GESTATIONAL PHTHALATE/REPLACEMENT EXPOSURE: A GLIMPSE INTO MATERNAL RISK FACTORS, BIOLOGICAL TARGETS, AND GESTATIONAL CARDIOMETABOLIC HEALTH

By

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ABSTRACT

Pregnancy is a period of heightened susceptibility to numerous modifiable environmental stressors, especially to one diverse class of endocrine and metabolic disrupting chemicals, ortho-phthalate diesters (commonly known as phthalates) and their replacements. Evidence suggests prenatal phthalate exposure is associated with adverse maternal and child health outcomes in pregnancy, but these health consequences may also persist well beyond gestation. This is concerning, as pregnant women are ubiquitously exposed to phthalates, but also their plasticizer replacements di(isononyl) cyclohexane-1,2-dicarboxylate (DiNCH) and di(2-ethylhexyl) terephthalate (DEHTP) to which exposure is increasing. Based on recent experimental and observational evidence, these replacements may have similar or worse health consequences than the original phthalates. Additionally, pregnant women are not exposed to one phthalate or replacement at a time, but a mixture of these chemicals, which necessitates evaluating associations of multiple phthalates/replacements with our outcomes of interest to understand the potential true impact of real-life exposures. Therefore, the studies presented in this dissertation were designed to identify potential maternal risk factors, gestational hormonal targets, and cardiometabolic health consequences of prenatal exposure to phthalates and their replacements in women enrolled in the Illinois Kids Development Study. Specifically, we evaluated determinants of maternal phthalate/ replacement exposure (**Chapter 2**), as well as associations of phthalates/replacements with maternal sex-steroid hormones (**Chapter 3**) and gestational weight gain (**Chapter 4**). We developed an approach for measuring urinary sex-steroid hormones at multiple gestational timepoints to capture longitudinal changes in associations of phthalates/

replacements with hormones. Given the roles of gestational hormones in coordinating critical pregnancy metabolic adaptations, we also addressed the potential involvement of these biological processes in associations between phthalates/replacements and maternal cardiometabolic health, with a focus on gestational weight gain as one clinically-relevant metabolic endpoint. Throughout, various statistical mixtures approaches, including weighted quantile sum regression, quantile-based g-computation, *k*-means clustering, and principal component analysis, were highlighted as ways to evaluate phthalate/replacement mixtures. The overarching goal of this dissertation was to underscore the importance of considering maternal pregnancy health, highlighting regrettable substitution, and emphasizing the utility of statistical mixtures approaches to address our research questions of interest.

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“Najpiękniejszych chwil w życiu nie zaplanujesz. One przyjdą same.”

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LIST OF ABBREVIATIONS

AA	Anti-androgenic
AHEI-2010	Alternative Healthy Eating Index
AMH	anti-Müllerian hormone
BBzP	Benzylbutyl phthalate
BKMR	Bayesian Kernel Machine Regression
BMI	Body mass index
BPA	Bisphenol A
BPF	Bisphenol F
BPS	Bisphenol S
CDC	Centers for Disease Control and Prevention
CESD	Center for Epidemiological Studies Depression
CI	Confidence interval
CVD	Cardiovascular Disease
DAG	Directed acyclic graph
DBP	Di-n-butyl phthalate
DEHTP	Di(2-ethylhexyl) terephthalate
DEP	Diethyl phthalate
DiBP	Di-iso-butyl phthalate
DiNCH	Di(isononyl) cyclohexane-1,2-dicarboxylate
DiNP	Di-isononyl phthalate
DOP	Di-n-octyl phthalate
EDC	Endocrine disrupting chemical

EDPS	Edinburgh Postnatal Depression Scale
ELISA	Enzyme-linked immunosorbent assay
FFQ	Food Frequency Questionnaire
FSH	Follicle-stimulating hormone
GWG	Gestational weight gain
GWGz	Gestational weight gain z-scores
HDL	High-density lipoprotein
HighMWP	High-molecular-weight phthalate
HPG	Hypothalamic-pituitary-gonadal
HPLC-MS/MS	High-performance liquid chromatography mass spectrometry
I-KIDS	Illinois Kids Development Study
IOM	Institute of Medicine
IQR	Interquartile range
LDL	Low-density lipoprotein
LH	Luteinizing hormone
LOD	Limit of detection
LOQ	Limit of quantification
LowMWP	Low-molecular-weight phthalate
MBP	Mono-n-butyl phthalate
MBzP	Monobenzyl phthalate
MCNP	Monocarboxynonyl phthalate
MCOCH	Cyclohexane-1,2-dicarboxylic acid-mono(carboxyoctyl) ester
MCOP	Monocarboxyoctyl phthalate

MCPP	Mono(3-carboxypropyl) phthalate
MECPP	Mono(2-ethyl-5-carboxypentyl) phthalate
MECPTP	Mono(2-ethyl-5-carboxypentyl) terephthalate
MEHP	Mono(2-ethylhexyl) phthalate
MEHHP	Mono(2-ethyl-5-hydroxyhexyl) phthalate
MEHHTP	Mono(2-ethyl-5-hydroxyhexyl) terephthalate
MEOHP	Mono(2-ethyl-5-oxohexyl) phthalate
MEP	Monoethyl phthalate
MHBP	Mono-hydroxybutyl phthalate
MHiBP	Mono-hydroxy-isobutyl phthalate
MHiNCH	Cyclohexane-1,2-dicarboxylic acid-monohydroxy isononyl ester
MiBP	Mono-isobutyl phthalate
MiNP	Mono-isononyl phthalate
MONP	Monooxononyl phthalate
MRI	Magnetic resonance imaging
MWHS	Midlife Women's Health Study
NHANES	National Health and Nutrition Examination Survey
OR	Odds Ratio
PC	Principal component
PCA	Principal component analysis
PCP	Personal care products
P_{int}	*P*-value for moderation tested using multiplicative interactions
$P_{linear\ trend}$	*P*-value for linear trend test across quartiles

Q1	Quartile 1
Q2	Quartile 2
Q3	Quartile 3
Q4	Quartile 4
QGComp	Quantile-based G-computation
RIA	Radioimmunoassay
RR	Risk ratio
SES	Socioeconomic status
SG	Specific gravity
SHBG	Sex hormone binding globulin
STRAW+10	Stages of Reproductive Aging Workshop + 10
∑AA	Sum of phthalate metabolites with anti-androgenic biological activity
∑DBP	Sum of di-n-butyl phthalate metabolites
∑DEHP	Sum of di(2-ethylhexyl) phthalate metabolites
∑DEHTP	Sum of di(2-ethylhexyl) terephthalate metabolites
∑DiBP	Sum of di-iso-butyl phthalate metabolites
∑DiNCH	Sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites
∑DiNP	Sum of di-isononyl phthalate metabolites
∑PCP	Sum of phthalate metabolites of parents in personal care products
∑Phthalates	Sum of all phthalate metabolites
∑Plastics	Sum of phthalate metabolites of parents in plastics
SumDBP	Sum of di-n-butyl phthalate metabolites
SumDEHP	Sum of di(2-ethylhexyl) phthalate metabolites

SumDEHTP	Sum of di(2-ethylhexyl) terephthalate metabolites
SumDiBP	Sum of di-iso-butyl phthalate metabolites
SumDiNCH	Sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites
SumDiNP	Sum of di-isononyl phthalate metabolites
SumEstrogens	Sum of the eight major urinary estrogen metabolites
SumPCP	Sum of phthalate metabolites of parents in personal care products
SumPlastics	Sum of phthalate metabolites of parents in plastics
SumTestosterones	Sum of the two major testosterone metabolites
TDS	Total Diet Study
U.S.	United States
VLDL	Very-low-density lipoprotein
WQSR	Weighted Quantile Sum Regression

CHAPTER 1: INTRODUCTION

As many as 86% of women in the United States (U.S.) will have given birth at least once by the time they reach their forties (1, 2). Pregnancy is a time of positive energy balance requiring weight gain to support not only the growing fetus and placenta, but also to increase maternal tissue deposition and plasma volume for nutrient storage and fetal transfer (3). These processes are coordinated via physiological adaptations in hormone synthesis and signaling (4), in glucose and lipid homeostasis (3), and in inflammation and immune response (5). For example, substantial increases in estrogens (i.e., estrone, estradiol, and estriol) and minor increases in androgens (i.e., testosterone) support implantation, placental angiogenesis, parturition, and metabolic adaptations (3, 4, 6, 7). However, this also makes pregnancy a sensitive window since disruptions in the above-mentioned adaptations can lead to adverse pregnancy and birth outcomes, including pre-term birth, altered fetal growth, pregnancy hypertensive disorders, and gestational diabetes (7-9). Consequently, perturbations during pregnancy can have lasting consequences for child health (2, 10, 11). For example, adverse pregnancy and birth outcomes have been associated with the development of later life diseases in the offspring, including cardiovascular disease, metabolic syndrome, osteoporosis, infertility, and cognitive dysfunction (10). This is in accordance with the developmental origins of health and disease hypothesis (10, 12). Pregnancy is also an important determinant of maternal lifelong health, as more recent preliminary evidence indicates that pregnancy itself (and especially having adverse pregnancy or birth outcomes) may have lasting consequences for maternal later life cardiometabolic disease risk (2, 11). Therefore, it is important to identify the modifiable risk factors associated with adverse pregnancy health

to protect maternal and child lifelong health.

Pregnancy is a period of heightened susceptibility to numerous modifiable environmental stressors (2, 13), especially to one diverse class of endocrine and metabolic disrupting chemicals, ortho-phthalate diesters (commonly known as phthalates) and their replacements. Greater than 90% of pregnant women have measurable levels of urinary phthalate metabolites, which are used as biomarkers for predicting exposure to phthalates, suggesting that pregnant women are ubiquitously exposed to these chemicals (14). This widespread exposure is due to the use of phthalates in common daily used consumer products. For example, some phthalates, such as di(2-ethylhexyl) phthalate (DEHP), are plasticizers used during food processing and in food packaging materials, but can also be found in medical tubing and in the coating of certain medications and supplements (15). Other phthalates, such as diethyl phthalate (DEP), are solvents used to stabilize scents in personal care products and cosmetics (16). As a result, ingestion, dermal absorption, and inhalation are major routes of exposure to these chemicals during pregnancy. Because these chemicals are found in personal care products and cosmetics, women generally have higher urinary phthalate metabolite concentrations than men (14). Additionally, a pregnant woman's phthalate exposure largely depends on her socioeconomic status, lifestyle, and health factors (17, 18). This is important to note since knowing the major risk factors associated with higher or even lower phthalate exposure is not only necessary for understanding exposure patterns, but also critical for identifying at risk groups of pregnant women. The current literature suggests that pregnant women who self-identify as Black and/or Hispanic, have lower socioeconomic status, and/or

engage in unhealthy lifestyle behaviors are likely to have higher phthalate exposure than non-Hispanic White women, those with higher socioeconomic status, and/or those who engage in healthier lifestyle behaviors (17, 18). Further research is necessary to corroborate these findings, but also identify other critical determinants, particularly modifiable factors, of phthalate exposure that may be exclusive to pregnancy.

Phthalates are traditionally classified as endocrine and metabolic disrupting chemicals based on evidence from experimental models in non-pregnant animals, although there are limited and inconsistent findings in pregnancy experimental and observational studies. Specifically, *in vitro* studies suggest that phthalates are weakly estrogenic (19, 20), and *in vivo* studies in male offspring of exposed dams showed that certain phthalates have anti-androgenic effects (21, 22). The metabolic disrupting properties are supported by studies *in vitro* and non-pregnancy *in vivo* models showing that phthalates can interact with peroxisome proliferator-activated receptor gamma, liver X receptors, and retinoid X receptors (23) – these receptors are important mediators for regulating glucose and lipid homeostasis, adipogenesis, but also hormone synthesis and signaling. Because pregnancy is a sensitive window with coordinated dynamic changes in hormone and metabolic homeostasis, the endocrine and metabolic disrupting potential of phthalates and widespread exposure to these chemicals is concerning. Unfortunately, it is also these pregnancy-specific physiological adaptations that makes it challenging to translate the above mentioned experimental findings to human pregnant women. Pregnancy adaptations are also trimester-specific, making it challenging for human epidemiologic studies to identify consistent associations across various cohorts. Studies in pregnancy

cohorts are needed to determine if the endocrine and metabolic properties of phthalates translate to human pregnant women.

The above-discussed phthalate mechanisms of action are especially detrimental in pregnancy since they may be responsible for adverse pregnancy and birth outcomes, including altered gestational weight gain – a clinically relevant marker of gestational health outcomes. In pregnancy cohort studies, prenatal phthalate exposure is associated with increased odds of pregnancy loss (24), pregnancy hypertension (25, 26), gestational diabetes (27), pre-term birth (28-30), altered birth weight (26), and inappropriate gestational weight gain (31-33). The deleterious effects of phthalates on pre-term birth, altered birth weight, and inappropriate gestational weight gain has also been reported in several *in vivo* pregnancy experimental animal models (34-39). Clinically, gestational weight gain is used as an easily measured marker of pregnancy health and fetal growth because it is a complex phenotype with contributions from both the mother and the developing fetus (3). Institute of Medicine (IOM) recommendations for pregnancy weight gain were implemented based on the robust evidence that deviations from appropriate weight gain are predictive of the same above-mentioned poor pregnancy and birth outcomes (40-42). Inappropriate weight gain may also have long lasting consequences for both mother and baby (43, 44), which are also potential adverse endpoints of phthalate exposure. Conducting further studies to determine whether phthalates are associated with altered gestational weight gain will be important to elucidate the potential maternal and child health repercussions of prenatal phthalate exposure.

In response to growing public concerns over prenatal phthalate exposure for child health and the subsequent regulation of these chemicals in certain consumer products (58), purportedly safe replacements were introduced. Specifically, replacements di(isononyl) cyclohexane-1,2-dicarboxylate (DiNCH) and a terephthalate diester, di(2-ethylhexyl) terephthalate (DEHTP) were developed and introduced into the U.S. market before or in early 2000s to replace plasticizer phthalates such as DEHP (59-61). Unfortunately, the safety and toxicity of these ortho-phthalate replacements are relatively unknown, but recent observational evidence indicates that prenatal exposure to DiNCH and DEHTP may be associated with increased risk of pre-term birth (62) and inappropriate gestational weight gain (54). This highlights the concept of regrettable substitution where a chemical with unknown or unforeseen hazard is used to replace a chemical identified as problematic (63). Unfortunately, findings from the National Health and Nutrition Survey (NHANES) and other U.S. biomonitoring studies show that urinary ortho-phthalate metabolite concentrations are decreasing, while DiNCH and DEHTP metabolite concentrations are increasing over time (64, 65). Additional studies in pregnancy are needed to understand the implications of prenatal DiNCH and DEHTP exposure.

Pregnant women are not exposed to one phthalate or replacement at a time, but a mixture of these chemicals. Unfortunately, most prior studies have focused on assessing associations of single phthalates/replacements with health outcomes, which makes it challenging to know the true impact of exposures to real-life chemical mixtures (66). Some studies suggest that the identified risks related to chemical exposures may be greater when evaluating multiple chemicals together rather than one at a time (67). Additionally,

assessing chemicals as mixtures may better identify pregnant women who will benefit most from interventions targeted at reducing chemical exposures. Therefore, to better simulate real life exposure, the environmental epidemiology field has turned to utilizing traditional, but also developing novel methods for evaluating chemicals as mixtures (66, 67). For example, traditional unsupervised (pattern identification methods, including clustering (i.e., *k*-means clustering) and dimension reduction (i.e., principal component analysis, PCA) approaches can be used to identify sub-groups of pregnant women based on their chemical exposures or identify patterns among chemicals that track together, respectively (68, 69). Novel supervised statistical mixtures methods, including weighted quantile sum regression (WQSR) and quantile-based g-computation (QGComp), can be used to estimate the cumulative or joint effect of multiple chemicals on an outcome of interest, but also identify "bad actors", or which chemicals contribute most to the overall chemical mixture effect (66, 70-73). Studies focusing on assessing associations of phthalates and their replacements as a mixture are needed to further understand gestational exposure patterns to these chemicals, but also better model the potential true effect of phthalate/replacement exposure on pregnancy health.

Given the gaps outlined above, the main objectives of this dissertation were to further understand the maternal risk factors, pregnancy hormonal targets, and cardiometabolic consequences of exposure to individual and a mixture phthalates and their replacements in pregnancy. Specifically, we identified the major maternal characteristics associated with prenatal phthalate/replacement exposure and determined if phthalate/replacement exposure was associated with altered gestational hormone concentrations and

gestational weight gain. These knowledge gaps were addressed in three aims corresponding to **Chapters 2 – 4** of this dissertation (presented in **Figure 1**) using data collected as part of the Illinois Kids Development Study (I-KIDS) – an ongoing prospective pregnancy and birth cohort of women from Champaign-Urbana, Illinois following 720 pregnant women and their child from early pregnancy through childhood. In Aim 1 (**Chapter 2**), we used pattern identification methods, *k*-means and PCA, to identify phthalate/replacement exposure patterns along with major seasonal, time, and maternal sociodemographic, lifestyle, and health determinants of these exposure patterns. In Aim 2 (**Chapter 3**), we evaluated gestational timepoint- and fetal sex-specific associations of individual phthalates/replacements with maternal estrogen and testosterone concentrations. In Aim 3 (**Chapter 4**), we evaluated phthalates/replacements individually and as a mixture using WQSR and QGComp to determine if these chemicals are associated with altered gestational weight gain. The overarching goal of this dissertation was to stress the importance of considering maternal pregnancy health, highlight regrettable chemical substitution, and emphasize the utility of statistical mixtures approaches for addressing these research questions.

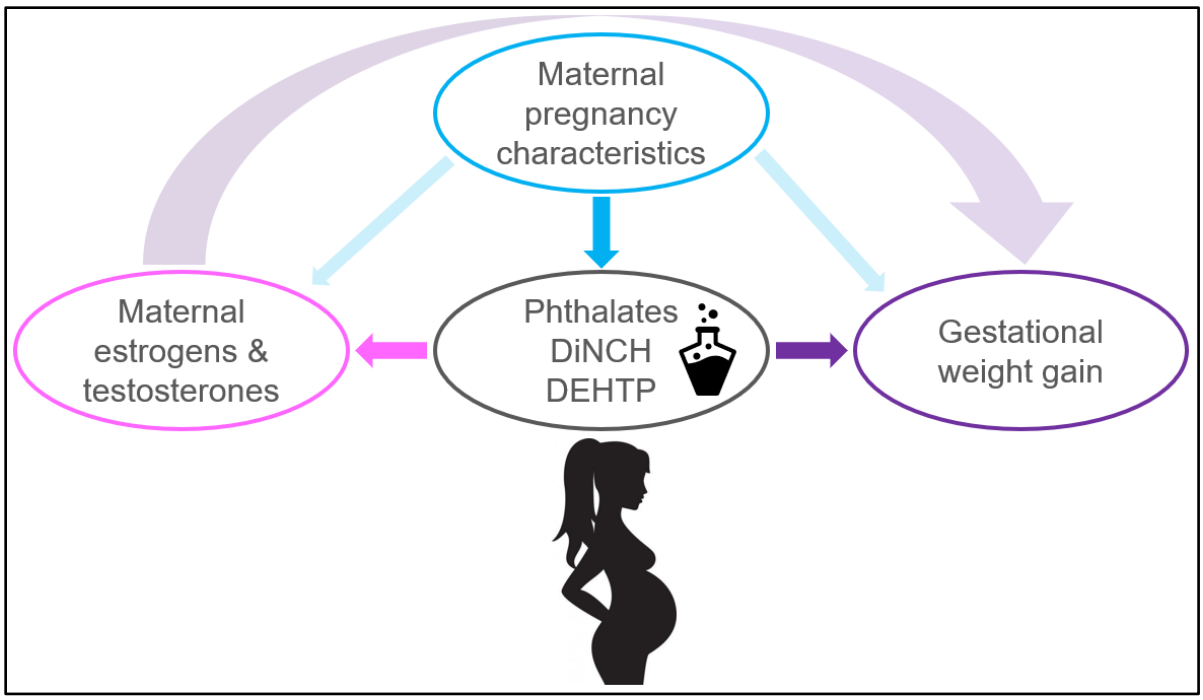

Figure 1. Summary of three aims evaluated in dissertation. The colors represent the following dissertation aims/chapters: blue = Aim 1/Chapter 2, pink = Aim 2/Chapter 3, and purple = Aim 3/Chapter 4. The darker shaded arrows indicate the specific relationships evaluated, while the lighter arrows show how the dissertation aims are related.

