Urinary Phthalate Metabolites in Pregnant Women: Occurrences, Predictors, and Association with Maternal Hormones

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Research Article

Keywords: Pregnancy, phthalates, predictors, thyroid hormone

Posted Date: September 27th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-886141/v1

Abstract

Phthalate have been detected in human urine and may cause endocrine disruption. However, research on the prediction of exposure and the association with thyroid hormones during gestation is limited. We recruited 463 pregnant women and collected urine and blood samples, and questionnaire data at initial maternity examination. We analyzed ten phthalate metabolites: mono-isobutyl phthalate (MiBP), mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-*n*-butyl phthalate (MnBP), mono-*n*-octyl phthalate (MOP), mono-benzyl phthalate (MBzP), as well as metabolite of di-2-ethylhexyl phthalate (DEHP): mono (2-ethylhexyl) phthalate, mono-(2-ethyl-5-oxohexyl) phthalate, and mono-(2-ethyl-5-carboxypentyl) phthalate. Multivariable generalized estimating equation models and liner mixed models were used to predict urinary biomarker concentrations and assessed associations between phthalate exposure and thyroid hormones. We observed negative associations between phthalate metabolites and old age (MMP, MnBP), and physical activity (MiBP, MOP). Positive associations with lower education (MEP, MOP), living near the road (MEP, MnBP, ∑DEHP), living space (MiBP, MnBP, ∑DEHP), consuming more puffed food (MEP, MBzP), and take-out food (MEP, MnBP,∑DEHP). MnBP (percent change [%△] = 4.25; 95% CI = 0.32, 8.18) and ∑DEHP (%△=5.12; 95% CI = 1.25, 8.99) were positively associated with thyroid stimulating hormone, and this suggested MEP and MnBP were inversely associated with free thyroxine (%△=-1.26; 95% CI=-2.34, -0.18) and total triiodothyronin (%△=-2.62; 95% CI=-3.17, -2.07). Our findings suggest that lower consumption of puffed food, cosmetics use and moderate physical activity were predictive of lower phthalate biomarker concentration. Certain phthalate esters are associated with altered thyroid hormone levels.

Introduction

Phthalates easters (PAEs) is a high production volume chemical used in a variety of common consumer products (Frery et al., 2020). In general, low molecular weight phthalates (metabolites > 250 Da), such as Dimethyl phthalate (DMP), Diethyl phthalate (DEP) and Dibutyl phthalates (DBP), are primarily used in personal care products, while high molecular weight phthalates, such as di-2-ethylhexyl phthalate (DEHP), Benzylbutyl phthalate, Di-isononyl phthalate, and Di-*n*-Octyl Phthalate (DnOP), are commonly used in the production of polyvinyl chloride and building products (Wittassek and Angerer, 2008). PAEs have been identified as global pollutants and have received increasing public attention in recent years (Johns et al., 2015).

As the phthalate plasticizers are not chemically bound to products, they can leak, migrate or evaporate into the indoor atmosphere, foodstuff, other materials (Wang et al., 2018). Humans are exposed through ingestion, inhalation, and dermal exposure during their whole lifetime, including intrauterine development (Andersen et al., 2018). Pregnant women are considered to be a population that is vulnerable to phthalates, and there have been very little research on phthalate levels and related factor about in China (Zhu et al., 2016). Predictors of phthalate exposure in maternal population are urgently needed.

Previous studies suggest that maternal exposure to some PAEs is associated with endormetriosis, weight gain, insulin resistance, but also with adverse birth outcomes, including gestational length or preterm birth (Praveena et al., 2020; Shoshtari-Yeganeh et al., 2019). However, the underlying mechanism remains unclear. Previous studies have demonstrated that these chemical may disrupted thyroid hormones from in *vitro* and animal studies (Zhang et al., 2021). Additionally, give the close association between thyroid hormones and pubertal development, male infertility, diminished female fecundity, and childhood adiposity (Krassas et al., 2010). It is suspected that PAEs are linked a series of adverse health effect that may potentially be mediated by the thyroid hormonal system,

In this work, we measured the concentration of phthalate metabolites in maternal urine of 457 pregnant Chinese women. We aimed to: 1) determine levels of urinary biomarkers of phthalate in China pregnant women; 2) explore the personal characteristic associated with phthalate metabolite concentration, 3) examine associations between urinary phthalate metabolite and thyroid hormones. The results of this study will help identify phthalate of potential risk and develop appropriate mitigation measure for pregnant women.

Materials And Methods

Study participants

The data used were collected form an ongoing longitudinal birth cohort study in the city of Ezhou, Hubei Province, China. Pregnant women were invited to participant in the study at their first antenatal examination (<12 weeks of gestational) in Ezhou Maternal and Child Health hospital from November 2018 to November 2019. The women were eligible for enrollment if the following criteria were met: (1) resident of Ezhou city, (2) willingness to complete questionnaires at first antenatal examination, (3) willing to urine samples and serum during regular prenatal care visit, and (4) willingness to give birth to their babies in the hospital where the study was being conducted.

A total of 568 pregnant women donated their urine and serum samples at their first antenatal examination (8.2±2.4 weeks), and 498 participants complete the survey questionnaire. Thirty-five pregnant women had participant in our previous intervention study (Wu et al., 2021),

resulting in 463 pregnant women for analysis. This study was approved by the Ethics Committee of Hubei University of Chinese Medicine. Written informed consent was obtained from all participants.

