Association Between Prenatal Phthalate Exposure and Anthropometric Measures of Newborns in a Sample of Iranian Population

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Abstract

Background: This study aimed to examine the associations between the urinary concentrations of phthalate metabolites among a sample of Iranian pregnant women with anthropometric measures of neonates.

Methods: Urine samples were obtained from 121 pregnant women at their first trimester of pregnancy; the levels of monobutyl phthalate (MBP), mono-benzyl phthalate (MBzP), mono-2-ethylhexyl phthalate (MEHP), and mono (2-ethyl-5hydroxyhexyl) phthalate (MEHHP) metabolites were determined by gas chromatography mass spectrometry (GC/MS). The correlation between the urinary concentration of these metabolites and some socio-demographic factors of the participants (maternal education, age, family income, pre-pregnancy body mass index), their lifestyle variables (smoking habit, food pattern, and physical activity), cleaning products use data (cosmetic and household cleaning products) with anthropometric measures of neonates were investigated.

Results: MBzP, MBP, MEHP, and MEHHP were detected in 100% of participants with the concentration ranged from 120 to 860 μg/g creatinine. Significant correlations were observed between the urinary levels of maternal MBzP (adjusted β =0.3 (0.001), p=0.03) and MEHHP and (adjusted β =0.3 (0.001), p=0.04) with the birth weight of female neonates. MBP (adjusted β = -0.3(0.02), p=0.04) and MBzP (adjusted β =-0.3 (0.001), p=0.02) were found negatively associated with head circumference in male and female newborns, respectively. Significant differences existed in the birth weight of infants whose mothers used plastic packaging for pickle and those who had history of passive smoking (p-value< 0.05).

Conclusions: the studied metabolites had higher concentration in the Iranian pregnant women urine compared to the other countries. Higher levels of prenatal exposure to phthalate metabolites may adversely impact the health status of newborns.

Background

Phthalates or phthalic acid esters (PAEs) are a group of compounds widely used in a broad range of industrial products. Low molecular-weight phthalates such as diethyl phthalate (DEP), and dibutyl phthalate (DBP) are commonly used as additives and stabilizing agents in cosmetics, perfumes, lotions, pesticides as stabilizing agents, while high molecular-weight phthalates (with ≥ 8 carbons in the alkyl chain) including di-2-ethyl hexyl phthalate (DEHP) and butyl benzyl phthalate (BBzP) are mainly applied as plasticizers in the manufacture of packaging materials, polyvinylchloride (PVC), as well as in the manufacture of blood transfusion devices and catheters [1]. Although, phthalates can easily be released from materials containing these hazardous compounds, and thus may be entered into the body through inhalation, ingestion or dermal adsorption, but dietary intake has been regarded to contribute as the main route of human exposure to phthalates [2]. Upon the absorption, the diester phthalates have been assumed to quickly hydrolyze to monoester phthalates (mono-2-ethylhexyl phthalate, MEHP), which are subsequently oxidized into the more simple products such as MEHHP (mono2-ethyl-5-hydroxyhexyl), and therefore excreted into the urine and feces [3].

Previous studies have detected substantial amounts of phthalates in the cord blood and amniotic fluid, indicating that these compounds can cross the placenta and may harmfully affect the growing fetus. In this regard, several epidemiological studies have shown a potential relationship between maternal exposure to phthalates and the risk of poor birth outcomes [4]. Due to these potential adverse effects on human health, many developed countries have prohibited the use of di-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), and butyl benzyl phthalate (BBzP) in industrial products [5]. However, there is no strict regulation set up yet to manage the use of these chemicals in most of the developing countries, including Iran. Since several physiological changes occur during the pregnancy, pregnant women and their fetus may be more prone to negative health effects by the exposure to environmental chemicals [6]. To the best of our knowledge, there is no previous publication have investigated the association of exposure to phthalates and birth outcomes among Iranian pregnant women. Therefore, in the present study, we aimed to evaluate the urinary concentrations of phthalate metabolites in a sample of Iranian pregnant women at their first trimester and examine the possible association between phthalate exposures during pregnancy with the anthropometric measures of neonates. The findings of this study can also provide an insight into common source exposure to phthalates among Iranian pregnant women which can help decision makers to take the appropriate measures.

Methods

Study population

This cross-sectional study was conducted between the years 2018–2019 on 121 pairs mother-newborn who lived in Isfahan, Iran. The participants were selected randomly among those pregnant women at their first trimester who attended at health care centers in Isfahan city in the context of the PERSIAN birth cohort. The distributions of participants' location are given in Fig. 1. The participants were completely informed about the objectives, methodology as well as the voluntary nature of the survey, and a signed consent letter was taken from them. It should be mentioned that, the protocols and ethical issues related to this study were approved and observed by Ethics Committees of Isfahan university of Medical sciences.

The early morning urine samples were taken from the participants, collected in borosilicate containers and transferred to the laboratory to be kept at -20 °C until the future experiments. During the sample collection, the PERSIAN Birth Cohort questionnaires were used to gather the data on socio-demographic variables (maternal education, age, family income), lifestyle factors (pre-pregnancy BMI, smoking habit, and physical activity (PA)), and food habits and household cleaning products use [7]. The total physical activity (MET-minutes/week) score were computed by IPAQ (International Physical Activity Questionnaire) [8]. To obtain the data regarding the food intake habits of the participants, food frequency questionnaire (FFQ) was applied. The questionnaire included the questions asking about the frequency of consuming fried foods (as daily, 1–2 per week, 1–3 per month, seldom, and never), and the use of plastics for packaging certain foods including bread, lemon juice, pickle, leftover and water.