CHAPTER 2: IDENTIFICATION OF PROFILES AND DETERMINANTS OF MATERNAL PREGNANCY URINARY BIOMARKERS OF PHTHALATES AND REPLACEMENTS IN THE ILLINOIS KIDS DEVELOPMENT STUDY

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2.1. ABSTRACT

Pregnant women are exposed to multiple phthalates and their replacements, which are endocrine disrupting chemicals associated with adverse maternal and child health outcomes. Identifying maternal characteristics associated with phthalate/replacement exposure during pregnancy is important. We evaluated 13 maternal sociodemographic and lifestyle factors, enrollment year, and conception season as determinants of exposure biomarkers of phthalates and their replacements in 482 pregnant women from the Illinois Kids Development Study (I-KIDS, enrolled 2013-2018). We quantified 19 phthalate/replacement metabolites in pools of five first-morning urines collected across pregnancy. K-means clustering identified women with distinct patterns of biomarker concentrations and principal component analysis (PCA) identified principal component (PC) profiles of biomarkers that exist together. We used multivariable regression models to evaluate associations of predictors with identified k-means clusters and PCs. K-means clustering identified two clusters of women: 1) low phthalate/di(2-ethylhexyl) terephthalate

(∑DEHTP) and 2) high phthalate/∑DEHTP biomarker concentrations. PCA identified four PCs with loadings heaviest for biomarkers of plasticizer phthalates [di-isononyl, di-isodecyl, di-n-octyl phthalates] (PC1), of other phthalates [dibenzyl, di-n-butyl, di-iso-butyl phthalates] (PC2), of phthalate replacements [∑DEHTP, di(isononyl) cyclohexane-1,2-dicarboxylate (∑DiNCH)] (PC3), and of monoethyl phthalate [MEP] (PC4). Overall, age, marital status, income, parity, pre-pregnancy BMI, caffeine intake, enrollment year, and conception season were independently associated with k-means cluster membership and at least one PC. Additionally, race/ethnicity, education, employment, pregnancy intention, smoking status, alcohol intake, and diet were associated with at least one PC. For instance, women who conceived in the spring, summer, and/or fall months had lower odds of high phthalate/∑DEHTP cluster membership and had lower plasticizer phthalate, phthalate replacement, and MEP PC scores. Conception season, enrollment year, and several sociodemographic/lifestyle factors were predictive of phthalate/replacement biomarker profiles. Future studies should corroborate these findings, with a special focus on replacements to which pregnant women are becoming increasingly exposed.

2.2. KEYWORDS

DEHTP, determinants, DiNCH, endocrine disruptors, phthalate, pregnancy.

2.3. INTRODUCTION

Ortho-phthalate diesters, or phthalates, are a class of chemicals widely used in the production of plastics for food contact materials and in some personal care products to which humans are exposed through ingestion, dermal absorption, and inhalation (14, 74).

An increasing body of evidence points to phthalates as endocrine disrupting chemicals that interact with multiple hormones and hormone-regulated processes (75-79), which is concerning given that pregnancy is a hormonally sensitive window and pregnant women are ubiquitously exposed to these chemicals (14). Higher maternal urinary phthalate metabolite concentrations have been associated with adverse pregnancy outcomes, including preeclampsia and pregnancy hypertensive disorders (25, 26), glucose intolerance and gestational diabetes (32, 80, 81), and preterm birth (82). In response to the growing concerns over the endocrine disrupting properties and subsequent regulation of ortho-phthalate diesters (58), purportedly safe replacements were developed and introduced into the U.S. market before or in the early 2000s, including di(isononyl) cyclohexane-1,2-dicarboxylate (DiNCH) (59) and a terephthalate diester, di(2-ethylhexyl) terephthalate (DEHTP) (60, 61). Recent observational evidence indicates that DiNCH and DEHTP exposure may be associated with adverse health outcomes, including increased risk of uterine fibroids and pre-term birth (62, 83), as well as altered sex steroid hormone and oxidative stress levels (84-87). Therefore, additional studies are needed to identify maternal characteristics associated with phthalate and replacement exposures, which can be used as covariates in studies evaluating the health implications of increasing and decreasing exposure to these chemicals during pregnancy.

To identify sub-populations of pregnant women with higher phthalate/replacement exposures, numerous prior studies evaluated important seasonal, sociodemographic, and lifestyle predictors of phthalate metabolite (and to a lesser extent phthalate replacement) concentrations. In general, these studies evaluated bivariable and/or

multivariable associations between predictors and concentrations of individual phthalate biomarkers (as individual biomarkers or molar sums of biomarkers from common parents) and found that the following characteristics most often remained as important determinants of phthalate biomarker concentrations: age, race/ethnicity, education, income, marital status, social class, parity, pre-pregnancy body mass index (BMI), smoking status, diet, and study year (17, 18, 88-93). However, these studies only assessed determinants of individual biomarkers (or molar sums representing single parent compounds) in single-pollutant models. Given that pregnant women are exposed to numerous phthalates and their replacements, some studies also assessed predictors of maternal exposure to numerous chemicals (including phthalates) using unsupervised learning methods such as *k*-means clustering and principal component analysis (PCA) (94-97). One study using *k*-means paired with logistic regression analyses found that race/ethnicity and diet were associated with clusters of women who had higher biomarker concentrations of personal care product and plasticizer phthalates, respectively (95). Another study observed associations of parity, pre-pregnancy BMI, and job type with high phthalate cluster membership (97). The few studies using PCA paired with linear regression analyses reported that age, birthplace, race/ethnicity, income, job type, parity, pre-pregnancy BMI, smoking status, and diet were associated with principal components (PCs) heavily loaded for phthalate metabolites, including mono(3-carboxypropyl) phthalate (MCPP), mono-n-butyl phthalate (MBP), monobenzyl phthalate (MBzP), mono-iso-butyl phthalate (MiBP), and monoethyl phthalate (MEP), and metabolites of di(2-ethylhexyl) phthalate (DEHP) (94, 95, 97). Such approaches that identify determinants of biomarker concentration patterns/profiles may better identify unique characteristics of

women who may benefit most from interventions targeted at decreasing phthalate/replacement exposure.

Given that phthalates and their replacements are a diverse class of chemicals with multiple exposure sources, our study focused on identifying whether maternal sociodemographic characteristics, lifestyle factors, enrollment year, and conception season are predictors of phthalate/replacement biomarker concentrations. Our first objective was to ascertain patterns of phthalate/replacement biomarker concentrations using both *k*-means clustering and PCA, which can identify groups of pregnant women with similar phthalate/replacement biomarker concentration profiles (*k*-means) and groups of phthalates/replacements that likely exist together (PCA). Our second objective was to evaluate associations of maternal sociodemographic characteristics, early gestation lifestyle factors, conception season, and study enrollment year with the patterns of phthalate/replacement biomarker concentrations identified in *k*-means and PCA.

2.4. MATERIALS AND METHODS

2.4.1. Illinois Kids Development Study (I-KIDS) recruitment and enrollment

The current study includes pregnant women from I-KIDS, an ongoing prospective pregnancy cohort designed to evaluate the impacts of prenatal environmental chemical exposures on infant neurodevelopment. Pregnant women were recruited at their first prenatal care appointment from two local obstetric clinics in Champaign-Urbana, IL. Women who expressed interest in the study were eligible to participate if they were ≥ 10 but < 15 weeks pregnant, 18-40 years old, fluent in English, in a low-risk singleton

pregnancy, living within a 30-minute drive of the University of Illinois campus, and not planning to move out of the area before their child's first birthday. The current study includes the first 482 women who enrolled in I-KIDS between December 2013 and August 2018, and remained in the study through the birth of their infant. These women provided written informed consent and the study was approved by the Institutional Review Board at the University of Illinois. The analysis of de-identified specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

2.4.2. Collection of maternal sociodemographic, lifestyle, and conception season information

Immediately after enrollment, an I-KIDS staff member visited each participant's home to obtain information about sociodemographic and lifestyle characteristics. We collected information about the following sociodemographic characteristics using an interviewer-administered questionnaire: age, race/ethnicity, education level, marital status, employment status, and household annual income. Women additionally reported whether they planned their current pregnancy. To determine conception season, we used the estimated due date based on the first day of the last menstrual period reported at baseline and confirmed after the first trimester ultrasound. Each woman reported the following information since conception: smoking status, the number of eight-ounce cups of caffeinated beverages consumed on a typical day, and the number of servings of alcoholic beverages consumed per week. Self-reported pre-pregnancy weight and height were used to calculate pre-pregnancy BMI (in kg/m^2). Self-reported pre-pregnancy BMI is highly correlated with first trimester measured BMI in other pregnant populations (98-

100), as well as ours (r = 0.99, data not shown). Participants completed a semi-quantitative food frequency questionnaire (FFQ) at enrollment that was adapted for pregnant women from the full-length Block-98 FFQ (NutritionQuest, Berkeley, CA) and asked about maternal diet during the previous three months (101). Reported dietary intakes were used to calculate first trimester Alternative Healthy Eating Index 2010 (AHEI-2010) – an 11-component diet quality measure (scored out of 110) based on food/nutrients predictive of chronic disease risk and mortality; higher scores reflect better diet quality (102, 103).

2.4.3. Assessment of urinary phthalate/replacement biomarker concentrations

I-KIDS participants provided up to five first-morning urine samples at the following gestational timepoints: 8-15, 13-22, 19-28, 25-33, 32-40 weeks gestation (median 13, 17, 23, 28, and 34 weeks gestation, respectively) as described previously (86), which corresponded with study home visits (at median 13, 17, and 34 weeks gestation) or routine prenatal care visits (median 23 and 28 weeks gestation). Most women contributed all five urine samples (94.4%), whereas 5.2% and 0.4% contributed four and three urine samples, respectively. Urine samples were collected in polypropylene urine cups and refrigerated immediately. Within 24 hours of collection, urine samples were aliquoted for long-term storage or pooled from each timepoint. Beginning with the first visit's sample, we added 900 μL of urine to a 5 mL cryovial tube. Each time women provided a sample, we layered fresh urine onto frozen urine from prior gestational timepoints before immediately freezing it at -80° C. At the end of pregnancy, we thawed and vortex all pooled samples to measure specific gravity. For quality assurance and control, we also

collected duplicates and purified water blanks every 10 samples to be analyzed at the CDC. We stored all aliquoted urine at -80° C and sent pooled samples on dry ice to the CDC laboratory in three batches in chronological order of enrollment (batch one enrolled December 2013 - February 2015, batch two enrolled February 2015 - July 2016, and batch three enrolled July 2016 - August 2018). The following phthalate/replacement metabolites were quantified in all batches using previously published methods (104, 105): 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), monoisononyl phthalate (MiNP), monocarboxyoctyl phthalate (MCOP), monocarboxynonyl phthalate (MCNP), MCPP, MBzP, MEP, MBP, mono-hydroxybutyl phthalate (MHBP), MiBP, mono-hydroxy-isobutyl phthalate (MHiBP), cyclohexane-1,2-dicarboxylic acid-mono(carboxyoctyl) ester (MCOCH), and cyclohexane-1,2-dicarboxylic acid-monohydroxy isononyl ester (MHiNCH). Three additional metabolites were added to the CDC analytical panel for women in batches two and three (monooxononyl phthalate (MONP), mono(2-ethyl-5-hydroxyhexyl) terephthalate (MEHHTP), and mono(2-ethyl-5-carboxypentyl) terephthalate (MECPTP)) (104, 106). The CDC laboratory has rigorous quality control/quality assurance protocols with excellent long-term reproducibility of most phthalate metabolite biomarkers over 3 and 8 month periods and intra- and inter-day coefficients of variation < 14% for most biomarkers (104-106).

2.4.4. Statistical analysis

For phthalate/replacement metabolite concentrations below the limit of detection (LOD),

we used instrumental-reading values to avoid bias associated with imputing values below the LOD (107). Across the individual and molar sum biomarkers described below, only one woman had a zero concentration for the sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites (∑DiNCH) (meaning that her urinary concentrations of both MCOCH and MHiNCH were zero). In final statistical models we added a constant (1.0) to ∑DiNCH before ln-transformation to avoid undefined estimates (108). To account for urine dilution, we adjusted all urinary phthalate/replacement metabolite concentrations using the following formula: $P_c = P[(1.016 - 1)/(SG - 1)]$, where P_c is the specific gravity-adjusted metabolite concentration, P is the measured metabolite concentration (ng/mL), 1.016 is the population median specific gravity, and SG is the specific gravity of each individual urine sample (109). We summed the molar concentrations (in nmol/mL) of the following metabolites to create biomarkers of exposure to phthalate/replacement parent compounds that are metabolized and excreted as multiple urinary metabolites (86): MEHP, MEHHP, MEHOP, and MECPP for the sum of di(2-ethylhexyl) phthalate metabolites (∑DEHP); MiNP and MCOP for the sum of di-isononyl phthalate metabolites (∑DiNP); MBP and MHBP for the sum of di-n-butyl phthalate metabolites (∑DBP); MiBP and MHiBP for the sum of di-iso-butyl phthalate metabolites (∑DiBP); MHiNCH and MCOCH for ∑DiNCH; and MEHHTP and MECPTP for the sum of di(2-ethylhexyl) terephthalate metabolites (∑DEHTP). We also created another ∑DiNP (∑DiNP2) limited to women enrolled between February 2015 and August 2018 to include MONP. These molar concentrations were converted to ng/mL by multiplying ∑DEHP, ∑DiNP (both versions), ∑DBP, ∑DiBP, ∑DiNCH, and ∑DEHTP by the molecular weights of MECPP, MCOP, MBP, MiBP, MHiNCH, and MECPTP, respectively. We estimated exposure to di-

isodecyl phthalate, di-n-octyl phthalate, benzylbutyl phthalate, and diethyl phthalate using ng/mL concentrations of their corresponding urinary metabolites MCNP, MCPP, MBzP, and MEP, respectively.

To understand how phthalate/replacement metabolite concentrations in I-KIDS compare to those in the general U.S. population, we used data from the National Health and Nutrition Examination Survey (NHANES) survey cycles 2013-14, 2015-16, and 2017-18 (110-112). These NHANES survey cycles correspond with urine collection years in I-KIDS. Though most women in NHANES were not pregnant, we subset the NHANES sample to only include 18 – 40 year-old females with data on urinary phthalate/replacement metabolite concentrations. Finally, because NHANES does not provide specific gravity information, we reported median (25^{th}, 75^{th} percentiles) unadjusted phthalate/replacement metabolite concentrations for both samples (**Table 2**)**.**

Our analyses included 15 maternal characteristics that have been previously shown to predict phthalate/replacement biomarker concentrations or were hypothesized to be critical determinants of phthalate/replacement exposure in our population (17, 88, 91, 113-115). These included age, race/ethnicity, education, marital status, employment status, household annual income, parity, conception season, enrollment year, smoking in the first trimester, consumption of alcohol and caffeine in the first trimester, pregnancy intention, pre-pregnancy BMI, and diet quality. Almost all predictors were assessed as categorical variables, with the exception of enrollment year, which we evaluated as a continuous variable that can be interpreted for every 1 year increase. Details about

variable operationalization are provided in **Table 1**. Of note, an additional category for smoking in the first trimester ("unknown") was created to account for missingness due to an ambiguous skip pattern in the first iteration of the survey. Pre-pregnancy BMI was categorized based on standard U.S. clinical cut-offs (116).

We selected methods to evaluate chemical mixtures appropriate for the specific research question (66). We used the following two unsupervised methods (objective 1): *k*-means clustering and PCA (68, 69). *K*-means clustering identifies subgroups of women with distinct biomarker concentration profiles, which is useful for identifying pregnant women who may experience relatively high or low chemical exposures. We used *k*-means clustering to group pregnant women into *k* number of distinct, non-overlapping clusters (identified using Euclidean geometry) based on their similarities across all individual phthalate/replacement biomarker concentrations. To identify the optimal number of clusters, we compared 1, 2, 3, and 4 cluster solutions using the pseudo f-statistic index (the ratio of between-cluster variance to within cluster variance) and confirmed the ideal number of clusters using elbow plots of R^2 values. PCA identifies linear combinations of biomarker concentration patterns among highly correlated biomarker that explain most of the variance in biomarker concentrations in a population. These resulting patterns can be related to common exposure sources or behaviors in the study population. We used PCA with a Varimax rotation to identify biomarkers of highly correlated phthalate/replacements to which pregnant women are likely exposed and created distinct, uncorrelated PC scores that explain most of the variance in phthalate/replacement biomarker concentrations in our participants. To determine the ideal number of PCs, we assessed elbow plots of

eigenvalues (total variance explained by each component) and used the total variance explained to confirm the optimal number of components that best represents the data. We considered biomarkers with loadings ≥ 0.3 to be notable. For both *k*-means and PCA, we included specific gravity-adjusted phthalate/replacement biomarker concentrations in ng/mL that were ln-transformed and z-transformed.

We used logistic and linear regression models to evaluate associations of 15 maternal characteristics with the identified clusters and PCs, respectively (objective 2). Evaluating associations of characteristics with identified *k*-means clusters using logistic regression models provides information about characteristics of pregnant women with specific phthalate/replacement biomarker concentration profiles. Assessing relationships of maternal characteristics with identified PCs using linear regression models provides information about characteristics that likely result in exposure to certain phthalates/replacements from common exposure sources or behaviors. We evaluated both unadjusted models (bivariable analyses) and models simultaneously adjusted for all 15 predictors (multivariable analyses). A total of 9 women had missing data on at least one predictor. Therefore, 473 women who enrolled between December 2013 and August 2018 (referred to as the full sample) were included in final multivariable analyses. To assess MONP and both DEHTP metabolites, we conducted additional analyses limited to women enrolled between February 2015 and August 2018 (referred to as the sub-sample). A total of 305 women were included in these multivariable analyses. There was high agreement between the full and sub-samples with regards to *k*-means cluster membership (Kappa statistic = 0.82) and PC scores ($r > 0.8$). However, we reported

results from both samples to provide information about phthalate/∑DiNCH biomarker concentrations across the whole study period and to report results related to phthalate replacement DEHTP.