Survey instrument

We used a self-administered questionnaire, which was developed based on a previous questionnaire used in the US, Peru, and Democratic Republic of Congo and further adapted to the setting in China (Bai et al., 2016). It has also been demonstrated validation in our previous study (Wu et al., 2017). The questionnaire was divided into three sections: sociodemographic information, living conditions, and daily lifestyle. Socio-demographic information included age, education (college, high school, middle school or below), house income (<5000$, 5000-10000$, >10000$), first born status (yes, no), and occupation (unemployed, employed). Living environment included building type (reinforced concrete, brick-wood), distance from the road (<30, 30-100, >100 m), average living space (<20, 20-40, >40 m^2), kitchen fuel (goal, natural gas, wood), decorating materials (bricks, wall paints, tiles), second-hand smoke (yes, no). Lifestyle included frequency of diary product consumption (<2, 2-5, >5 times/week), puffed food consumption (<2, 2-5, >5 times/week), take out (<2, 2-5, >5 times/week), eat fruits (<5, >5 times/week), eat vegetable (<5, >5 times/week), water source (tap water, well water, pond water), frequency of use plastic bag (<2, 2-5, >5 times/week), frequency of cosmetic use (<2, 2-5, >5 times/week), frequency of use electronic products (<2, 2-5, >5 hours/day), physical activity (<1, 1-2, >2 hours/day) and traffic type (bus, car, walk).

Phthalate metabolite measurements

The participant provided the first morning urine sample in 30-mL brown glass bottles. Samples were divided into aliquots and stored at -80°C until analysis was performed. These included the following: mono-isobutyl phthalate (MiBP) and mono-methyl phthalate (MMP) from DMP; mono-ethyl phthalate (MEP) from DEP; mono-*n*-butyl phthalate (MnBP) from di-n-butyl phthalate; mono-*n*-octyl phthalate (MOP) from DnOP; mono-benzyl phthalate (MBzP) from butyl benzyl phthalate, and mono (2-ethylhexyl) phthalate (MEHP) from DEHP. The analyses also involved three secondary oxidation metabolites of DEHP: namely mono-(2-ethyl-5-hydroxyhexyl)-phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP). Then, we used ΣDEHP represents the sum of the molar concentrations of MEHP, MEHHP, MEOHP, and MECPP.

The metabolites were analyzed in the School of Laboratory Medicine, Hubei University of Chinese Medicine using liquid chromatography-mass spectrometry (LC-MS/MS; Agilent, USA), according to the method for urine phthalates measurement described in Specht et al. (Specht et al., 2015). The detailed method was presented in our previous study (Wu et al., 2018). The calibration curve covered the range 0.100–200 ng/mL, and each batch of samples analyzed involved blank and quality controlsamples. The intra- and inter-day relative standard deviations were 11.7% and 13.2%, respectively.

The metabolite concentrations were corrected for the urine dilution using specific gravity (SG) as follows: $Pc = P\ [(SG_M–1)/(SG–1)]$, where Pc is the SG-adjusted urine concentration (ng/mL), P is the measured metabolite concentration, SG is the specific gravity of the urine sample, and SG_M is the median SG of the samples for the studied population (Upson et al., 2013). The SG of each sample was measured using a handheld refractometer (PAL10-S; Atago, Tokyo, Japan) at room temperature. For PAE concentrations below the limit of detection (LOD), a value equal to was imputed.

Thyroid hormone measurement

Serum sample were collected at first antenatal examination. The samples were frozen at -20°C until shipped overnight on dry ice to the analytical laboratory. Blood plasma was analyzed for thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), total triiodothyronine (TT3), total thyroxine (TT4) and thyroglobulin (TG) at Ezhou Maternal and Child Health hospital by automated chemiluminescence immunoassay (Roche, Germany). The intra-assay coefficients of variation (CV) for all hormones ranged from 3.2% (for FT3) to 9.8% (for TSH), and inter-assay CV were ranged from 2.4% (for TT3) to 7.4% (for FT4).

Statistic analysis

Descriptive statistics were used to describe our study population characteristic with the expression of a number (%) or mean±SD. Geometric means and selected percentiles were calculated to describe the distributions of urinary phthalate metabolites (and SG-adjusted) and thyroid hormones.

We fitted generalized estimating equation (GEE) models, using an identity link and exchangeable correlation, to explore the characteristic that predict phthalate exposure (Reeves et al., 2019). We initially fitted single predictor model for each phthalate biomarker concentration including all single covariate. Multivariable regression models were fitted considering all predictors with a p value less than 0.20 from the bivariate GEE models. We used a backward selection approach to select a final, parsimonious model for each phthalate biomarker where all variables significant at $p<0.10$ level were retained. We calculated predicted means and 95% confidence intervals (CI) for each variable based on the final

parsimonious model, with all covariates held at their means. For categorical variables, we calculated the mean at each level of the variable, for continuous variables, we calculated means at the midpoint value of each quartile.