Measuring urinary metabolites of phthalate

All the measurements were done along with the standard solutions of MBP, MBzP, MEHP, and MEHHP in methanol, which were provided separately, collected in Teflon capped amber glass containers, and stored at temperatures below zero. To measure the phthalate content of urine samples the following steps were taken: at first, 10 ml from each of the urine samples were defreezed and digested by 20 µl of β-glucuronidase enzyme for 18 h incubation at 37 C in order to extract the to extract MBP, MBzP, MEHP, and MEHHP metabolites. Then, 0.2 g of sodium chloride was added to the preparation and shaked for 24 h at 37 °C. Afterwards, 5 ml of the obtained mixture was diluted with the same volume of distilled water and the solution pH was adjusted to 2 using 10% sulfuric acid solution. In the next step, 1 mL acetone and 20 µl of chlorobenzene were added to the previously obtained mixture, and centrifuged for 5 min at 5000 rpm. Then, the sediments were withdrawn from the bottom of the tubes using a micro-syringe and collected in different microtubes and dried under nitrogen gas stream. Thereafter, 10 µL of MSTFA was mixed with the sediment and centrifuged. At the final, 10 µl of the prepared solution was injected into the GC/MS (Model A 7890 of Agilent technologies, USA) to measure the concentration of investigated phthalate metabolites [9]. To minimize the bias of the dilution difference between the samples, the phthalate concentrations were adjusted using creatinine levels. The limits of detection (LOD) were 0.017, 0.0126, 0.018, and 0.019 µg/L for MBP, MBzP, MEHP, and MEHHP, respectively. For the metabolite concentrations below the LOD, LODs divided by 2 was considered in the statistical analyzes [10].

Anthropometric measures of neonates

Data on the anthropometric indices (weight, length, and head circumference) and other related information of neonates of corresponding mothers who participated in this study were extracted from the hospital records which had been measured by experienced obstetric nurses using standardized procedures.

Quality assurance and quality control (QA/QC)

To confirm the reliability of the analytical data and to increase confidence in the relevance of obtained responses, the quality assurance and quality control (QA/QC) were performed. Accordingly, the linear regression gave a good fit (R2 ≥ 0.98) with high precision (≤ 13.2% RSD). The limit of detection (LOD) and limit of quantification (LOQ) were based on the signal-to-noise ratio of 3 and 10, respectively. For the metabolite concentrations below the LOD, LODs divided by 2 was considered in the statistical analyzes [10]. Furthermore, to minimize the bias of the dilution difference between the samples, the phthalate concentrations were adjusted using creatinine levels. The R2, precision (% RSD), LOD, LOQ, and mean recovery are summarized in Table 1.

Table 1
QA/QC parameters of Phthalate metabolites.

	MBP	MBzP	MEHP	MEHHP
R^2	0.99	0.99	0.99	0.98
RSD (%)	7	6.9	13.2	6.2
LOD (µg/L)	0.017	0.013	0.018	0.019
LOQ (µg/L)	0.06	0.04	0.06	0.07
Recovery (%)	83	94	90	69

Data analysis

Continuous variables have been presented as mean ± SD and median (minimum-maximum) while categorical variables were expressed as percentages. Normality of continuous data was evaluated by using Kolmogorov-Smirnov test and Q-Q plot. One-way Analysis of variance (ANOVA) or independent samples t-test was used to compare the mean values of birth outcomes cross categories of possible demographic and lifestyle determinants while Pearson or non-parametric correlation coefficients were used for evaluation the bivariate association of metabolites with infants' anthropometric measures and continuous determinants. Multiple linear regression was used for evaluation of the association of metabolites with infants' anthropometric measures and adjustment was done for mothers' basic demographic and clinical characteristics. All statistical analyses were done using SPSS software version 23 (IBM SPSS Inc., Chicago, IL). $P < 0.05$ was considered as statistically significant.

Results And Discussion

The characteristics of the participants are summarized in Table 2. Descriptively, 59% (n = 71) of participants were ≥ 30-years old, and 41% (n = 50) of them were ≤ 30 years old, 69% of subjects (n = 83) were categorized into overweight, while 29% of them (n = 35) had normal weight and only 2% (n = 3) were classified as underweight. The main part of the participants were educated academically (n = 105, 86.8%). The majority of the participants had family income of 100–300 $ per month and grouped into the middle income category (n = 69, 57%). Household cleaning products and cosmetic products were found as the most common products used by 92.6% (112) and 100% (121) of the participants, respectively. Moreover, more than half (51.26%) of the pregnant women have reported the use of plastics for packaging of bread (n = 76, 62.8%), lemon juice (n = 67, 55.4%), pickle (n = 55, 45.5%), leftover (n = 52, 43%) and water (n = 60, 49.6%). The data showed that 31.4% (n = 38) of the participants have used fried foods more than 1 time per week (1–2 times/week), however 4.1% (n = 5) of them had never used fried food item. The majority of the participants did not have enough physical activity, where 53.7% of study population (n = 65) were grouped into the low physical activity category. Among the studied pregnant women only 6.6% of (n = 8) had high physical activity.

The mean (± SD) of birth weight, birth length, and head circumference of infants of the corresponding mother who participated in this survey was 3204.04 ± 480.94 g, 50.35 ± 3.16 cm, and 34.5 ± 1.71 cm, respectively.

Table 2
Maternal characteristics of 121 pregnant women enrolled in the study

Variables	**Mean, n (%)**	**Variables**			**Mean, n (%)**
Maternal age (years)		**Cosmetic usage**		No	9(7.4)
				Yes	112(92.6)
< 25	8(6)	**Smoking during pregnancy**		No	121(100)
				Yes	0(0)
25–29	42(35)	**Passive smoking during pregnancy**		No	56(46.3)
				Yes	65(53.7)
30–34	47(39)	**Using household cleaning products**		No	0(0)
				Yes	121(100)
> 34	24(20)	**Plastic packaging usage**	Bread	No	45(37.2)
Pre-pregnancy BMI (kg/m^2)				Yes	76(62.8)
Underweight (< 18.5)	3(2)		Lemon juice	No	54(44.6)
				Yes	67(55.4)
Normal weight (18.5–23.9)	35(29)		Pickle	No	66(54.5)
				Yes	55(45.5)
Overweight (≥ 24)	83(69)		Leftover	No	69(57)
				Yes	52(43)
Education			Water	No	61(50.4)
				Yes	60(49.6)
Less than high school	7(5.8)		Total	No	(48.74)
				Yes	(51.26)
High school	9(7.4)	**Physical activity**			
College	105(86.8)	High			8(6.6)
Family income ($/month)		Moderate			48(39.7)
High (> 300)	12(9.9)	Low			65(53.7)
Middle (100–300)	69(57)	**Eating fried foods**			
Low (< 100)	40(33.1)	daily			30(24.8)
Birth weight (g)	3204.04(480.94)	1–2 per week			38(31.4)
Head circumference(cm)	34.5(1.71)	1–3 per month			35(28.9)
Birth length (cm)	50.35(3.16)	Seldom (< once a month)			13(10.7)
		Never			5(4.1)

