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Mediation by hormone concentrations on the associations between repeated measures of phthalate mixture exposure and timing of delivery

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Abstract

Background: Phthalates are used in manufacturing of consumer products, resulting in ubiquitous human exposure to phthalate mixtures. Previous work has suggested that phthalates display endocrine disrupting capabilities, and exposure is associated with early delivery.

Objective: To assess mediating effects of hormone concentrations on associations between phthalate mixtures and preterm birth (PTB).

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Author contributions

A. Cathey: formal analysis, investigation, methodology, visualization, writing - original draft, writing - review and editing; M. Aung: methodology, writing - review and editing; D. Watkins: supervision, writing - review and editing; Z. Rosario: data curation, resources; C. Vélez-Vega: funding acquisition; A. Alshawabkeh: funding acquisition, project administration; J. Cordero: funding acquisition; B. Mukherjee: conceptualization, methodology, supervision, writing - review and editing; J. Meeker: conceptualization, funding acquisition, project administration, supervision, writing - review and editing.

Competing Interests

The authors declare no conflicts of interest.

Data Sharing

The data dictionary for all variables used in this analysis is publicly available and can be found here: https://arecibo.ece.neu.edu/dictionary/. Any data requested will be made available by contacting the authors, who will forward to request to the PROTECT DMAC database manager. The request will be reviewed with the data owners, who will provide an export of the requested data after approval. All data from the database will be deidentified, individual-level, participant data. All data is available now and will remain available until at least January 1, 2025. Any data requester must agree to acknowledge the NIEHS P42 Center grant that supported the collection of this data. Additional files, including analysis code, will also be provided by contacting the first and corresponding authors.

Methods: Repeated urinary phthalates and serum hormones were measured among 1011 women in the PROTECT Puerto Rico birth cohort from 2011–2019. We utilized ridge regression to create phthalate environmental risk scores (ERS), which represent weighted summaries of total phthalate exposure. Mediation analyses were conducted on a subset of 705 women. We additionally conducted fetal sex-specific analyses.

Results: Free thyroxine (FT4) mediated 9.6% of the association between high molecular weight (HMW) ERS at 18 weeks and reduced gestational age at delivery (95%CI:1.07–29.9). Progesterone at 26 weeks mediated 21.1% and 16.2% of the association between HMW ERS at 18 and 22 weeks, and spontaneous PTB, respectively. Among male fetuses, corticotropin releasing hormone (CRH) at 18 weeks mediated 28.2% of the association between low molecular weight ERS and spontaneous PTB.

Significance: We provide introductory evidence of hormone disruption on the causal pathway between phthalate exposure and early delivery. We also show differences by fetal sex, but a larger sample size is necessary to validate our findings.

INTRODUCTION

Humans are exposed to a myriad of environmental contaminants from diverse sources on a daily basis. The result is a consistent body burden of a mixture of many different toxicants which have multiple downstream effects on human physiology, largely through endocrine disrupting pathways. Many epidemiology and toxicology studies have explored health effects of single pollutants, but very few have attempted to understand the biological effects of complex mixtures. Pregnant women are especially susceptible to adverse health outcomes resulting from environmental exposures, particularly those with endocrine disrupting capabilities. Hormone concentrations through pregnancy are important for proper fetal development, maintenance of the uterine wall, and initiation of pro-labor events[1–4]. Understanding how exposures to environmental chemical mixtures may interfere with hormone regulation in pregnant women is critically important for protection of this vulnerable population.

Phthalates are synthetic plasticizers used in production of many consumer products such as vinyl flooring, plastic food packaging, and personal care products[5]. Humans are never exposed to single phthalate compounds; exposure rather occurs in complex mixtures which differ based on an individual's use of consumer products, socioeconomic status, and diet[6]. Each parent phthalate compound is metabolized within the body, and sometimes several different metabolites result from one parent compound[7], furthering the need to study mixtures of phthalates rather than individual metabolites. Phthalate metabolites are often highly correlated with one another, and so methods which accommodate issues of multicollinearity are preferred over those which assess associations with many individual metabolites.

Previous research has shown phthalate metabolites to be associated with preterm and spontaneous preterm birth (PTB), as well as earlier gestational age at delivery[8–11]. Phthalates are also known endocrine disruptors, and greater exposures to phthalates have been associated with altered concentrations of various hormones that are important for

pregnancy such as corticotropin releasing hormone (CRH), estriol, progesterone, thyroid hormones, and testosterone[12–15]. Given the hormonal activity of phthalates and their association with early delivery, we have hypothesized that phthalate exposure may lead to adverse pregnancy outcomes via disruption of hormone concentrations throughout pregnancy.

To test this hypothesis, we utilize a novel data analysis pipeline which incorporates repeated measures of phthalate mixture exposure and hormone concentrations, in addition to causal mediation analyses. We use ridge regression to construct environmental risk scores (ERS), which are weighted sums of one's overall exposure to a mixture of phthalate metabolites, to assess exposure to high and low molecular weight phthalate mixtures at an individual level over multiple time points during gestation. ERS were then used in causal mediation analysis to determine the mediating effect of hormone concentrations on the associations between phthalate mixtures and adverse birth outcomes.

METHODS

Study Population

Data for the present study was obtained from the PROTECT (Puerto Rico Testsite for Exploring Contamination Threats) cohort, a longitudinal birth cohort in the northern karst region of Puerto Rico designed to investigate environmental contaminants in relation to adverse pregnancy outcomes. Details of the study design and recruitment protocols have been previously described[16]. Briefly, women were recruited at 14±2 weeks gestation and were eligible to participate if they were between the ages of 18 and 40 years, participated in their first clinic visit before their 20th week of pregnancy, had not taken oral contraceptives within 3 months of getting pregnant, had not used *in vitro* fertilization to get pregnant, and had no known preexisting medical or obstetric conditions. This study was approved by the research and ethics committees of the University of Michigan School of Public Health (IRB exemption #HUM00037064), University of Puerto Rico (IRB# A8570110), Northeastern University (IRB# 10-03-03a). All study participants provided full informed consent prior to participation.

Phthalate Exposure Assessment

Study participants provided urine samples at up to three time points during pregnancy: median 18, 22, and 26 weeks. All spot urine samples were frozen at −80°C and shipped over night on dry ice to the Centers for Disease Control (Atlanta, GA, USA) for analysis. All samples were analyzed for 13 phthalate metabolites: mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MBP), monobenzyl phthalate (MBzP), mono-isobutyl phthalate (MiBP), mono-hydroxyisobutyl phthalate (MHiBP), mono-3-carboxypropyl phthalate (MCPP), mono carboxyisononyl phthalate (MCNP), mono carboxyisooctyl phthalate (MCOP), and mono-hydroxybutyl phthalate (MHBP). Urine samples were analyzed using solid phase extraction high-performance liquid chromatography-isotope dilution tandem mass spectrometry, the details of which are described elsewhere[17]. Values detected below

the limit of detection (LOD) were assigned a value of the LOD divided by the square root of two[18].