To make our results from these models including ln-transformed continuous biomarkers and/or outcome more interpretable, we transformed regression coefficients to percent changes (and associated 95% confidence intervals) in hormones concentration in relation to the interquartile range (IQR) increase in urinary biomarker concentrations. Liner Mixed models was used for associations between phthalate exposure and thyroid hormones (Aker et al., 2018). Models included covariates that were significantly associated with one or more thyroid hormones as well as one or more urinary phthalate metabolites. All analyses were performed using R version 3.5.3 (http://www.r-project.org).

Results

The basic characteristics of the 463 participants in this study are presented in Table 1. The mean age of the study participants was 27.6 years, 40% attained at least a college education. A total of 46.4% live in households with income between 5000 and 10000 USD (Table 1). Most of them living in reinforced concrete building (85.4%), use natural gas as cooking fuel (66.9%), always eat fruits (76.1%), often use cosmetics (78.2%), and use the bus (48.3%).

Table 1
Character of the participants in this study (n = 463)

Sociodemographic	N	% of cohort
Age		
< 25	99	21.3
25–30	225	48.6
> 30	139	30.0
Education		
Middle school or below	31	7.48
High school	217	52.4
College	166	40.1
Income (USD per year)		
< 5000	111	31.0
5000–10000	166	46.4
10000	81	22.6
First born status		
Yes	157	37.0
No	267	62.9
Occupation		
Unemployed	102	26.6
Employed	281	73.3
Living conditions		
Building type		
Reinforced concrete	340	85.4
Brick-wood	58	14.6
Distance from the road(m)		
< 30	62	15.2
30–100	123	30.2
> 100	222	54.5
Average living space (m^2)		
< 20	56	14.4
20–40	139	35.6
> 40	195	50.0
Kitchen fuel		
Natural gas	293	66.9
Goal	89	20.3
Wood	56	12.8
Decorating materials		
Brick	203	47.9
Wall paints	122	28.8

Sociodemographic	N	% of cohort
Tiles	99	23.3
Second-hand smoking		
Yes	222	53.0
No	197	47.0
Lifestyle		
Diary product consumption		
< 2 (times/week)	87	20.5
2–5 (times/week)	236	55.7
> 5 (times/week)	101	23.8
Puffed food consumption		
< 2 (times/week)	64	14.7
2–5(times/week)	109	25.0
> 5(times/week)	263	60.3
Frequency of take out		
< 2 (times/week)	81	18.3
2–5(times/week)	104	23.5
> 5(times/week)	257	58.1
Frequency of eat fruit		
< 5(times/week)	102	23.9
> 5(times/week)	324	76.1
Frequency of eat vegetable		
< 5(times/week)	90	21.2
> 5(times/week)	335	78.8
Water source		
Tap water	132	30.7
Well water	228	53.0
Pond water	70	16.3
Frequency of use plastic bag		
< 2 (times a week)	68	15.1
2–5 (times a week)	325	72.4
> 5(times a week)	56	12.5
Frequency of use cosmetics		
< 2 (times/week)	45	11.0
2–5 (times/week)	44	10.8
> 5 (times/week)	319	78.2
Frequency of use electronic product		
< 2 (hours/day)	123	28.5
2–5 (hours/day)	167	38.7

Sociodemographic	N	% of cohort
> 5 (hours/day)	142	32.9
Physical activity		
< 1(hours/day)	108	25.8
1–2(hours/day)	168	40.1
> 2(hours/day)	143	34.1
Traffic type	187	48.3
Bus		
Car	111	28.7
Walk	89	23.0

The descriptive statistics for phthalate metabolites concentration and SG-corrected are presented in Table 2. The levels of all metabolites examined were greater than 90%, except for that of MBzP and MOP, which were 39.8% and 38.5% lower than the LOD, respectively. The median value of detection was greatest for MnBP (mean = 64.9 ng/mL) and ∑DEHP (mean = 162.3 ng/mL). Among the DEHP metabolites, MEOHP was detected at the highest concentration, followed by MEHHP, MECP and MEHP, in descending order. TT4 (mean = 132.8 ng/mL) was detected at the highest levels among all thyroid hormones, followed by FT4 (mean = 15.1ng/mL).

Table 2
Urine levels of phthalate metabolites (unadjusted and SG-adjusted) and thyroid hormones in the population of pregnant women