We used SAS 9.4 (version 15.1, SAS Institute) for all statistical analyses. We used PROC FASTCLUS and PROC LOGISTIC to assign *k*-means clusters and for bivariable and multivariable logistic regression models, respectively. We used PROC FACTOR for the PCA and PROC GENMOD for bivariable and multivariable linear regression models. Based on recommendations from the American Statistical Association and others (117, 118), rather than using *P*-values, we used the magnitude of associations and 95% confidence intervals (CIs) to identify potentially meaningful results. We used RStudio Version 1.3.1093 (RStudio, Boston, MA) to generate figures.

2.5. RESULTS

2.5.1. I-KIDS characteristics and urinary phthalate/replacement biomarker concentrations

Sociodemographic and lifestyle characteristics of I-KIDS women have been previously described (86) and are outlined in **Table 1**. Briefly, most women were non-Hispanic white, of high socioeconomic status, and engaged in healthy lifestyle behaviors. Greater than 97% of I-KIDS women had detectable urinary concentrations of at least one metabolite per phthalate parent compounds (including DEHTP), while only 77% had detectable urinary concentrations of at least one DiNCH metabolite (**Table 2**). Most phthalate/replacement biomarkers were weakly-to-moderately correlated ($r < 0.4$),

although strong correlations were observed between MCPP, ∑DiNP, and ∑DiNP2 ($r > 0.8$; **Figure 2**). I-KIDS pregnant women had similar median urinary phthalate and DiNCH metabolite concentrations as those from a nationally representative sample of 18 – 40 year-old pregnant or non-pregnant U.S. women from the 2013 - 2018 National Health and Nutrition Examination Survey (NHANES) cycles (**Table 2**). However, I-KIDS women had higher median concentrations of DEHTP metabolites, but lower median concentrations of MEP (with overlapping 25^{th} and 75^{th} percentiles) compared to NHANES women.

Table 1. Characteristics of I-KIDS women in the full and sub-samples.

	Full sample enrolled 12/2013 - 8/2018 (n=482)	Sub-sample enrolled 2/2015 - 8/2018 (n=309)
	n (%)	n (%)
Maternal age		
< 30 years	197 (41.9)	115 (37.2)
≥ 30 years	285 (59.1)	194 (62.8)
Race/ethnicity	*1 missing*	
Non-Hispanic white	385 (80.0)	251 (81.2)
Non-Hispanic black	26 (5.4)	16 (5.2)
Asian	26 (5.4)	20 (6.5)
Other[1]	44 (9.2)	22 (7.1)
Education		
Some college or less	90 (18.7)	52 (16.8)
College grad or higher	392 (81.3)	257 (83.2)
Marital status		
Married	426 (88.4)	273 (88.4)
Unmarried	56 (11.6)	36 (11.7)
Employment status		
Unemployed	67 (13.9)	39 (12.6)
Employed	415 (86.1)	270 (87.4)
Household income	*4 missing*	
<$60,000	138 (28.6)	84 (27.2)
$60,000-$99,999	182 (37.8)	114 (36.9)
≥$100,000	158 (32.8)	109 (35.3)
Enrollment year		
12/2013 – 02/2015	173 (35.9)	--
02/2015 – 07/2016	174 (36.1)	174 (56.3)
07/2016 – 08/2018	135 (28.0)	135 (43.7)
Conception season		
Winter	122 (25.3)	80 (25.9)
Spring	134 (27.8)	101 (32.7)
Summer	107 (22.2)	78 (25.2)
Fall	119 (24.7)	50 (16.2)
Parity		
0 children	246 (51.0)	164 (53.1)
≥ 1 child	236 (49.0)	145 (46.9)
Active smoker		
No	423 (87.8)	294 (95.2)
Yes	24 (5.0)	15 (4.9)
Missing	35 (7.3)	--
Alcohol intake	*1 missing*	
No serving/week	281 (58.3)	180 (58.3)
≥ 1 servings/week	200 (41.5)	129 (41.8)
Pregnancy intention		
Planned	321 (66.6)	207 (67.0)
Unplanned	161 (33.4)	102 (33.0)
Pre-pregnancy BMI		
< 25 kg/m^2	258 (53.5)	162 (52.4)
≥ 25 kg/m^2	224 (46.5)	147 (47.6)
Caffeine intake		
None	196 (40.7)	124 (40.1)
< 1 cups/week	145 (30.1)	93 (30.1)
≥ 1 cups/week	141 (29.3)	92 (29.7)
Overall diet quality	*3 missing*	*2 missing*
AHEI < 55.1	239 (49.6)	153 (49.5)
AHEI ≥ 55.1	240 (49.8)	154 (49.8)

[1]Hispanic white, American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, multiracial, and other. I-KIDS, Illinois Kids Development Study.

Table 2. Unadjusted phthalate/replacement metabolite concentrations (ng/mL).

		I-KIDS (n=482)		NHANES (n=1076)
Parent Compound	**Metabolite(s)**	**% ≥ LOD**	**Median (25th, 75th pctl) 2013-2018**	**Median (25th, 75th pctl) 2013-2018**
DEHP	MEHP	74.3	1.3 (0.8, 2.2)	1.1 (0.6, 2.3)
	MEHHP	100.0	6.0 (3.8, 9.2)	5.3 (2.5, 10.8)
	MEOHP	100.0	4.6 (3, 6.9)	3.7 (1.7, 7.3)
	MECPP	100.0	9.2 (6.1, 14.8)	8.6 (4.1, 16.8)
DiNP	MCOP	100.0	11.0 (5.4, 25.7)	8.0 (3.5, 23.6)
	MiNP	41.7	0.7 (0.4, 1.5)	0.6 (0.6, 1.1)
	MONP	100.0	2.7 (1.7, 4.7)[1]	1.6 (0.7, 3.2)[3]
DiDP	MCNP	100.0	2.1 (1.4, 3.3)	1.6 (0.8, 3.4)
DOP	MCPP	97.1	1.5 (0.9, 2.6)	1.1 (0.5, 2.6)
BBzP	MBzP	99.6	5.3 (2.8, 12)	4.6 (1.6, 12.0)
DEP	MEP	100.0	25.0 (12.6, 46.5)	34.4 (14.6, 85.8)
DBP	MBP	100.0	12.6 (8.1, 19.5)	11.2 (5.1, 20.2)
	MHBP	90.0	1.2 (0.7, 2)	0.9 (0.3, 1.7)[2]
DiBP	MiBP	99.8	9.1 (5.5, 14.1)	8.7 (4.0, 17.9)
	MHiBP	99.8	3.3 (2, 5.1)	2.9 (1.4, 6.0)[2]
DiNCH	MHiNCH	77.4	0.8 (0.4, 1.6)	0.4 (0.3, 1.1)
	MCOCH	50.4	0.5 (0.3, 1)	0.4 (0.4, 0.8)[3]
DEHTP	MEHHTP	100.0	8.7 (3.7, 19.7)[1]	6.0 (2.2, 17.1)[3]
	MECPTP	100.0	60.5 (24.3, 140)[1]	20.7 (8.5, 67.6)[3]

Urinary phthalate/replacement metabolite concentrations were obtained for 18-40 year-old pregnant and non-pregnant females from NHANES survey years 2013-14, 2015-16, and 2017-18 I-KIDS reports numeric values for all concentrations below the LOD, while NHANES replaces all values below the LOD with the LOD/√2 for that metabolite. Concentrations do not account for urine dilution. [1]n=309, [2]n=1074, [3]n=682. I-KIDS, Illinois Kids Development Study; NHANES, National Health and Nutrition Examination Survey.

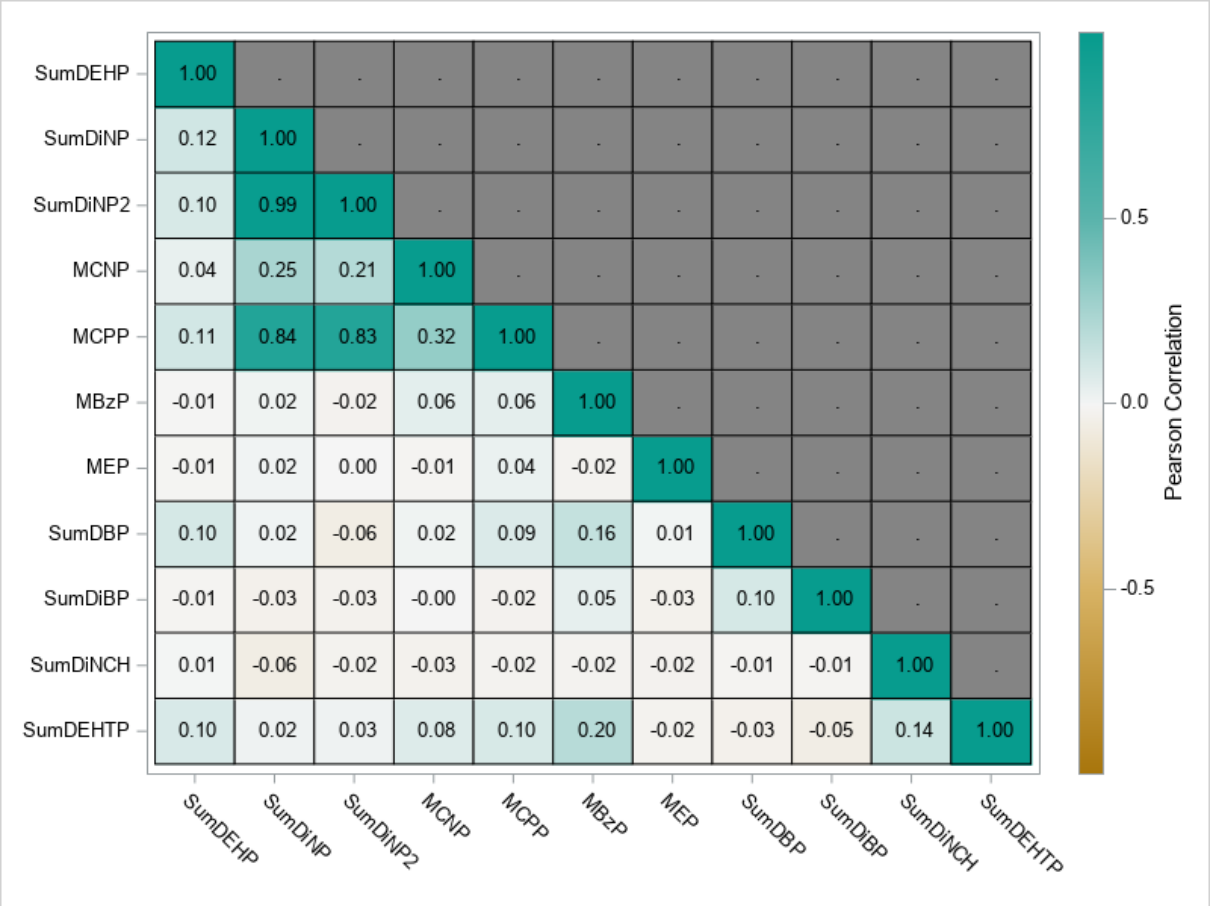

Figure 2. Correlations between urinary specific gravity-adjusted phthalate/replacement biomarker concentrations. Heat map presents Pearson correlations between all biomarkers. Yellow and turquoise indicate negative and positive correlations, respectively, where lighter shades represent weaker correlations and darker shades represent stronger correlations.

2.5.2. K-means clusters of women with distinct phthalate/replacement biomarker concentration profiles

Our goal with using k-means clustering was to identify groups of women with distinct profiles of urinary phthalate/replacement biomarker concentrations. In the full sample (excluded MONP and the DEHTP metabolites), we identified the following two clusters: cluster 1 included women with concentrations of all phthalate biomarkers below the sample median, while cluster 2 included women with concentrations of all phthalate biomarkers above the sample median (**Figure 3**). ∑DiNCH concentrations were similar between the two clusters, and therefore did not drive cluster membership. In the sub-sample (includes MONP and DEHTP metabolites), the k-means procedure identified similar clusters as those identified in the full sample. Therefore, the two clusters of women in this sub-sample included women with all phthalate biomarker concentrations (including ∑DEHTP) below the sample median (cluster 1) and those with all phthalate biomarker concentrations (including ∑DEHTP) above the sample median (cluster 2) (**Figure 3**).

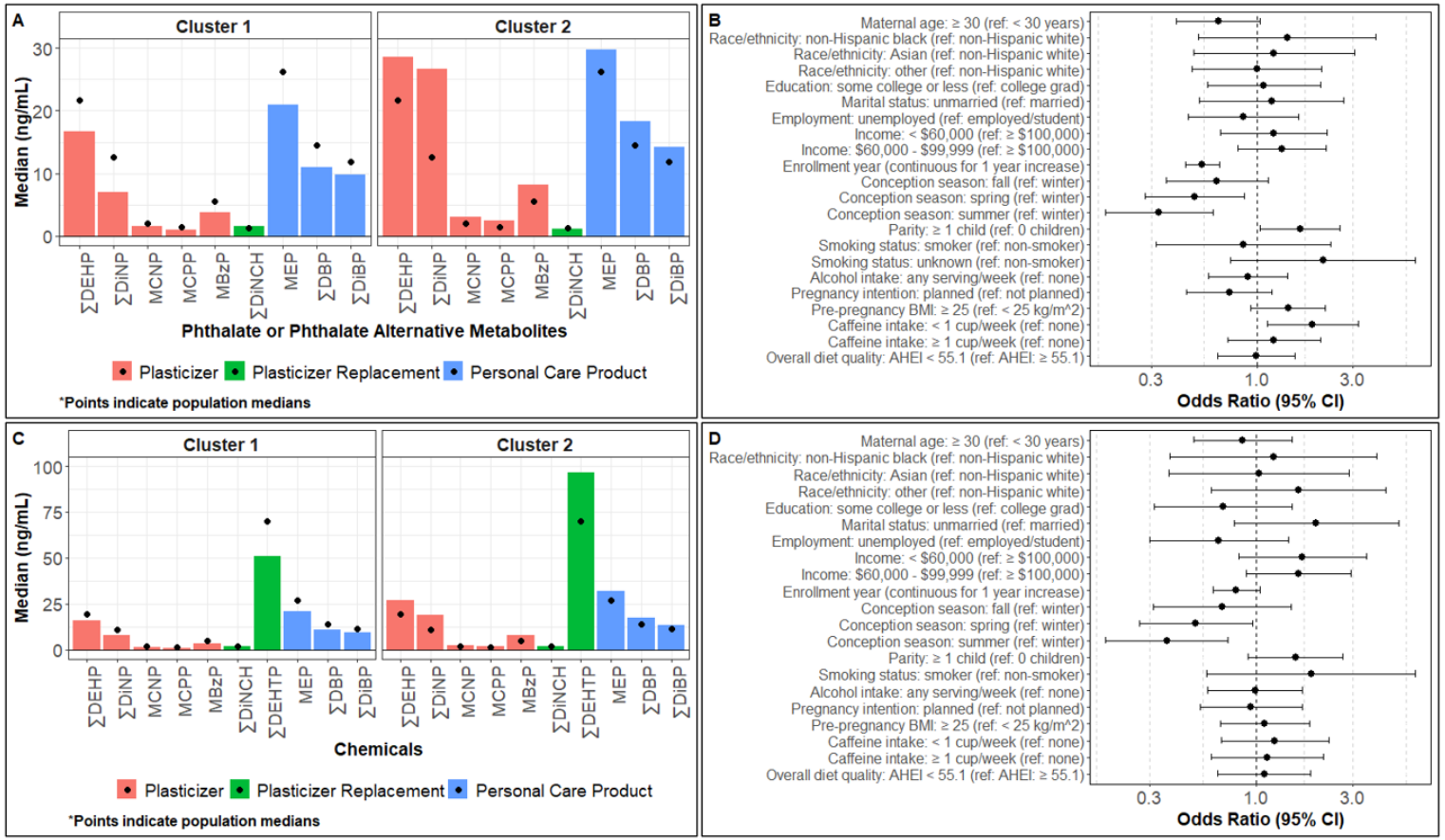

Figure 3. Multivariable associations of sociodemographic, lifestyle, enrollment year, and conception season predictors with *k*-means clusters. Median urinary specific gravity-adjusted phthalate/replacement biomarker concentrations by *k*-means cluster in the **A)** full sample (enrolled between 12/2013 and 8/2018) or **C)** sub-sample (enrolled between 2/2015 and 8/2018). Logistic regression models simultaneously adjusted for all listed predictors evaluated associations of 15 predictors with the odds of having **B)** high phthalate (cluster 2, n=230) versus low phthalate (cluster 1, n=243) in the full sample or **D)** high phthalate including ∑DEHTP (cluster 2, n=131) versus low phthalate including ∑DEHTP (cluster 1, n=174) biomarker concentrations in the sub-sample. BMI, body mass index; CI, confidence interval.

2.5.3. Associations of maternal characteristics with identified k-means clusters

Bivariable analyses evaluating associations of characteristics with k-means clusters for the full and sub-samples are presented in **Table 3**. In multivariable logistic regression models simultaneously adjusted for all characteristics, women had higher odds of high phthalate cluster membership if they had ≥ 1 child prior to the I-KIDS pregnancy (ref = no children; OR: 1.6; 95% CI: 1.0, 2.6), had overweight or obesity before pregnancy (ref = under-/normal weight; OR: 1.4; 95% CI: 0.9, 2.2), and consumed < 1 cup of caffeine per week (ref = no caffeine consumption; OR: 1.9; 95% CI: 1.1, 3.2) (**Figure 3** and **Table 3**). Conversely, women had lower odds of high phthalate cluster membership if they were ≥ 30 years old (ref = < 30 years; OR: 0.6; 95% CI: 0.4, 1.0), enrolled earlier in the study (for every 1 year increase in study year; OR: 0.5; 95% CI: 0.4, 0.7), and conceived in the spring (OR: 0.5; 95% CI: 0.3, 0.9), summer (OR: 0.3; 95% CI: 0.2, 0.6), or fall (OR: 0.6; 95% CI: 0.4, 1.1) compared to winter. In multivariable logistic regression models in the sub-sample, associations of enrollment year, conception season, and parity with cluster membership were similar to those observed in the full sample (**Figure 3** and **Table 3**). However, additional associations of marital status and annual household income with cluster membership emerged in the multivariable logistic regression models in the sub-sample (**Figure 3** and **Table 3**). Specifically, women had a higher odds of high phthalate (including ∑DEHTP) cluster membership if they were unmarried (ref = married, OR: 2.0; 95% CI: 0.8, 5.1) and had annual household incomes < $100,000 (ref = ≥ $100,000; < $60,000 OR: 1.7; 95% CI: 0.8, 3.5; $60,000 - $99,999 OR: 1.6; 95% CI: 0.9, 2.9).

Table 3. Associations of maternal sociodemographic and lifestyle characteristics with *k*-means clusters.