Hormone Measurement

All women provided serum samples at their first and third clinic visits, aligning with median 18 (16–20) and 26 (24–28) weeks' gestation. Serum samples were analyzed at the Central Ligand Assay Satellite Services laboratory in the Department of Epidemiology at the University of Michigan School of Public Health. Progesterone, sex hormone-binding globulin (SHBG), testosterone, total triiodothyronine (T3), total thyroxine (T4), free thyroxine (fT4) and thyroid-stimulating hormone (TSH) were measured using a chemiluminescence immunoassay. Estriol (E3) and corticotropin releasing hormone (CRH) were measured using an enzyme immunoassay. Some hormone concentrations were not available for all participants due to sample volume limitations. The ratios of progesterone to estriol (Prog/E3) and T3 to T4 (T3/T4) were assessed in addition to measured hormones because of previous research indicating that the ratios may be better indices of adverse pregnancy outcomes than single hormone measurements[19,20]. All hormone concentrations below the limit of detection (LOD) were replaced by the LOD divided by the square root of two.

Birth Outcome Assessment

Self-reported date of the last menstrual period was collected at the first study visit and used in combination with early ultrasound measurements to determine gestational age at birth, based on recommendations from the American College of Obstetricians and Gynecologists[21]. We abstracted gestational age at birth from medical records postpartum, and PTB was defined as delivery before 37 weeks' gestation. We also assessed spontaneous PTB, defined as PTB presenting with premature rupture of membranes, spontaneous preterm labor, or both[22].

Calculation of Phthalate ERS

A schematic of our analytical pipeline is depicted in Supplementary Figure 1. Study participant's exposures to mixtures of phthalates were estimated utilizing ridge regression to calculate environmental risk scores (ERS)[23], which represent a weighted sum of each individual's overall phthalate exposure profile based on individual phthalate associations with the outcome of interest. Ridge employs only one tuning parameter which shrinks the coefficients of unimportant predictors towards zero (but never to exact zero) and stabilizes selection in the presence of highly correlated predictors. Five-fold cross validation and optimization of prediction errors were used to estimate lambda. Ridge returns a vector of coefficients which represent the relative importance of each predictor for the outcome of interest. These coefficients were then multiplied by each study participant's measured phthalate metabolite concentrations, giving weighted concentrations of each metabolite. Weighted concentrations were then summed to arrive at the ERS. Effects of high versus low molecular weight phthalates were assessed by running ridge analysis on metabolite mixtures separated into high versus low molecular weight groups, and then constructing a high molecular weight (HMW) ERS and a low molecular weight (LMW) ERS.

Statistical Analyses

Distributions of demographic characteristics and other relevant health information were tabulated. Environmental risk scores were calculated for all women in the study sample for whom we had full exposure data and data on at least one birth outcome (N=1011). Mediation analyses were conducted on a subset of those women for whom we also had mediator data (N=705).

Ridge analysis and ERS calculation were conducted utilizing a cumulative average approach over up to 3 study visits. ERS at visit 1 were derived from only phthalate concentrations measured at study visit 1. ERS at visit 2 were derived using the geometric mean of phthalate concentrations at the first and second study visits, and ERS at visit 3 were derived using the geometric mean of phthalate concentrations measured at all 3 study visits. All analyses included categorical maternal age and maternal education as unpenalized covariates. We additionally conducted exploratory analyses stratified by fetal sex to investigate potential differences in hormone-mediated pathways unique to women who had a male or female fetus. All phthalate concentrations were corrected for specific gravity to account for differences in urinary dilution between study subjects. Ridge regression was conducted utilizing the *glmnet* package in R (version 3.5.1).

Causal Mediation Analyses—In the causal mediation framework, the relationship between exposures and outcomes can be framed in several ways. The mediated effect, also known as the natural indirect effect (NIE), is the change in outcome when the exposure is held constant and the mediator is changed to the level it would have been with an increase in exposure. The natural direct effect (NDE) corresponds to the change in the outcome in association with a change in exposure while keeping the mediator at the level it would have been at the original exposure level. Finally, the total effect (TE) corresponds to a change in the outcome associated with a change in exposure without any consideration or adjustment for the mediator. The TE is also equal to the sum of the NDE and NIE. We can then calculate the proportion of mediation by dividing the NIE by the TE. These effects are identified through two sets of counterfactuals for the outcome and mediator as a function of the "changed level of the exposure."

These effects can be estimated by fitting regression models to the observed data only if the following assumptions hold true: 1) there is no unmeasured confounding for the relationship between the exposure and outcome, 2) there is no unmeasured confounding for the relationship between the mediator and outcome, after controlling for the exposure, 3) there is no unmeasured confounding on the relationship between the exposure and the mediator, and 4) there is no downstream effect of the exposure which confounds the relationship between the mediator and the outcome. The temporal ordering assumption must also be met, such that the exposure precedes the mediator, which precedes the outcome. Causal diagrams depicting these relationships are shown in Supplementary Figure 2. If all of these assumptions are met, the following statistical models can be used to estimate mediating effects:

$$Model\,1: g[E(Y \mid a, m, c)] = \beta_{y0} + \beta_{ya}\overline{a_t} + \beta_{ym}m_t + \beta_{yc}^{T}c$$
$$Model\,2: E[M \mid a, c] = \beta_{m0} + \beta_{ma}\overline{a_t} + \beta_{mc}^{T}c$$

where g is *probit* for binary outcomes and *identity* for gestational age; E is the linear model for the mediator conditional on the exposure; $\overline{a_t}$ represents the phthalate ERS calculated from the cumulative average approach at study visit t, corrected for specific gravity; m_t represents the observed hormone concentrations at study visit t; c represents observed values of covariates which are constant over time; and Y represents the outcome. Resulting coefficients from the outcome model (model 1) and the mediator model (model 2) are used to calculate the NIE.

Mediation methods applied in the present analysis were adapted from those described in Aung et al[24]. Visit-specific phthalate ERS were used as exposure variables, and visit-specific hormone concentrations were used as mediators, in causal mediation analyses. Using ERS provides an advantage over individual phthalate metabolites because it reduces the potential for bias due to correlation between metabolites, and it allows for risk assessment and ascertainment of the biological pathways implicated with exposure to a whole class of environmental contaminants. All models adjusted for categorical maternal age and maternal education, and fetal sex. Exploratory mediation analyses were also conducted which were stratified by fetal sex. Mediation effect estimates correspond to a change in the exposure (phthalate ERS) from the first to the third quartile. All mediation analyses were conducted using the *mediation* package in R (version 3.5.1).

Sensitivity Analyses—The sequential ignorability assumption – that there is no downstream effect of the exposure, or unmeasured pre-treatment covariate, which confounds the relationship between the mediator and the outcome – is a strong assumption and necessitates employing sensitivity analyses. The *medsens* function within the *mediation* package allows us to check the robustness of natural indirect effects to the possible existence of unobserved pre-treatment covariates. A sensitivity parameter, ρ, is calculated which represents the correlation between the residuals of the mediator and outcome regressions. The sequential ignorability assumption is violated when pre-treatment confounders exist which affect both the mediator and the outcome, giving ρ which is not equal to zero. *Medsens* tests how the NIE varies as a function of different values of ρ, and returns a range of ρ values within which the NIE is sensitive to pre-treatment confounding and becomes null. A second sensitivity analysis allows us to test how the significance of natural indirect effects change when unique combinations of covariates are included in the model. Testing each unique combination of covariates included in this analysis (maternal age, maternal education, and fetal sex) results in 7 combinations.