Urinary biomarkers (ng/mL)	< % LOD (LOD)	Mean	5th	25th	50th	75th	95th
MMP	3.2(0.4)	30.0	2.39	11.56	24.51	45.97	71.0
SG-MMP		33.7	2.49	13.35	31.92	50.9	74.7
MEP	2.4(0.4)	33.8	5.43	16.93	29.33	48.42	74.3
SG-MEP		36.1	9.09	19.6	40.8	56.7	85.7
MiBP	3.6 (0.6)	19.9	1.80	8.50	17.00	28.11	51.5
SG-MiBP		22.7	2.46	9.46	19.5	30.9	55.6
MnBP	0(0.7)	59.3	7.89	33.08	52.01	83.61	129.7
SG-MnBP		64.9	9.34	36.5	56.6	86.8	141.8
MBzP	39.8(0.4)	2.68	-	-	2.16	4.58	7.44
SG-MBzP		2.74	-	-	2.15	4.56	7.32
MOP	38.5(0.7)	2.18	-	-	1.66	2.16	3.34
SG-MOP		2.36	-	-	1.97	2.63	3.17
MEHP	0(0.5)	7.55	-	2.11	6.46	12.45	21.81
SG-MEHP		8.2	-	2.16	8.64	13.6	22.9
MECP	2.6(0.4)	14.3	1.11	6.39	13.21	20.12	32.98
SG-MECP		15.6	1.01	7.15	15.5	23.8	38.6
MEHHP	3.2(0.6)	62.3	6.48	29.3	54.5	88.9	143.3
SG-MEHHP		65.8	6.54	31.9	62.6	111.4	160.8
MEOHP	2.8(0.7)	66.9	4.50	28.92	58.18	95.51	159.9
SG-MEOHP		70.7	4.08	25.3	66.1	100.2	165.6
∑DEHP		151.9	53.9	102.8	141.3	195.7	267.5
SG-∑DEHP		162.3	55.4	117.1	157.4	223.0	292.5
Thyroid hormones							
TSH (mIU/L)		1.68	0.91	1.43	1.68	2.15	2.92
FT3 (pmol/L)		4.98	2.51	4.41	5.03	5.46	6.81
FT4 (pmol/L)		15.1	10.9	13.9	14.9	15.8	17.5
TT3 (nmol/L)		1.31	0.95	1.15	1.29	1.48	1.94
TT4 (nmol/L)		132.8	126.3	130.4	133.0	135.8	142.8
TG (nmol/L)		14.7	12.4	13.6	14.6	15.8	17.2

Table 3 presents the results of our multivariable regression modeling of MMP, MEP, MiBP, MnBP, MBzP, MOP, and ∑DEHP concentrations. Predictors of MMP concentration were age (lower among those > 30 years old), high puffed food consumption, vegetable (lower with among 5 times/week), and transporting by car. MEP concentration were positively associated with lower education, brick-wood building type, living near the road, higher frequency of consumption of puffed food, consumption of take out-food, use of cosmetics, as well as transportation by bus and car. MiBP concentration was inversely related to high income and being asecond-born. Small living space, drinking well water and pond water, lower physical activity were positively associated with MiBP. Increased age were negatively associated with MnBP, while living near the road, higher frequency of take-out consumption, use cosmetics, and electronic product were positively associated with MnBP. Living near the road were associated with higher MBzP concentration, as well as puffed food consumption, plastic bag, and drinking pond water. Participants with lower education had higher MOP compared with those with higher education. Higher puffed food consumption, lower physical activity,

drink pond water were associated with high MOP concentration. The total metabolite concentration of DEHP was positively associated with low educational status, being the first born, living near the road, small living space, and a higher frequency of use of electronic products.

Table 3
Predictors of urinary concentration of MMP, MEP, MiBP, MnBP, MBzP, MOP and ∑DEHP in generalized estimated equation models

Socio-demographic	MMP	MEP	MiBP	MnBP	MBzP	MOP	∑DEHP
Age							
< 25	30.8(26.2, 35.4)	35.9(31.3, 40.5)		61.1 (51.4, 69.5)			151.0(135.8, 166.2)
25–30	31.6(27.4, 35.8)	35.7(31.9, 39.5)		59.5(53.3, 65.7)			151.9(140.0, 163.8)
> 30	27.6* (23.6, 31.6)	31.9(27.5, 36.3)		53.8* (47.6, 60)			149.8(136.6, 163.0)
Education (years)							
< 9		35.2* (32.4, 39.0)	19.8 (17.5, 22.4)		3.1 (1.95,4.1)	1.43 * (1.20, 1.65)	151.4* (139.9, 162.8)
9–12		33.9 (30.7, 37.1)	19.2 (17.2,21.2)		2.7 (2.3, 3.1)	1.18 (1.01,1.35)	154.5 (144.6, 164.3)
> 12		31.9 (25.7, 38.1)	16.8 (11.2, 22.4)		2.5 (2.0, 3.0)	1.20 (0.70, 1.90)	136.5 (109.4, 163.4)
Income (USD per year)							
< 5000	30.8 (24.0, 37.6)		21.2 (17.0, 25.4)			2.40 (1.92, 2.90)	
5000–10000	31.9 (27.5, 36.1)		17.9 (14.9, 20.9)			2.69 (2.26, 3.14)	
> 10000	28.1 (24.2, 31.9)		16.4 * (14.2, 18.6)			2.79 (1.99, 3.59)	
First born status							
First born		38.0 (30.1, 45.9)	19.4 (14.4, 24.4)		1.93 (1.51,2.35)	1.59 (0.94, 2.24)	153.8* (127.8, 179.8)
Second born		34.8 (22.8, 46.8)	13.3* (5.9, 20.7)		1.83 (1.15, 2.51)	1.16 (0.71, 1.62)	125.2 (89.7, 164.5)
Occupation							
Unemployed	29.8(25.0, 34.6)			56.1(49.2, 63.0)			144.9(130.9, 158.9)
Employed	29.1(26.5, 31.7)			58.4(54.2, 62.6)			152.4(144.2, 160.6)
Living conditions							
Building type							
Reinforced concrete	27.9 (19.8, 36.0)	16.6(11.9, 21.3)	37.8 (29.9, 45.7)		2.28(1.35, 3.21)		
Brick-wood	31.7 (12.8, 50.6)	25.4* (15.8, 35.0)	36.5 (12.5, 60.5)		1.69 (0.98, 2.40)		
Distance from the road (m)							
< 50	29.7 (17.6,41.8)	41.5* (31.3, 51.7)	20.3 (12.1, 28.5)	54.5 * (43.6, 65.4)	2.27* (1.51, 3.03)	1.41 (0.93, 1.89)	164.8* (136.0, 193.6)