	Unadjusted OR (95% CI) n=482	Unadjusted OR (95% CI) n=309
Maternal age		
< 30 years	ref	ref
≥ 30 years	**0.6 (0.4, 0.9)**	0.7 (0.5, 1.2)
Race/ethnicity		
Non-Hispanic White	ref	ref
Non-Hispanic Black	1.7 (0.8, 3.9)	1.8 (0.7, 5.0)
Asian	0.9 (0.4, 2.1)	0.9 (0.4, 2.4)
Other	1.2 (0.6, 2.2)	2.0 (0.8, 5.0)
Education		
Some college or less	**1.6 (1.0, 2.6)**	1.1 (0.6, 2.1)
College grad or higher	ref	ref
Marital status		
Married	ref	ref
Unmarried	**1.7 (1.0, 3.0)**	**2.3 (1.1, 4.6)**
Employment status		
Unemployed	1.2 (0.7, 1.9)	0.8 (0.4, 1.6)
Employed	ref	ref
Household income		
<$60,000	**1.9 (1.2, 3.0)**	**2.0 (1.1, 3.7)**
$60,000-$99,999	**1.6 (1.0, 2.5)**	**1.7 (1.0, 2.9)**
≥$100,000	ref	ref
Enrollment year		
Continuous (1 year increase)	**0.5 (0.5, 0.6)**	0.8 (0.7, 1.1)
Conception season		
Winter	ref	ref
Spring	**0.4 (0.3, 0.7)**	**0.5 (0.3, 0.9)**
Summer	**0.3 (0.2, 0.6)**	**0.3 (0.2, 0.6)**
Fall	**0.7 (0.4, 1.1)**	**0.7 (0.3, 1.4)**
Parity		
0 children	ref	ref
≥ 1 child	**1.7 (1.2, 2.4)**	**1.5 (0.9, 2.3)**
Active smoker		
No	ref	ref
Yes	1.4 (0.6, 3.2)	2.0 (0.7, 5.8)
Skip Pattern Missing	**7.1 (2.7, 18.6)**	--
Alcohol intake		
No serving/week	ref	ref
≥ 1 servings/week	0.8 (0.6, 1.2)	1.1 (0.7, 1.7)
Pregnancy intention		
Planned	**0.6 (0.4, 0.9)**	**0.6 (0.4, 1.0)**
Unplanned	ref	ref
Pre-pregnancy BMI		
< 25 kg/m^2	ref	ref
≥ 25 kg/m^2	1.2 (0.8, 1.7)	1.1 (0.7, 1.8)
Caffeine intake		
None	ref	ref
< 1 cups/week	**1.6 (1.0, 2.4)**	1.3 (0.8, 2.3)
≥ 1 cups/week	1.1 (0.7, 1.8)	1.3 (0.7, 2.2)

Table 3 (cont'd).

	Unadjusted OR (95% CI) n=482	Unadjusted OR (95% CI) n=309
Overall diet quality		
AHEI < 55.1	**1.3 (0.9, 1.8)**	1.3 (0.8, 2.1)
AHEI ≥ 55.1	ref	ref
Data are presented as odds ratios (95% CI) from unadjusted and adjusted logistic regression models evaluating the probability of being in cluster 2 (high phthalate/∑DEHTP) compared to cluster 1 (low phthalate/∑DEHTP). Adjusted models control for all the maternal characteristics listed in the table. CI, confidence interval. Bold indicate potentially meaningful findings. Full sample n=243 and n=230 for clusters 1 and 2, respectively; sub-sample n=174 and n=131 for clusters 1 and 2, respectively.		

2.5.4. PCs of phthalate/replacement biomarker concentrations

Our goal with PCA was to identify phthalate/replacement biomarker concentrations that exist together due to common exposure sources or lifestyle factors. In the full sample, four PCs accounted for 71.2% of the total variance (32.2%, 17.2%, 11.2%, and 10.6% of the total variance explained by components 1-4, respectively). The heaviest loadings for each PC were as follows: ∑DiNP, MCNP, and MCPP with component 1 (referred to as phthalate plasticizer component); MBzP, ∑DBP, and ∑DiBP with component 2 (referred to as other phthalate component); ∑DEHP and ∑DiNCH with component 3 (referred to as ∑DEHP/∑DiNCH component); and MEP with component 4 (referred to as MEP component) (**Table 4**). In the sub-sample, four PCs accounted for 66.9% of the total variance (27.6%, 16.3%, 13.1%, and 9.9% of the total variance explained by components 1-4, respectively). Components 2 (other phthalate component) and 4 (MEP component) were similar to those discussed above, whereas component 1 was heavily loaded by ∑DiNP2, MCNP, and MCPP (referred to as plasticizer phthalate component) and component 3 was heavily loaded by ∑DiNCH and ∑DEHTP (referred to as phthalate replacement component) (**Table 4**). In the full sample and the sub-sample, the biomarkers that loaded most heavily were positively correlated with the four component scores indicating that as urinary concentrations of those biomarkers increase, component scores

increase (**Table 4**).

Table 4. Standardized scoring coefficients from principal component analyses.

Biomarker	Component 1	Component 2	Component 3	Component 4
Full sample (enrolled 12/2013 - 08/2018, n=482)				
∑DEHP	0.18615	0.12433	**0.30419**	-0.01715
∑DiNP	**0.38358**	-0.11581	-0.04215	-0.00094
MCNP	**0.32727**	-0.07695	-0.08505	-0.01991
MCPP	**0.35948**	-0.02074	0.0854	-0.00803
MBzP	-0.05799	**0.39207**	-0.20075	0.02746
MEP	-0.0205	-0.05858	-0.00947	**0.99756**
∑DBP	-0.0426	**0.45189**	0.03671	0.01679
∑DiBP	-0.09515	**0.44958**	0.04137	-0.14473
∑DiNCH	0.02608	-0.04767	**0.90648**	-0.0018
Sub-sample (enrolled 02/2015 - 08/2018, n=309)				
∑DEHP	0.09557	0.17086	0.02263	0.15387
∑DiNP2	**0.43855**	-0.13006	-0.04165	-0.00371
MCNP	**0.33061**	-0.01817	-0.04642	-0.02653
MCPP	**0.39854**	-0.00695	-0.03763	-0.08151
MBzP	-0.05536	**0.34434**	0.01587	0.14104
MEP	-0.04751	-0.04257	-0.02487	**0.91181**
∑DBP	-0.05685	**0.44587**	0.01462	-0.00131
∑DiBP	-0.06325	**0.44524**	-0.04736	-0.30297
∑DiNCH	-0.09997	0.03464	**0.6192**	-0.09097
∑DEHTP	0.001	-0.03457	**0.56354**	0.06057
Bold indicate biomarkers with notable loadings (≥ 0.3).				

2.5.5. Associations of maternal characteristics with identified PCs

Results of bivariable analyses evaluating the relationships between characteristics and PC scores for the full and sub-samples are presented in **Tables 5** and **6**, respectively. In multivariable linear regression models in the full sample (**Figure 4** and **Table 5**), phthalate plasticizer component scores were lower in Asian women (ref = non-Hispanic white), those with annual household incomes < $60,000 (ref = ≥ $100,000), those who conceived in the spring, summer, or fall (ref = winter), and those who planned their pregnancy (ref = unplanned pregnancy). Conversely, plasticizer component scores were higher in women who had lower educational attainment (ref = college graduates), enrolled earlier in the

study, those who had overweight or obesity before pregnancy (ref = under-/normal weight), and who consumed < 1 cups of caffeine/week (ref = no caffeine consumption). Other phthalate scores were lower in women ≥ 30 years old (ref = < 30 years old) and those with lower educational attainment (ref = college graduates), but higher in Asian women or women of other race/ethnicity (ref = non-Hispanic white), those with annual household incomes < $100,000 (ref = ≥ $100,000), and those who had ≥ 1 child prior to the I-KIDS pregnancy (ref = no children). ∑DEHP/∑DiNCH component scores were lower in women who conceived in the spring or summer months (ref = winter), in those with annual household incomes < $60,000 (ref = ≥ $100,000), and in women with unknown smoking status (ref = non-smokers). However, ∑DEHP/∑DiNCH component scores were higher in Asian women or those of other race/ethnicity (ref = non-Hispanic white), in women who enrolled later in the study, in those who smoked in the first trimester (ref = non-smokers), and in those that had overweight or obesity before pregnancy (ref = under-/normal weight). Lastly, MEP scores were higher in black women or those of other race/ethnicity (ref = non-Hispanic white), in unmarried women (ref = married), and among those who consumed some amount of caffeine/week (ref = no caffeine consumption), while MEP scores were lower in women who conceived in the spring or fall months (ref = winter), who consumed ≥ 1 servings/week of alcohol in the first trimester (ref = no alcohol consumption), who had overweight or obesity before pregnancy (ref = under-/normal weight), and had poor first trimester diet quality (ref = better diet quality).

Table 5. Associations of predictors with 4 principal component scores in the full sample.

	Component 1	Component 2	Component 3	Component 4
	Unadjusted β (95% CI)	**Unadjusted β (95% CI)**	**Unadjusted β (95% CI)**	**Unadjusted β (95% CI)**
Maternal age				
< 30 years	ref	ref	ref	ref
≥ 30 years	-0.1 (-0.3, 0.1)	**-0.2 (-0.4, 0.0)**	**0.2 (0.0, 0.4)**	0.0 (-0.2, 0.2)
Race/ethnicity				
Non-Hispanic White	ref	ref	ref	ref
Non-Hispanic Black	0.1 (-0.3, 0.5)	0.1 (-0.3, 0.5)	-0.1 (-0.5, 0.3)	**1.3 (0.9, 1.7)**
Asian	**-0.5 (-0.9, -0.1)**	**0.5 (0.1, 0.9)**	0.2 (-0.2, 0.6)	0.1 (-0.3, 0.4)
Other	0.1 (-0.2, 0.4)	**0.2 (-0.1, 0.5)**	**0.4 (0.1, 0.7)**	**0.5 (0.2, 0.8)**
Education				
Some college or less	**0.2 (0.0, 0.5)**	0.1 (-0.1, 0.3)	-0.1 (-0.3, 0.1)	**0.2 (0.0, 0.4)**
College grad or higher	ref	ref	ref	ref
Marital status				
Married	ref	ref	ref	ref
Unmarried	0.1 (-0.2, 0.4)	0.2 (-0.1, 0.5)	0.0 (-0.3, 0.3)	**0.6 (0.3, 0.9)**
Employment status				
Unemployed	0.0 (-0.3, 0.2)	**0.3 (0.0, 0.5)**	0.0 (-0.3, 0.3)	0.1 (-0.1, 0.4)
Employed	ref	ref	ref	ref
Household income				
< $60,000	0.0 (-0.3, 0.2)	**0.6 (0.3, 0.8)**	**-0.3 (-0.5, -0.1)**	**0.2 (0.0, 0.4)**
$60,000-$99,999	0.0 (-0.2, 0.2)	**0.2 (0.0, 0.4)**	**-0.2 (-0.4, 0.0)**	0.1 (-0.1, 0.3)
≥ $100,000	ref	ref	ref	ref
Enrollment year				
Continuous	**-0.3 (-0.4, -0.3)**	0.0 (-0.1, 0.1)	**0.3 (0.2, 0.4)**	0.0 (-0.1, 0.1)
Conception season				
Winter	ref	ref	ref	ref
Spring	**-0.6 (-0.8, -0.3)**	0.1 (-0.1, 0.4)	0.0 (-0.3, 0.2)	-0.2 (-0.4, 0.1)
Summer	**-0.6 (-0.9, -0.4)**	0.0 (-0.2, 0.3)	-0.1 (-0.4, 0.1)	0.0 (-0.2, 0.3)
Fall	**-0.3 (-0.5, -0.1)**	0.1 (-0.1, 0.4)	0.0 (-0.3, 0.2)	-0.2 (-0.4, 0.1)
Parity				
0 children	ref	ref	ref	ref
≥ 1 child	**0.2 (0.0, 0.3)**	**0.3 (0.1, 0.5)**	0.1 (-0.1, 0.3)	0.1 (-0.1, 0.2)
Active smoker				
No	ref	ref	ref	ref
Yes	**0.4 (-0.1, 0.8)**	0.1 (-0.3, 0.5)	0.2 (-0.2, 0.6)	**0.4 (-0.1, 0.8)**
Skip Pattern Missing	**0.7 (0.3, 1.0)**	0.1 (-0.2, 0.5)	**-0.8 (-1.1, -0.4)**	-0.1 (-0.4, 0.3)
Alcohol intake				
No serving/week	ref	ref	ref	ref
≥ 1 servings/week	0.0 (-0.2, 0.2)	-0.1 (-0.3, 0.1)	0.1 (-0.1, 0.3)	-0.1 (-0.3, 0.1)
Pregnancy intention				
Planned	**-0.2 (-0.4, 0.0)**	**-0.2 (-0.4, 0.0)**	-0.1 (-0.3, 0.1)	-0.1 (-0.3, 0.1)
Unplanned	ref	ref	ref	ref
Pre-pregnancy BMI				
< 25 kg/m^2	ref	ref	ref	ref
≥ 25 kg/m^2	0.1 (-0.1, 0.2)	0.1 (-0.1, 0.2)	0.1 (-0.1, 0.3)	-0.1 (-0.3, 0.1)

Table 5 (cont'd).

	Component 1	Component 2	Component 3	Component 4
	Unadjusted β (95% CI)	**Unadjusted β (95% CI)**	**Unadjusted β (95% CI)**	**Unadjusted β (95% CI)**
Caffeine intake				
None	ref	ref	ref	ref
< 1 cups/week	**0.2 (0.0, 0.4)**	0.0 (-0.2, 0.2)	0.1 (-0.1, 0.3)	**0.2 (0.0, 0.4)**
≥ 1 cups/week	0.1 (-0.1, 0.3)	0.0 (-0.3, 0.2)	0.2 (-0.1, 0.4)	**0.2 (0.0, 0.4)**
Overall diet quality				
AHEI < 55.1	0.1 (-0.1, 0.3)	0.0 (-0.2, 0.2)	0.0 (-0.2, 0.1)	0.0 (-0.2, 0.2)
AHEI ≥ 55.1	ref	ref	ref	ref

Data are presented as β-estimates (95% CI) from unadjusted (n=482) and adjusted (n=473) linear regression models. Adjusted models control for all the maternal characteristics listed in the table. CI, confidence interval. Bold indicate potentially meaningful findings.

In multivariable analyses in the sub-sample (**Figure 4** and **Table 6**), associations of race/ethnicity, enrollment year, and conception season with the phthalate plasticizer component, of race/ethnicity, annual household income, and parity with the other phthalate component, of annual household income, enrollment year, conception season, and pre-pregnancy BMI with the phthalate replacement component, and of race/ethnicity, marital status, and conception season with the MEP component were similar to those reported above in the full sample. However, unique associations of maternal characteristics with each component also emerged in this sub-sample. Phthalate plasticizer scores were higher among women who were unemployed (ref = employed) and those who smoked in the first trimester (ref = non-smoker). Other phthalate scores were higher among women who enrolled later in the study, but were lower in those who had lower educational attainment (ref = college graduates) and had poor first trimester diet quality (ref = better diet quality). Phthalate replacement scores were lower among women who were unemployed (ref = employed), but higher among women ≥ 30 years old (ref = < 30 years old). Lastly, MEP scores were higher among women ≥ 30 years old (ref = < 30 years old), those who enrolled earlier in the study, those who smoked in the first

trimester (ref = non-smoker), and those who planned their current pregnancy (ref = unplanned pregnancy).

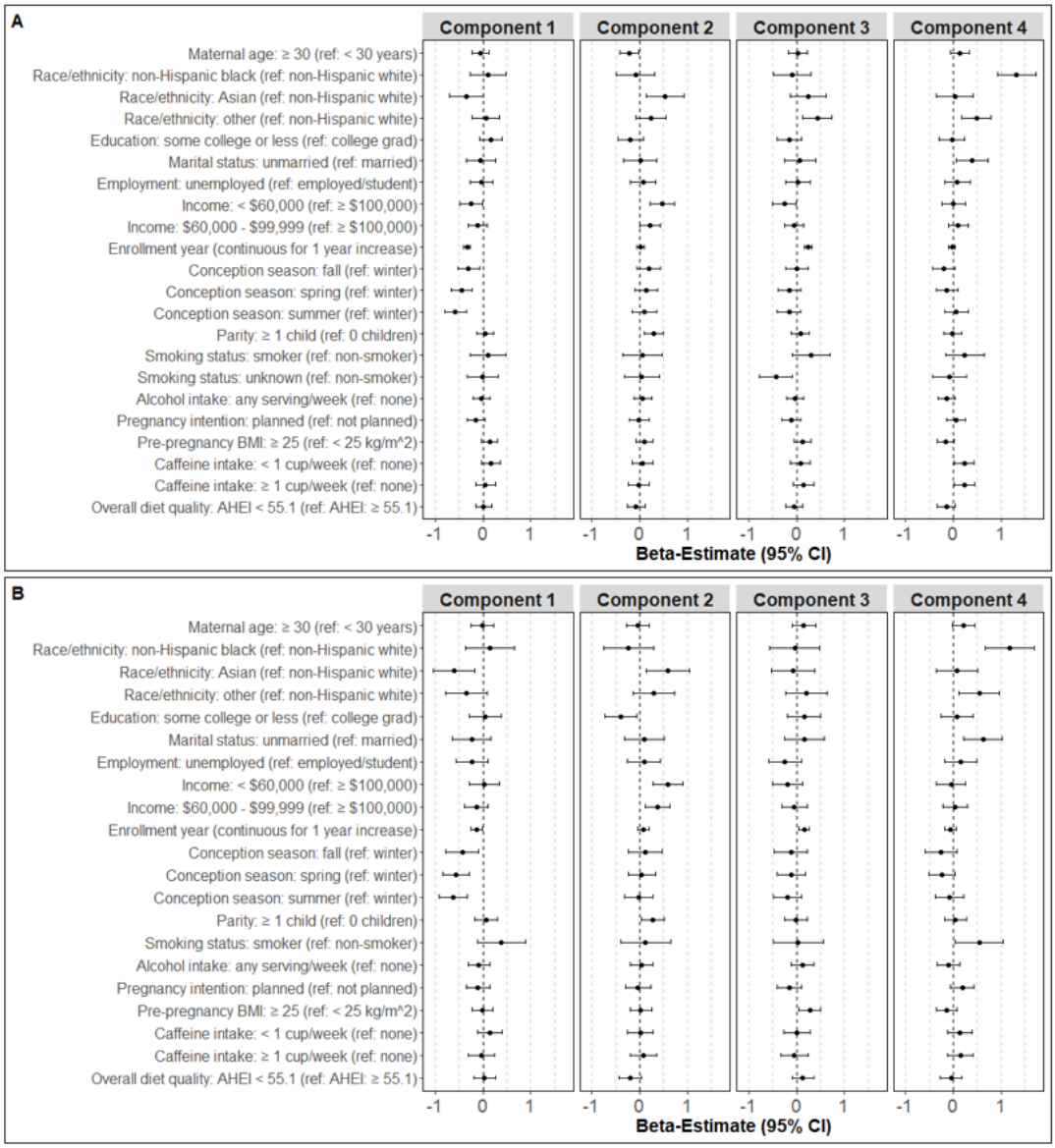

Figure 4. Multivariable associations of sociodemographic, lifestyle, enrollment year, and conception season predictors with principal component scores. Linear regression models simultaneously adjusted for all listed predictors evaluated associations of 15 maternal predictors with change in component scores in the **A)** full sample (enrolled between 12/2013 and 8/2018, n=473) and **B)** sub-sample (enrolled between 2/2015 and 8/2018, n=305). Component 1 = phthalate plasticizer component, component 2 = other phthalate component, component 3 = ∑DEHP/∑DEHTP (full sample) or phthalate replacement (sub-sample) component, and component 4 = MEP component. BMI, body mass index; CI, confidence interval.

Table 6. Associations of predictors with 4 principal component scores in the sub-sample.