Table 3 shows the mean, minimum, and maximum concentration of phthalate metabolites adjusted by creatinine. All the urine samples (n = 121) were positive for phthalate metabolites. MEHHP had the highest concentration (866.5 ± 307.6 μg/g creatinine), while MEHP was detected in the all samples with the lowest levels (126.5 ± 118.3 μg/g creatinine). The mean concentration of MBP, MBzP, MEHP, and MEHHP found in this study were 13, 30, 20, and 44 times greater than those levels have reported by other studies in the US and European countries [6, 11, 12]. These results are in accordance with the findings of Amin et al. who previously reported a higher levels of exposure to phthalates in Iranian population [9]. Several factors including the socio-demographic, environmental, regional, size of study population and etc. can be attributed to the different levels of exposure obtained in this survey and those reported by other countries.

Table 3
Mean concentration of phthalate metabolites (μg/g creatinine) in pregnant women urine

Creatinine-adjusted phthalate metabolites(μg/g)	MBP	MBzP	MEHP	MEHHP
LOD (μg/L)	0.017	0.013	0.018	0.019
%> LOD*	100	100	100	100
Min	8.5	15.6	4.2	37.8
Max	602.2	705.4	455.9	1285.9
Mean (SD)	342.5 (193.8)	308.5 (229.4)	**126.5 (118.3)**	**866.5 (307.6)**
* Counts of detectable sample] / [Total sample counts] × 100(%).				

The results of correlation analysis between the creatinine-adjusted urinary levels of phthalate metabolites and birth outcome measures are presented in Table 4. None of the phthalate metabolites exhibited significant correlation with the birth outcomes, and only the correlation between MBzP with birth weight was of borderline significance (β = 0.2 (0.16), p = 0.06). Based on the results, it is obvious that the concentration of phthalates in first trimester maternal urine is positively correlated to the birth weight of neonates; however negative correlation is existed between urinary phthalate levels with birth length and head circumference of newborns.

Table 4
Correlation between urinary phthalate concentrations and anthropometric measures of neonates

Phthalate Metabolites	Birth weight				Birth length				Head circumference			
	Crude		Adjusted		Crude		adjusted		Crude		adjusted	
	β* (SE**)	p	β*(SE**)	P	β*(SE*)	p	β*(SE**)	p	β*(SE**)	p	β*(SE**)	p
MBP	-0.2(0.2)	0.02	0.1(0.001)	0.9	-0.08(0.001)	0.4	-0.03(0.001)	0.8	-0.13(0.2)	0.2	-0.06(0.001)	0.6
MBzP	-0.3(0.2)	0.006	0.2(0.16)	**0.06**	-0.1(0.001)	0.1	-0.11(0.001)	0.3	-0.07(0.001)	0.4	-0.15(0.001)	0.2
MEHP	-0.2(0.3)	0.02	0.01(0.0004)	0.9	-0.2(0.002)	0.07	-0.14(0.002)	0.2	-0.07(0.001)	0.4	-0.2(0.001)	0.09
MEHHP	-0.2(0.1)	0.06	0.1(0.001)	0.8	-0.2(0.001)	0.04	-0.16(0.001)	0.1	-0.14(0.3)	0.2	-0.07(0.001)	0.5

Adjusted for maternal age, Pre-pregnancy BMI, gestational age, educational level, Family annual income, Total exposure to chemical products, plastic packaging.

* β: Regression coefficient

** SE: Standard error

In line with the results found in this study, Suzuki et al. [13] and Philippat et al. [14] have also found no statistically significant associations between phthalate exposure and birth outcomes. Furthermore, Huang et al. have reported negative association between the maternal urinary phthalates concentration with the head circumference of their newborns, however the significant correlations was only noted in female neonates [15]. Consistent with our results, Zhu et al. has also shown no significant relationship between maternal urinary phthalates and birth length[16].

On the contrast, Shoaff et al. previously showed that a ten-fold increase in the maternal urinary MEP levels is associated with a 0.23 standard deviation reduction (95% CI: −0.46, − 0.01) in birth weight z-score, however after adjustment for confounding factors this relationship was attenuated towards the null [17]. In a Spanish cohort, Casas et al. have assessed the effect of prenatal exposure to eight phthalates on fetal growth, but found no significant association between the maternal urinary ∑DEHP concentrations and any of the growth outcome measures [18]. In another study conducted by Wolff et al. they investigated the association between maternal urinary phthalates concentration at third-trimester with the body size measures of infants at birth. They found that low molecular weight phthalates had a positive, but not statistically significant association with the head circumference of newborns. [19].

Despite the mentioned studies, there are several other reports have shown inverse [20, 21] or negative associations [22, 23] between maternal urinary levels of phthalate metabolites and birth outcomes. In this regard, Lenters et al. [20] and Zhang et al. [21] have shown that maternal urinary levels of some DEHP metabolites during pregnancy is associated with low birth weight or increased risk of low birth weight. The discrepancy between the results might be explained by the potential contamination with phthalate di-esters or the difference in the levels of exposure to these compounds among different populations [24].

