Association of phthalate exposure with thyroid function during pregnancy

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ABSTRACT

Background: The extent of thyroid disruptive effects of phthalates during pregnancy remains unclear.
Aim: To investigate the association of maternal urinary phthalates with markers of the thyroid system during early pregnancy.
Methods: Urinary concentrations of phthalate metabolites and serum concentrations of thyroid stimulating hormone (TSH), free and total thyroxine (FT4 and TT4) and free and total triiodothyronine (FT3 and TT3) were measured in pregnant women in early pregnancy in the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy study (2007-ongoing), a population-based prospective cohort.
Results: In the 1,996 included women, higher di-ethyl-hexyl phthalate (DEHP) metabolites were associated with a lower FT4 (β [SE] for the molar sum: −0.13 [0.06], *P* = 0.03) and a higher TSH/FT4 ratio (0.003 [0.001], *P* = 0.03). Higher concentrations of di-*iso*-nonyl phthalate (DINP) metabolites were associated with a lower TT4 (β [SE] for the molar sum: 0.93 [0.44], *P* = 0.03) as well as with lower TT4/FT4 and TT4/TT3 ratios. Higher metabolites of both dibutyl and butyl-benzyl phthalate (DBP and BBzP) were associated with lower T4/T3 ratio (free and total) and higher FT4/TT4 and FT3/TT3 ratios. A higher diisononyl cyclohexane dicarboxylate (DINCH) metabolite concentration was associated with a higher TT3.
Conclusions: These results translate results from experimental studies suggesting that exposure to phthalates may interfere with the thyroid system during pregnancy. This is also true for compounds that have been introduced to replace known disruptive phthalates. Further experimental studies should take into account the human evidence to better investigate the potential underlying mechanisms of thyroid disruption by phthalates.

1. Introduction

Phthalates are pseudo-persistent synthetic chemical compounds widely used in plastic and personal care products such as food packaging and cosmetics. Human beings are ubiquitously exposed to phthalates, which enter the body through oral, respiratory and dermal routes, being detected in all body fluids, including the fetal compartment (Benjamin et al., 2017). Experimental studies have shown that phthalates interfere with the thyroid system by altering expression of genes involved in

hypothalamic-pituitary-thyroid (HPT) axis, thyroid hormone transport, metabolism and action (Sun et al., 2018; Kim et al., 2018; Ye et al., 2017; Dong et al., 2017; Jia et al., 2016; Liu et al., 2015; Wenzel et al., 2005; Duan et al., 2018; Ishihara et al., 2003; O'Connor et al., 2002). In addition, some experimental studies have suggested that exposure to phthalates might aggravate or exacerbate thyroid autoimmunity (Duan et al., 2019; Duan et al., 2018; Wu et al., 2017). During pregnancy, there is an increased demand for thyroid hormone due to alterations in thyroid hormone metabolism and binding proteins, as well as placental thyroid

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hormone transfer and consumption by the fetus (Korevaar et al., 2017). Adequate thyroid hormone availability during pregnancy is required for an optimal pregnancy outcome as thyroid hormones play an essential role in the regulation of metabolism as well as fetal growth and development, including early fetal neurodevelopment (Korevaar et al., 2017; Mughal et al., 2018; Ghassabian and Trasande, 2018).

Prenatal exposure to phthalates has been associated with adverse neurodevelopmental outcomes of the offspring, such as lower nonverbal intelligent quotient score (van den Dries et al., 2020) or an increased risk of attention-deficit hyperactivity disorder (Stephanie et al., 2018), as well as adverse metabolic outcomes such as obesity (Harley et al., 2017). Several epidemiological studies have investigated the association of phthalate exposure with gestational thyroid function, showing that higher phthalate exposure is associated with lower maternal free thyroxine (FT4) and total thyroxine (TT4) concentrations (Romano et al., 2018; Villanger et al., 2020; Yao et al., 2016; Huang et al., 2016; Kuo et al., 2015; Johns et al., 2015), and either lower or higher triiodothyronine (T3) concentrations (Villanger et al., 2020; Johns et al., 2015). However, it is difficult to relate these findings to observations from experimental studies, due to a lack of data on the full spectrum of thyroid measurements or a small sample size. The aim of this study is to fill this gap by investigating associations of maternal phthalate exposure with a wide spectrum of maternal thyroid function measurements during early pregnancy.

2. Methods

2.1. Study population

This study was embedded within the Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) study, a population-based prospective pregnancy cohort. SELMA was established to investigate the effects of early life exposure to environmental toxicants, in particular potential endocrine disrupting chemicals, on the mothers health, pregnancy outcomes and child health and development (Bornehag et al., 2012). Pregnant women were enrolled at median gestational week of 10 (with 95% of the women recruited before week 14) in the county of Värmland (Sweden) between September 2007 and March 2010. Participating families gave written consent for collection of blood and urine samples and participation in the SELMA study. The SELMA study has been approved by the regional ethical committee, Uppsala, Sweden (2007-05-02, Dnr: 2007/062) (Bornehag et al., 2012). For the current study, we included all women with data on the exposures and/or outcomes and excluded those with pre-existing thyroid disease or who used thyroid medications.

2.2. Maternal thyroid function measurements

Maternal blood samples were obtained and centrifuged during the first prenatal visit at the antenatal care centers. Serum samples were frozen at $-80°$ Celsius and stored in a biobank at the Central Hospital in Karlstad. Serum TSH, FT4, TT4, FT3, TT3, thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb) were measured using electrochemoluminescence assays (Cobas® e601; Roche Diagnostics, Mannheim, Germany) at the Department of Clinical Chemistry, Máxima Medical Center (Veldhoven, The Netherlands). Between-run coefficients of variation were 2.1%, 3.5%, 3.8%, 3.8%, and 7.7% for TSH, FT4, TT4, FT3 and TT3, respectively. TPOAb positivity and TgAb positivity were defined as TPOAb > 34 IU/ml or TgAb > 115 IU/l (manufacturer cutoffs), and the coefficients of variation were 12.4% and 7.1% for TPOAb at 33 or 100 IU/l, respectively, 10.9% and 8.6% for TgAb at 76 and 218 IU/l, respectively. The reference ranges of TSH and thyroid hormones in the SELMA study are reported previously (Derakhshan et al., 2018).