Results

Characteristics of the study population are shown in Supplementary Table 1. Preterm and spontaneous preterm birth occurred in about 9% and 5% of the cohort, respectively. Pregnancies were about 53% male and 46% female. Most women were under the age of

30, had at least some college education, were employed, lived in a home earning less than $30k per year, were either married or cohabitating, did not smoke and reported never being exposed to environmental tobacco smoke, did not consume alcohol during pregnancy, had less than two previous live births, and had a pre-pregnancy BMI below 30 kg/m^2. Pregnancy and demographic characteristics did not differ appreciably between the full population and the mediation subset.

Weights derived from ridge regression for each birth outcome are shown Figure 1, separated into LMW and HMW phthalate groups. Among LMW phthalates, weights for metabolites of DBP and DiBP were mostly positive and stronger compared to MEP, the metabolite of DEP. These metabolites also possessed the strongest weights at visit 3 for PTB and at visit 2 for spontaneous PTB and gestational age at birth. Unexpectedly, both DBP and DiBP at visit 2 had one metabolite with a strong positive weight (MHBP, MiBP) and one metabolite with a strong negative weight (MBP, MHiBP) for gestational age at birth. Among HMW phthalates, most weights were relatively weak, with the exception of MCNP, which was positive and strongest at visit 3 for PTB and spontaneous PTB.

Weights from ridge regression for exploratory analyses stratified by fetal sex are shown in Supplementary Figure 3. For PTB, the strongest weights were assigned to metabolites of DBP and DiBP, and weights were particularly strong at visit 2 among pregnancies with a male fetus. Like results among all pregnancies, for both DBP and DiBP, the weight for one metabolite was positive (MBP and MHiBP) while the other was negative (MHBP and MiBP). Weights were similar for spontaneous PTB, except that DBP and DiBP metabolite weights were also very strong at visit 3 among pregnancies with a male fetus. Weights for gestational age at birth were generally weaker than those for PTB and spontaneous PTB, but DBP and DiBP metabolites still had the strongest weights among male fetuses.

Total Effects of Phthalate Mixtures on Timing of Delivery

Associations between interquartile range (IQR) increases in phthalate ERS and birth outcomes across the study period, subset to mothers with mediator data, are shown in Table 1. Among all pregnancies, visit 1 HMW phthalates were associated with increased odds of spontaneous PTB (OR:1.70, 95%CI: 1.04,2.78) and reduced gestational age at birth (β: −0.37 weeks, 95%CI:−0.58,−0.15). LMW phthalates at visits 2 and 3 were associated with reduced gestational age at birth (β:−0.32 weeks, 95%CI:−0.50,−0.14, and β:−0.34 weeks, 95%CI:−0.59,−0.10, respectively).

Among pregnancies with a female fetus, LMW phthalate ERS at all 3 study visits showed an increase in the odds of PTB (v1 OR:1.85, 95%CI:1.00,3.41; v2 OR:2.88, 95%CI:1.30,6.38; v3 OR:2.72, 95%CI:1.22,6.08), while only first and second visit HMW phthalate ERS were associated with higher odds of PTB (OR:2.00, 95%CI:1.13,3.52 and OR:2.47, 95%CI:1.30,4.72, respectively). Increased odds of spontaneous PTB were observed with increases in both LMW phthalate ERS (OR:2.19, 95%CI:1.02,4.72) and HMW phthalate ERS (OR:1.96, 95%CI:1.06,3.60) at visit 1. Increased LMW phthalate ERS at the second and third study visits were associated with reduced gestational age at birth (β:−0.46 weeks, 95%CI:−0.86,−0.07 and β:−0.51 weeks, 95%CI:−0.90,−0.12, respectively), while the HMW phthalate ERS at all three study visits was associated with reduced gestational age at birth

(v1 β:−0.63 weeks, 95%CI:−1.00,−0.26; v2 β:−0.42 weeks, 95%CI:−0.76,−0.08; v3 β:−0.39 weeks, 95%CI:−0.73,−0.04).

Among pregnancies with a male fetus, PTB was associated with HMW phthalate ERS at the first study visit (OR:2.32, 95%CI:1.20,4.50) and LMW phthalate ERS at the second study visit (OR:1.86, 95%CI:1.02,3.39). Odds of spontaneous PTB were associated with LMW phthalate ERS at the second (OR:4.46, 95%CI:1.52,13.1) and third study visit (OR:2.76, 95%CI:1.25,6.10), and with HMW phthalate ERS at the first study visit (OR:2.49, 95%CI:1.14,5.44). Finally, reductions in gestational age at birth were observed with increasing HMW phthalate ERS at the first study visit (β:−0.39 weeks, 95%CI:−0.75,−0.03) and increasing LMW phthalate ERS at the second study visit (β:−0.42 weeks, 95%CI: −0.69,−0.15).

Mediation Effects

Estimations of natural indirect effects and percent mediated across the study among all pregnancies are shown in Table 2. In contrast to results shown in Table 1, NIE estimates for binary outcomes are shown on the absolute probability scale. These estimates indicate the change in absolute probability of the outcome, rather than the odds of the outcome, when the exposure is held constant and the mediator is changed to a level it would have been with an IQR increase in exposure. In other words, the NIE can be conceptualized as the absolute change in probability of the outcome which results only from the effect that the exposure has on the mediator. Corresponding p-values for natural indirect effects are depicted in Figure 2. The association between visit 1 LMW phthalate ERS and odds of spontaneous PTB was not significant, however, the indirect effect of the ratio of testosterone to SHBG at visit 1 was significant (NIE:0.004, 95%CI: 2.14e-5,0.010; 16.0% mediated). Results were similar for visit 1 testosterone. Visit 1 fT4 also mediated 10.2% of this association (NIE:0.003, 95%CI: −1.83e-4,0.008). Visit 1 HMW phthalate ERS significantly increased odds of spontaneous PTB (OR:1.70, 95%CI:1.04,2.78), and 8.81% of this association was mediated by visit 1 fT4 (NIE:0.003, 95%CI:1.1e-4,0.008). Though the total effects of HMW phthalate ERS at visits 2 and 3 on odds of spontaneous PTB were not significant, various indirect effects were notable. Effects of exposure at visit 2 were significantly mediated by visit 3 progesterone (NIE:0.005, 95%CI:3.95e-4,0.011; 21.2% mediated) and marginally mediated by visit 3 fT4 (NIE:0.003, 95%CI:−3.07e-4,0.008; 11.3% mediated), while effects of exposure at visit 3 were significantly mediated by visit 3 progesterone (NIE:0.004, 95%CI:3.72e-4,0.010; 16.2% mediated). Finally, the significant reduction in gestational age at birth resulting from an IQR increase in visit 1 HMW phthalate ERS (β:−0.37 weeks, 95%CI:−0.58,−0.15) was mediated by visit 1 fT4 (9.6% mediated), which accounted for 0.036 weeks of the total reduction in gestational age at birth.

Sensitivity Analyses

Sensitivity analyses were conducted on main mediation models which returned significant or marginally significant results. Robustness of natural indirect effects to pre-treatment confounding, tested using the *medsens* function in the *mediation* package, are shown in Supplementary Table 2. When sensitivity of the NIE was tested for values of ρ between −0.9 and 0.9 (corresponding to strong inverse and positive correlations between the residuals of

the mediator and outcome models, respectively) the NIE was most robust for three models: the association between HMW phthalate ERS at visit 1 and spontaneous PTB, mediated by visit 1 fT4 (ρ sensitivity region: 0.1–0.2), and the associations between visit 2 and visit 3 HMW phthalate ERS and spontaneous PTB, mediated by visit 3 progesterone (ρ sensitivity region: 0.1–0.3 for both). These three models were further assessed for differences in natural indirect effects with every unique combination of the three covariates utilized in the study, maternal age, maternal education, and fetal sex, results for which are shown in Supplementary Figure 4.

