0 (51.8-94.9)
E2 (ng/ml)	124	4.77 (3.33-7.55)	154	4.66 (3.11-6.52)
T/E2	124	18.5 (14.0-25.9)	154	15.9 (12.1-21.3)
P4 (ng/ml)	124	228.8 (185.6-298.7)	154	209.0 (165.1-276.1)
LH (mIU/ml)	123	<LOQ (<LOD-0.87)	149	<LOQ (<LOQ-<LOQ)
FSH (mIU/ml)	123	<LOQ (<LOD-0.67)	148	<LOQ (<LOQ-<LOQ)
SHBG (nmol/l)	124	16.4 (13.3-19.3)	154	15.5 (12.8-18.4)
PRL (ng/ml)	123	86.5 (63.4-117.0)	151	86.0 (60.4-119.0)
Inhibin B (pg/ml)	124	44.4 (35.0-61.1)	154	<LOD (<LOD-12.7)
INSL3 (ng/ml)	123	0.29 (0.25-0.34)	24	0.18 (0.17-0.24)
TSH (μ U/ml)	127	2.2 (1.2-4.0)	156	2.2 (1.4-3.9)
FT4 (ng/dl)	127	2.0 (1.9-2.3)	156	2.0 (1.8-2.3)

Abbreviations: T: Testosterone, E2: Estradiol, P4: Progesterone, LH: Luteinizing Hormone, FSH: Follicle Stimulating Hormone, SHBG: Sex Hormone Binding Globulin, PRL: Prolactin, INSL3: Insulin-like factor 3, TSH: Thyroid Stimulating Hormone, FT4: Free Thyroxine, Med: Median, IQR: Inter Quartile Range, ND: Not Determined, LOQ: Limit of quantification.

Table 3 Linear regression coefficients (β) of hormone levels in relation to fetal BPA levels.

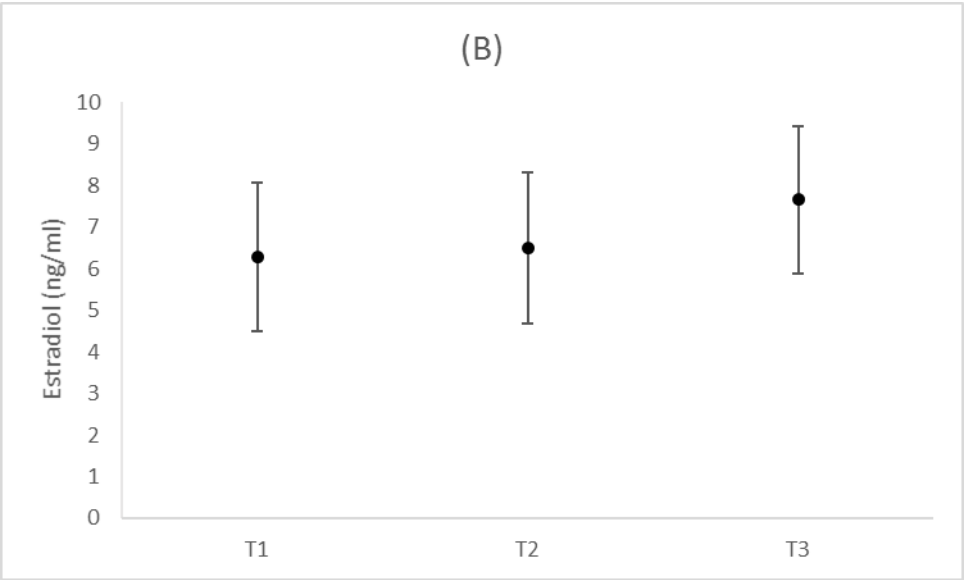
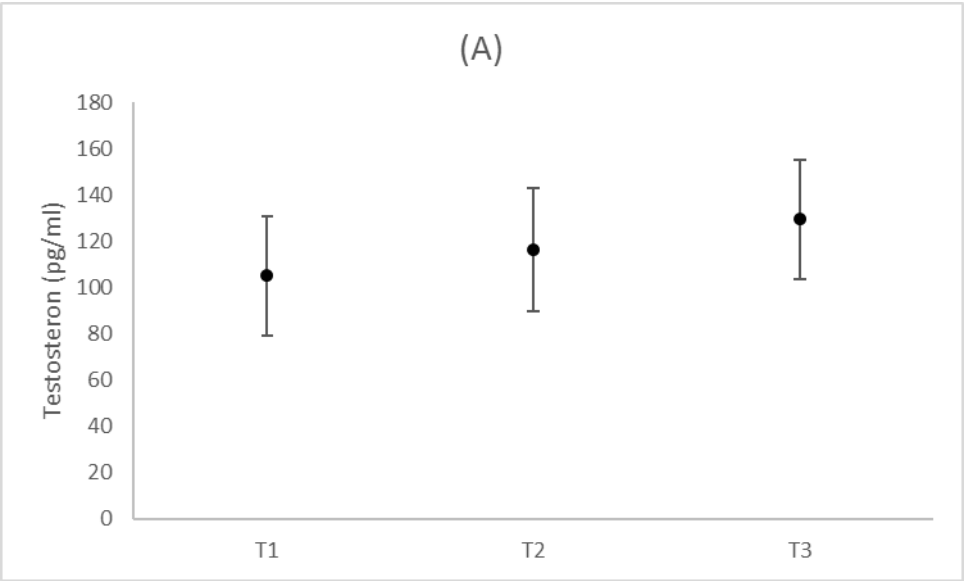
Hormone	All β^a (95% CI)	P _{interaction} ^b	Boys β^c (95% CI)	Girls β^c (95% CI)
T (pg/ml)	0.25 (-0.16, 0.66)	0.420	0.15 (-0.02, 0.32)	0.05 (-0.13, 0.22)
E2 (ng/ml)	0.26 (-0.11, 0.64)	0.341	0.15 (-0.03, 0.34)	0.04 (-0.10, 0.18)
T/E2	-0.01 (-0.37, 0.34)	0.941	-0.01 (-0.16, 0.15)	0.00 (-0.14, 0.15)
P4 (ng/ml)	0.17 (-0.29, 0.62)	0.983	0.16 (0.00, 0.32)	0.16 (-0.04, 0.36)
SHBG (nmol/l)	-0.07 (-0.32, 0.19)	0.291	0.02 (-0.06, 0.09)	0.10 (-0.02, 0.22)
PRL (ng/ml)	-0.38 (-0.71, -0.05)	0.010	-0.12 (-0.24, 0.01)	0.14 (0.00, 0.28)
InhibinB (pg/ml)	ND	0.285	0.07 (-0.06, 0.20)	ND
INSL3 (ng/ml)	ND	0.505	0.05 (-0.04, 0.14)	ND
TSH (μ U/ml)	-0.04 (-0.54, 0.45)	0.819	-0.06 (-0.32, 0.20)	-0.12 (-0.29, 0.05)
FT4 (ng/dl)	0.01 (-0.10, 0.12)	0.969	0.01 (-0.04, 0.06)	0.01 (-0.04, 0.05)