We defined various outcomes to cover several aspects of the thyroid system. First, we studied absolute serum concentrations of TSH, FT4, TT4, FT3 and TT3. Second, thyroid autoimmunity, as reflected by

TPOAb and/or TgAb positivity, was studied based on experimental data (Duan et al., 2019; Duan et al., 2018; Wu et al., 2017). Third, the ratios of FT4/FT3 or TT4/TT3 were calculated as a marker of peripheral T4 metabolism by deiodinase enzymes (Liu et al., 2015; Zhai et al., 2014). Fourth, the TSH/FT4 ratio was calculated as a proxy of the negative feedback system at the level of the pituitary (Dong et al., 2017; Jia et al., 2016; Rothacker et al., 2016). Finally, the TT4/FT4 or TT3/FT3 ratios were calculated as a marker of the binding of thyroid hormones to thyroid hormone binding proteins, mainly thyroxine binding globulin (Ishihara et al., 2003; Zhai et al., 2014).

2.3. Analyses of phthalates in urine

At the first prenatal visit, morning urine samples were collected and stored at -20 °C and subsequently analyzed for 14 phthalate metabolites at the Laboratory of Occupational and Environmental Medicine at Lund University, Lund, Sweden using a method presented by Gyllenhammar et al. (Gyllenhammar et al., 2017) using liquid chromatography - hybrid triple quadrupole linear ion trap mass spectrometry (QTRAP 5500, AB Sciex, Framingham, MA, USA ; LC-MS/MS). The 14 measured phthalate metabolites per parent compound are listed in Supplemental Table 1. Briefly, aliquots of 0.2 mL of urine were incubated at 37 °C for 30 min with β-glucoronidase (Escherichia coli). Labeled internal standards for all analyzed compounds were used. In-house prepared quality control samples and chemical blank samples were analyzed within each sampling batch. The samples were analyzed in a randomized order. Creatinine concentrations were determined using an enzymatic method, as described by Mazzachi et al (Mazzachi et al., 2000), and all samples were corrected for urine dilution by creatinine adjustment. The laboratory participates in the HBM4EU QA/QC programme, and has qualified as HBM4EU laboratory for the analysis of: MBzP, MEHP, MEHHP, MEOHP, MECPP, MCiOP, MOiNP, MHiNP. The LOD for all phthalate metabolites ranged from 0.01 to 0.1 ng/mL.

All measured values were reported and used in these analyses. We calculated the weighted molar sum of DEHP, DINP and DIDP metabolites (each group separately) by the following formula: ((metabolite 1 concentration in µg/g creatinine)÷(molecular weight of metabolite 1 in g/mol)) + ((metabolite 2 concentration in µg/g creatinine)÷(molecular weight of metabolite 2 in g/mol)) + etc. (expressed as µmol/g).

2.4. Analysis of cotinine in serum

Cotinine, a biomarker of tobacco smoke exposure, was analyzed in serum using LC-MS/MS (Dürr et al., 2015). Briefly, aliquots of 0.1 mL serum were added with labelled internal standard and precipitated using acetonitrile. The analyses of cotinine are part of the Round Robin intercomparison program with results within the tolerance limits.

2.5. Covariates

Maternal ethnicity, education level and height were assessed using questionnaires. Data on weight and height were used to calculate BMI (kg/length^2) and were ascertained from the Swedish National Birth Register. Serum cotinine levels with the following cut-offs, below 0.2 ng/mL, 0.2–15 ng/mL or higher than 15 ng/mL, were used to categorize participants as non-smoker, passive smoker or active smoker, respectively.

2.6. Statistical analysis

TSH and phthalates concentrations were natural log-transformed because of outlier effects and to deal with skewed model. We used Spearman's correlation coefficients to assess the correlations between urinary phthalates concentrations (adjusted for urinary creatinine). We utilized multivariable linear regression, and restricted cubic splines with three knots to assess nonlinearity, to study the association of urinary

phthalate metabolites as well as the molar sums (with a separate model for each) with the thyroid system outcomes. All analyses were adjusted for the following potential confounders which were selected a priori according to the directed acyclic graph (Textor et al., 2016) presented in the Supplemental Fig. 1: maternal age, BMI, parity, smoking status (according to serum cotinine concentrations), education level, ethnicity, gestational age at the time of blood sampling, TPOAbs and TgAbs. Urinary phthalate concentrations were standardized for urinary creatinine and urinary creatinine was included as a covariate in all models, to reach an optimal adjustment for urinary dilution (O'Brien et al., 2016).

We utilized multiple imputation by chained equations to impute missing data of covariates, pooling 25 imputed datasets for analyses (Buuren and Groothuis-Oudshoorn, 2011). All statistical analyses were performed using R statistical software version 3.6.1 (packages *mice*, *rms* and *corrplot*; https://www.r-project.org/).

3. Results

The study population comprised 1,996 women (Fig. 1) with a mean (SD) age of 30.9 (4.9) years and median (95% range) gestational age of 10 (6–14) weeks (Table 1). Creatinine adjusted urinary concentrations and percentage > LOD of the 14 measured phthalate metabolites are reported in Supplemental Table 2. The Spearman correlation coefficients between phthalate metabolites ranged from −0.01 to 0.98 (Fig. 2).