Socio-demographic	MMP	MEP	MiBP	MnBP	MBzP	MOP	∑DEHP
50–100	29.1 (19.0, 39.2)	38.0 * (30.3, 45.7)	19.5(11.6, 27.4)	54.8* (38.8, 70.8)	1.86* (1.30, 2.42)	1.46 (1.18, 1.74)	142.8* (124.2, 161.4)
> 100	25.8 (17.4, 34.2)	28.9 (17.7, 40.1)	13.7 (10.8, 16.6)	46.7 (35.8, 57.6)	1.01 (0.51, 1.51)	1.13 (0.82, 1.44)	105.3 (75.8, 131.5)
Average living space (m^2)							
< 20			24.5* (15.6, 33.6)	63.2* (47.5, 78.9)		2.33 (1.54, 3.12)	179.5 * (128.1, 230.9)
20–40			14.9 (9.7, 20.1)	53.8 (40.3, 67.3)		1.63 (1.12, 2.14)	153.7 (122.7, 184.7)
> 40			13.2 (9.1, 17.3)	40.9 (29.6, 52.2)		0.96 (0.64, 1.28)	126.1 (105.2, 147.0)
Kitchen fuel							
Natural gas	29.5 (24.5, 34.5)	32.9 (28.4, 37.4)		50.0(42.6, 57.4)			137.5(123.1, 151.9)
Goal	29.2(26.4, 32.0)	34.3(31.5, 37.1)		62.0(57.2, 66.8)			156.0(147.6, 164.4)
Wood	33.9 (23.1, 44.7)	29.8 (21.8, 37.8)		57.5(41.5, 73.5)			152.6(126.2, 179.0)
Decorating materials							
Wallpaper	31.0(26.0, 36.0)	33.2(28.4, 38.0)			2.45(1.89, 3.01)		151.8(136.8, 166.8)
Wall paints	30.3(26.9, 33.7)	32.1(28.7,35.8)			2.83(2.39, 3.27)		153.3(142.1, 164.5)
Tiles	28.1(22.1, 34.1)	34.8(29.2, 40.4)			2.48(2.12, 2.84)		151.8(143.6,160.0)
Second-hand smoking							
Yes			19.5(17.5, 21.5)	60.9(54.9, 66.9)			152.4(142.6, 162.2)
No			19.1(16.8, 21.4)	57.6(52.4, 62.8)			150.3(140.1, 160.5)
Life style							
Diary product consumption							
< 2 (times/week)	26.9(21.9, 31.9)		20.0(16.6, 23.4)		3.12(2.44, 3.76)		156.3(139.7, 172.9)
2–5 (times/week)	30.1(26.9, 33.3)		20.4(18.3, 22.5)		2.70(2.30,3.10)		152.7(142.9, 162.5)
> 5 (times/week)	31.4(26.8, 36.0)		18.3(15.7, 20.9)		2.64 (2.13, 3.15)		142.7(130.1, 155.3)
Puffed food consumption							
< 2 (times/week)	17.8 (9.1, 26.5)	24.6(20.0, 29.2)		64.3(54.5, 74.1)	1.50(1.10, 1.90)	1.26(1.0, 1.52)	146.8(138.8, 154.8)