Table 5
The crude and adjusted associations of phthalate metabolite concentrations in first-trimester maternal urines with anthropometric measures of neonates

Phthalate Metabolites			**Birth weight**		**Birth length**		**head circumference**	
			Girl	**Boy**	**Girl**	**Boy**	**Girl**	**Boy**
MBP	Crude	β (SE)	-0.2(0.2)	-02(0.3)	-0.1(0.001)	-0.05(0.002)	-0.07(0.001)	-0.1(0.001)
		p	0.1	0.1	0.4	0.7	0.6	0.4
	adjusted	β (SE)	0.1(0.2)	-0.1(0.4)	-0.1(0.002)	0.06(0.002)	-0.2(0.001)	-0.3(0.2)
		p	0.2	0.6	0.2	0.7	0.1	**0.04**
MBzP	Crude	β (SE)	-0.3(0.2)	-0.2(0.2)	-0.16(0.001)	-0.1(0.001)	-0.2(0.001)	0.08(0.001)
		p	0.007	0.2	0.2	0.4	0.1	0.6
	adjusted	β (SE)	0.3(0.001)	-0.1(0.3)	-0.1(0.001)	-0.1(0.002)	-0.3(0.001)	0.08(0.001)
		p	**0.03**	0.6	0.3	0.5	**0.02**	0.7
MEHP	Crude	β (SE)	-0.3(0.3)	-0.1(0.5)	-0.1(0.003)	-0.2(0.003)	-0.1(0.002)	-0.02(0.001)
		p	0.02	0.3	0.4	0.1	0.3	0.9
	adjusted	β (SE)	0.1(0.1)	0.04(0.6)	-0.1(0.003)	-0.2(0.004)	-0.2(0.002)	-0.09(0.002)
		p	0.3	0.8	0.5	0.2	0.08	0.6
MEHHP	Crude	β (SE)	-0.2(0.1)	-0.2(0.2)	-0.2(0.001)	-0.1(0.001)	0.03(0.001)	-0.04(0.001)
		p	0.2	0.2	0.06	0.4	0.8	0.8
	adjusted	β (SE)	0.3(0.001)	-0.2(0.2)	-0.2(0.001)	-0.1(0.002)	-0.2(0.001)	-0.1(0.001)
		p	**0.04**	0.2	0.1	0.4	0.2	0.6

Adjusted for maternal age, Pre-pregnancy BMI, gestational age, educational level, Family annual income, Total exposure to chemical products, plastic packaging.

* β: Regression coefficient

** SE: Standard error

The results of crude and adjusted associations between maternal urinary phthalates concentration with the birth outcomes in boys and girl neonates are presented in Table 5. According to these findings, MBP (adjusted β = -0.3(0. 2), p = 0.04) and MBzP (adjusted β =-0.3 (0.001), p = 0.02) were negatively associated with head circumference in boys and girls, respectively. Furthermore, positive associations were observed between maternal urinary levels of MBzP (adjusted β = 0.3 (0.001), p = 0.03) and MEHHP (adjusted β = 0.3 (0.001), p = 0.04) with birth weight in girls after adjusting for potential confounding factors.

The correlation between prenatal exposure to MBzP and MEHHP with higher birth weight in female neonates may suggest that exposure to these chemicals during pregnancy can influence the function of feminine hormones, thus increase fetal growth or induce fat accumulation. Consistently, it has been demonstrated that phthalates have weak estrogenic activities [25, 26] and can promote adipocyte differentiation by activation of peroxisome proliferator-activated receptor gamma (PPARs) [27]. Furthermore, due to their estrogenic activities, phthalates may engage nuclear receptors and induce the expression of several other genes involved in obesity. Additionally, it is possible that prenatal phthalate exposure may affect thyroid axis function, disrupt energy balance and metabolism, and thus result in the fat accumulation [28]. These compounds may also affect the pituitary-adrenal axis, which is crucial for fetal growth [29]. Since we observed a sex difference in the associations of maternal urinary phthalate metabolite levels with birth weight of infants, hence it can be concluded that the most potential mechanism by which these compounds have resulted in higher birth weight might be related to their influence on sexual hormones or sex related biological variables [30]. However, further studies are warranted to shed insights into this claim.

We also examined the effects of some potential factors which may be associated with prenatal exposure to phthalates including maternal education level, family income per month, plastic packaging (bread, lemon juice, pickle, leftover, water) on their birth outcomes (Table 6).

Table 6
Association of some maternal factors with possible influence on prenatal exposure to phthalates with the neonatal anthropometric measures

Variables			Birth weight		Birth length		Head circumference	
			Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Education		College	3120 ± 315	0.8	49.5 ± 2.5	0.9	33.9 ± 2	0.4
		High school	3160 ± 252		49.9 ± 1.6		33.7 ± 1	
		<high school	3271 ± 305		50.6 ± 2.5		33.4 ± 1	
Family income per month ($)		< 100	3161 ± 429	0.5	49.6 ± 2.6	0.9	33.9 ± 1	0.7
		100–300	3124 ± 322		49.5 ± 1.9		33.7 ± 1.5	
		> 300	3014 ± 388		49.9 ± 4		33.8 ± 1	
Plastic packaging	Bread	Yes	3115 ± 378	0.7	49.3 ± 2.2	0.1	33.8 ± 1.3	0.8
		No	3142 ± 348		50 ± 2.6		33.8 ± 1.4	
	Lemon juice	Yes	3103 ± 412	0.5	49.4 ± 2.4	0.3	33.8 ± 1.4	0.9
		No	3153 ± 302		49.8 ± 2.4		33.8 ± 1.3	
	Pickle	Yes	3051 ± 401	**0.04***	49.3 ± 2.4	0.3	33.7 ± 1.4	0.6
		No	3188 ± 324		49.8 ± 2.4		33.8 ± 1.3	
	Leftover	Yes	3096 ± 306	0.4	49.7 ± 2.7	0.7	34 ± 1.6	0.3
		No	3148 ± 406		49.5 ± 2.1		33.7 ± 1.1	
	Water	Yes	3065 ± 337	0.07	49.3 ± 2.1	0.3	33.9 ± 1.4	0.5
		No	3185 ± 386		49.8 ± 2.6		33.7 ± 1.3	
Passive smoking during pregnancy		Yes	3050 ± 326	**0.01***	49.3 ± 2.2	0.2	34 ± 1.2	0.1
		No	3213 ± 393		50 ± 2.6		33.6 ± 1.5	
Physical activity		Low	3109 ± 322	0.08	49.5 ± 1.9	0.07	33.7 ± 1	0.8
		Moderate	3188 ± 368		50 ± 2.8		33.9 ± 1.7	
		High	2885 ± 586		47.9 ± 2.9		33.8 ± 1.3	