For the fT4 mediation model, only 2 out of 7 covariate combinations resulted in an NIE p-value <0.05 (a model including all covariates, p=0.036; and a model containing only maternal age and fetal sex, p=0.035), while all covariate combinations gave p-values <0.05 for both progesterone mediation models.

Fetal Sex Stratified Analyses

Estimations of natural indirect effects and percent mediated across the study among pregnancies with a male fetus are shown in Supplementary Table 3. Corresponding p-values for natural indirect effects are depicted in Supplementary Figure 5. The association between visit 1 LMW phthalate ERS and spontaneous PTB was mediated by visit 1 CRH (28.2%), progesterone (17.6%), testosterone (30.6%), and the ratio of testosterone to SHBG (31.1%). Visit 3 CRH also marginally mediated the associations between visit 2 and visit 3 LMW phthalate ERS (10.8% and 13.9%, respectively) and odds of spontaneous PTB. Visit 1 testosterone marginally mediated the association between visit 1 LMW phthalate ERS and gestational age at birth (15.5%). Finally, visit 3 CRH mediated the association between visit 3 HMW phthalate ERS and gestational age at birth (38.3%). There were no significant mediating effects observed among pregnancies with a female fetus (data not shown).

Discussion

In this novel analysis, we explored the mediating effects of hormone concentrations on the associations between gestational exposure to a mixture of phthalates and adverse birth outcomes. This work makes significant innovations on previously published research by combining novel mixtures methods[24] with repeated measures analyses to provide causal mediation analyses using repeated biomarker data within an exposure mixtures framework. We provide evidence that significant associations exist between gestational exposure to a mixture of phthalates and increased odds of PTB and spontaneous PTB, and gestational age at birth, and that these associations differ by molecular weights of phthalates and gestational age at exposure assessment. We also provide introductory evidence of significant differences between fetal sexes, and mediation by various hormones on the associations between phthalate mixtures and these adverse birth outcomes.

A previous review of reviews has demonstrated that there is evidence of a positive association between exposure to various phthalate metabolites and odds of preterm birth[25]. A review by Radke and colleagues showed strong evidence of this positive association, and also concluded that there was moderate evidence for a positive association between increased DEHP and DBP exposure and odds of preterm birth[26]. Another review assessed

only DEHP exposures in relation to adverse delivery outcomes and observed inconsistent findings likely due to variation in sample sizes, exposure assessment, and confounder adjustment across studies[27]. A third review cited many of the same studies as the aforementioned reviews and came to a similar conclusion that, despite differences in study designs and phthalate metabolites included, significant associations between moderate levels of phthalate exposure and preterm birth are robustly documented[28]. Our work adds to the previous human studies on phthalates and preterm birth by exploring which metabolites within a phthalate mixture are most important for timing of delivery and how the mixtures may disrupt hormones to affect pregnancy.

Among all pregnancies, we observed significant total effects of visit 1 HMW phthalate ERS on increased odds of spontaneous PTB and reduced gestational age at birth which were significantly mediated by visit 1 fT4. Thyroid hormones are important in early pregnancy for proper development of the fetal brain and skeleton, and throughout the rest of pregnancy for growth of the fetus[29]. Previous studies have demonstrated associations between phthalate exposure and altered thyroid hormone concentrations[12–14,30], as well as associations between thyroid hormones and increased risk of PTB[31], but mechanistic human studies are lacking. Importantly, our previous work has shown a significant association between increased fT4 and risk of spontaneous PTB which is not modified by fetal sex within this same cohort (Cathey et al, submitted). These results provide further evidence for thyroid hormones acting on the casual pathway between phthalate exposure and PTB, but future work is needed to understand the mechanisms of this pathway. These findings also have important clinical implications because they underscore thyroid hormones as potential early markers of phthalate toxicity on preterm birth.

We observed suggestive evidence of mediation by visit 1 testosterone and fT4 on the association between visit 1 LMW phthalate ERS and spontaneous PTB, and by visit 3 progesterone on the associations between visit 2 and 3 HMW phthalate ERS and spontaneous PTB. Previous work has shown some of these hormones to be important for regulation of the timing of labor. During the first 9 weeks of pregnancy, the corpus luteum is responsible for secreting the necessary progesterone for maintenance of the fetus. After that, the placenta becomes the main source of progesterone. A previous *in silico* study found strong binding affinity between phthalate metabolites and the progesterone receptor[32]. Accordingly, another *in vitro* study found that treatment of human placental cells with phthalate metabolites resulted in an inhibition of the progesterone receptor gene via negative feedback from an increase in progesterone concentrations[33]. Thus, phthalate exposure at this time could stimulate progesterone production by the placenta via interaction with the progesterone receptor. Elevated circulating progesterone could then inhibit the progesterone receptor gene, which could result in reduced expression of the progesterone receptor gene and thus reduced progesterone function. Together, these data suggest that maternal exposure to mixtures of phthalates during mid gestation could result in increased production of progesterone by the placenta, which then participates in a negative feedback loop with the progesterone receptor, resulting in a reduction of the anti-labor effects of progesterone on the pregnancy, possibly contributing to increased risk of preterm birth.

There is also a biological basis for the proposed mediating effect of testosterone on the association between phthalate exposures and preterm delivery. Despite existing evidence that phthalates possess anti-androgenic biological effects, previous work has shown a positive association between testosterone concentrations during pregnancy and exposure to LMW phthalates[12]. Higher circulating concentrations of testosterone may act on the endometrium to produce lower levels of PP14, an endometrial secretory protein which is inversely associated with risk of preterm birth as early as 6–18 weeks' gestation[34]. Decreased production of PP14 is associated with abnormal development of the endometrium and greater likelihood of downstream pregnancy complications[35–37]. Therefore, gestational exposure to LMW phthalates may result in elevated testosterone production, which could then adversely affect the endometrium to produce less PP14 and cause endometrial dysfunction leading to elevated risk of preterm delivery.

When exploratory analyses were employed to investigate differences between fetal sexes, various significant mediating effects were observed among only pregnancies with a male fetus (CRH, progesterone, and testosterone), though corresponding total effects did not reach significance. Similar to results among all pregnancies, significant mediating effects of progesterone and testosterone were observed. Interestingly, mediating effects of these hormones were important only for LMW phthalates. In contrast to results among all pregnancies, mediating effects of CRH were observed for both spontaneous PTB and gestational age at birth among males. Concentrations of CRH begin to exponentially rise around midgestation and peak at the initiation of labor, possibly acting as a placental clock for the timing of delivery. The initial rise in CRH has been observed to occur earlier during pregnancy among women who eventually deliver preterm, leading researchers to believe that CRH may be an early marker for pregnancies at risk for preterm delivery[38]. This physiological role, coupled with past observations of significant positive associations with phthalate exposure[12], suggests that CRH could in fact mediate the association between phthalates and preterm delivery. Additionally, it has been postulated that CRH may signal to the fetal zone of the fetal adrenal gland to stimulate production of DHEA-S, a precursor of androgens and estrogens, to activate pro-labor events[39].