3.1. Phthalate metabolites and maternal TSH, thyroid hormones and thyroid autoimmunity

There was no association of phthalate metabolites with TSH (Table 2). A higher concentration of MEP or DEHP metabolites (MEHHP, MEOHP and MECPP) was associated with a lower FT4 (Table 2). In addition, there was a L-shaped association of MBP with FT4 (Table 2, Fig. 3). A higher MECPP, MHiNP, MCiOP, MHiDP or MCiNP concentration was associated with a lower TT4 (Table 2). Furthermore, there was an inverted U-shaped association of all DEHP metabolites (individually) with FT3 (Table 2, Fig. 4 and Supplemental Fig. 2), but there was no association of other phthalate metabolites with FT3. While a higher MBP, MBzP, and MOiNCH (DINCH metabolite) were associated with a higher TT3, a higher MECPP was associated with a lower TT3 (Table 2). There was no association of phthalates with thyroid autoimmunity (Supplemental Table 3).

Table 1
Characteristics of the study population of 1,996 pregnant women in the SELMA study.

Characteristics	N = 1,996
Thyroid-stimulating hormone (mU/L)	1.30 (0.11–4.13)
Free thyroxine (pmol/L)	15.0 (11.4–19.5)
Total thyroxine (nmol/L)	118 (81–166)
Free triiodothyronine (pmol/L)	4.67 (3.72–5.96)
Total triiodothyronine (nmol/L)	1.93 (1.27–2.90)
Thyroid peroxidase antibodies (IU/mL)	12.2 (6.3–246)
Thyroglobulin antibodies (IU/mL)	10.9 (10–413)
Urinary creatinine (g/L)	1.09 (0.37–2.47)
Gestational age (weeks)	10 (6–14)
Age (years)	30.9 (4.9)
BMI (kg/m2)	24.8 (4.5)
Parity, n (%)	
0	894 (45)
1	713 (36)
≥2	389 (19)
Ethnicity, n (%)	
Western	1,935 (97)
Non-Western	61 (3)
Serum cotinine, n (%)	
Non-smoker: <0.2 ng/mL	1,699 (85.1)
Passive smoker: 0.2–15 ng/mL	117 (5.9)
Active smoker: >15 ng/mL	180 (9)
Education level, n (%)	
Low	83 (4)
Medium	724 (36)
High	1,189 (60)

Data are median (95% range), mean (SD) or number (percentage) as appropriate.

3.2. Phthalate metabolites and thyroid hormone metabolism

A higher MBP concentration was associated with a lower FT4/FT3 ratio up to the concentrations of around 80 µg/g (L-shaped, Fig. 3) and a lower TT4/TT3 ratio (Table 3). In addition, a higher MBzP concentration was associated with a lower FT4/FT3 and TT4/TT3 ratio (Table 3). Higher MHiNP and MOiNP (both metabolites of DINP) were associated with a lower TT4/TT3 ratio (Table 3). The same association was seen for a higher MOiNCH, a DINCH metabolite (Table 3). There was no association of other phthalate metabolites with FT4/FT3 or TT4/TT3 ratios.

3.3. Phthalate metabolites and the ratio of TSH/FT4, TT4/FT4 and TT3/FT3

A higher MEHHP, MEOHP and MECPP as well as DEHP were associated with a higher TSH/FT4 ratio (Table 4). There was no association of other phthalates with the TSH/FT4 ratio. A higher MBP and MBzP was associated with both a higher TT4/FT4 and a higher TT3/FT3 ratio (Table 4). Moreover, a higher MCiOP and MCiNP was associated with a higher TT4/FT4 ratio, while a higher MECPP and MOiNCH was associated with a lower TT3/FT3 ratio (Table 4).

4. Discussion

The consistency and patterns of the association of phthalate metabolites with markers of the thyroid system in the current study provide evidence for thyroid system disruptive effects of exposure to particularly DEHP, but also DINP, DINCH, DBP and BBzP metabolites during early pregnancy. Higher exposure to all DEHP metabolites was associated with lower absolute FT4 and FT3 concentrations while higher MBP and MBzP were associated with a higher TT3 and a lower free or total T4/T3 ratio. A similar pattern was identified for the DINCH metabolite MOiNCH, higher concentrations of which was associated with a higher TT3 and a lower TT4/TT3 ratio.

Phthalates are diesters of phthalic acid and their individual characteristics depend on the length of the side branches. Phthalates that are
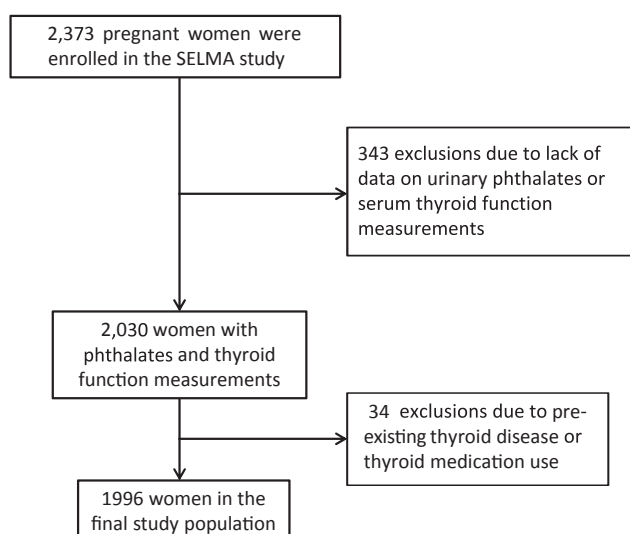

Fig. 1. Flowchart of the study population.