Socio-demographic	MMP	MEP	MiBP	MnBP	MBzP	MOP	∑DEHP
2–5(times/week)	25.6* (21.4, 29.8)	33.9* (29.7, 38.1)		59.8(51.6, 68.0)	2.46* (1.89, 3.05)	1.29 (1.15, 1.43)	151.8(145.4, 158.2)
> 5(times/week)	31.1* (28.3, 33.9)	33.7* (31.1, 36.3)		58.4(54.2, 62.6)	2.73* (2.41, 3.05)	2.72* (1.85,3.59)	166.9(152.3, 181.5)
Frequency of eat fruit							
< 5(times/week)	29.6(27.0, 32.2)		21.8(18.6, 25.0)		2.77(2.47, 3.10)		
≥ 5(times/week)	29.7(24.7, 34.7)		18.7(17.0, 20.4)		2.31(1.79, 2.83)		
Frequency of eat vegetable							
< 5(times/week)	36.5* (29.3, 43.7)	35.5(28.7, 42.3)			2.74(1.94, 3.54)		136.2(126.6, 145.8)
≥ 5(times/week)	28.7(26.3, 31.1)	33.3(30.9, 35.37)			2.65(2.35, 2.95)		143.5(135.6, 151.4)
Frequency of take-out food							
< 2 (times/week)		33.4(28.2, 38.6)	20.1(16.6, 23.6)	41.8(32.5, 51.1)			125.5(96.2, 151.8)
2–5(times/week)		33.7(31.1, 36.3)	19.1(17.4, 20.8)	58.5(50.1, 66.9)			148.8(140.9, 156.5)
> 5(times/week)		42.8* (34.8, 50.8)	24.3(17.9, 30.7)	59.3 * (54.8, 63.7)			160.8* (144.0, 177.6)
Use plastic bag							
< 2 (times a week)	28.9(24.1, 33.7)	32.2(27.6, 36.8)		58.9(53.3, 64.5)	2.00(1.50, 2.50)	1.16(1.00,1.32)	148.4 (136.4, 160.4)
2–5 (times a week)	30.6(26.4, 34.8)	35.7(31.5, 39.9)		62.9(54.8, 71.0)	2.70* (2.28, 3.12)	1.34(1.08, 1.60)	150.9(139.9, 161.9)
> 5(times a week)	30.3(26.7, 33.9)	35.3(31.7, 38.9)		64.6(57.3, 71.9)	2.85* (2.33, 3.37)	1.48(1.18, 1.78)	158.4(143.8, 173.0)
Frequency of use cosmetics							
< 2 (times/week)	28.4 (24.2, 32.6)	31.1 (27.0, 35.2)	18.3 (16.1, 20.5)	55.1 (50.6, 59.6)		1.16 (0.94, 1.38)	
2–5 (times/week)	27.6 (21.0, 34.2)	34.7 (27.1, 44.8)	20.2 (17.1, 23.3)	61.9 (53.5, 70.3)		1.19 (0.88, 1.50)	
> 5 (times/week)	30.4(26.8, 34.0)	36.9* (33.7, 40.1)	22.3 (16.7, 27.9)	64.9* (52.5, 77.3)		1.38* (1.18, 1.58)	
Use electronic product							
< 2 (hours/day)		35.3(30.3, 40.3)	18.1(15.3, 20.9)	51.2(43.2, 59.2)			140.1(127.9, 152.3)
2–5 (hours/day)		31.3(27.4, 35.2)	19.7(17.1, 22.3)	56.3(49.5, 63.1)			154.9(140.3, 169.5)

Socio-demographic	MMP	MEP	MiBP	MnBP	MBzP	MOP	∑DEHP
> 5 (hours/day)		34.5(30.5, 38.5)	19.9(17.0, 22.8)	68.7* (62.3, 75.1)			157.9* (145.3, 170.5)
Physical activity							
< 1(hours/day)	30.7(27.3, 34.1)		21.7* (18.8, 24.6)	61.5(54.1, 68.69)	2.62(2.20, 3.04)	1.34* (1.14,1.54)	
1– 2(hours/day)	31.5(26.9, 36.1)		18.6(16.4,20.8)	63.7(57.2, 70.2)	2.77(2.25, 3.29)	1.40* (1.18,1.62)	
> 2(hours/day)	30.4(26.9, 33.9)		19.9(17.3, 22.5)	56.6(51.2, 62.0)	2.78(2.34, 3.22)	1.04(0.87, 1.21)	
Water source							
Tap water			14.2(10.4, 18.0)		2.52(1.81, 3.23)	1.15(0.88, 1.42)	160.5(144.7, 176.3)
Well water			18.9* (15.9, 21.9)		2.41(2.05, 2.77)	1.17(1.01, 1.33)	148.2(139.0, 157.4)
Pond water			20.5* (18.5, 22.5)		3.22* (2.66, 3.74)	1.51* (1.27,1.75)	160.6(146.4, 174.8)
Traffic type							
Bus	32.2(28.0, 36.4)	32.3* (28.7, 35.9)	17.9(15.5, 20.3)				153.1(142.3, 163.9)
Car	36.3* (32.3, 40.3)	32.6* (27.2, 38.0)	19.2(16.4, 22.0)				147.5(131.7, 163.3)
Walk	33.4(29.3, 37.5)	26.5(22.5, 30.5)	18.5(15.9, 21.1)				154.9 (142.3, 167.5)

Figure 1 showed the association between the urinary levels of phthalate metabolites and thyroid hormones. After adjustment for covariates, MnBP and ∑DEHP were positively associated with TSH. An IQR increase in MnBP and ∑DEHP were suggestively associated with 4.25% (percent change [%△] = 4.25; 95% CI = 0.32, 8.18) and 5.12 % (%△=5.12; 95% CI = 1.25, 8.99) increase in TSH, respectively. MBzP was inversely associated with FT3, with an IQR increase in MBzP associated with a 2.14% (%△=-2.14; 95% CI= -3.21, -1.07) decrease. There was a significant 1%-2% decreases in FT4 in relation to an IQR increase in MEP (%△= -1.26; 95% CI=-2.34, -0.18), and ∑DEHP (%△=-2.04; 95% CI= -3.50, -0.58). MnBP was associated with a reduced TT3 (%△=-2.62; 95% CI= -3.17, -2.07). ∑DEHP was also inversely associated with TT4 (%△= -1.86; 95% CI= -3.24, -0.48). MnBP and MOP were not associated with any thyroid hormones. None of phthalate metabolite significantly associated with TG.

Discussion:

In this present study, we assessed phthalate exposure, related factors and associate with thyroid hormones in maternal, we found that 1) pregnancy women were commonly exposure to PAEs, 2) higher frequency of puffed food consumption, take- outs, and use plastic bag were predicted higher mPAEs, 3) MnBP and ∑DEHP were positive associate with TSH, while some phthalate was inversely associated with other thyroid hormones.