According to the results, maternal education level, family income per month, and physical activity had not significant influences on the investigated birth outcome measures (p-value > 0.05). However, birth weight of infants was significantly different (p-value < 0.05) among those participants who used plastic packaging for pickle and passively exposed to smoke. This can be explained by the fact that, phthalates are still widely used as plasticizers in materials used for food and water packaging for many years, therefore the plastic packaging of food items can be associated with higher exposure to these chemicals [31]..

Although the main part of the participants in the present work were non-smokers, but more than half of them (n = 65, 53.7%) were passively exposed to smoke. Likewise, previous studies have also linked smoking habits to higher exposure levels to phthalates[11, 32]. In this regard, Casas et al. observed that smoking has a direct association with the elevated urinary phthalates [33]. It has been demonstrated that cigarette smoke and filters contain phthalates in the form of di-2-methoxyethyl phthalate [34]. Furthermore, it was assumed that low-quality cigarettes may also further increase the urinary concentration of phthalates metabolites both in first- and second-hand smokers. Table 7 presents the results of correlations between quantitative determinants and birth outcome measures.

Table 7
The effect of some maternal determinates on the neonatal anthropometric measures

Variables	Birth weight		Birth length		Head circumference	
	r	p-value	r	p-value	r	p-value
Age	0.09	0.3	0.01	0.9	0.04	0.6
BMI	0.18	**0.04**	0.03	0.7	0.1	0.1
Cosmetic	0.21	**0.02**	0.03	0.7	0.04	0.6
Household Cleaning	0.24	**0.007**	0.05	0.6	0.12	0.2
Fried Foods	0.01	0.9	0.04	0.6	0.07	0.4
Physical Activity	0.12	0.2	0.06	0.5	0.09	0.3
r: Correlation coefficient						

Here, we found positive significant correlations between prenatal BMI, and the use of cosmetics and household cleaning products with the neonatal birth weight (p-value < 0.05 and r = 0.18, r = 0.21, and r = 0.24, respectively). However, there was no significant relationships observed between maternal age, consumption of fried foods and physical activity with the birth measures (p-value > 0.05).

There are a large body of evidences indicating that urinary phthalate metabolites in pregnant women is positively associated with the amount and frequency of cosmetics and household cleaning products they use [35]. On contrast with our findings, Valvi et al. showed no association between the use of cosmetics and urinary levels of phthalates [11]. The legislative actions regarding the production of cosmetics and care products in various countries are thoroughly different. For instance, the use of some phthalates including DEHP, di-n-butyl phthalate, di-iso-butyl phthalate and BBP in the production of cosmetics have been prohibited by the EU (European Union)[36], however there is no strict regulation on the use of these chemicals in the production of various industrial products in developing countries, including Iran. Besides, Iran has been ranked as the second country in the Middle East with the highest use of personal care products [37]. This is in line with our observation that a large proportion of the participants in this study have reported higher amounts of cosmetics use. On the other hands, since the majority of the women participated in this survey were of low or middle income families, therefore it can be assumed that the main part of this population may use inexpensive with low-quality cosmetic products containing higher grades of phthalates.

Household cleaning products have also been regarded as another important source of phthalate exposure, especially among pregnant women [38]. Accordingly, Valvi et al. have shown that the use of household chemical products is associated with the higher urinary concentrations of MEHHP, MEHP and MBzP [11]. In countries like Iran, since females are mainly involved in household works, thus they may expose to higher levels of phthalates through the use of cleaning chemical products. Furthermore, pregnant women and their growing fetus are prone to more adverse effects in part due to the physiological changes during the pregnancy [39].

In this study, higher levels of MBzP, MEHHP, and MBP were found in urinary samples of obese individuals compared to women with normal body weight.

Prenatal maternal BMI is considered as one of the most important determinants of fetal weight, growth and body composition [40]. It is clear that the maternal BMI is not play as biological effector, but the mechanism by which prenatal maternal BMI affects the fetal growth remains largely unknown [41]. Several studies have demonstrated that fetal growth mainly is influenced by placental capacity in transferring nutrients to fetus, the genetics of parents as well as by maternal supply of nutrients. Since maternal BMI may influence nutrients supply and the capacity of placenta in transporting nutrient from mother to fetus, thus it can affect the fetal growth measures. Previous studies have also linked maternal BMI positively to the levels of circulating glucose. Additionally, higher maternal BMI and plasma glucose levels have been associated with the longer gestational age and greater body fat of newborns [42]. In spite of influencing adipogenesis, phthalates have lipophilic properties enable these chemical to be stored in adipose tissue. Therefore, it is expected that obese mothers may exhibit higher urinary levels of phthalates and potentially have more affected newborns [39].

Limitations and Strengths

One of the important limitations of the present study was its cross-sectional nature. Furthermore, this study has assessed the maternal urinary phthalates concentration only at first trimester period. To the best of our knowledge, no or only limited number of studies have been conducted in Iran to address this issue, thus these findings can provide a basis for the future studies and helps decision makers to implement proper actions. Taken together, higher exposure to neglected chemicals such as phthalates, especially during pregnancy could threaten the health of both mothers and newborns. We recommend further investigations to evaluate the prenatal exposure to phthalates during second and third trimesters as well. Such data on the whole period of pregnancy can be more meaningful.