	Component 1	Component 2	Component 3	Component 4
	Unadjusted β (95% CI)	Unadjusted β (95% CI)	Unadjusted β (95% CI)	Unadjusted β (95% CI)
Maternal age				
< 30 years	ref	ref	ref	ref
≥ 30 years	0.0 (-0.3, 0.2)	0.0 (-0.3, 0.2)	0.1 (-0.1, 0.4)	0.1 (-0.1, 0.4)
Race/ethnicity				
Non-Hispanic White	ref	ref	ref	ref
Non-Hispanic Black	0.1 (-0.4, 0.6)	-0.1 (-0.5, 0.5)	0.2 (-0.3, 0.7)	**1.2 (0.8, 1.7)**
Asian	**-0.6 (-1.0, -0.1)**	**0.7 (0.3, 1.1)**	**-0.3 (-0.7, 0.2)**	0.1 (-0.4, 0.5)
Other	-0.2 (-0.6, 0.2)	**0.4 (0.0, 0.8)**	**0.3 (-0.1, 0.8)**	**0.7 (0.3, 1.1)**
Education				
Some college or less	0.0 (-0.3, 0.3)	-0.1 (-0.4, 0.2)	0.2 (-0.1, 0.5)	**0.3 (0.01, 0.6)**
College grad or higher	ref	ref	ref	ref
Marital status				
Married	ref	ref	ref	ref
Unmarried	0.0 (-0.4, 0.3)	0.1 (-0.2, 0.5)	0.2 (-0.1, 0.6)	**0.7 (0.4, 1.1)**
Employment status				
Unemployed	**-0.3 (-0.6, 0.1)**	**0.3 (-0.1, 0.6)**	**-0.3 (-0.6, 0.0)**	0.2 (-0.2, 0.5)
Employed	ref	ref	ref	ref
Household income				
<$60,000	-0.1 (-0.3, 0.2)	**0.6 (0.3, 0.8)**	-0.1 (-0.4, 0.2)	0.2 (-0.1, 0.5)
$60,000-$99,999	-0.1 (-0.4, 0.2)	**0.3 (0.04, 0.6)**	-0.1 (-0.3, 0.2)	0.1 (-0.2, 0.3)
≥$100,000	ref	ref	ref	ref
Enrollment year				
Continuous	**-0.1 (-0.2, 0.0)**	0.0 (-0.1, 0.2)	**0.2 (0.1, 0.3)**	0.0 (-0.2, 0.1)
Conception season				
Winter	ref	ref	ref	ref
Spring	**-0.5 (-0.8, -0.3)**	0.1 (-0.2, 0.4)	-0.1 (-0.4, 0.2)	**-0.3 (-0.6, 0.0)**
Summer	**-0.6 (-0.9, -0.3)**	-0.1 (-0.4, 0.2)	**-0.3 (-0.6, 0.0)**	-0.1 (-0.4, 0.2)
Fall	**-0.4 (-0.7, -0.1)**	0.1 (-0.2, 0.5)	-0.1 (-0.5, 0.3)	-0.2 (-0.5, 0.2)
Parity				
0 children	ref	ref	ref	ref
≥ 1 child	0.1 (-0.1, 0.3)	**0.3 (0.1, 0.5)**	0.0 (-0.2, 0.3)	0.1 (-0.1, 0.3)
Active smoker				
No	ref	ref	ref	ref
Yes	**0.4 (-0.1, 0.9)**	0.0 (-0.5, 0.5)	0.2 (-0.3, 0.7)	**0.6 (0.1, 1.1)**
Alcohol intake				
0 drinks	ref	ref	ref	ref
> 1 drink	0.0 (-0.2, 0.3)	-0.1 (-0.3, 0.2)	**0.2 (0.01, 0.5)**	-0.1 (-0.3, 0.2)
Pregnancy intention				
Planned	-0.1 (-0.4, 0.1)	-0.2 (-0.4, 0.1)	-0.2 (-0.4, 0.1)	-0.1 (-0.4, 0.1)
Unplanned	ref	ref	ref	ref
Pre-pregnancy BMI				
< 25 kg/m^2	ref	ref	ref	ref
≥ 25 kg/m^2	0.0 (-0.2, 0.2)	0.0 (-0.2, 0.3)	**0.3 (0.1, 0.5)**	-0.1 (-0.3, 0.2)

Table 6 (cont'd).

	Component 1	Component 2	Component 3	Component 4
	Unadjusted β (95% CI)	**Unadjusted β (95% CI)**	**Unadjusted β (95% CI)**	**Unadjusted β (95% CI)**
Caffeine intake				
None	ref	ref	ref	ref
<1 cups/week	0.2 (-0.1, 0.5)	0.1 (-0.2, 0.3)	0.1 (-0.2, 0.3)	0.1 (-0.2, 0.4)
≥1 cups/week	0.0 (-0.3, 0.3)	0.1 (-0.2, 0.4)	0.1 (-0.2, 0.3)	0.2 (-0.1, 0.5)
Overall diet quality				
AHEI < 55.1	0.1 (-0.1, 0.3)	-0.2 (-0.4, 0.1)	**0.2 (-0.1, 0.4)**	0.1 (-0.2, 0.3)
AHEI ≥ 55.1	ref	ref	ref	ref

Data are presented as β-estimates (95% CI) from unadjusted (n=309) and adjusted (n=305) linear regression models. Adjusted models control for all the maternal characteristics listed in the table. CI, confidence interval. Bold indicate potentially meaningful findings.

2.6. DISCUSSION

In the current study, we identified two distinct clusters of women: those with low phthalate (including ∑DEHTP) and those with high phthalate (including ∑DEHTP) biomarker concentrations. We also identified four components representative of phthalate/replacement biomarker concentrations from common exposure sources or those that track with certain behaviors. We identified age, marital status, annual household income, parity, pre-pregnancy BMI, caffeine intake, conception season, and enrollment year as important predictors of *k*-means clusters and at least one PC. Additionally, race/ethnicity, education, employment, pregnancy intention, smoking and consuming alcohol in the first trimester, and first trimester diet quality were identified as important determinants of at least one PC. Overall, our findings contribute information about predictors of phthalate/replacement mixtures that may be important confounding factors in studies evaluating associations of chemical mixtures with pregnancy-related health outcomes. Furthermore, our results may inform future perinatal health recommendations by providing insights into characteristics of pregnant women who are most likely to be exposed to phthalates and their replacements.

2.6.1. K-means clustering identified two groups of women with distinct phthalate/replacement biomarker profiles

K-means clustering is useful for identifying subgroups of pregnant women with distinct patterns of biomarker concentrations. A major strength of this approach is that the identified clusters of women can be used in future studies to evaluate the relationships between population exposure patterns and adverse health outcomes. In our study, we identified two groups of women: those who had low phthalate (including ∑DEHTP) and those who had high phthalate (including ∑DEHTP) biomarker concentrations. A study of pregnant women from Ohio used *k*-means clustering to characterize patterns of exposure to numerous chemical classes, including phthalates, and identified three clusters of women that had different phthalate biomarker concentration patterns than those identified in our study (95). However, somewhat consistent with our results, another study of pregnant women from Wuhan, China evaluated trimester-specific population profiles of multiple chemical classes and observed that in any one trimester, groups of pregnant women had either high or low phthalate biomarker concentrations (97). This highlights a limitation of *k*-means because identified biomarker concentration patterns are population-specific and may not be generalizable to other cohorts. Additionally, *k*-means analyses that account for multiple classes of chemicals may yield different conclusions than those that focus on one chemical class. Therefore, additional studies using these approaches are needed to determine whether these patterns persist in other populations. Nevertheless, in our relatively homogenous population, *k*-means identified two relatively even clusters of women with concentrations of all phthalate biomarkers (including DEHTP) that were consistently higher or lower than the sample median. Interestingly, our

results also suggests that the plasticizer replacement biomarker ∑DiNCH was not related to high vs. low chemical cluster membership. In other words, DiNCH was uniformly distributed in our population. Because DiNCH was developed specifically for use in so-called sensitive applications, including medical tubing and children's toys, it is possible that sources of DiNCH are less avoidable in our population than other phthalates/replacements.

2.6.2. Several maternal characteristics were important predictors of k-means clusters

Although previous studies evaluated predictors of phthalate/replacement biomarker concentrations, most studies did not account for exposures to chemical mixtures. Therefore, we paired *k*-means clustering with logistic regression to identify characteristics associated with the two identified clusters of women. Overall, we observed that age, marital status, annual household income, parity, pre-pregnancy BMI, caffeine intake, enrollment year, and conception season remained the strongest important independent predictors of phthalate/replacement biomarker concentrations. Our findings that pregnant women who are younger, unmarried, have lower incomes, and had pre-pregnancy overweight/obesity have higher phthalate biomarker concentrations are in line with findings from several previous studies of pregnant women from the contiguous U.S. and Puerto Rico, Canada, Mexico, Europe, and China (18, 33, 90, 92, 93, 119-121). To our knowledge, this is the first study to identify caffeine consumption as a predictor of phthalate biomarker concentrations in pregnant women, although recent coffee consumption was associated with higher MCPP biomarker concentrations in an

adolescent population (122) and higher MEHHP-to-MECPP and MEOHP-to-MECPP ratios in U.S adults from NHANES 2001 – 2012 survey cycles (123). Whether this relationship is confounded by lifestyle factors that track with caffeine consumption in pregnancy, or is due to the diuretic nature of caffeine, or contamination by phthalates in food packaging used to prepare or consume caffeinated beverages will need to be further investigated. Our findings that women with at least one child have higher phthalate concentrations are consistent with some prior studies, but the literature related to phthalates and parity is generally mixed (92, 93, 124, 125). We also observed that women who conceived in the spring/summer/fall had lower odds of high phthalate/∑DEHTP. Given that women who conceived in the spring/summer provided most of their urine samples in fall/winter, our findings are somewhat consistent with studies of Swedish and Chinese pregnant women reporting higher phthalate biomarker concentrations in urine samples collected during spring/summer than fall/winter (88, 114). These studies suggest that these trends are likely due to seasonal differences in diet or personal care product use. Our findings related to enrollment period are supported by those from NHANES and other U.S. biomonitoring studies showing that phthalate biomarker concentrations are decreasing, especially phthalate plasticizer biomarker concentrations, while DiNCH and DEHTP metabolite concentrations are increasing over time (64, 65). These trends have also been confirmed in studies that include more recent sampling periods (126, 127) and suggest that women may be choosing to change their product use over time or that the use of phthalates/replacements in consumer products may be changing.

2.6.3. PCA identified four components of phthalate/replacement biomarker concentrations

PCA is a dimension reduction method useful for identifying distinct, uncorrelated patterns of biomarker concentrations among highly correlated chemicals. Consistent with previous human biomonitoring studies (128), we observed that phthalate/replacement biomarkers were correlated along exposure sources, as the four identified components were heavily loaded by phthalates from plastics (∑DiNP or ∑DiNP2, MCNP, and MCPP), other phthalates (MBzP, ∑DBP, and ∑DiBP), major plasticizer phthalate and its replacements (∑DEHP, ∑DiNCH, and ∑DEHTP), and a major personal care product-related phthalate metabolite, MEP. While we only assessed phthalates, previous studies included additional chemical classes to identify broader exposure patterns during pregnancy (94-97, 129). Using PCA, a few studies in pregnant populations from the USA (Ohio), Canada, and Europe reported that MEP always represented a unique component (94, 95, 129), which is consistent with our findings. In most populations, including ours, pregnant women have highest MEP concentrations relative to other phthalate metabolites likely due to their use of fragranced/perfumed products or cosmetics that contain diethyl phthalate, MEP's parent compound (130, 131). Though a major limitation of PCA is that the identified components are unique to each population (similar to *k*-means), these consistent MEP findings in predominately white Western populations suggest that there may be certain exposure patterns that are consistent in pregnant women, although this needs to be further corroborated in non-Western populations. Most importantly, PCA in our study also identified a separate component heavily weighted for biomarkers of plasticizer replacements, DiNCH and DEHTP. This suggests that the replacements either have

shared exposure sources or exist together through behaviors aimed at reducing exposure to phthalates. These results are concerning since phthalate replacement concentrations are increasing over time (64), and their health impacts during pregnancy are poorly understood (132, 133). Additional studies in more diverse populations are needed to confirm these exposure biomarker patterns.

2.6.4. Several maternal characteristics were important predictors of PCs

Pairing PCA results with linear regression helped us identify maternal characteristics that tracked most with the four identified exposure biomarker profiles/clusters. Similar to our results from *k*-means clustering, this approach also identified age, marital status, income, parity, pre-pregnancy BMI, caffeine intake, enrollment year, and conception season as important independent predictors of phthalate/replacement biomarker concentrations. However, these analyses additionally identified race/ethnicity, education, employment status, pregnancy intention, smoking status, alcohol intake, and diet quality as determinants of at least one PC. Our findings pertaining to sociodemographic characteristics are consistent with previous studies from the contiguous U.S. and Puerto Rico showing that women who are younger, have lower income, and are unmarried have higher “other” phthalate metabolite concentrations (MBzP, ∑DBP and ∑DiBP), and that women who are older, have higher incomes, and are employed have higher phthalate replacement biomarker concentrations (17, 18, 119, 134). Our findings also confirmed that black women, those who were older, and those who smoked during pregnancy have higher MEP concentrations, those with lower educational attainment have lower “other” phthalate biomarker concentration, and that women who had overweight or obesity before

pregnancy had higher phthalate plasticizer biomarker concentrations (18, 90, 91, 119, 121, 124, 134). However, inconsistent with results the Puerto Rican study (113), we observed that women with pre-pregnancy overweight or obesity had higher phthalate replacement biomarker concentrations. Urinary DEHTP metabolite concentrations in our population are at least two times higher than those in the Puerto Rico cohort, which may explain discrepancies in findings. Additionally, our findings regarding pregnancy intention are somewhat in line with those from a multi-center cohort of U.S. pregnant women finding that women with unplanned pregnancies generally had higher MCPP and lower MEP first trimester biomarker concentrations (115). We also observed that women with lower diet quality had lower "other" phthalate PC scores (strongest correlations with MBzP, ∑DBP and ∑DiBP). One possible explanation is that women with lower diet quality may be less likely to take supplements (135), which are a source of DBP (136). Alternatively, it is possible that our diet quality measure does not account for the use of plastic food storage containers or the use of plastic water bottles – dietary habits that have been associated with urinary BBzP and DBP metabolite biomarker concentrations (137).

With regard to other studies using PCA, our findings are somewhat in line with those in pregnant women from Ohio, Canada, and China (discussed above). Race/ethnicity, household income, educational attainment, parity, pre-pregnancy BMI and diet were important predictors of phthalate biomarker PCs in the Ohio cohort (95), parity and smoking status were predictors of phthalate biomarker PCs in the Canadian cohort (94), while income, pre-pregnancy BMI, and employment status were important predictors of phthalate biomarkers PCs in the China cohort (97). However, two of these studies also

reported that birthplace was associated with phthalate biomarker PCs (94, 95), which was not evaluated in our study. Conversely, the multi-cohort European study found no associations of education or employment status with phthalate biomarker PCs (96). Given that only a few studies paired PCA with linear regression models, additional studies are needed to corroborate our findings. Nevertheless, our results show that phthalate/replacement exposure biomarker patterns in pregnant women are associated with sociodemographic-related lifestyle characteristics that may predict consumption of products containing phthalates/replacements.

2.6.5. Strengths and limitations

Our study has several strengths. First, we systematically assessed a large number of *a priori* hypothesized maternal characteristics, some of which are not extensively studied in the literature (e.g. caffeine consumption, pregnancy intention, overall diet quality), providing novel information about predictors of phthalate/replacement biomarker concentrations in pregnancy. Second, we focused only on phthalates and their replacements to better understand biomarker profiles and predictors of this large class of endocrine disrupting chemicals. Additionally, we assessed associations of predictors with phthalate/replacement metabolites using mixture methods. It has been demonstrated that the use of mixture methods to evaluate association of phthalate biomarkers with preterm birth identified preterm birth risk beyond what was expected based on single-pollutant models. Our approach may help us better identify potential confounding factors of associations between cumulative phthalate/replacement exposure and pregnancy-related health outcomes (138). Third, phthalate/replacement biomarker concentrations

were quantified in pooled samples of up five first-morning urines, which is considered best practice for the assessment of non-persistent chemicals (139-141). Additionally, most women (94%) contributed all five urine samples, indicating that chemical biomarker concentrations measured in pooled samples in our population likely represent exposure across gestation. Lastly, our study is one of few to evaluate maternal predictors of DiNCH and DEHTP metabolite concentrations – plasticizer replacements to which pregnant women are becoming increasingly exposed. Interestingly, women in our study had higher DEHTP concentrations than women from NHANES, but also higher concentration than those reported by other pregnancy cohorts (121, 134). We are either capturing more recent trends or I-KIDS women (who represent women with higher socioeconomic status than the representative sample in NHANES) may be choosing products labeled as "phthalate free" that may contain replacements. This will need to be investigated in future studies.

However, our study also has limitations. First, data on three urinary metabolites (DiNP metabolite MONP and DEHTP metabolites MEHHTP and MECPTP) were not available from our earliest participants, which limits our available sample size for assessing predictors of ∑DiNP (with the additional MONP metabolite) and ∑DEHTP, as well as ∑DiNCH that had lower detection frequencies compared to the other phthalate/replacement metabolites evaluated in our study. The loss of power and introduction of additional metabolites in multivariable analyses may account for differences in associations in the full versus sub-samples. Second, the timing for assessing early pregnancy lifestyle factors, which may change across a pregnancy,

relative to phthalate/replacement biomarker concentrations analyzed from a pooled sample to represent total gestational concentrations, may not be appropriate given the non-persistent nature of these chemicals. Third, the I-KIDS population is primarily comprised of non-Hispanic white women of high socioeconomic status, which may limit the generalizability of our findings to more diverse populations and our capacity to use refined categories for our sociodemographic variables. For example, our Asian category falsely homogenizes a diverse set of ethnicities, so additional studies may be needed to expand on this category. Lastly, although *k*-means clustering and PCA can be informative, the number and types of identified *k*-means clusters or PCs varies by population, making it challenging to use these data to generate universal recommendations for reducing prenatal phthalate exposure.

2.7. CONCLUSION

In this midwestern U.S. population, we identified two distinct groups of pregnant women with specific phthalate/replacement biomarker concentration profiles, and four uncorrelated profiles of phthalate/replacement biomarkers that likely track together due to shared exposure sources. We also observed that several sociodemographic characteristics, early pregnancy lifestyle characteristics, enrollment year, and seasonality were associated with biomarker concentration profiles identified in *k*-means clustering and PCA. These findings contribute to the growing body of literature reporting confounding factors that should be considered in statistical models evaluating associations of biomarkers of phthalate/replacement mixtures with pregnancy-related health outcomes. Additionally, pairing unsupervised pattern identification methods like *k*-

means clustering or PCA with analyses evaluating predictors of phthalate/replacement biomarker concentration patterns is valuable for making recommendations targeted at limiting women's consumption/use of phthalate-containing products during pregnancy. Future studies in more diverse populations are needed to confirm our findings (especially the magnitude of associations for each predictor) and to continue evaluating replacements such as DEHTP and DiNCH to fill in knowledge gaps about the determinants of these supposedly safer alternatives during and beyond pregnancy.