In our study, MBP was the highest concentration and was detected in all samples, which might be attributed to the wide use of DBP. DBP is used as a solvent, fixative or alcohol denaturant, and is widely used in many personal care products and cosmetics, such as fragrances, skin lotions, nail polish, and eye shadow. Studies from United States (Cantonwine et al., 2014) and Kuwait (Guo et al., 2011a) have reported that in 14 phthalate metabolites, MnBP was the dominant compound, with geometric mean concentration of 62.0 ng/mL and 59.7 ng/mL. The concentration of MnOP concentrations was the lowest and was detected in only 50% of the samples. This is similar to a previous study in Netherlands (van den Dries et al., 2020) and Sweden (Shu et al., 2018), perhaps because of the less frequent use of DnOP and its ready degradation(Zhang et al., 2018). Compared to a previous study in China (Guo et al., 2011b), MMP was less 1.5 times less than previous reported. Over last decade, alternative chemical have been increasingly substituted for polyvinyl chloride and/or certain phthalates (e.g., DMP) in cosmetic, children's toys and medical device (Zhang et al., 2020). The concentration of DEHP more than twice as high among pregnant

women compared to studies in Germany (Wittassek et al., 2009) and Taiwan (Lin et al., 2011). Their detected time is delivery, our investigate time is in the first trimester, when they have more sensitive against endocrine-disrupting chemicals.

In the current study, we found age is negative association with MMP and MnBP, which is consist with prior study of postmenopausal women in the USA which found out that MBzP, MnBP, MiBP were inversely associated with age (Reeves et al., 2019). We also found participants with lower education statuses have higher concentration of MEP, MOP, and $\sum$DEHP than those with higher education. The living environment, especially living near the road, was a significant predictor of most phthalate biomarkers. Consistent with previous study(Hubinger, 2010), our results showed that eating puffed food and take-outs, could increase the concentration of MMP, MEP, MnBP, and $\sum$DEHP. Indeed, in a Belgian study (Fierens et al., 2012), DBP and DEHP were detected in food products and packaging materials at the highest concentrations, among eight phthalate compounds measured. Phthalate can migrate into food from plasticized PVC materials such as the tubing typically used in the milking process, lid gaskets, food-packaging films, gloves used in the preparation of foods and conveyor belts(Hubinger and Havery, 2006). A study in children also found that the frequency of eating out and consumption of hamburgers were identified to be positively correlated with urinary MEHP concentration (Lee et al., 2019). In addition, cosmetic was also been demonstrated another main exposure sources of phthalates. Exposure to PAEs commonly used in dry cleaning shops may occur through skin absorption, eye contact, or inhalation of the vapors (Fisher et al., 2019). A study on girls aged 8–13 years also reported urinary concentration of MnBP, MiBP, and DEHP metabolites were positively associated with the use of colored cosmetics and hair products (Lewis et al., 2013). Importantly, we further found that pregnant women with adequate physical activity, such as walking and yoga, had inversely associations with the concentration of MiBP and MOP. This is similar to a study in Australian (Bai et al., 2015), slightly higher total phthalate metabolite concentration were associated with insufficient activity.

Normal thyroid function is important in maintain normal reproduction, and could be impacted by the endocrine disrupting effects of PAEs (Krassas et al., 2010). Recent studies in animals (Dong et al., 2019) and in vitro (Kambia et al., 2021) screening assays provide evidence that phthalate exposure might affect fetal neurodevelopment through thyroid signaling mechanisms. Our results showed that MEP and MBzP were negatively associated with FT4 and FT3. The findings were consistent with previous study that among 845 children aged 4–9 years, MnBP and $\sum$DEHP were positively associate with TSH, but inversely associated with FT3, FT4, TT4 (Boas et al., 2010). A previous study that contained 1675 participants (> 20 year) have reported the urine of DEHP were inversely associated with TT4 and FT4, but were positively associated with TSH concentration (Axelsson et al., 2015). A possible reason is that thyroid hormones, TT3 and TT4, are produced in thyroid gland, and their levels are negatively controlled by TSH from the pituitary gland via the hypothalamic-pituitary-thyroid axis (de Oliveira et al., 2020). Our findings suggest that elevated phthalate biomarker concentration are due an effect of thyroid hormones.

Strengths of this study include its focus on pregnant women and potentially at-risk population, a fairly large sample size for assessing predictors of metabolite concentration, important contribution for informing future exposure assessment and epidemiology studies, and the collection of self-reported daily lifestyles, which allowed for a statistically significant analysis. In addition, we detected the varying levels of thyroid hormones during pregnancy. This provided evidence that thyroid hormones could be impacted by the endocrine disrupting effects of PAEs. However, our study has several limitations. Firstly, our study investigate time is during their first antenatal examination, that is in the first trimester prenatal, we did not repeated measurements throughout the pregnancy, which would result in less measurement error and stronger association. However, previous documents have identified the fetal thyroid only begins to produce hormones at 8–10 weeks gestation, and is completely dependent on maternal thyroid hormones for neurodevelopment in its first weeks of life (Mastorakos et al., 2007). Additionally, our study lack of information about other environmental chemical exposures (e.g., polycyclic aromatic hydrocarbon, or bisphenol A), which may be unmeasured confounders in our analysis. Finally, this study based on a hospital design and most of participants are the Han nationality, findings may limit generalizability to other countries and socioeconomic groups.