Conclusion

This study was conducted to determine the possible relationship between prenatal phthalate exposure and neonatal anthropometric measures in association with maternal lifestyle variables and characteristics of pregnant women. According to the findings, the following conclusions can be made:

- MBzP, MBP, MEHP, and MEHHP were detected in 100% of urines obtained from pregnant women at their first-trimester.
- Investigated metabolites had higher concentration in the Iranian pregnant women urines compared to the other countries.
- Our findings revealed a positive association between the maternal concentrations of MBzP and MEHHP with increased birth weight in girl neonates.
- Urinary concentration of phthalates were significantly higher among those pregnant women who had higher pre-pregnancy BMI, as well as among the users of cosmetics and household cleaning products, and those subjects who routinely used plastic packaging for pickle storage.
- Pregnant women who were passive smokers had significantly higher levels of urinary phthalates.

Declarations

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Authors' contributions

M. Darvishmotevalli , M. Moradnia, R. Hosseini surveyed the studies for data extraction, inclusion, assessing the study quality, and wrote the first draft of the manuscript, B. Bina supervised this study, A Feizi performed the data-analysis, K. Ebrahimpour and H Pourzamani provided critical input for the manuscript, Roya Kelishadi did critical revision of the manuscript. All authors have contributed considerably, and all authors are in agreement with respect to the manuscript content. The authors read and confirmed the final manuscript.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee, Isfahan University of Medical Sciences (Code: IR.MUI.RESEARCH.REC.1397.441) and project number #397573.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Gao D-W, Wen Z-D: Phthalate esters in the environment: A critical review of their occurrence, biodegradation, and removal during wastewater treatment processes. *Science of the total Environment* 2016, 541:986-1001.
2. Bui TT, Giovanoulis G, Cousins AP, Magnér J, Cousins IT, de Wit CA: Human exposure, hazard and risk of alternative plasticizers to phthalate esters. *Science of the total environment* 2016, 541:451-467.
3. Lee K-M, Kho Y, Kim P-g, Park S-h, Lee J-H: Urinary levels of phthalate metabolites and associations with demographic characteristics in Korean adults. *Environmental Science and Pollution Research* 2017, 24(17):14669-14681.
4. Li X, Sun H, Yao Y, Zhao Z, Qin X, Duan Y, Wang L: Distribution of Phthalate Metabolites between Paired Maternal–Fetal Samples. *Environmental science & technology* 2018, 52(11):6626-6635.
5. Becker K, Güen T, Seiwert M, Conrad A, Pick-Fuß H, Müller J, Wittassek M, Schulz C, Kolossa-Gehring M: GerES IV: phthalate metabolites and bisphenol A in urine of German children. *International journal of hygiene and environmental health* 2009, 212(6):685-692.
6. Wenzel AG, Brock JW, Cruze L, Newman RB, Unal ER, Wolf BJ, Somerville SE, Kucklick JR: Prevalence and predictors of phthalate exposure in pregnant women in Charleston, SC. *Chemosphere* 2018, 193:394-402.
7. Kelishadi R, Majdzadeh R, Motlagh ME, Heshmat R, Aminaee T, Ardalan G, Esmaillzadeh A, Azadbakht L, Poursafa P, Movahedian M *et al*: Development and evaluation of a questionnaire for assessment of determinants of weight disorders among children and adolescents: The CASPIAN-IV study. *International Journal of Preventive Medicine* 2012, 3(10):699-705.