CHAPTER 3: MATERNAL PHTHALATE AND PHTHALATE ALTERNATIVE METABOLITES AND URINARY BIOMARKERS OF ESTROGENS AND TESTOSTERONES ACROSS PREGNANCY

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3.1. ABSTRACT

Pregnant women are ubiquitously exposed to phthalates from food packaging materials and personal care products. Phthalates alter estrogen and testosterone concentrations in experimental models, but their ability to impact these hormones in human pregnancy is not well characterized. We recruited women ages 18-40 into the Illinois Kids Development Study (I-KIDS) in early pregnancy. Participants provided up to 5 first-morning urine samples across pregnancy (8-40 weeks gestation) that we pooled for quantification of 19 phthalate or phthalate alternative metabolites. Either individual (ng/mL) or molar sums (nmol/mL) of metabolites were used as exposure biomarkers. We summed urinary concentrations (ng/mL) of eight major estrogen (SumEstrogens) and two major testosterone (SumTestosterones) metabolites measured at median 13, 28, and 34 weeks gestation. We also estimated the ratio of estrogens-to-androgens. Linear mixed-effects models assessed relationships of phthalates/alternatives as continuous measures or as concentration quartiles with SumEstrogens, SumTestosterones, and the Estrogen/Androgen ratio in 434 women. In our models, we controlled for age, race,

education, parity, smoking in the first trimester, pre-pregnancy body mass index, diet quality, conception season, fetal sex, and gestational age at hormone assessment. We also explored whether gestational age at hormone assessment or fetal sex modified these associations. All biomarkers and outcomes were specific gravity-adjusted, and continuous exposures and outcomes were also natural log-transformed. Most participants were non-Hispanic white (80.9%), college educated (82.2%), and had urinary phthalate/alternative metabolite concentrations similar to those of reproductive-aged U.S. women. Overall, select phthalate metabolites were positively associated with SumEstrogens and SumTestosterones, but negatively associated with the Estrogen/Androgen ratio. For example, SumEstrogens was 5.1% (95%CI: 1.8, 8.5) higher with every 2-fold increase in sum of di(2-ethylhexyl) phthalate metabolites, while SumTestosterones was 7.9% (95%CI: 1.0, 15.3) higher and Estrogen/Androgen ratio was -7.7% (95%CI: -13.6, -1.4) lower with every 2-fold increase in monoethyl phthalate. However, phthalate alternatives were only positively associated with SumEstrogens, which was 2.4% (95%CI: 0.4, 4.5) and 3.2% (95%CI: 0.7, 5.8) higher with every 2-fold increase in sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites and sum of di(2-ethylhexyl) terephthalate metabolites, respectively. Gestational age- and fetal sex-specific associations were only consistently observed for associations of phthalates/alternatives with SumEstrogens, where associations were strongest in mid-to-late pregnancy in women carrying females. Phthalates/alternatives may impact gestational hormones, with potential for gestational age- and fetal sex-specific associations. Whether maternal urinary estrogens and testosterones mediate

associations of phthalates/alternatives with pregnancy and fetal outcomes merits further investigation.

3.2. KEYWORDS

Phthalate replacements, phthalates, pregnancy, urinary estrogen metabolites, urinary testosterone metabolites.

3.3. INTRODUCTION

Estrogens and androgens (e.g. testosterone) are sex-steroid hormones that support pregnancy progression and fetal development (142). While maternal ovaries and adrenal glands contribute to maternal circulating sex-steroid hormones in early pregnancy, by the second trimester, the maternal-fetal-placental unit is almost solely responsible for synthesizing sex-steroid hormones from maternal and fetal cholesterol (4, 143). Longitudinal studies demonstrate that estrogens and testosterone increase across gestation to support numerous pregnancy-related events (6, 144). For example, substantial increases in major circulating estrogens (i.e. estrone, estradiol, estriol) are responsible for supporting implantation, placental angiogenesis, maternal metabolism, and parturition (6). Although less understood, minor increases in gestational testosterone support cervical remodeling and myometrial function responsible for parturition (7). As a result, deviations from normal gestational estrogen and testosterone patterns may result in adverse maternal and fetal outcomes. To that end, observational studies suggest that disrupted estrogens and androgens are associated with increased risk of pre-eclampsia, gestational diabetes, and pre-term birth (7-9). To protect maternal and child health, it is

critical to understand factors that could disrupt the patterns of estrogens and testosterone in pregnancy.

Pregnant women are widely exposed to phthalates, which are endocrine disrupting chemicals that modify the synthesis and function of sex-steroid hormones. In the 2015-16 National Health and Nutrition Examination Survey (NHANES), >90% of reproductive-aged 18-40 year old U.S. women had detectable concentrations of at least one phthalate metabolite in their urine, which may be related to the use of phthalates in common consumer products, including food packaging materials and personal care products (145, 146). Prenatal phthalate exposure has been associated with poor pregnancy and birth outcomes, including early pregnancy loss and pre-term birth (24, 29), as well as adverse child outcomes, including cognitive and metabolic problems (45, 50). Evidence for the endocrine disrupting potential of phthalates originates from in vitro and in vivo studies showing that certain phthalates can bind to estrogen and androgen receptors and may have weak estrogenic or anti-androgenic properties (19-22). Phthalates may also indirectly impact estrogen and testosterone synthesis and function by altering follicle stimulating hormone concentrations or by interacting with peroxisome proliferator-activated receptors or thyroid receptors, which are part of estrogen and androgen regulatory pathways (147). Consistently, studies in animal models and humans have also demonstrated adverse associations of prenatal phthalate exposure with reproductive capacity or sex hormone-mediated outcomes (148, 149). However, few epidemiological studies have directly evaluated the impact of prenatal phthalate exposure on maternal hormone levels during pregnancy (77, 150-152). Additionally, while policies have led to

reduced use of phthalates in certain consumer products (153, 154), phthalate alternatives have been introduced to replace phthalates in some plastic materials, with limited data related to the consequences of exposure to these replacements on human health.

To our knowledge, five prospective cohort studies have evaluated associations of maternal phthalate exposure with serum or plasma sex-steroid hormones in pregnant women (77, 150-152, 155), and only one evaluated relationships of phthalate alternatives with these hormones (152). Furthermore, only two studies evaluated fetal sex-specific associations, which may be important given that maternal phthalate exposures are sex-specifically associated with fetal and child health outcomes (151, 155). Additionally, previous studies related to phthalates and maternal hormones only evaluated associations of phthalates with sex-steroid hormones during a single trimester – either in the first trimester (150) or the second/third trimesters (77, 151, 152), although a recent study evaluated maternal sex-steroid hormones in the first trimester and at term upon arrival to the hospital for delivery (155). Given the dynamic cross-pregnancy changes in estrogen and, to a lesser extent, testosterone levels, and the fact that previous studies collectively suggest that associations of phthalates/alternatives with hormones differ across pregnancy (77, 150-152, 155), it is critical to evaluate these associations at multiple timepoints across gestation.

Repeated blood sampling across gestation may not be feasible in large cohorts of pregnant women, but sex-steroid hormones can be measured in urine (143), which in some cases may be easier to obtain than blood. This approach was developed in non-

pregnant women as a proxy biomarker of circulating sex-steroid hormones (143, 156). More importantly, there is some evidence that urinary hormones may recapitulate observed associations of plasma/serum hormones with certain reproductive and lifestyle factors (157, 158). For example, urinary estrogen metabolites correlate with age at first birth and smoking status analogously to correlates of serum estrogen (157, 158), and urinary testosterone metabolite concentrations decrease with women's age similar to what is observed with serum testosterone (159). Conversely, other studies suggest that urinary hormones or their metabolites may not reflect circulating hormone concentrations, but rather represent hormone metabolism (160, 161). To circumvent these discrepancies with assessing hormones in blood versus urine, previous studies created sums of multiple urinary estrogen and testosterone metabolites to provide an estimate of total circulating estrogen or testosterone concentrations (160, 161). While this approach does not allow for evaluating associations between phthalates/alternatives and individual hormones, it does make it easier to conduct cross-pregnancy assessment of hormone status in large pregnancy cohorts.

Given the importance of estrogens and testosterone in pregnancy, our first objective was to evaluate associations of gestational urinary biomarkers of phthalates/alternatives exposures with urinary estrogens, testosterones, and the estrogen/androgen ratio. Unlike previous studies, we evaluated hormones across gestation in urine and explored whether these relationships are dose-dependent. Because estrogens/androgens change across pregnancy and some differ by fetal sex, our second objective was to evaluate whether associations of gestational phthalate/alternative metabolite concentrations with urinary

estrogens/testosterones differ across three gestational timepoints, or if they differ depending on the sex of the fetus. Results from our analyses provide additional insights into the endocrine disrupting potential of phthalates and phthalate alternatives across pregnancy, which may have long-term implications for maternal and child health.

3.4. MATERIALS AND METHODS

3.4.1. Illinois Kids Development Study (I-KIDS) recruitment and enrollment

This study includes pregnant participants from I-KIDS, an ongoing prospective pregnancy cohort with the overarching goal of evaluating the impacts of prenatal environmental chemical exposures on infant neurodevelopment. We recruited pregnant women from two local obstetric clinics in Champaign-Urbana, IL at their first prenatal care appointment. Women who expressed interest in the study were contacted by I-KIDS staff and were eligible to participate if they were ≥10 but <15 weeks pregnant, 18-40 years old, fluent in English, not in a high-risk pregnancy or carrying multiple fetuses, living within a 30-minute drive of the University of Illinois campus, and not planning to move out of the area before their child's first birthday. The current study includes the first 439 women who enrolled in I-KIDS between December 2013 and February 2018. For the current study (and all chemical/hormone analyses) women must have remained in the study through the birth of their infant. Enrolled women provided written informed consent according to the Institutional Review Board at the University of Illinois. The analysis of de-identified specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

3.4.2. Collection of maternal sociodemographic and lifestyle information at enrollment

Immediately after enrollment, we visited each pregnant I-KIDS participant at home to obtain information about their demographics, lifestyle, and health. We interviewed participants approximately monthly after this initial visit to ascertain important pregnancy-related updates, but only used information collected at the initial visit in the current analysis. Specifically, the following relevant demographic and lifestyle variables were self-reported by women at baseline: maternal age, race/ethnicity, highest educational level attained, smoking status since conception, and parity. Women also reported pre-pregnancy weight in pounds and height in feet and inches, which we used to calculate pre-pregnancy body mass index (BMI in kg/m^2). Self-reported pre-pregnancy BMI has been shown to be highly correlated with measured first trimester BMI (98-100), which we also confirmed in a subset of women from our study ($r = 0.93$; data not shown). Estimated due date based on the last menstrual period was collected at baseline and confirmed after the first trimester ultrasound, while information about fetal sex was collected at birth. At 8-15 and 32-40 weeks gestation, women completed a semi-quantitative food frequency questionnaire (FFQ) adapted from the full-length Block-98 FFQ (NutritionQuest, Berkeley, CA). The FFQ asked about maternal diet during the previous three months (101) and was used to calculate the Alternative Healthy Eating Index 2010 (AHEI-2010) in early and late gestation. AHEI-2010 is an 11 component diet quality measure (scored out of 110) based on foods/nutrients predictive of chronic disease risk, where a higher score is reflective of better overall diet quality (102). Because overall diet quality was relatively stable from early to late pregnancy in the I-KIDS population (data not shown), we used the mean of

AHEI-2010 scores at the two timepoints to estimate maternal diet quality across gestation.

3.4.3. Collection and processing of urine samples for chemical and hormone analyses

Pregnant women provided first-morning urine samples at 8-15, 14-22, 19-28, 25-33, and 32-40 weeks gestation (median 13, 17, 23, 28, and 34 weeks, respectively). Urine samples were collected in polypropylene urine cups. All samples were refrigerated immediately after collection and transported on ice to the I-KIDS laboratory. Within 24 hours of collection, urine samples were warmed for 30 minutes to room temperature, vortexed, and assessed for specific gravity using a handheld refractometer (TS400; Reichert Technologies, Depew, NY). Each urine sample was aliquoted into polypropylene cryovial tubes (Nalgene, Rochester, NY) using disposable polyethylene bulb transfer pipettes (Fisher Scientific, Ann Arbor, MI). Duplicates and purified water blanks were collected and analyzed for every 10 samples. In addition to creating individual aliquots of urines at each timepoint, we also pooled all five urines for each participant by adding 900 µL of fresh urine from each timepoint to a 5 mL cryovial tube containing frozen urine from previous gestational timepoints. Specific gravity of pooled samples was measured at the end of pregnancy, when each pooled sample was thawed and vortexed. All urine was stored at -80 °C.

3.4.4. Quantification of urinary phthalate/alternative metabolites

Because phthalates/phthalate alternatives have short half-lives and high within-individual exposure variability (162), the current study assessed phthalate and phthalate alternative metabolites in pooled samples of up to five first morning urines to approximate maternal

phthalate/alternative exposure across pregnancy. We shipped frozen pooled urine samples to the CDC on dry ice in three batches in the order of participant enrollment (batch one enrolled December 2013-February 2015, batch two enrolled February 2015-July 2016, and batch three enrolled July 2016-February 2018). Urinary phthalate and phthalate alternative metabolite concentrations were quantified at the CDC using on-line solid phase extraction coupled with isotope dilution-high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (104). The following metabolites were quantified in all batches: 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), mono-isononyl phthalate (MiNP), monocarboxyoctyl phthalate (MCOP), monocarboxynonyl phthalate (MCNP), mono(3-carboxypropyl) phthalate (MCPP), monobenzyl phthalate (MBzP), monoethyl phthalate (MEP), mono-n-butyl phthalate (MBP), mono-hydroxybutyl phthalate (MHBP), mono-isobutyl phthalate (MiBP), mono-hydroxy-isobutyl phthalate (MiHBP), cyclohexane-1,2-dicarboxylic acid-monohydroxy isononyl ester (MHiNCH), and cyclohexane-1,2-dicarboxylic acid-mono(carboxyoctyl) ester (MCOCH). Three additional metabolites were added to the CDC analytical panel for women in batches two and three (monooxononyl phthalate (MONP), mono(2-ethyl-5-hydroxyhexyl) terephthalate (MEHHTP), and mono(2-ethyl-5-carboxypentyl) terephthalate (MECPTP)).

3.4.5. Measurement of urinary estrogen and testosterone metabolites

We measured eight major estrogens (estrone, estradiol, estriol, 16α-hydroxyestrone, 2-hydroxyestrone, 2-methoxyestrone, 4-hydroxyestrone, and 4-methoxyestrone) and two

major testosterones (testosterone and 5α-dihydrotestosterone) in three individual first-morning urine samples collected at median 13, 28, and 34 weeks gestation, corresponding to early, middle, and late gestational plasma estrogen and testosterone concentrations (metabolites are bolded in **Figure 5**, adapted from: (4, 143)). All samples were analyzed in one analytical batch using methods adapted from Xu et al. (143). Urine samples for hormone analyses were prepared as follows: 500 µL of urine was mixed with 100 µL 1 M acetate buffer (pH 4.0), 20 µL of 0.5 µg/mL D3-testosterone (Sigma Aldrich Co. St. Louis, MO), and 20 µL beta-glucuronidase (Roche through Sigma Aldrich). The mixture was then vortexed, incubated in a heat block at 63 °C for 30 minutes, and centrifuged for 5 minutes at 8,000 rpm. Standards for all hormones were purchased from Steraloids Inc, Newport, RI. Solid-phase extraction (SPE) cleanup was performed using polymeric reverse phase cartridges (StrataTM-X, Phenomenex, Torrence, CA). Prepared and cleaned samples were analyzed with a 5500 QTRAP LC/MS/MS system (AB Sciex, Framingham, MA) with 1200 series HPLC system (Agilent Technologies) in the Roy J. Carver Biotechnology Center Metabolomics Lab at the University of Illinois at Urbana-Champaign.

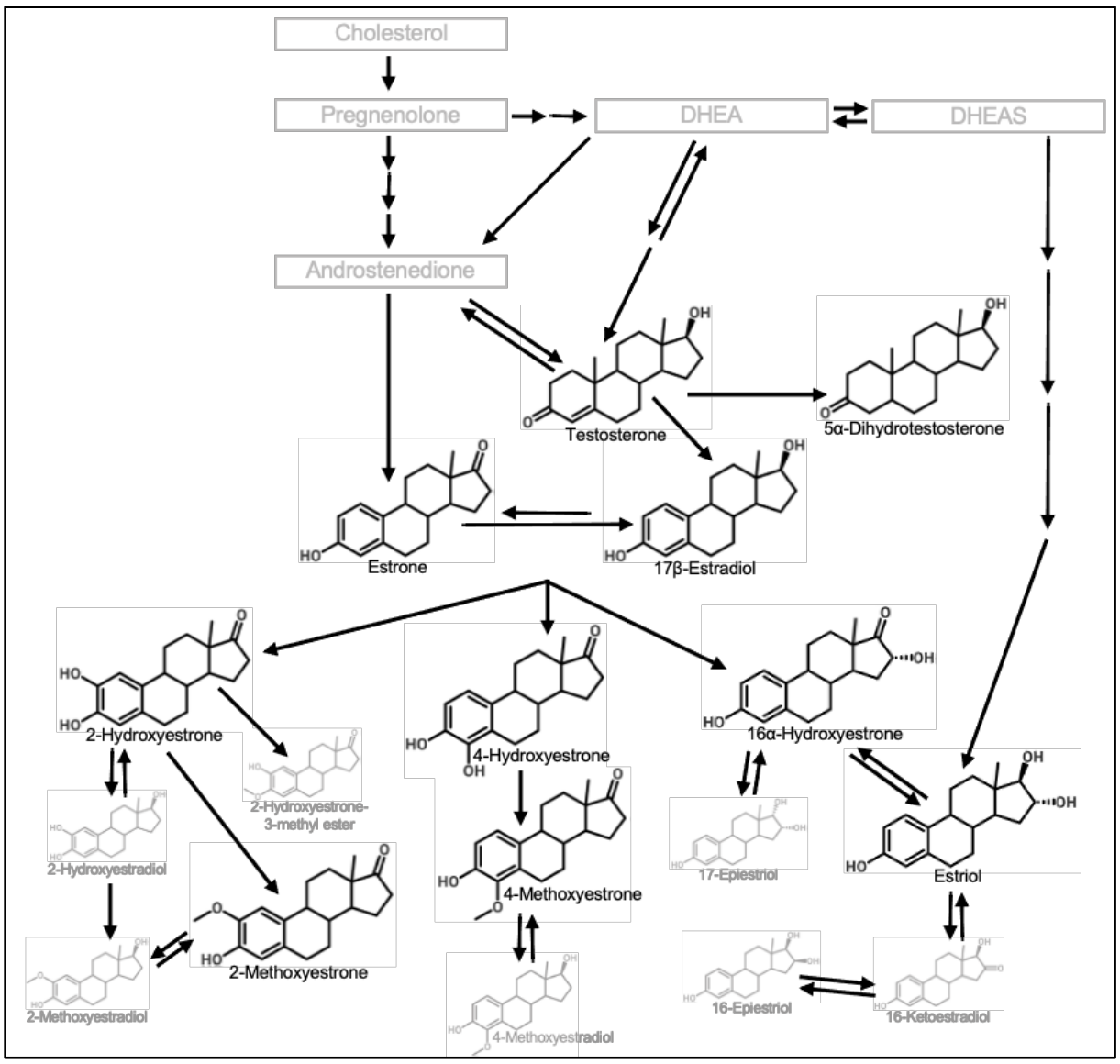

Figure 5. Estrogen and testosterone metabolism pathway. Maternal estrogens and testosterones are cholesterol-derived hormones that are primarily synthesized from maternal cholesterol in the ovaries, adrenal glands, and adipose tissue (to a lesser extent) in the first trimester. The maternal-fetal-placental unit becomes the primary source of hormones after the first trimester. Estrogens and testosterones are metabolized in the maternal liver, and the parent compounds as well as resulting metabolites are excreted in urine. The bolded compounds are the eight major urinary estrogen and two major urinary testosterone parent compounds or metabolites measured in this study. DHEA, dihydroepiandrostenediene; DHEAS, dihydroepiandrostenediene sulfate.