2,373 pregnant women were enrolled in the SELMA study

343 exclusions due to lack of data on urinary phthalates or serum thyroid function measurements

2,030 women with phthalates and thyroid function measurements

34 exclusions due to pre-existing thyroid disease or thyroid medication use

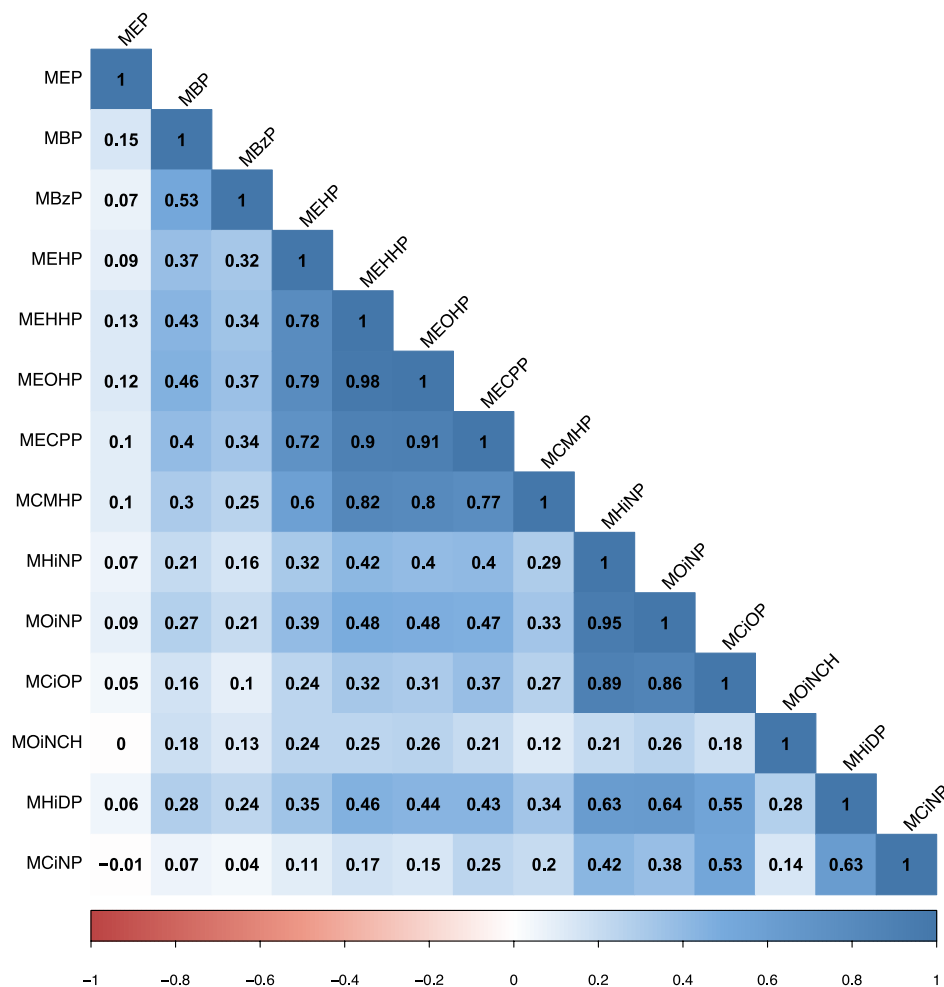1996 women in the final study population

Fig. 2. Spearman correlation coefficients between phthalate metabolites.

short-branched (such as DEP and DBP) and commonly used in cosmetic products, have a low molecular weight and are metabolized into a single isoform. Long-branched high molecular weight phthalates such as DEHP and DINP are used in a variety of plastic products but can be metabolized into several isoforms (Frederiksen, Skakkebaek, and Andersson, 2007). The use of several phthalates, such as DEHP, DBP and BBzP had been restricted in the European Union (Ec, 2018). Nonetheless, widespread use in other parts of the world as well as the long life-cycle of plastic products and recycling of these plastics validate studies on their potential harmful effects (Ionas et al., 2014; Pivnenko et al., 2016).

Although originating from different parent compounds, we identified consistent results for both MBP and MBzP, metabolites of DBP and BBzP, respectively. Higher exposure to both was associated with a higher TT3, a lower FT4/FT3 and TT4/TT3 ratio, and a higher TT4/FT4 and TT3/FT3 ratio. In vitro studies have identified several thyroid disruption capabilities of high levels ($>10^{-6}$ M) of MBP and MBzP including thyroid receptor (TR) antagonist activity (Sugiyama et al., 2005; Shen et al., 2009), down-regulation of TRβ and TRα gene expression (Shimada and Yamauchi, 2004; Sugiyama et al., 2005) and down-regulation of sodium/iodide symporter (NIS) gene promoter (Breous et al., 2005). Despite these experimental data, we did not find any association of MBP or MBzP with TSH or TSH/FT4 ratio that could reflect any interference with TRs and/or the HPT-axis. Our results showing a higher TT3 and lower T4/T3 ratios are more indicative of an increased T4 metabolism. Interestingly, we also identified that exposure to MBP and MBzP was associated with a higher TT4/FT4 and TT3/FT3 ratio. These outcomes reflect an increase in thyroid hormone binding protein concentrations and saturation which could be affected through

endocrine disruption effects on estrogen receptor activity (Ghisari and Bonefeld-Jorgensen, 2009) and increased β-hCG production (Adibi et al., 2017). It is difficult to extract further evidence for these working mechanisms from other studies in pregnant women as results have been heterogeneous, reporting associations of phthalates with either TSH (Villanger et al., 2020), FT4 or FT3 (Souter et al., 2020; Johns et al., 2016), FT4 and TT4 (Yao et al., 2016; Huang et al., 2016) or no association at all (Huang et al., 2018; Romano et al., 2018; Johns et al., 2015) and the majority of studies lacked data that reflects important components of the thyroid system. Interestingly, in our study, the association of MBP with FT4 and the FT4/FT3 ratio was L-shaped, meaning that up to a certain concentration (~80 μg/g) a higher MBP was associated with a lower FT4 or FT4/FT3 ratio after which the association seemed to plateau. Despite the fact that endocrine disrupting chemicals can have non-monotonic and low-dose effects on the endocrine system (Gore et al., 2015; Vandenberg et al., 2012), the potential for non-monotonic associations in humans, especially during pregnancy has not been investigated thoroughly. We can speculate that the underlying mechanism by which MBP affects FT4 concentrations and thus also the FT4/FT3 ratio could be due to TR antagonist activity or increased T4 metabolism, mechanisms that could get saturated or become neutralized by downstream homeostasis mechanisms. An overview of urinary phthalates concentrations in other pregnant populations shows a diverse pattern of exposure based on the time period and location (Supplemental Table 4) which can contribute to the heterogeneity within studies.