8. Cleland C, Ferguson S, Ellis G, Hunter RF: Validity of the International Physical Activity Questionnaire (IPAQ) for assessing moderate-to-vigorous physical activity and sedentary behaviour of older adults in the United Kingdom. *BMC medical research methodology* 2018, 18(1):176.
9. Amin MM, Ebrahimpour K, Parastar S, Shoshtari-Yeganeh B, Hashemi M, Mansourian M, Poursafa P, Fallah Z, Rafiei N, Kelishadi R: Association of urinary concentrations of phthalate metabolites with cardiometabolic risk factors and obesity in children and adolescents. *Chemosphere* 2018, 211:547-556.
10. Schuhmacher M, Nadal M, Domingo JL: Environmental monitoring of PCDD/Fs and metals in the vicinity of a cement plant after using sewage sludge as a secondary fuel. *Chemosphere* 2009, 74(11):1502-1508.
11. Valvi D, Monfort N, Ventura R, Casas M, Casas L, Sunyer J, Vrijheid M: Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. *International journal of hygiene and environmental health* 2015, 218(2):220-231.
12. Berman T, Hochner-Celnikier D, Calafat AM, Needham LL, Amitai Y, Wormser U, Richter E: Phthalate exposure among pregnant women in Jerusalem, Israel: results of a pilot study. *Environment international* 2009, 35(2):353-357.
13. Suzuki Y, Niwa M, Yoshinaga J, Mizumoto Y, Serizawa S, Shiraishi H: Prenatal exposure to phthalate esters and PAHs and birth outcomes. *Environment international* 2010, 36(7):699-704.
14. Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, Silva MJ, Brambilla C, Pin I, Charles M-A: Exposure to phthalates and phenols during pregnancy and offspring size at birth. *Environmental health perspectives* 2011, 120(3):464-470.
15. Huang Y, Li J, Garcia JM, Lin H, Wang Y, Yan P, Wang L, Tan Y, Luo J, Qiu Z: Phthalate levels in cord blood are associated with preterm delivery and fetal growth parameters in Chinese women. *PloS one* 2014, 9(2):e87430.
16. Zhu Y, Wan Y, Zhang B, Zhou A, Huo W, Wu C, Liu H, Jiang Y, Chen Z, Jiang M: Relationship between maternal phthalate exposure and offspring size at birth. *Science of the Total Environment* 2018, 612:1072-1078.
17. Shoaff JR, Romano ME, Yolton K, Lanphear BP, Calafat AM, Braun JM: Prenatal phthalate exposure and infant size at birth and gestational duration. *Environmental research* 2016, 150:52-58.
18. Casas Sanahuja M, Valvi D, Ballesteros-Gomez A, Gascon Merlos M, Fernandez MF, García Esteban R, Iñiguez C, Martínez Muriano D, Murcia M, Monfort Mercader N: Exposure to bisphenol A and phthalates during pregnancy and ultrasound measures of fetal growth in the INMA-Sabadell cohort. 2016.
19. Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, Wetmur J, Calafat AM: Prenatal phenol and phthalate exposures and birth outcomes. *Environmental health perspectives* 2008, 116(8):1092-1097.
20. Lenters V, Portengen L, Rignell-Hydbom A, Jönsson BA, Lindh CH, Piersma AH, Toft G, Bonde JP, Heederik D, Rylander L: Prenatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. *Environmental health perspectives* 2015, 124(3):365-372.
21. Zhang Y, Lin L, Cao Y, Chen B, Zheng L, Ge R-S: Phthalate levels and low birth weight: a nested case-control study of Chinese newborns. *The Journal of pediatrics* 2009, 155(4):500-504.
22. Botton J, Philippat C, Calafat AM, Carles S, Charles M-A, Slama R, Group EM-CCS: Phthalate pregnancy exposure and male offspring growth from the intra-uterine period to five years of age. *Environmental research* 2016, 151:601-609.
23. Ferguson KK, Meeker JD, Cantonwine DE, Chen Y-H, Mukherjee B, McElrath TF: Urinary phthalate metabolite and bisphenol A associations with ultrasound and delivery indices of fetal growth. *Environment international* 2016, 94:531-537.
24. Kato K, Silva MJ, Brock JW, Reidy JA, Malek NA, Hodge CC, Nakazawa H, Needham LL, Barr DB: Quantitative detection of nine phthalate metabolites in human serum using reversed-phase high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. *Journal of analytical toxicology* 2003, 27(5):284-289.
25. Ghisari M, Bonefeld-Jorgensen EC: Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. *Toxicology letters* 2009, 189(1):67-77.
26. Kiyama R, Wada-Kiyama Y: Estrogenic endocrine disruptors: Molecular mechanisms of action. *Environment International* 2015, 83:11-40.
27. Hao C, Cheng X, Xia H, Ma X: The endocrine disruptor mono-(2-ethylhexyl) phthalate promotes adipocyte differentiation and induces obesity in mice. *Bioscience reports* 2012, 32(6):619-629.
28. Boas M, Feldt-Rasmussen U, Main KM: Thyroid effects of endocrine disrupting chemicals. *Molecular and cellular endocrinology* 2012, 355(2):240-248.
29. Liu T, Li N, Zhu J, Yu G, Guo K, Zhou L, Zheng D, Qu X, Huang J, Chen X: Effects of di-(2-ethylhexyl) phthalate on the hypothalamus-pituitary-ovarian axis in adult female rats. *Reproductive toxicology* 2014, 46:141-147.
30. Grün F, Blumberg B: Endocrine disrupters as obesogens. *Molecular and cellular endocrinology* 2009, 304(1-2):19-29.
31. European Food Safety Authority: Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request related to a 18th list of substances for food contact materials. . In*.*
32. Arbuckle TE, Davis K, Marro L, Fisher M, Legrand M, LeBlanc A, Gaudreau E, Foster WG, Choeurng V, Fraser WD: Phthalate and bisphenol A exposure among pregnant women in Canada—results from the MIREC study. *Environment international* 2014, 68:55-65.

33. Casas L, Fernández MF, Llop S, Guxens M, Ballester F, Olea N, Irurzun MB, Rodríguez LSM, Riaño I, Tardón A: Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. *Environment international* 2011, 37(5):858-866.
34. Jackson Jr WJ, Darnell WR: Process for foaming cellulose acetate rod. In. US Patent 4,507,256. Eastman Kodak.; 1985: 72.
35. Darvishmotevalli M, Bina B, Feizi A, Ebrahimpour K, Pourzamani H, Kelishadi R: Monitoring of urinary phthalate metabolites among pregnant women in Isfahan, Iran: the PERSIAN birth cohort. *Journal of Environmental Health Science and Engineering* 2019, 17(2):969-978.
36. Wittassek M, Koch HM, Angerer J, Brüning T: Assessing exposure to phthalates–the human biomonitoring approach. *Molecular nutrition & food research* 2011, 55(1):7-31.
37. Volpe M, Nazzaro M, Coppola R, Rapuano F, Aquino R: Determination and assessments of selected heavy metals in eye shadow cosmetics from China, Italy, and USA. *Microchemical Journal* 2012, 101:65-69.
38. Harley KG, Kogut K, Madrigal DS, Cardenas M, Vera IA, Meza-Alfaro G, She J, Gavin Q, Zahedi R, Bradman A: Reducing phthalate, paraben, and phenol exposure from personal care products in adolescent girls: findings from the HERMOSA intervention study. *Environmental health perspectives* 2016, 124(10):1600-1607.
39. Philips EM, Jaddoe VW, Trasande L: Effects of early exposure to phthalates and bisphenols on cardiometabolic outcomes in pregnancy and childhood. *Reproductive Toxicology* 2017, 68:105-118.
40. Sewell MF, Huston-Presley L, Super DM, Catalano P: Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. *American journal of obstetrics and gynecology* 2006, 195(4):1100-1103.
41. Roland MCP, Friis CM, Voldner N, Godang K, Bollerslev J, Haugen G, Henriksen T: Fetal growth versus birthweight: the role of placenta versus other determinants. *PLoS one* 2012, 7(6):e39324.
42. Nathanielsz PW, Poston L, Taylor PD: In utero exposure to maternal obesity and diabetes: animal models that identify and characterize implications for future health. *Obstetrics and gynecology clinics of North America* 2007, 34(2):201-212.