3.4.6. Statistical analysis

3.4.6.1. Final sample size and covariate selection

A total of 439 women were available for statistical analyses. In all statistical models, we included the following covariates selected a priori using previous literature: maternal age, race/ethnicity, education, parity, smoking since conception, pre-pregnancy BMI, AHEI-2010, season of conception, fetal sex, and timepoint of hormone assessment. We evaluated correlations between all potential confounders to test for multicollinearity. Three women had missing information about pre-pregnancy BMI, and two others had missing information about race/ethnicity or AHEI-2010 (**Table 7**). Therefore, 434 pregnant women were included in final statistical models. For hormone analysis, a total of 433, 424, and 426 women contributed urine samples at median 13, 28, and/or 34 weeks gestation, respectively, with 414 contributing urine samples at all three timepoints and 20 contributing urine samples at only two timepoints. Maternal age, AHEI-2010, and pre-pregnancy BMI were included as continuous variables, while the remaining covariates were categorical (**Table 7**).

3.4.6.2. Exposure and outcome variables

To avoid bias associated with imputing values below the limit of detection (LOD) (107), we used instrument-read values for all samples. Across the chemical and hormone analyses, only 5 values were zero (n=1 for SumDiNCH; n=3 and n=1 for SumTestosterones at median 13 and 28 weeks gestation, respectively), so in final statistical models we added a constant 0.0001 to these zero values before natural log-transformation (ln-transformation) to avoid undefined estimates (108). To account for

urine dilution, we used the following formula to adjust all urinary chemical and hormone metabolite concentrations: $P_c = P[(SG - 1)/(SG_i - 1)]$, where P_c is the specific gravity adjusted chemical or hormone metabolite concentration, P is the measured chemical or hormone metabolite concentration (ng/mL), SG is the median specific gravity of the pooled samples used for chemical analysis (1.016) and three urine samples used for hormone analysis (1.015), and SG_i is the specific gravity of each individual urine sample (109).

We approximated exposure to phthalate and phthalate alternative parent compounds that are metabolized and excreted as multiple urinary metabolites using the following molar-sum (in nmol/mL) equations: sum of di(2-ethylhexyl) phthalate metabolites (SumDEHP) = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308), sum of di-isononyl phthalate metabolites (SumDiNP) = (MiNP/292) + (MCOP/322), sum of di-n-butyl phthalate metabolites (SumDBP) = (MBP/222) + (MHBP/238), sum of di-iso-butyl phthalate metabolites (SumDiBP) = (MiBP/222) + (MHiBP/238), sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites (SumDiNCH) = (MHiNCH/314) + (MCOCH/328), and sum of di(2-ethylhexyl) terephthalate metabolites (SumDEHTP) = (MEHHTP/294) + (MECPTP/308). We excluded MONP from SumDiNP because this metabolite was only measured in batches 2 and 3, and we did not observe marked differences in associations between SumDiNP and hormones when MONP was included in the sum (data not shown). We approximated exposure to di-isodecyl phthalate, di-n-octyl phthalate, benzylbutyl phthalate, and diethyl phthalate using the concentrations (in ng/mL) of their non-molar converted major urinary metabolites MCNP, MCPP, MBzP, and

MEP, respectively. Additionally, we molar-summed (nmol/mL) some phthalate metabolites based on common exposure sources by calculating the sum of phthalate metabolites of parent compounds found in plastics (SumPlastics) and personal care products (SumPCP) as follows (163): SumPlastics = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308) + (MiNP/292) + (MCOP/322) + (MCNP/336) + (MCPP/252) + (MBzP/256) and SumPCP = (MEP/194) + (MBP/222) + (MHBP/238) + (MiBP/222) + (MHiBP/238). SumPlastics and SumPCP are reflective of high and low molecular weight phthalate metabolites, respectively.

Urine and blood likely capture different hormone forms, such that unconjugated (biologically active) hormones are measured in blood, whereas conjugated (biologically inactive) hormones are generally measured in urine (161). Therefore, individual urinary hormones, especially the downstream metabolites of the parent hormones, may not be representative of hormones in circulation, but rather provide insights into steroid hormone metabolism. This is particularly evident when comparing studies that quantified maternal parent estrogen concentrations (estrone, estradiol, and estriol) across pregnancy in blood versus urine, where estradiol and estriol tend to have the highest concentrations in blood and urine, respectively, relative to the other parent estrogens (4, 164). To circumvent this, we created hormone sums at each of the three gestational timepoints to represent total estrogens (SumEstrogens of eight major urinary estrogen metabolites) and testosterones (SumTestosterones of two major testosterone metabolites) in early, mid, and late gestation – an approach that has been previously used to characterize hormone status using hormones measured in urine (160, 161). Because the estrogen-to-androgen ratio

may be an important indicator of pregnancy health (165), we additionally created an Estrogen/Androgen ratio by dividing SumEstrogens by SumTestosterones at each gestational timepoint. The Estrogen/Androgen ratio was only created for women who had non-zero values for both SumEstrogens and SumTestosterones.

3.4.6.3. Linear mixed model approach

We used linear mixed models to accommodate our longitudinal prospective design with hormone outcomes at three timepoints in gestation. SumEstrogens, SumTestosterones, and Estrogen/Androgen ratio were analyzed separately. An unstructured covariance 3×3 matrix was specified for the model's residuals. All statistical analyses were conducted in SAS Software, version 9.4 (SAS Institute Inc, Cary, NC). Associations were considered significant at $P < 0.05$, and we did not adjust for multiple comparisons (166).

The first part of our first objective was to evaluate associations of continuous phthalate/alternative biomarkers with maternal sums of urinary hormone metabolites across pregnancy. We ln-transformed our exposures and outcomes to fit normality assumptions and added 0.0001 to phthalate/alternative or hormones that had zero as a minimum value (see Section 3.4.6.2 for specific details). The second part of our first objective was to assess potential dose-response relationships between phthalates/alternatives and hormones. Phthalates were categorized based on quartiles of urinary phthalate/alternative biomarker concentrations, while hormones were ln-transformed as described previously. For our second objective, because previous studies suggest that associations of phthalate and phthalate alternative biomarkers with

hormones may be gestational age- or fetal sex-specific (77, 151, 152, 155), we also evaluated gestational age- and fetal sex-specific relationships between phthalate/alternative biomarkers and hormones by including a three-way interaction (and all relevant two-way interactions) between exposure, gestational age at hormone sampling, and fetal sex. We only evaluated gestational age- and fetal sex-specific associations of continuous phthalate/alternative biomarkers with hormones.

3.4.6.4. Reporting of results

All gestational age- and fetal sex-specific results were reported regardless of the significance of the three-way interaction term. All β-estimates and 95% confidence intervals (CIs) were back transformed and presented as a percent change in hormones in tables and figures. In models with both ln-transformed phthalate/alternative biomarkers and hormones (objectives 1 and 2), β-estimates and 95% CIs were back transformed using the equation $[((2.00)^{\beta} – 1)*100]$ to represent a percent change in hormones for each 2-fold increase in phthalate/alternative (**Table 8**). For models with ln-transformed hormones and phthalate/alternatives biomarkers in quartiles (objective 1), β-estimates and 95% CIs were back transformed using the equation $[(e^{\beta} – 1)*100]$ to represent the percent change in hormones among women in quartiles two (Q2), three (Q3), and four (Q4) of urinary phthalate/alternative biomarkers concentrations, which were each compared to quartile one (Q1) (**Table 8**, **Figures 8-10**).

3.5. RESULTS

3.5.1. Demographic and lifestyle characteristics of the I-KIDS population

Baseline characteristics of 439 pregnant I-KIDS women are reported in **Table 7**. Participants had a median (range) age of 30 years (18-40), and the majority were non-Hispanic white (81%) and college educated (82%). Approximately 52% of women were nulliparous, while 48% were primiparous or multiparous. The majority of women did not smoke since conception (88%), 52% were normal weight and 45% were overweight or had obesity before pregnancy, and median (range) diet quality score measured by the AHEI-2010 was 55.8 (28.1-82.8) out of 110. Season of conception was equally distributed across all seasons, and fetal sex was also relatively evenly distributed, with 52% and 48% of women carrying a female or male fetus, respectively.

Table 7. Baseline demographic and lifestyle characteristics of I-KIDS pregnant women.

Demographic or lifestyle characteristic	I-KIDS women (n=439)[1]
	median (range) or n (%)
Age	30.0 (18.0 – 40.0)
Alternative Healthy Eating Index-2010 *(1 missing)*	55.8 (28.1 – 82.8)
Race/ethnicity *(1 missing)*	
Non-Hispanic White	355 (80.9)
Others[2]	83 (18.9)
Education	
Some college of less	78 (17.8)
College graduate or higher	361 (82.2)
Parity	
Nulliparous	228 (51.9)
Primiparous	139 (31.7)
Multiparous	72 (16.4)
Smoking during 1st trimester	
No	385 (87.7)
Yes	21 (4.8)
Unknown	33 (7.5)
Pre-pregnancy BMI *(3 missing)*	
Underweight (<18.5 kg/m^2)	10 (2.3)
Normal weight (18.5-24.9 kg/m^2)	227 (51.7)
Overweight (25-29.9 kg/m^2)	103 (23.5)
Obese (≥30 kg/m^2)	96 (21.9)
Season of conception	
Winter	108 (24.6)
Spring	114 (25.9)
Summer	100 (22.8)
Fall	117 (26.7)
Fetal sex	
Female	229 (52.2)
Male	210 (47.8)

[1]Percentages may not add up to 100% due to missing.[2]Includes Hispanic Whites, Native American or Alaska Natives, Asians, Blacks or African Americans, Native Hawaiians or other Pacific Islanders, and others. BMI, body mass index; I-KIDS, Illinois Kids Development Study.

3.5.2. Urinary phthalate and phthalate alternative metabolite concentrations

The concentrations of most phthalate and phthalate alternative metabolites were detectable in 100% of women, except for the following metabolites (% of women with metabolite concentrations >LOD): MEHP (74%), MiNP (42%), MCPP (97%), MBzP (99%), MHBP (90%), MiBP (99%), MHiBP (99%), MHiNCH (77%), and MCOCH (49%) (**data not shown**). Urinary phthalate/alternative metabolite concentrations in I-KIDS women were generally comparable to those in 18-40-year-old women from NHANES

cycles 2013-14 and 2015-16 (**Figure 6**).

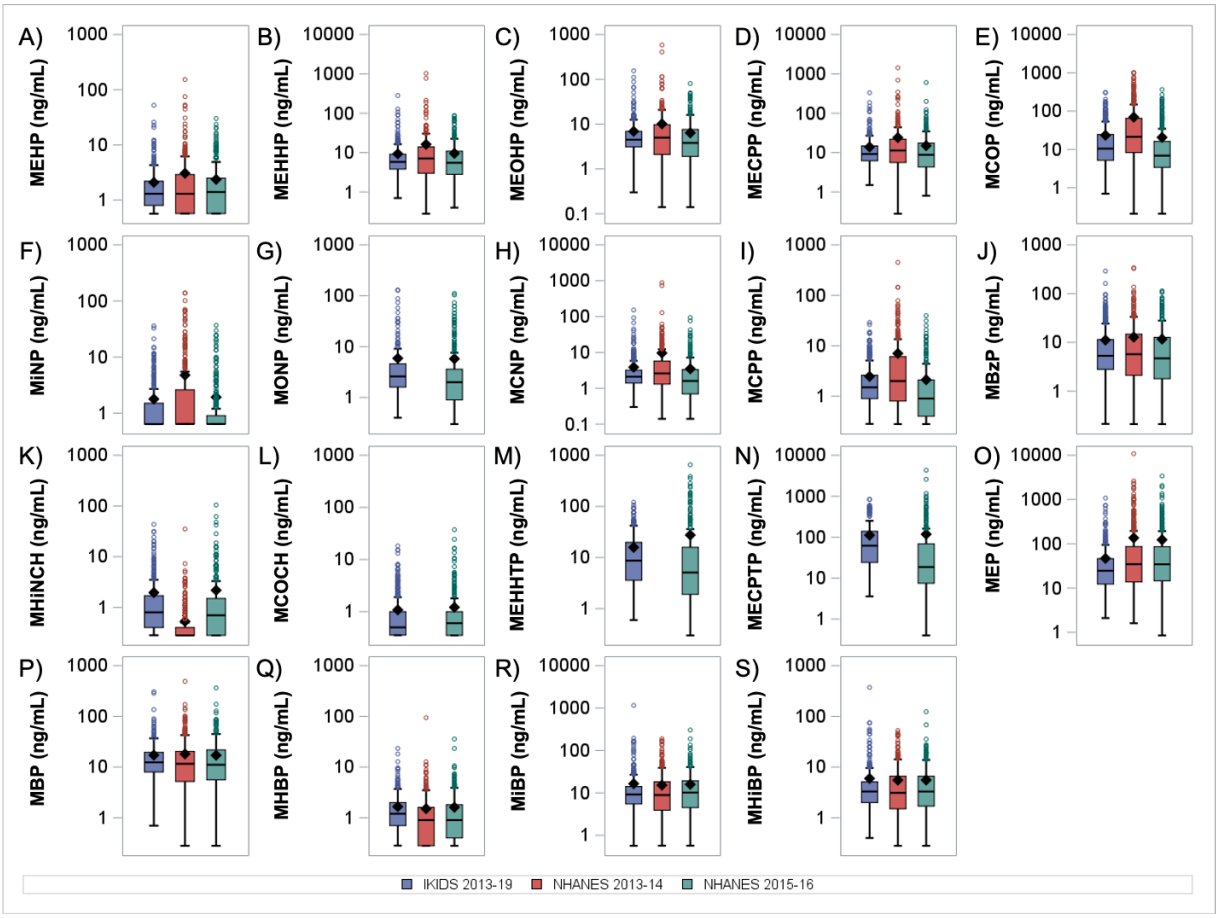

Figure 6. Urinary phthalate and phthalate alternative metabolite concentrations in pregnant women from I-KIDS 2013-18 compared to women from the NHANES 2013-16. Urinary phthalate (A-J, O-S) and phthalate alternative (K-N) metabolite concentrations were assessed from pooled sample of five urine samples per participant (n=439). Results are presented as 1.5 times the interquartile range below and above the 25th and 75th percentiles (lower and upper endpoints of whisker), the 25th and 75th percentiles (lower and upper edges of box), median (line inside box), and mean (diamond). Urinary phthalate and phthalate alternative metabolite concentrations assessed from a spot urine sample per participant were obtained for 18-40-year-old reproductive-aged women from NHANES survey years 2013-14 (n=394 or 392) and 2015-16 (n=348). I-KIDS, Illinois Kids Development Study; NHANES, National Health and Nutrition Examination Survey.

3.5.3. Urinary sex-steroid hormone concentrations

As expected, urinary SumEstrogens, SumTestosterones, and the Estrogen/Androgen ratio increased across gestation (**Figure 7**; $P_{time} < 0.0001$). Median (range) urinary specific gravity-adjusted hormone concentrations at 8-15, 25-33, and 32-40 weeks gestation were as follows: SumEstrogens: 3064.4 ng/mL (313.5-57,879.9), 11,168.9 ng/mL (2,845.3-40,420.4), and 14350.1 ng/mL (4,063.1-41,561.0), respectively (**Figure 7A**); SumTestosterones: 3.6 ng/mL (0.0-61.9), 3.4 ng/mL (0.0-84.6), and 4.3 ng/mL (0.1-73.2), respectively (**Figure 7B**); and Estrogen/Androgen ratio: 848.3 (75.5-236,684.0), 3,720.2 (43.3-332,635.5), and 3,659.1 (176.7-106,960.2), respectively (**Figure 7C**).

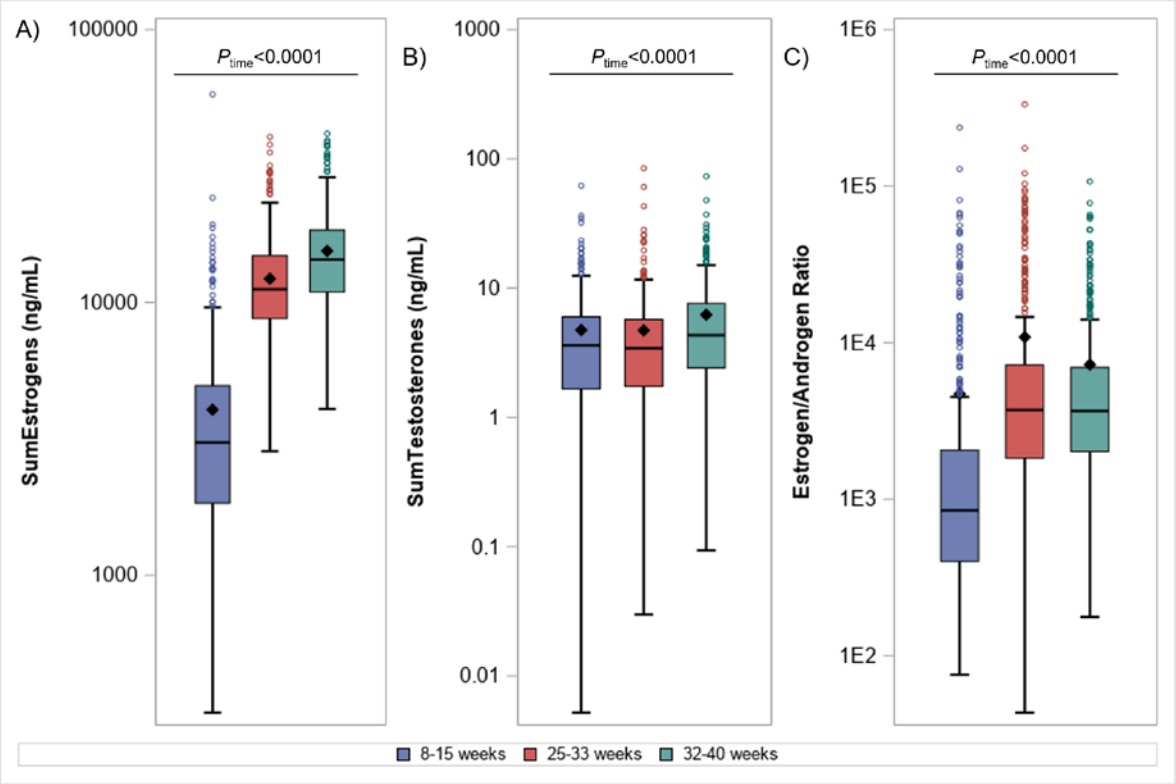

Figure 7. Distribution of urinary (A) SumEstrogen (ng/mL), (B) SumTestosterone (ng/mL), and (C) Estrogen/Androgen ratio at 8-15, 25-33, and 32-40 weeks gestation (n=439). Results are presented as 1.5 times the interquartile range below and above the 25th and 75th percentiles (lower and upper endpoints of whisker), the 25th and 75th percentiles (lower and upper edges of box), median (line inside box), and mean (diamond). Linear mixed models were used to assess whether hormone concentrations differed across the three gestational timepoints (P_{time}).