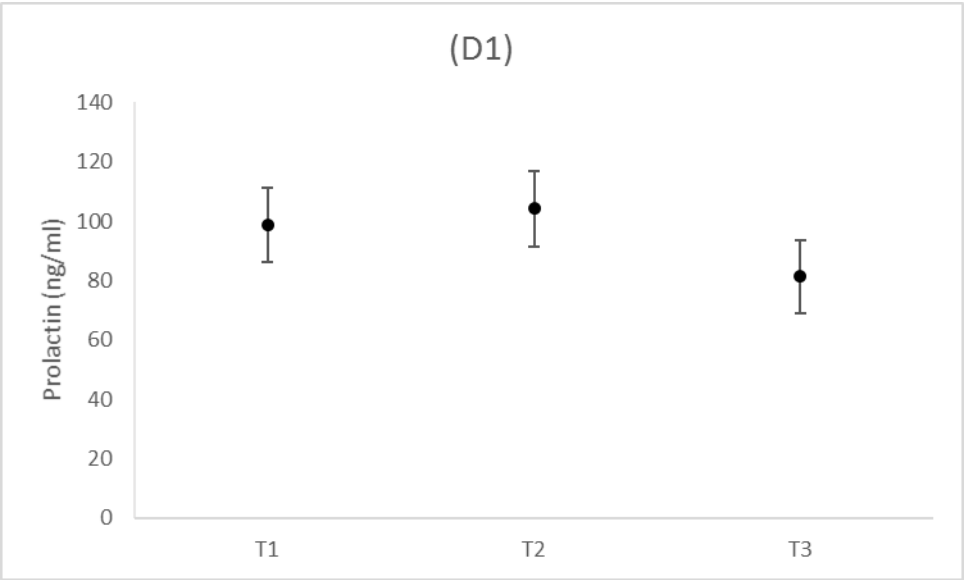
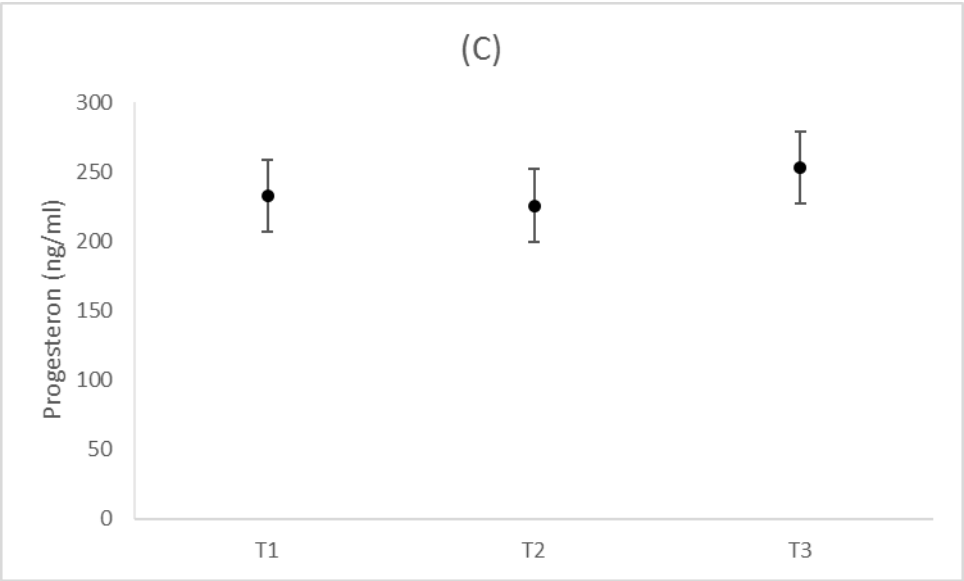
Abbreviations: T: Testosterone, E2: Estradiol, P4: Progesterone, SHBG: Sex Hormone Binding Globulin, PRL: Prolactin, INSL3: Insulin-like factor 3, TSH: Thyroid Stimulating Hormone, FT4: Free Thyroxine, CI: Confidence Interval, ND: Not Determined.