Figures

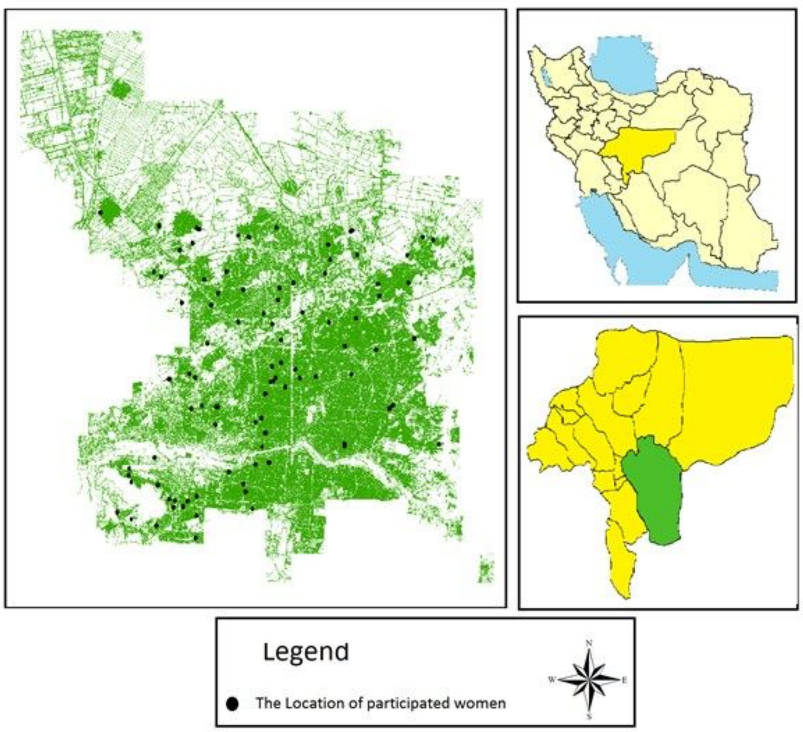
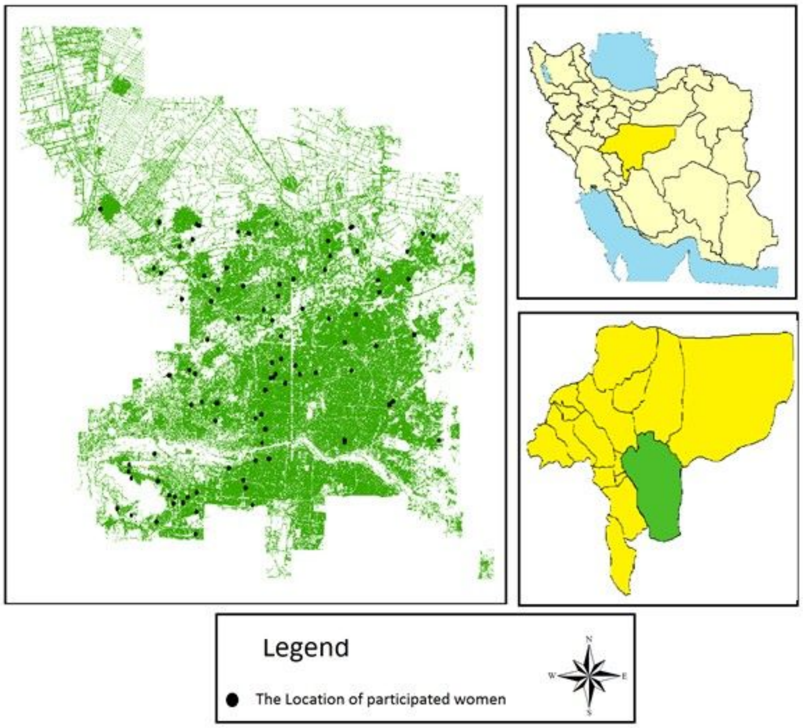


Figure 1

The distribution of participants' location in Isfahan city. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.



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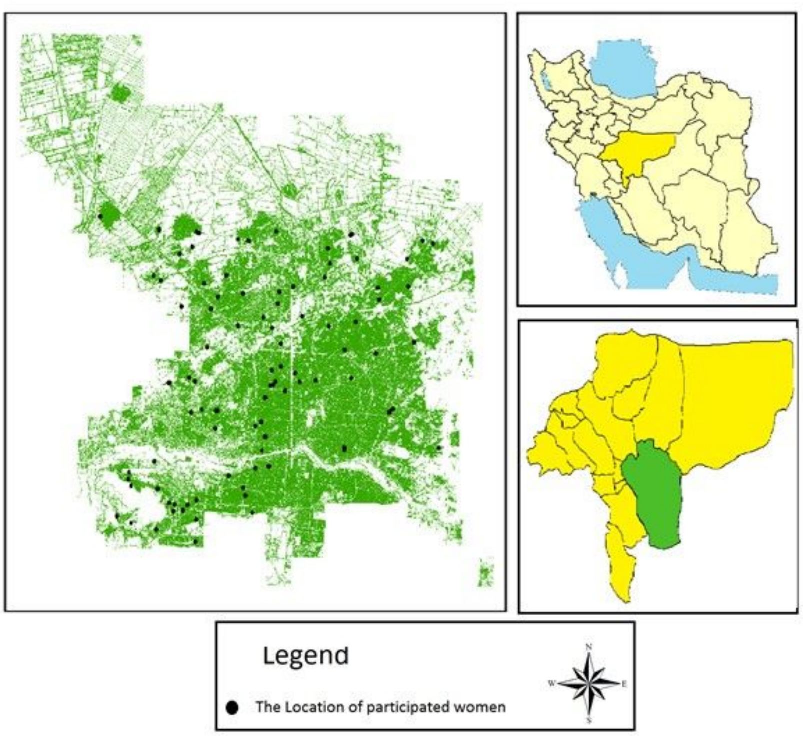

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