In the current study we identified a negative association of DEHP metabolites with FT4, FT3 and TT4 concentrations, which is in line with

Table 2
The association of phthalate metabolites with thyroid function.

Parent Phthalate Compound	Phthalate Metabolites	TSH β (SE)	TSH P value	FT4 β (SE)	FT4 P value	TT4 β (SE)	TT4 P value	FT3 β (SE)	FT3 P value	TT3 β (SE)	TT3 P value
DEP	MEP	0.02 (0.02)	0.22	−0.08 (0.04)	0.03	−0.28 (0.46)	0.53	−0.004 (0.01)	0.75	−0.005 (0.008)	0.54
DBP	MBP	0.01 (0.02)	0.55	Non-linear	0.02	0.97 (0.76)	0.20	0.04 (0.02)	0.07	0.04 (0.01)	0.006
BBzP	MBzP	−0.01 (0.02)	0.63	−0.09 (0.05)	0.06	0.65 (0.52)	0.21	0.02 (0.01)	0.23	0.03 (0.01)	0.002
DEHP	Molar sum	0.04 (0.02)	0.09	−0.13 (0.06)	0.03	−1.04 (0.66)	0.11	Non-linear	0.01	−0.02 (0.01)	0.09
	MEHP	0.02 (0.02)	0.38	−0.08 (0.05)	0.11	−0.04 (0.57)	0.94	Non-linear	0.006	−0.006 (0.01)	0.57
	MEHHP	0.05 (0.02)	0.06	−0.12 (0.05)	0.03	−1.05 (0.61)	0.09	Non-linear	0.011	−0.01 (0.01)	0.13
	MEOHP	0.05 (0.02)	0.056	−0.15 (0.05)	0.006	−0.53 (0.61)	0.38	Non-linear	0.014	−0.01 (0.01)	0.42
	MECPP	0.05 (0.02)	0.08	−0.13 (0.06)	0.02	−1.74 (0.66)	0.008	Non-linear	0.013	−0.03 (0.01)	0.02
	MCMHP	0.03 (0.02)	0.26	−0.11 (0.06)	0.06	−0.52 (0.66)	0.43	Non-linear	0.036	−0.02 (0.01)	0.09
DINP	Molar sum	−0.02 (0.01)	0.28	−0.03 (0.04)	0.47	−0.93 (0.44)	0.03	0.007 (0.01)	0.53	−0.002 (0.008)	0.73
	MHiNP	−0.01 (0.02)	0.49	−0.03 (0.03)	0.42	−0.77 (0.38)	0.04	0.006 (0.01)	0.54	−0.002 (0.007)	0.78
	MOiNP	−0.02 (0.02)	0.33	−0.06 (0.04)	0.14	−0.46 (0.44)	0.29	0.002 (0.01)	0.83	0.004 (0.008)	0.56
	MCiOP	−0.02 (0.02)	0.20	−0.01 (0.04)	0.74	−1.13 (0.47)	0.01	0.009 (0.01)	0.45	−0.006 (0.009)	0.44
DINCH	MOiNCH	−0.006 (0.01)	0.67	−0.02 (0.03)	0.50	0.03 (0.36)	0.93	0.005 (0.009)	0.58	0.01 (0.007)	0.03
DIDP	Molar sum	0.01 (0.02)	0.56	−0.04 (0.05)	0.47	−1.31 (0.57)	0.02	−0.001 (0.01)	0.91	−0.01 (0.01)	0.39
	MHiDP	0.01 (0.02)	0.44	−0.04 (0.04)	0.31	−1.07 (0.51)	0.03	0.007 (0.01)	0.57	−0.002 (0.01)	0.84
	MCiNP	0.002 (0.02)	0.91	−0.008 (0.05)	0.88	−1.37 (0.59)	0.02	−0.02 (0.01)	0.19	−0.02 (0.01)	0.08

Betas (SE) are calculated with a multivariable linear regression for each phthalate metabolite and the molar sums separately, adjusted for gestational age at the time of sampling, maternal age, urinary creatinine, smoking status (according to serum cotinine), body mass index, education, ethnicity, parity, thyroid peroxidase antibodies and thyroglobulin antibodies.

most (Johns et al., 2015; Villanger et al., 2020; Souter et al., 2020; Huang et al., 2018; Yao et al., 2016; Huang et al., 2016), but not all (Romano et al., 2018; Huang et al., 2016; Kuo et al., 2015) other studies in pregnant women. We have also identified that DEHP exposure is associated with a higher TSH/FT4 ratio, a marker of the HPT-axis feedback system. This confers with results from experimental studies, that show that DEHP upregulates thyrotropin-releasing hormone receptor expression (Sun et al., 2018; Dong et al., 2017; Liu et al., 2015), downregulates TSH receptor expression in the thyroid gland (Sun et al., 2018; Dong et al., 2017), and antagonizes TRs (Ghisari and Bonefeld-Jorgensen, 2009; Sugiyama et al., 2005; Shen et al., 2009) or limits their expression (Yu et al., 2018; Liu et al., 2015). On the other hand, we could not translate any of the experimental evidence of DEHP induced deiodinase type 2 and