Conclusion

Our study identified a number of factors associated with lower phthalate biomarker concentrations, including less puffed food consumption, less cosmetics use, moderate physical activity. Additionally, our results provided evidence for associations between PAEs with thyroid hormones, which may be important for pregnancy outcomes that are mediated by hypothalamic-pituitary-thyroid mechanisms. Our result is critical for identifying potential high-risk group and for protecting public health.

Declarations

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Hubei University of Chinese Medicine (No. 2018-IEC-010). Written informed consent was obtained from individual or guardian participants.

Consent to participate

Not applicable

Consent to Publish

Not applicable

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that there is no conflict of interest in this manuscript.

Funding

This work was supported by the Youth Program of the National Natural Science Foundation of China (21806032).

Authors Contributions

Wei Wu: Editing and Supervision. Zhi-li Ma: Original draft preparation. Fang Yang: Investigation. Ping Wu: Provision of study materials. De-xin Zhang: Software. Rong Zeng: Methodology. Dan-ling Sun: Visualization. Liu Cao: Reviewing. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to all participants and collaborators for their contribution to the cohort study.

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Figures

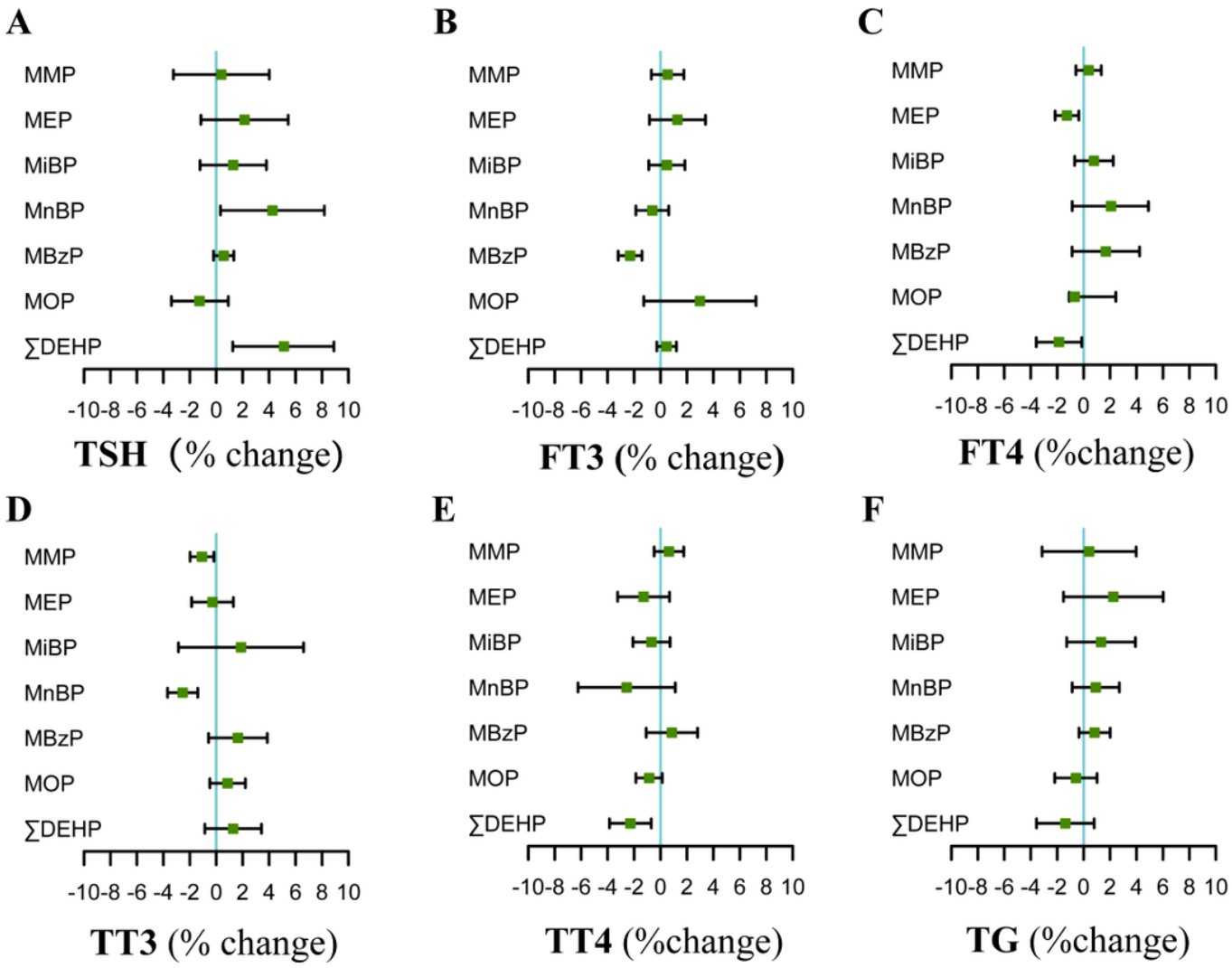

Figure 1

Liner mixed models regressing thyroid hormones versus phthalate metabolites % change in thyroid hormone per IQR change in urinary biomarker concentration.