This study was subject to several limitations. Some phthalate metabolite weights from ridge analysis were strongest at the second study visit, at which time we did not have access to hormone measurements, and so we may have missed important associations at that time point. We also detected some significant mediating effects which did not correspond to significant total effects. Detection of significant mediation signals could have been an artifact of strong associations between our exposure and mediator measures, to which the total effect would be robust. However, despite our large sample size, the small number of PTB and spontaneous PTB cases could also be interfering with our ability to detect truly significant total effects. The small number of PTB cases also limited our statistical power in exploratory analyses assessing differences by fetal sex. We did not have access to measurements for thyroid autoantibody status, which could confound associations with thyroid hormones. Some critical changes in the maternal endocrine environment occur earlier or later in gestation than we were able to measure, which could shed additional light on the various endocrine pathways implicated in adverse birth outcomes. Women with preexisting conditions were excluded from the analysis, which may limit the generalizability

of our findings. It is likely that all models with ERS are overfit because we did not use separate training and testing data sets for creating the ERS and running subsequent mediation analyses. Finally, the mediation analyses implemented here cannot accommodate situations where mediators confound one another, so it is possible that our results are biased if multiple mediators are operating on the same causal pathway. Future work will attempt to better understand the endocrine pathways implicated with phthalate exposures in order to create mediator risk scores that are reflective of entire pathways.

Despite these limitations, this study was also strong in many ways. To our knowledge, this is the first study to utilize this analysis pipeline with repeated exposure and mediator data, and our sample size was larger than many other epidemiology studies which assessed only single pollutant associations. We included a wide panel of hormone measurements to test a variety of endocrine pathways, and we add to a very limited body of epidemiology literature supporting a role for CRH in adverse birth outcomes. Exclusion of women with preexisting conditions, though it limited our generalizability as stated previously, allowed us to better understand biological effects related only to environmental exposures and not confounded by other health conditions. We assessed the more likely homogenous spontaneous subtype of preterm birth, which may help in understanding the physiological pathways that make this subtype unique. We also provide novel evidence of differential toxicity pathways of high versus low molecular weight phthalate compounds, and that molecular weight may influence the gestational age at which exposure confers the greatest toxicity. Lastly, we added to a growing body of evidence suggesting differential biological pathways and risks associated with adverse birth outcomes between male and female pregnancies. Future studies with larger number of adverse birth outcome cases should seek to better understand physiological differences between pregnancies with male and female fetuses.

In conclusion, we provide innovative evidence of fT4 concentrations mediating the association between gestational exposure to a mixture of HMW phthalates and elevated risk for spontaneous PTB. We also provide novel suggestive evidence of mediation by testosterone and progesterone, as well as CRH being a mediator unique to pregnancies with a male fetus.

Importantly, we add to a limited body of evidence suggesting that environmental exposures and subsequent risk for adverse pregnancy outcomes are not equitable between male and female pregnancies, and that phthalate molecular weight may influence observed associations. Future work will aim to increase statistical power with more cases of adverse pregnancy outcomes, and to better understand the true physiological implications of altered hormone concentrations during pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Impact Statement

This study provides introductory evidence that an alteration of hormone concentrations occurs on the causal pathway between gestational phthalate mixture exposure and subsequent preterm birth. In addition to the novel application of repeated biomarker measurements and mixtures methods in causal mediation analyses, we also explored differences between classes of phthalate compounds and between fetal sexes. We show that differential endocrine pathways may be disrupted with exposures to low versus high molecular weight phthalate compounds, and that pregnancies with a male fetus may be more susceptible to endocrine disruption than those with a female fetus.

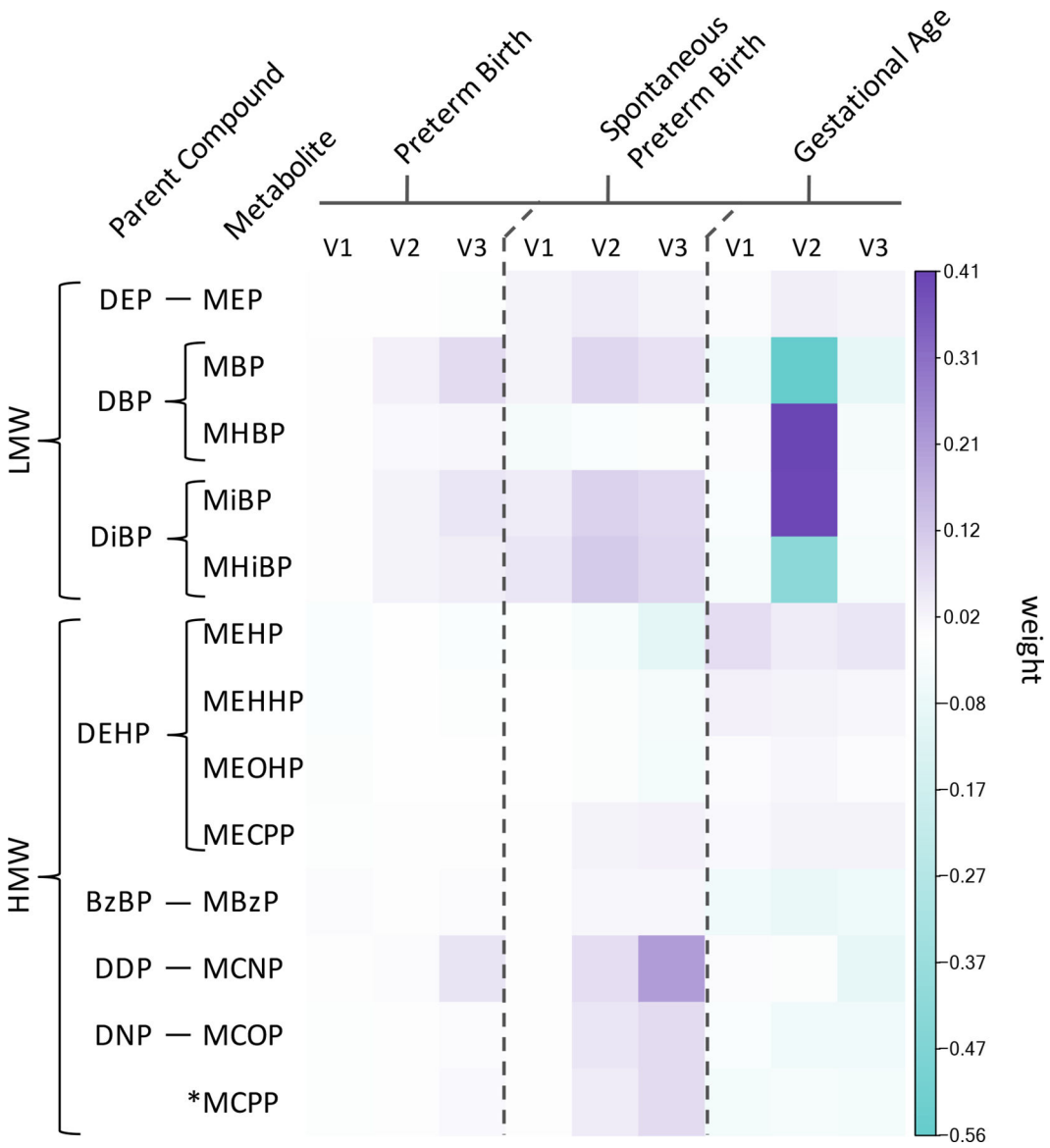

Figure 1.