3 (D2 and D3) gene expression (Liu et al., 2015; Jia et al., 2016). Epidemiological studies show that both lower maternal thyroid hormone availability as well as prenatal exposure to DEHP is associated with lower non-verbal IQ or a higher risk of developing Attention-Deficit Hyperactivity Disorder (van den Dries et al., 2020; Stephanie et al., 2018). Another interesting finding of our study is the inverted U-shaped association of DEHP metabolites with FT3. Currently, there is not enough experimental data available to enable adequate interpretation of these findings and further replication in human data is required. However, in general, non-monotonic and low-dose effects of endocrine disrupting chemicals on various endocrine systems have been found in experimental studies which is in line with the physiology of how hormones interact with their receptors (Gore et al., 2015; Vandenberg et al., 2012).
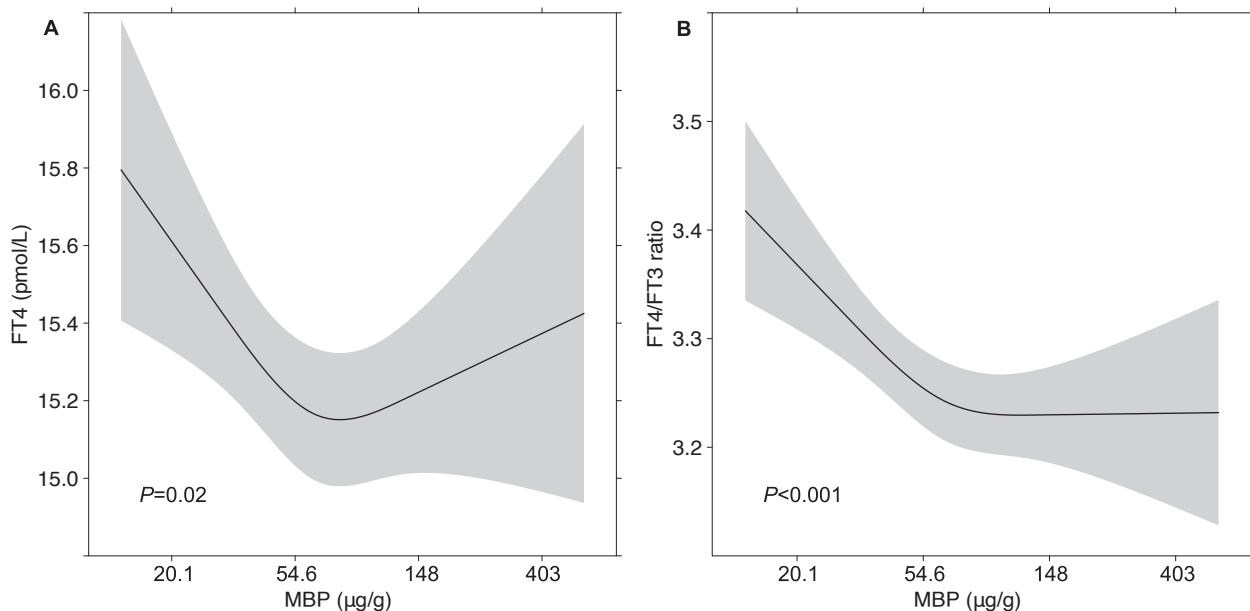

Fig. 3. Association of mono-butyl phthalate (MBP) with FT4 and FT4/FT3 ratio. Figure shows the association of maternal urinary concentrations of MBP with serum concentrations of FT4 (A) and FT4/FT3 ratio (B), based on multivariable linear regression models adjusted for gestational age at the time of sampling, maternal age, thyroid peroxidase antibodies, thyroglobulin antibodies, urinary creatinine, smoking status (according to serum cotinine), body mass index, education, ethnicity and parity.
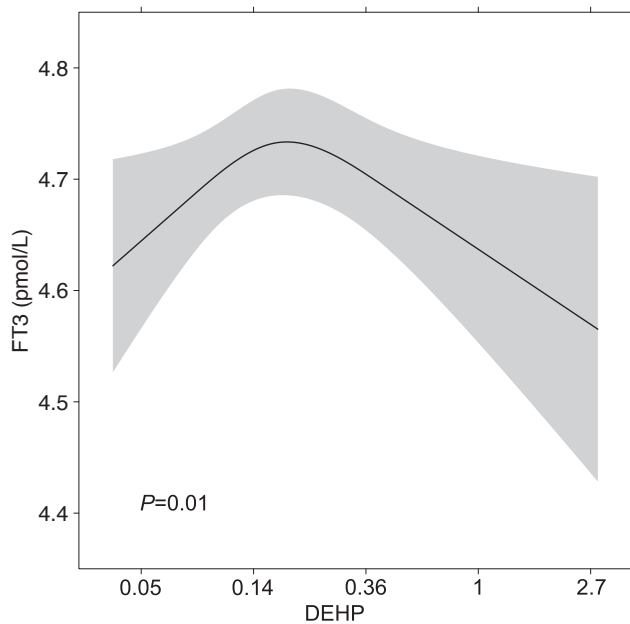
Fig. 4. Association of the molar sum of Di-ethyl-hexyl phthalate (DEHP) metabolites with FT3. Figure shows the association of the molar sum of maternal urinary concentrations of Di-ethyl-hexyl phthalate (DEHP) metabolites with serum concentrations of FT3, based on a multivariable linear regression model adjusted for gestational age at the time of sampling, maternal age, thyroid peroxidase antibodies, thyroglobulin antibodies, urinary creatinine, smoking status (according to serum cotinine), body mass index, education, ethnicity and parity.