^a Adjusted for sex, interaction of sex and BPA and days of mass screening test was conducted (only for TSH and FT4).

^b P for interaction of sex and BPA.

^c Adjusted for days of mass screening test was conducted (only for TSH and FT4). BPA and hormone levels were $\log_{10}$ transformed.





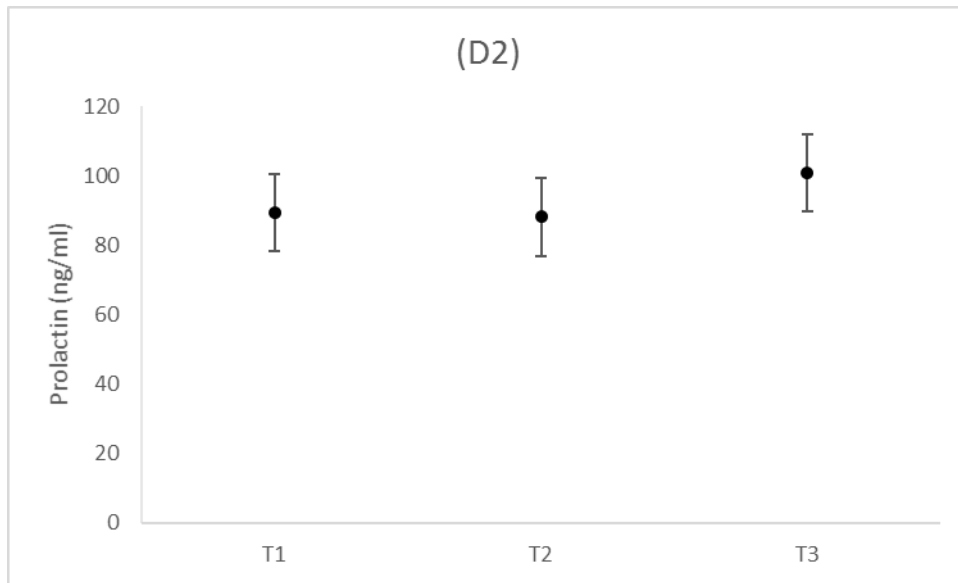


Figure 1 The dose-response relationship between the tertiles of BPA and reproductive and thyroid hormone levels of infants; (A) Testosterone (T) among boys (T1: N=42, T2: N=40, T3: N=42), (B) Estradiol (E2) among boys (T1: N=42, T2: N=40, T3: N=42), (C) Progesterone (P4) among boys (T1: N=42, T2: N=40, T3: N=42) , (D1) Prolactin (PRL) among boys (T1: N=41, T2: N=40, T3: N=42), (D2) Prolactin (PRL) among girls (T1: N=52, T2: N=49, T3: N=51). Values for the first, second and third tertiles were as follows: T1 = < limit of quantification (LOQ) to 0.041, T2 = 0.042 to 0.067, T3 = 0.068 < ng/ml. Estimated marginal mean (EMM)s are indicated in black circles and the error bars show the upper and lower 95% Confidence Intervals.