Weights assigned from ridge regression depicting the relative importance of each phthalate metabolite for predicting birth outcomes at each study visit.

Box color corresponds to the weight assigned to each metabolite from ridge regression. Darker colors indicate greater magnitude of weights, purple color indicates positive weights, and green color indicates inverse weights.

*the MCPP metabolite results from metabolism of multiple high molecular weight parent phthalates

Abbreviations: LMW: low molecular weight; HMW: high molecular weight; MEP: monoethyl phthalate; MBP: mono-*n*-butyl phthalate; MHBP: mono-hydroxybutyl phthalate; MiBP: mono-isobutyl phthalate; MHiBP: mono-hydroxyisobutyl phthalate; MEHP: mono-2-ethylhexyl phthalate; MEHHP: mono-2-ethyl-5-hydroxyhexyl phthalate; MEOHP: mono-2-ethyl-5-oxohexyl phthalate; MECPP: mono-2-ethyl-5-carboxypentyl phthalate;

MBzP: monobenzyl phthalate; MCNP: mono carboxyisononyl phthalate; MCOP: mono carboxyisooctyl phthalate; MCPP: mono-3-carboxypropyl phthalate.

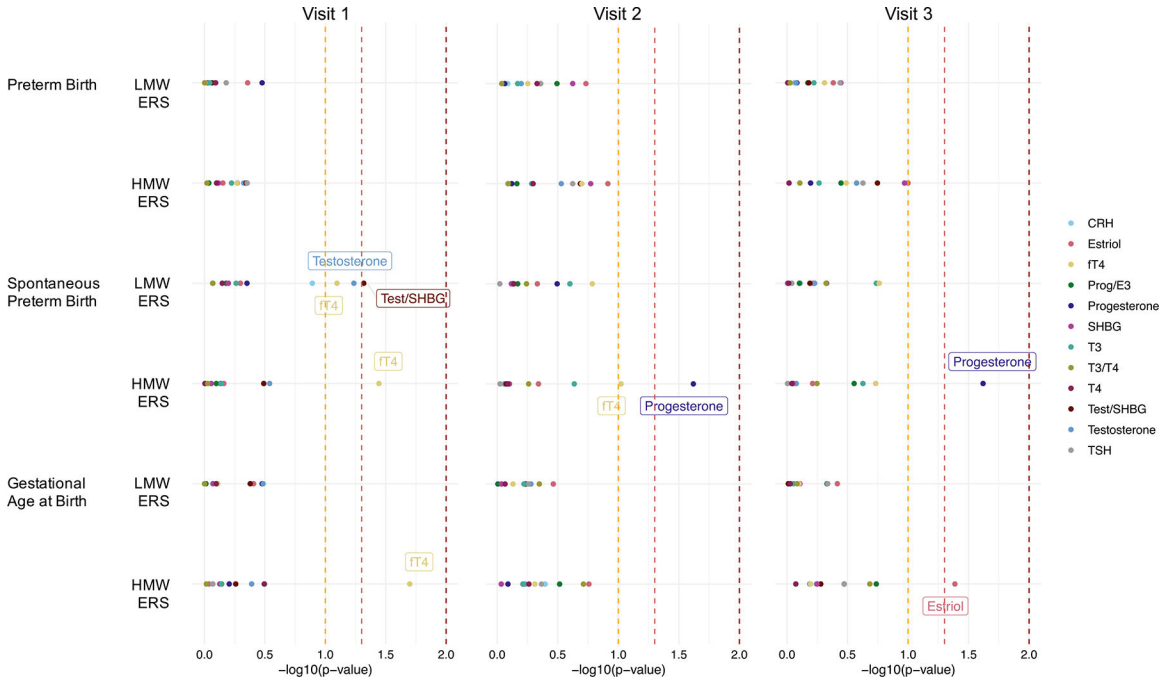

Figure 2.

Estimated -$\log_{10}$(p-values) of mediating effects by hormone concentrations on the associations between phthalate ERS and birth outcomes among all pregnancies.

From left to right within each chart, the vertical dashed lines represent p-values of 0.1, 0.05, and 0.01. All models were adjusted for continuous maternal age and categorical education.

Abbreviations: ERS: environmental risk score; LMW: low molecular weight; HMW: high molecular weight; CRH: corticotropin releasing hormone; fT4: free thyroxine; Prog/E3: ratio of progesterone to estriol; SHBG: sex hormone binding globulin; T3: total triiodothyronine; T3/T4: ratio of total triiodothyronine to total thyroxine; T4: total thyroxine; Test/SHBG: ratio of testosterone to sex hormone binding globulin; TSH: thyroid stimulating hormone.

Table 1.

Associations between phthalate ERS and birth outcomes across the study period, among women with mediator data.

	Visit 1				Visit 2				Visit 3			
	LMW		HMW		LMW		HMW		LMW		HMW	
	N	Est (95% CI)	N	Est (95% CI)	N	Est (95% CI)	N	Est (95% CI)	N	Est (95% CI)	N	Est (95% CI)
All Pregnancies												
Preterm Birth	526	1.18 (0.80, 1.76)	526	1.50 (0.98, 2.29)	405	1.35 (0.85, 2.13)	405	1.10 (0.70, 1.74)	424	1.36 (0.86, 2.16)	424	1.17 (0.75, 1.82)
Spont. Preterm Birth	511	1.40 (0.84, 2.35)	511	**1.70 (1.04, 2.78)**	392	1.57 (0.78, 3.18)	392	1.29 (0.64, 2.60)	411	1.38 (0.71, 2.68)	411	1.53 (0.81, 2.88)
Gest. Age (weeks)	531	−0.26 (−0.52, 0.01)	531	**−0.37 (−0.58, −0.15)**	405	**−0.32 (−0.50, −0.14)**	405	−0.14 (−0.38, 0.11)	424	**−0.34 (−0.59, −0.10)**	424	−0.15 (−0.40, 0.09)
Female Fetuses												
Preterm Birth	244	**1.85 (1.00, 3.41)**	244	**2.00 (1.13, 3.52)**	195	**2.88 (1.30, 6.38)**	195	**2.47 (1.30, 4.72)**	206	**2.72 (1.22, 6.08)**	206	1.40 (0.72, 2.74)
Spont. Preterm Birth	237	**2.19 (1.02, 4.72)**	237	**1.96 (1.06, 3.60)**	189	3.34 (0.97, 11.51)	189	2.20 (0.81, 5.97)	200	2.20 (0.64, 7.54)	200	1.39 (0.48, 4.00)
Gest. Age (weeks)	245	−0.32 (−0.70, 0.05)	245	**−0.63 (−1.00, −0.26)**	195	**−0.46 (−0.86, −0.07)**	195	**−0.42 (−0.76, −0.08)**	206	**−0.51 (−0.90, −0.12)**	206	**−0.39 (−0.73, −0.04)**
Male Fetuses												
Preterm Birth	282	1.36 (0.78, 2.37)	282	**2.32 (1.20, 4.50)**	210	**1.86 (1.02, 3.39)**	210	1.32 (0.70, 2.48)	218	1.11 (0.64, 1.95)	218	1.40 (0.80, 2.44)
Spont. Preterm Birth	274	1.66 (0.89, 3.08)	274	**2.49 (1.14, 5.44)**	203	**4.46 (1.52, 13.1)**	203	0.84 (0.28, 2.52)	211	**2.76 (1.25, 6.10)**	211	1.02 (0.42, 2.49)
Gest. Age (weeks)	286	−0.22 (−0.57, 0.14)	286	**−0.39 (−0.75, −0.03)**	210	**−0.42 (−0.69, −0.15)**	210	−0.02 (−0.34, 0.29)	218	−0.28 (−0.62, 0.05)	218	−0.11 (−0.46, 0.24)

Effect estimates refer to the odds of binary birth outcomes, or unit changes in continuous outcomes, with an interquartile range increase in phthalate ERS. ERS were calculated using a cumulative average approach; visit 2 was comprised of the geometric means of phthalate concentrations at visits 1 and 2, and visit 3 was comprised of the geometric means of phthalate concentrations from all 3 visits. All models adjust for categorical maternal age and education.