Interestingly, in a study in plastic recycling workers, there was an inverted U-shaped association of MEHHP, MEHP and monomethyl phthalate with total T3 (Wang et al., 2018). Further studies are required to investigate potential non-monotonic association of phthalates with thyroid function in pregnant women and general population.

DINP and DIDP are usually used as substitutes for DEHP, but we could only identify one human study on DINP (Villanger et al., 2020) and three experimental studies on the thyroid system disruption properties of these compounds. In experimental studies, thyroid system disruption activities of DINP and DIDP (at high concentrations between 10-3 and 10-4 M) include an increased iodine uptake (Wenzel et al., 2005), upregulation of NIS gene expression (Breous, Wenzel, and Loos, 2005) and aggravation of thyroid autoimmunity in rats (DINP only) (Duan et al., 2019). We showed that DINP exposure is associated with a lower TT4 and consequently also a lower TT4/TT3 and TT4/FT4 ratio, a combination which likely reflects alterations in T4-specific binding to thyroid hormone binding proteins. On the other hand, DIDP was associated with a lower TT4, but not with any other outcomes indicating this is most likely a spurious finding. The availability of the full range of clinical thyroid function measurements not only allows for studying various components of the thyroid system, but also allows for more critical interpretation of potential thyroid disrupting effects. The results on DINP and DIDP in the current study can generate hypotheses and feed into experimental studies to further elucidate the potential thyroid disrupting effects of these compounds.

DINCH is a more recent phthalate substitute (Demeneix and Slama, 2019), but studies have shown that exposure to DINCH or its metabolites can result in the induction of hepatic enzymes and thyroid hyperplasia (Mughal et al., 2018; Campioli et al., 2017). To the best of our knowledge, no other study has yet investigated the association of DINCH with thyroid function in humans. In the current study, DINCH exposure was associated with a higher TT3 and consequently, a higher TT3/FT3 but a lower TT4/TT3 ratio, which is indicative of effects on peripheral thyroid hormone metabolism.

Table 3
The association of phthalate metabolites with T4 to T3 ratios.

Parent Phthalate Compound	Phthalate Metabolites	FT4/FT3 ratio		TT4/TT3 ratio	
		β (SE)	P value	β (SE)	P value
DEP	MEP	−0.01 (0.009)	0.10	−0.02 (0.20)	0.91
DBP	MBP	−0.05 (0.01)	<0.001	−0.82 (0.34)	0.01
BBzP	MBzP	−0.03 (0.01)	0.002	−0.56 (0.34)	0.01
DEHP	Molar sum	−0.02 (0.01)	0.10	0.14 (0.29)	0.61
	MEHP	−0.01 (0.01)	0.27	0.19 (0.26)	0.47
	MEHHP	−0.02 (0.01)	0.06	0.03 (0.27)	0.89
	MEOHP	−0.02 (0.01)	0.06	0.04 (0.27)	0.88
	MECPP	−0.02 (0.009)	0.05	0.003 (0.29)	0.99
	MCMHP	−0.007 (0.01)	0.59	0.47 (0.29)	0.11
DINP	Molar sum	−0.01 (0.009)	0.26	−0.41 (0.20)	0.04
	MHiNP	−0.009 (0.007)	0.24	−0.35 (0.17)	0.04
	MOiNP	−0.01 (0.008)	0.14	−0.40 (0.20)	0.04
	MCiOP	−0.008 (0.009)	0.35	−0.41 (0.21)	0.05
DINCH	MOiNCH	−0.007 (0.007)	0.28	−0.44 (0.16)	0.006
DIDP	Molar sum	−0.003 (0.01)	0.74	−0.30 (0.26)	0.24
	MHiDP	−0.01 (0.01)	0.16	−0.41 (0.23)	0.07
	MCiNP	0.01 (0.01)	0.18	−0.04 (0.26)	0.85

Betas (SE) are calculated with a multivariable linear regression for each phthalate metabolite and the molar sums separately, adjusted for gestational age at the time of sampling, maternal age, urinary creatinine, smoking status (according to serum cotinine), body mass index, education, ethnicity, parity, thyroid peroxidase antibodies and thyroglobulin antibodies.

In the current study, MEP exposure was associated with lower FT4. While our study replicates previous studies in pregnant women showing that MEP is only poorly correlated with other phthalate metabolites (Villanger et al., 2020; Souter et al., 2020; Romano et al., 2018), the association with thyroid function remains to be determined. Some studies identified a negative association with TT4 (Romano et al., 2018), a positive association with TT3 and the TT3/TT4 ratio (Johns et al., 2016) or no association with thyroid function at all (Villanger et al., 2020; Yao et al., 2016; Huang et al., 2018; Huang et al., 2016; Kuo et al., 2015; Johns et al., 2015). Experimental data on MEP (or its parent compound DEP) has mainly focused on reproductive outcomes and only little endocrine disruptor activity was identified, probably due to the different position and smaller length of the carbon chain of its diester portion compared to other phthalates (Witorsch and Thomas, 2010). We could not identify experimental studies on the thyroid system disrupting potential of DEP (MEP), but the heterogeneous results from human studies in pregnant women cannot provide a meaningful direction for further experimental work on these compounds.

We did not find any association of phthalates with thyroid antibodies. We tested this hypothesis based on the results of two studies in rats showing the aggravation or exacerbation of autoimmune thyroid disease after exposure to DINP or DBP (Duan et al., 2019; Wu et al., 2017). A previous study in a cohort of women visiting a fertility center as well as studies in general populations also did not find any association of exposure to phthalates with TPOAb or TgAb concentrations (Souter et al., 2019; Choi et al., 2020). Human or experimental studies are still scares

Table 4

The association of phthalate metabolites with maternal TSH/FT4, total T4/FT4 and total T3/FT3 ratios.