Boldface text denotes significant findings with $p \leq 0.05$. LMW: low molecular weight; HMW: high molecular weight.

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Table 2.

Natural indirect effect estimates and percent mediated with an interquartile range increase in low or high molecular weight phthalate ERS over the study period among all pregnancies.

Low Molecular Weight ERS		$ERS_{v1} \rightarrow Hormones_{v1}$		$ERS_{v2} \rightarrow Hormones_{v3}$		$ERS_{v3} \rightarrow Hormones_{v3}$	
Outcome	**Mediator**	**NIE (95% CI)**	**Percent Mediated[a]**	**NIE (95% CI)**	**Percent Mediated[a]**	**NIE (95% CI)**	**Percent Mediated[a]**
PTB	CRH	0.000 (−0.002, 0.002)	0.14%	0.000 (−0.002, 0.004)	0.43%	0.000 (−0.002, 0.003)	0.19%
	Estriol	−0.001 (−0.006, 0.002)	NA	−0.003 (−0.009, 0.001)	NA	−0.001 (−0.006, 0.002)	NA
	Prog.	−0.002 (−0.006, 0.002)	NA	0.000 (−0.002, 0.003)	0.18%	0.000 (−0.002, 0.003)	0.27%
	Prog/E3	0.000 (−0.002, 0.002)	0.07%	−0.002 (−0.007, 0.002)	NA	−0.001 (−0.006, 0.004)	NA
	Test.	0.000 (−0.001, 0.002)	0.18%	−0.001 (−0.005, 0.002)	NA	0.000 (−0.004, 0.002)	NA
	Test./SHBG	0.000 (−0.002, 0.002)	0.16%	−0.001 (−0.006, 0.002)	NA	−0.001 (−0.004, 0.002)	NA
	SHBG	0.000 (−0.002, 0.002)	NA	−0.002 (−0.008, 0.001)	NA	−0.002 (−0.006, 0.001)	NA
	TSH	0.001 (−0.002, 0.004)	1.06%	0.001 (−0.001, 0.006)	2.42%	0.002 (−0.001, 0.007)	3.18%
	T3	0.000 (−0.001, 0.002)	0.05%	0.001 (−0.002, 0.004)	0.75%	0.001 (−0.002, 0.005)	1.15%
	fT4	0.000 (−0.004, 0.004)	0.13%	0.001 (−0.002, 0.006)	1.72%	0.001 (−0.001, 0.006)	2.18%
	T4	0.000 (−0.003, 0.004)	1.00%	−0.001 (−0.006, 0.002)	NA	0.000 (−0.004, 0.004)	0.03%
	T3/T4	0.000 (−0.002, 0.002)	0.01%	0.000 (−0.003, 0.003)	NA	0.000 (−0.003, 0.003)	NA
Spont. PTB	CRH	0.003 (−0.001, 0.009)	13.5%	0.001 (−0.002, 0.004)	1.39%	0.000 (−0.002, 0.004)	1.01%
	Estriol	−0.001 (−0.005, 0.002)	NA	0.001 (−0.002, 0.005)	3.02%	0.001 (−0.001, 0.004)	1.09%
	Prog.	0.001 (−0.002, 0.005)	3.57%	0.002 (−0.001, 0.006)	5.66%	0.001 (−0.002, 0.006)	4.51%
	Prog/E3	0.000 (−0.003, 0.002)	NA	−0.001 (−0.005, 0.003)	NA	0.000 (−0.004, 0.004)	NA
	Test.	**0.004 (0.000, 0.011)**	17.2%	0.000 (−0.003, 0.004)	1.30%	0.001 (−0.002, 0.005)	2.29%
	Test./SHBG	**0.004 (0.000, 0.010)**	16.0%	0.001 (−0.003, 0.005)	1.67%	0.001 (−0.002, 0.005)	1.84%
	SHBG	0.001 (−0.001, 0.004)	1.05%	0.000 (−0.003, 0.005)	1.37%	0.000 (−0.003, 0.003)	0.00%
	TSH	0.000 (−0.005, 0.004)	NA	0.000 (−0.003, 0.003)	NA	0.000 (−0.003, 0.003)	0.30%
	T3	0.001 (−0.002, 0.004)	2.44%	0.002 (−0.001, 0.006)	5.84%	0.002 (−0.001, 0.007)	8.24%
	fT4	**0.003 (0.000, 0.008)**	10.2%	0.002 (0.000, 0.007)	6.65%	0.002 (−0.001, 0.007)	7.05%
	T4	0.000 (−0.002, 0.003)	0.85%	0.000 (−0.004, 0.002)	NA	0.000 (−0.003, 0.003)	0.14%
	T3/T4	0.000 (−0.003, 0.004)	0.61%	0.001 (−0.002, 0.005)	2.48%	0.001 (−0.002, 0.005)	3.48%
Gest. Age	CRH	−0.009 (−0.037, 0.010)	2.61%	−0.004 (−0.021, 0.008)	0.77%	0.000 (−0.017, 0.016)	0.03%
	Estriol	0.011 (−0.012, 0.046)	NA	0.009 (−0.008, 0.037)	NA	0.011 (−0.013, 0.047)	NA