Parent Phthalate Compound	Phthalate Metabolites	TSH/FT4 ratio		TT4/FT4 ratio		TT3/FT3 ratio	
		β (SE)	*P* value	β (SE)	*P* value	β (SE)	*P* value
DEP	MEP	0.001 (0.001)	0.08	0.01 (0.02)	0.60	−0.001 (0.001)	0.44
DBP	MBP	0.001 (0.001)	0.35	0.13 (0.04)	0.002	0.005 (0.002)	0.03
BBzP	MBzP	0.00002 (0.001)	0.98	0.09 (0.03)	0.001	0.004 (0.001)	0.006
DEHP	Molar sum	0.003 (0.001)	0.03	−0.001 (0.03)	0.97	−0.003 (0.002)	0.10
	MEHP	0.002 (0.001)	0.11	0.04 (0.03)	0.22	−0.0007 (0.001)	0.72
	MEHHP	0.003 (0.001)	0.02	−0.002 (0.03)	0.94	−0.003 (0.002)	0.12
	MEOHP	0.003 (0.001)	0.01	0.04 (0.03)	0.17	−0.0005 (0.002)	0.80
	MECPP	0.0033 (0.001)	0.02	−0.05 (0.03)	0.15	−0.005 (0.002)	0.007
	MCMHP	0.002 (0.001)	0.10	0.02 (0.04)	0.55	−0.002 (0.002)	0.21
DINP	Molar sum	−0.0007 (0.001)	0.49	−0.05 (0.02)	0.02	−0.001 (0.001)	0.25
	MHiNP	−0.0004 (0.001)	0.63	−0.04 (0.02)	0.06	−0.001 (0.001)	0.42
	MOiNP	−0.0005 (0.001)	0.61	−0.009 (0.02)	0.71	0.0004 (0.001)	0.77
	MCiOP	−0.001 (0.001)	0.42	−0.08 (0.03)	0.002	−0.002 (0.002)	0.07
DINCH	MOiNCH	−0.0005 (0.0009)	0.53	0.02 (0.02)	0.31	0.003 (0.001)	0.01
DIDP	Molar sum	0.0003 (0.001)	0.80	−0.05 (0.03)	0.10	−0.001 (0.001)	0.58
	MHiDP	0.0006 (0.001)	0.62	−0.03 (0.03)	0.32	−0.0004 (0.002)	0.81
	MCiNP	−0.0003 (0.001)	0.82	−0.08 (0.03)	0.01	−0.002 (0.002)	0.33

Betas (SE) are calculated with a multivariable linear regression for each phthalate metabolite and the molar sums separately, adjusted for gestational age at the time of sampling, maternal age, urinary creatinine, smoking status (according to serum cotinine), body mass index, education, ethnicity, parity, thyroid peroxidase antibodies and thyroglobulin antibodies.

on this subject and further investigations are required to have a better understanding on whether phthalates result in thyroid autoimmunity.

In this study, all associations for the separate phthalate metabolites originating from the same parent compound were in the same direction for the various outcomes. This has several implications for the interpretation of our data. First, calculation of total exposure to a parent compound by means of the molar sum of the various metabolites seems more valid, although this does not take into account differences in the rate of metabolism or binding affinities for the various metabolites. Second, our results are more likely to reflect the potential effects of the parent compound. Third, same direction of association of the metabolites of the same parent compound with the outcomes indicates that our findings per individual metabolites are less likely due to chance.

We have previously shown that higher maternal urinary bisphenol A (BPA) was associated with lower FT4/FT3 and TT4/TT3 ratios which is in line with findings of experimental studies on effects of BPA on deiodinase enzymes gene expressions (Derakhshan et al., 2019). Using the same translational approach in the current study, exposure to several phthalate metabolites, such as MBP, MBzP and MOiNCH was also associated with a lower ratio of FT4/FT3 and/or TT4/TT3. Establishing the individual associations of each chemical compound with the maternal thyroid system on the background of experimental evidence is required to provide the best possible basis for investigating potential additive effects of multiple compounds as mixture effects.

In our effort to translate findings from experimental studies on phthalate exposure to data from pregnant women, we were able to analyze a large prospective dataset including a wide range of thyroid function tests. A potential limitation of our study is the cross-sectional nature of our data, since repeated measurements of urinary phthalates would provide a more accurate measure of overall exposure considering the short half-life of phthalates and also allow for studying more long-term effects of phthalates on the maternal thyroid system. Further studies with a longitudinal design and repeated measurements of exposures and thyroid function are required to replicate our findings. Another limitation of this study is that we did not have measurements of albumin, thyroid binding globulin or transthyretin to study the potential underlying mechanisms of changes in thyroid measurements in these pregnant women.

In conclusion, this study provides new evidence on the effects of phthalate exposure during early pregnancy on the thyroid system and its possible underlying mechanisms. Although further human studies should be performed to replicate our findings, also additional experimental studies are needed to replicate these results and to further investigate underlying mechanisms and the effects of thyroid system interference on the developing fetus.

CRediT authorship contribution statement

Arash Derakhshan: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Huan Shu:** Methodology, Resources, Data curation, Writing - review & editing. **Maarten A.C. Broeren:** Methodology, Resources, Data curation, Writing - review & editing. **Christian H. Lindh:** Methodology, Resources, Data curation, Writing - review & editing. **Robin P. Peeters:** Project administration, Supervision, Conceptualization, Methodology, Resources, Writing - review & editing, Funding acquisition. **Andreas Kortenkamp:** Project administration, Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **Barbara Demeneix:** Project administration, Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **Carl-Gustaf Bornehag:** Project administration, Conceptualization, Methodology, Writing - review & editing, Funding acquisition. **Tim I.M. Korevaar:** Supervision, Conceptualization, Methodology, Investigation, Writing - original draft, Writing - review & editing, Visualization, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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