	Prog.	0.019 (−0.017, 0.064)	NA	−0.003 (−0.020, 0.008)	0.68%	−0.001 (−0.022, 0.019)	0.08%
	Prog/E3	0.000 (−0.016, 0.017)	NA	0.000 (−0.028, 0.028)	NA	0.015 (−0.024, 0.060)	NA
	Test.	−0.011 (−0.043, 0.008)	3.15%	0.005 (−0.010, 0.026)	NA	0.000 (−0.014, 0.014)	0.02%
	Test./SHBG	−0.009 (−0.037, 0.009)	2.47%	0.004 (−0.009, 0.024)	NA	0.000 (−0.016, 0.013)	0.01%
	SHBG	0.001 (−0.013, 0.019)	NA	0.001 (−0.009, 0.013)	NA	0.003 (−0.012, 0.024)	NA
	TSH	−0.003 (−0.028, 0.020)	0.46%	−0.005 (−0.024, 0.011)	1.05%	−0.008 (−0.037, 0.011)	1.61%
	T3	−0.002 (−0.020, 0.012)	0.34%	0.004 (−0.009, 0.023)	NA	0.001 (−0.012, 0.017)	NA
	fT4	−0.005 (−0.041, 0.029)	1.55%	−0.002 (−0.017, 0.009)	0.26%	−0.002 (−0.021, 0.012)	0.23%
	T4	−0.003 (−0.031, 0.023)	0.88%	0.001 (−0.014, 0.018)	NA	−0.001 (−0.022, 0.021)	0.08%
	T3/T4	0.000 (−0.015, 0.015)	0.00%	0.006 (−0.007, 0.028)	NA	0.002 (−0.016, 0.024)	NA
High Molecular Weight ERS		**ERS_{v1} → $Hormones_{v1}$**		**ERS_{v2} → $Hormones_{v3}$**		**ERS_{v3} → $Hormones_{v3}$**	
Outcome	**Mediator**	**NIE (95% CI)**	**Percent Mediated[a]**	**NIE (95% CI)**	**Percent Mediated[a]**	**NIE (95% CI)**	**Percent Mediated[a]**
PTB	CRH	−0.002 (−0.008, 0.003)	NA	0.001 (−0.009, 0.013)	3.69%	0.000 (−0.012, 0.013)	1.28%
	Estriol	−0.001 (−0.005, 0.003)	NA	−0.006 (−0.015, 0.002)	NA	−0.007 (−0.016, 0.002)	NA
	Prog.	−0.001 (−0.005, 0.002)	NA	0.001 (−0.004, 0.007)	1.92%	0.001 (−0.004, 0.007)	2.61%
	Prog/E3	0.000 (−0.002, 0.002)	0.06%	−0.001 (−0.005, 0.003)	NA	−0.002 (−0.007, 0.002)	NA
	Test.	−0.002 (−0.007, 0.004)	NA	−0.005 (−0.016, 0.004)	NA	−0.006 (−0.020, 0.005)	NA
	Test./SHBG	−0.002 (−0.007, 0.003)	NA	−0.006 (−0.017, 0.003)	NA	−0.008 (−0.020, 0.003)	NA
	SHBG	0.000 (−0.003, 0.002)	NA	−0.004 (−0.012, 0.002)	NA	−0.005 (−0.013, 0.001)	NA
	TSH	−0.001 (−0.006, 0.002)	NA	0.003 (−0.002, 0.011)	7.64%	0.003 (−0.002, 0.009)	7.38%
	T3	−0.001 (−0.006, 0.003)	NA	0.002 (−0.005, 0.010)	5.31%	0.002 (−0.006, 0.011)	6.70%
	fT4	−0.001 (−0.005, 0.002)	NA	0.003 (−0.001, 0.009)	5.37%	0.004 (−0.003, 0.012)	11.0%
	T4	0.000 (−0.004, 0.003)	NA	−0.001 (−0.006, 0.002)	NA	0.000 (−0.004, 0.004)	0.03%
	T3/T4	0.000 (−0.004, 0.005)	0.28%	−0.001 (−0.009, 0.007)	NA	−0.001 (−0.010, 0.007)	NA
Spont. PTB	CRH	0.001 (−0.001, 0.004)	3.14%	0.001 (−0.008, 0.010)	2.75%	−0.001 (−0.010, 0.009)	NA
	Estriol	0.001 (−0.003, 0.004)	1.38%	0.002 (−0.004, 0.010)	8.78%	0.001 (−0.005, 0.009)	5.80%
	Prog.	0.000 (−0.004, 0.002)	NA	**0.005 (0.000, 0.011)**	21.2%	**0.004 (0.000, 0.010)**	16.2%
	Prog/E3	0.000 (−0.001, 0.002)	0.23%	0.000 (−0.004, 0.003)	NA	−0.002 (−0.006, 0.001)	NA
	Test.	0.001 (−0.001, 0.005)	3.72%	0.001 (−0.007, 0.009)	2.61%	0.001 (−0.008, 0.010)	3.89%
	Test./SHBG	0.001 (−0.001, 0.005)	3.30%	0.001 (−0.007, 0.009)	1.92%	0.001 (−0.008, 0.010)	1.86%
	SHBG	0.000 (−0.002, 0.002)	0.13%	0.001 (−0.004, 0.007)	2.62%	0.000 (−0.005, 0.005)	NA
	TSH	0.000 (−0.001, 0.001)	NA	0.000 (−0.006, 0.006)	NA	0.000 (−0.004, 0.005)	NA

	T3	0.000 (−0.001, 0.002)	0.33%	0.003 (−0.002, 0.010)	13.6%	0.003 (−0.002, 0.010)	13.6%
	fT4	**0.003 (0.000, 0.008)**	8.81%	**0.003 (0.000, 0.008)**	11.3%	0.003 (−0.002, 0.010)	13.7%
	T4	0.000 (−0.002, 0.002)	0.00%	0.000 (−0.003, 0.002)	NA	0.000 (−0.002, 0.003)	0.19%
	T3/T4	0.000 (−0.001, 0.002)	0.05%	0.002 (−0.004, 0.009)	6.97%	0.002 (−0.004, 0.008)	6.20%
Gest. Age	CRH	−0.009 (−0.033, 0.007)	1.98%	−0.015 (−0.056, 0.024)	6.78%	−0.018 (−0.086, 0.052)	8.54%
	Estriol	−0.001 (−0.023, 0.021)	0.18%	0.019 (−0.005, 0.059)	NA	**0.040 (0.001, 0.097)**	NA
	Prog.	0.008 (−0.023, 0.043)	NA	−0.002 (−0.025, 0.016)	0.49%	−0.011 (−0.041, 0.010)	4.20%
	Prog/E3	0.003 (−0.010, 0.021)	NA	0.021 (−0.016, 0.069)	NA	0.028 (−0.011, 0.077)	NA
	Test.	−0.007 (−0.032, 0.009)	1.43%	0.008 (−0.023, 0.046)	NA	0.017 (−0.044, 0.081)	NA
	Test./SHBG	−0.005 (−0.027, 0.011)	0.97%	0.007 (−0.020, 0.042)	NA	0.019 (−0.039, 0.084)	NA
	SHBG	0.002 (−0.010, 0.019)	NA	0.001 (−0.014, 0.019)	NA	0.010 (−0.023, 0.049)	NA
	TSH	−0.001 (−0.018, 0.014)	0.14%	−0.016 (−0.061, 0.024)	6.72%	−0.017 (−0.058, 0.018)	7.14%
	T3	−0.002 (−0.019, 0.010)	0.34%	0.007 (−0.020, 0.040)	NA	0.011 (−0.036, 0.063)	NA
	fT4	**−0.036 (−0.078, −0.004)**	9.60%	−0.007 (−0.036, 0.014)	2.62%	−0.010 (−0.058, 0.037)	4.56%
	T4	−0.010 (−0.039, 0.008)	2.46%	0.007 (−0.012, 0.035)	NA	0.002 (−0.019, 0.026)	NA
	T3/T4	0.000 (−0.011, 0.011)	NA	0.021 (−0.009, 0.065)	NA	0.030 (−0.015, 0.086)	NA

[a]Indication is NA when the TE and NIE are in different directions, rendering the percent mediated uninterpretable. Estimates refer to the increase in probability of experiencing binary outcomes, or the unit change in continuous outcomes, due to the resulting change in the mediator with an interquartile range increase in exposure, while holding the exposure constant. Boldface text indicates a p-value < 0.1.