3.5.4. Overall associations of phthalate/alternative biomarkers with hormones

In linear models, SumDEHP, MCNP, MCPP, SumPlastics, SumDiNCH, SumDEHTP, and SumDBP were positively associated with SumEstrogens along with less precise positive associations observed for SumDiBP and SumPCP with SumEstrogens (**Table 8**). For example, each 2-fold increase in SumDEHP and SumDEHTP was associated with 7.2% (95%CI: 3.9, 10.6) and 3.7% (95%CI: 1.2, 6.2) increase in SumEstrogens, respectively. Most analyses where phthalate/alternative biomarkers were modeled in quartiles generally supported the linear models. SumEstrogens were higher in women at the upper

quartiles of SumDEHP (Q3, Q4), MCPP (Q2, Q3, Q4), SumPlastics (Q3, Q4), SumDiNCH (Q4), SumDBP (Q2, Q3, Q4), SumDiBP (Q2, Q3, Q4), and SumPCP (Q2, Q3, Q4), as well as at MEP Q3 compared to those in Q1. For example, compared to Q1, SumEstrogens was 8.5% (95%CI: -0.1, 18.0) and 18.2% (95%CI: 8.4, 28.8) higher in SumDEHP Q3 and Q4.

Several phthalate and phthalate alternative biomarkers were also associated with urinary SumTestosterone concentrations across pregnancy (**Table 8**). In linear models, every 2-fold increase in MBzP and MEP was associated with 5.5% (95%CI: -1.1, 12.5) and 9.3% (95%CI: 1.8, 17.5) increase in SumTestosterones, respectively. In models where phthalate/alternative biomarkers were modeled as quartiles, compared to the lowest quartile, SumTestosterones were higher at higher quartiles of SumDEHP (Q3), MCPP (Q3), MBzP (Q4), SumPlastics (Q3), and MEP (Q3 and Q4). For example, SumTestosterones were 40.5% (95%CI: 7.4, 83.8) and 34.7% (95%CI: 2.7, 76.7) higher in MEP Q3 and Q4, respectively, compared to Q1. However, we additionally observed potential non-linear associations of SumDiBP and SumPCP, with SumTestosterones where strongest positive associations emerged at Q2 compared to the lowest quartile.

Some phthalate, but not phthalate alternative, biomarkers were associated with the Estrogen/Androgen ratio (**Table 8**). Specifically, in linear models, the ratio tended to be negatively associated with SumDEHP and MBzP, and was negatively associated with MEP and SumPCP, such that each 2-fold increase in MEP and SumPCP was associated with -10.0% (95%CI: -16.4, -3.0) and -10.1% (95%CI: -18.8, -0.6) lower

Estrogen/Androgen ratio. In quartile analyses, compared to those in Q1, the Estrogen/Androgen ratio tended to be lower for women in SumDEHP Q3 and MCPP Q3, and was lower in women at higher quartiles of MEP (Q3 and Q4) and SumPCP (Q4). For example, the Estrogen/Androgen ratio was -29.2% (95%CI: -46.4, -6.5) and -30.3% (95%CI: -47.4, -7.7) lower in MEP Q3 and Q4, respectively, compared to Q1.

Table 8. Overall associations of phthalate and phthalate alternative biomarkers with SumEstrogens, SumTestosterones, and Estrogen/Androgen ratio.

Biomarker	% Change (95%CI) in SumEstrogens	*P*	% Change (95%CI) in SumTestosterones	*P*	% Change (95%CI) in Estrogen/Androgen ratio	*P*
SumDEHP						
Linear association	**7.2 (3.9, 10.6)**	**<.0001**	-1.7 (-11.0, 8.5)	0.73	**10.7 (-0.1, 22.7)**	**0.05**
Q2 (ref=Q1)	-2.6 (-10.4, 5.8)	0.53	17.5 (-9.9, 53.2)	0.23	-17.5 (-37.4, 8.6)	0.17
Q3 (ref=Q1)	**8.5 (-0.1, 18.0)**	**0.05**	**42.6 (9.3, 86.0)**	**0.01**	**-26.5 (-44.2, -3.2)**	**0.03**
Q4 (ref=Q1)	**18.2 (8.4, 28.8)**	**0.0002**	10.8 (-15.8, 45.7)	0.46	9.9 (-17.3, 45.9)	0.51
SumDiNP						
Linear association	1.1 (-2.1, 4.4)	0.51	1.3 (-8.5, 12.2)	0.80	-2.6 (-12.3, 8.2)	0.62
Q2 (ref=Q1)	3.1 (-5.2, 12.1)	0.48	-6.1 (-28.1, 22.5)	0.64	3.9 (-21.2, 37.0)	0.79
Q3 (ref=Q1)	-2.6 (-10.8, 6.2)	0.55	7.8 (-18.2, 42.0)	0.59	-4.6 (-28.4, 27.1)	0.75
Q4 (ref=Q1)	8.3 (-0.9, 18.3)	0.08	3.3 (-21.9, 36.8)	0.82	7.5 (-19.7, 43.8)	0.63
MCNP						
Linear association	**3.2 (0.4, 6.1)**	**0.02**	5.8 (-3.1, 15.4)	0.21	-0.6 (-9.2, 8.9)	0.90
Q2 (ref=Q1)	-0.5 (-8.8, 8.5)	0.90	15.6 (-12.0, 51.7)	0.30	-16.9 (-37.4, 10.3)	0.20
Q3 (ref=Q1)	2.7 (-5.8, 12.0)	0.54	20.9 (-7.9, 58.5)	0.17	-16.0 (-36.7, 11.3)	0.22
Q4 (ref=Q1)	6.2 (-2.7, 16.0)	0.18	21.8 (-7.5, 60.4)	0.16	-11.2 (-33.3, 18.2)	0.41
MCPP						
Linear association	**4.4 (1.7, 7.1)**	**0.001**	3.6 (-4.6, 12.5)	0.40	0.3 (-8.0, 9.3)	0.95
Q2 (ref=Q1)	**9.8 (1.0, 19.5)**	**0.03**	25.0 (-4.2, 63.2)	0.10	-13.6 (-34.5, 13.9)	0.30
Q3 (ref=Q1)	**9.0 (0.3, 18.6)**	**0.04**	**36.7 (4.9, 78.0)**	**0.02**	**-23.9 (-42.2, 0.2)**	**0.05**
Q4 (ref=Q1)	**15.0 (5.7, 25.1)**	**0.001**	20.0 (-8.0, 56.5)	0.18	-2.0 (-25.6, 29.2)	0.89
MBzP						
Linear association	1.4 (-0.7, 3.5)	0.19	5.5 (-1.1, 12.5)	0.11	-5.1 (-11.3, 1.5)	0.12
Q2 (ref=Q1)	6.4 (-2.1, 15.7)	0.15	5.4 (-18.9, 37.1)	0.69	-6.4 (-28.8, 23.0)	0.63
Q3 (ref=Q1)	7.1 (-1.8, 16.8)	0.12	12.8 (-14.2, 48.1)	0.39	-13.3 (-34.6, 15.1)	0.32
Q4 (ref=Q1)	6.9 (-1.9, 16.5)	0.13	19.7 (-8.5, 56.7)	0.19	-15.5 (-36.1, 11.8)	0.24
SumPlastics						
Linear association	**5.4 (2.3, 8.7)**	**0.001**	1.9 (-7.5, 12.2)	0.71	5.2 (-4.8, 16.3)	0.32
Q2 (ref=Q1)	5.4 (-3.2, 14.7)	0.23	-10.0 (-31.2, 17.6)	0.44	21.3 (-8.3, 60.4)	0.18
Q3 (ref=Q1)	**15.6 (6.1, 26.0)**	**0.001**	**31.3 (0.1, 72.3)**	**0.05**	-7.1 (-30.0, 23.3)	0.61
Q4 (ref=Q1)	**14.1 (4.6, 24.5)**	**0.003**	3.6 (-21.3, 36.5)	0.80	18.6 (-11.0, 58.0)	0.24
SumDiNCH						
Linear association	**3.0 (1.0, 5.1)**	**0.003**	0.9 (-5.2, 7.4)	0.77	0.4 (-5.9, 7.2)	0.90
Q2 (ref=Q1)	-2.5 (-10.5, 6.3)	0.57	-15.2 (-35.3, 11.3)	0.24	2.9 (-22.4, 36.4)	0.84
Q3 (ref=Q1)	4.7 (-4.1, 14.3)	0.30	-17.7 (-37.6, 8.5)	0.17	23.4 (-7.4, 64.5)	0.15
Q4 (ref=Q1)	**9.7 (0.6, 19.7)**	**0.04**	-7.4 (-29.6, 21.9)	0.58	8.6 (-18.3, 44.4)	0.57
SumDEHTP						
Linear association	**3.7 (1.2, 6.2)**	**0.004**	3.6 (-4.1, 12.0)	0.37	0.5 (-7.3, 8.8)	0.91
Q2 (ref=Q1)	-2.0 (-12.2, 9.5)	0.72	4.5 (-26.5, 48.4)	0.81	2.1 (-28.9, 46.6)	0.91
Q3 (ref=Q1)	6.4 (-4.7, 18.8)	0.27	30.0 (-8.5, 84.6)	0.14	-19.1 (-43.6, 16.1)	0.25
Q4 (ref=Q1)	9.0 (-2.6, 22.0)	0.13	8.8 (-24.0, 55.7)	0.65	5.1 (-27.4, 52.0)	0.79

Table 8 (cont'd).

Biomarker	% Change (95%CI) in SumEstrogens	*P*	% Change (95%CI) in SumTestosterones	*P*	% Change (95%CI) in Estrogen/Androgen ratio	*P*
MEP						
Linear association	1.9 (-0.4, 4.3)	0.11	**9.3 (1.8, 17.5)**	**0.01**	**-10.0 (-16.4, -3.0)**	**0.01**
Q2 (ref=Q1)	5.8 (-2.8, 15.1)	0.19	29.0 (-1.0, 68.2)	0.06	-17.1 (-37, 9.2)	0.18
Q3 (ref=Q1)	**13.7 (4.4, 23.8)**	**0.003**	**40.5 (7.4, 83.8)**	**0.01**	**-29.2 (-46.4, -6.5)**	**0.02**
Q4 (ref=Q1)	2.9 (-5.6, 12.1)	0.52	**34.7 (2.7, 76.7)**	**0.03**	**-30.3 (-47.4, -7.7)**	**0.01**
SumDBP						
Linear association	**4.6 (1.3, 8.1)**	**0.01**	-3.2 (-12.8, 7.4)	0.54	0.7 (-9.6, 12.2)	0.90
Q2 (ref=Q1)	**12.1 (3.1, 22.0)**	**0.01**	4.6 (-20.0, 36.7)	0.74	-2.5 (-26.2, 28.7)	0.86
Q3 (ref=Q1)	**12.4 (3.4, 22.2)**	**0.01**	17.1 (-10.1, 52.6)	0.24	-13.9 (-34.6, 13.3)	0.28
Q4 (ref=Q1)	**11.0 (1.9, 21.0)**	**0.02**	6.5 (-18.8, 39.8)	0.65	-7.8 (-30.4, 22.3)	0.57
SumDiBP						
Linear association	2.7 (-0.1, 5.6)	0.06	-3.9 (-12.0, 4.8)	0.37	0.5 (-8.2, 10.1)	0.91
Q2 (ref=Q1)	**11.1 (2.2, 20.8)**	**0.01**	**31.2 (0.9, 70.7)**	**0.04**	-17.2 (-37.1, 9)	0.18
Q3 (ref=Q1)	7.6 (-1.2, 17.2)	0.09	-7.5 (-29.2, 20.9)	0.57	-2.9 (-26.5, 28.5)	0.84
Q4 (ref=Q1)	**11.1 (2.0, 20.9)**	**0.02**	-8.5 (-29.8, 19.4)	0.51	5.2 (-20.3, 38.9)	0.72
SumPCP						
Linear association	2.9 (-0.3, 6.1)	0.08	5.6 (-4.3, 16.5)	0.28	**-10.1 (-18.8, -0.6)**	**0.04**
Q2 (ref=Q1)	8.4 (-0.4, 18.1)	0.06	**31.2 (0.4, 71.6)**	**0.05**	-22.5 (-41.3, 2.3)	0.07
Q3 (ref=Q1)	7.4 (-1.4, 17.0)	0.10	21.6 (-7.1, 59.1)	0.15	-18.4 (-38.2, 7.7)	0.15
Q4 (ref=Q1)	6.6 (-2.3, 16.3)	0.15	24.6 (-5.2, 63.8)	0.11	**-31.9 (-48.7, -9.6)**	**0.01**

Linear mixed models evaluated overall associations of phthalates/phthalate alternatives with ln-transformed hormones controlling for age, race/ethnicity, education, parity, smoking during 1st trimester, pre-pregnancy body mass index, diet quality, season of conception, fetal sex, and gestational age at hormone assessment. Phthalates/phthalate alternatives are either included as ln-transformed continuous variables or variables categorized into quartiles of exposure with Q1 as the reference group. β-estimates and 95%CIs for associations of continuous phthalates/phthalate alternatives with hormones were back-transformed to represent a % change in hormones for every 2-fold increase in phthalate/alternative. Associations where $P \leq 0.05$ are bolded. CI, confidence interval; Q1-4, quartiles 1-4; Ref, reference. Phthalate/alternative concentrations in ng/mL or nmol/mL and hormone concentrations in ng/mL.

3.5.5. Gestational age- and fetal sex-specific associations of phthalate/alternative biomarkers with hormones

Some associations between phthalate/alternative biomarkers and urinary SumEstrogens did differ by gestational age at hormone assessment and/or fetal sex (**Figure 8**). Positive associations of SumDEHP, MCPP, SumPlastics, and SumDiNCH with SumEstrogens were observed in both sexes at 8-15 weeks gestation, but also at 25-33 or 32-40 weeks gestation (**Figures 8A**, **D**, **F**, **G**). Positive associations of MCNP with SumEstrogens were also consistent in both sexes and strongest at 32-40 weeks gestation (**Figure 8C**). Associations of SumDEHTP with SumEstrogens were positive in women carrying female fetuses and observed at all three gestational timepoints (**Figure 8H**), while associations of SumDiNP with SumEstrogens were positive in women carrying female fetuses and strongest at 8-15 weeks gestation (**Figure 8B**). Positive associations of MEP, SumDiBP, and SumPCP with SumEstrogens were only observed in women carrying female fetuses and were strongest at 8-15 weeks gestation, but also 25-33 or 32-40 weeks gestation (**Figures 8I**, **K**, **L**), while positive associations of SumDBP with SumEstrogens were not fetal sex-specific at 8-15 weeks gestation, but were only observed in women carrying females at 25-33 weeks gestation (**Figure 8J**). In these stratified analyses, urinary concentrations of MBzP were not associated with SumEstrogens at any gestational timepoint or by fetal sex (**Figure 8E**).

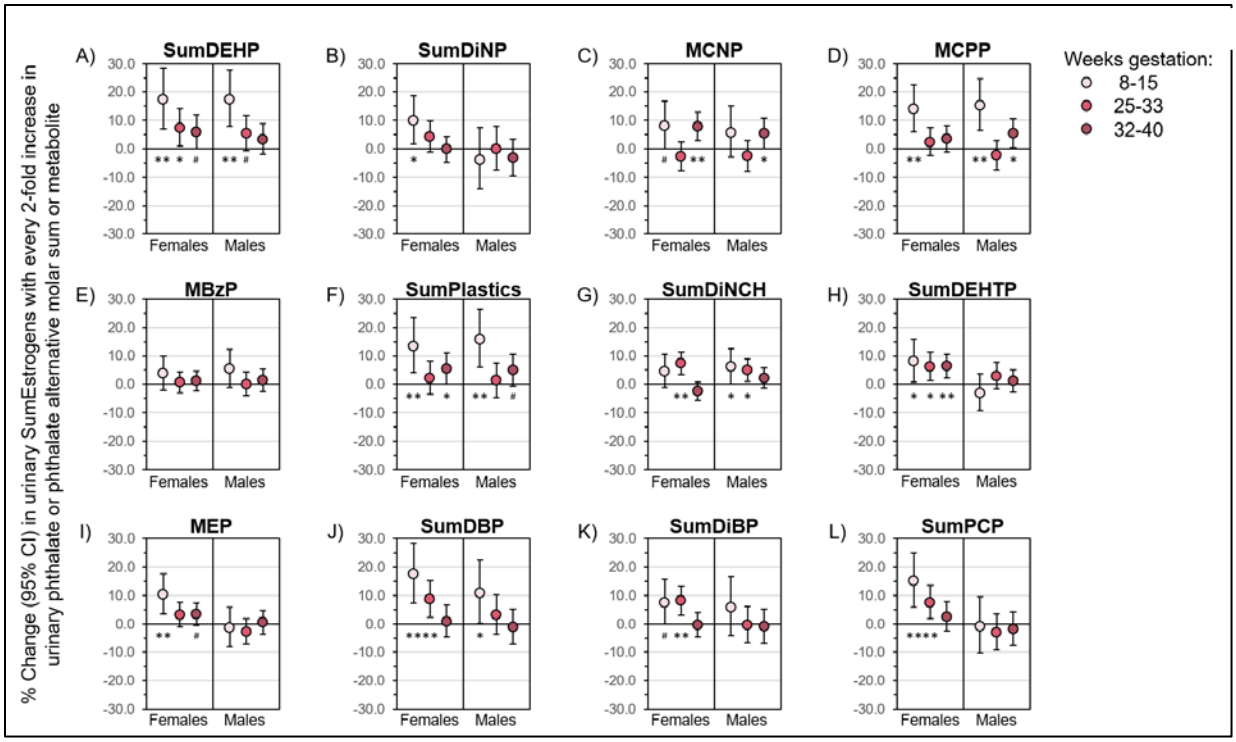

Figure 8. Gestational age- and fetal sex-specific associations of phthalate and phthalate alternative molar sums or metabolites with urinary SumEstrogens. Linear mixed models controlled for age, race/ethnicity, education, parity, smoking during 1st trimester, pre-pregnancy body mass index, diet quality, season of conception, fetal sex, and gestational age at hormone assessment. Models also included a three-way and all relevant two-way interactions between fetal sex, gestational age at hormone assessment, and chemicals. Data are presented as the percent change (filled circle) and 95% CI (solid lines) in urinary SumEstrogens with every 2-fold increase in urinary phthalate or phthalate alternative biomarker. CIs that do not cross the null are significant at #*P*<0.1, **P*<0.05, and ***P*<0.01. CI, confidence interval.

Some associations of phthalate (but not phthalate alternative) biomarkers with urinary SumTestosterones also differed by gestational age and fetal sex (**Figure 9**). Positive associations of MEP and SumPCP with SumTestosterones were strongest in women carrying female fetuses at 8-15 weeks gestation along with 25-33 or 32-40 weeks gestation (**Figure 9I**, **L**). Additionally, a positive association was observed between SumPlastics and SumEstrogens in women carrying females at 8-15 weeks gestation (**Figure 9F**), while MBzP was positively associated with SumEstrogens in women carrying

females at 25-33 weeks gestation (**Figure 9E**). However, SumDiBP was negatively associated with SumTestosterones in women carrying male fetuses, with strongest associations observed at 8-15 and 25-33 weeks gestation (**Figure 9K**). Maternal SumDEHP, SumDiNP, MCNP, MCPP, SumDiNCH, SumDEHTP, and SumDBP were not associated with SumTestosterones, regardless of gestational timepoint of hormone assessment or fetal sex (**Figures 9B**, **C**, **D**, **G**, **H**, **J**).

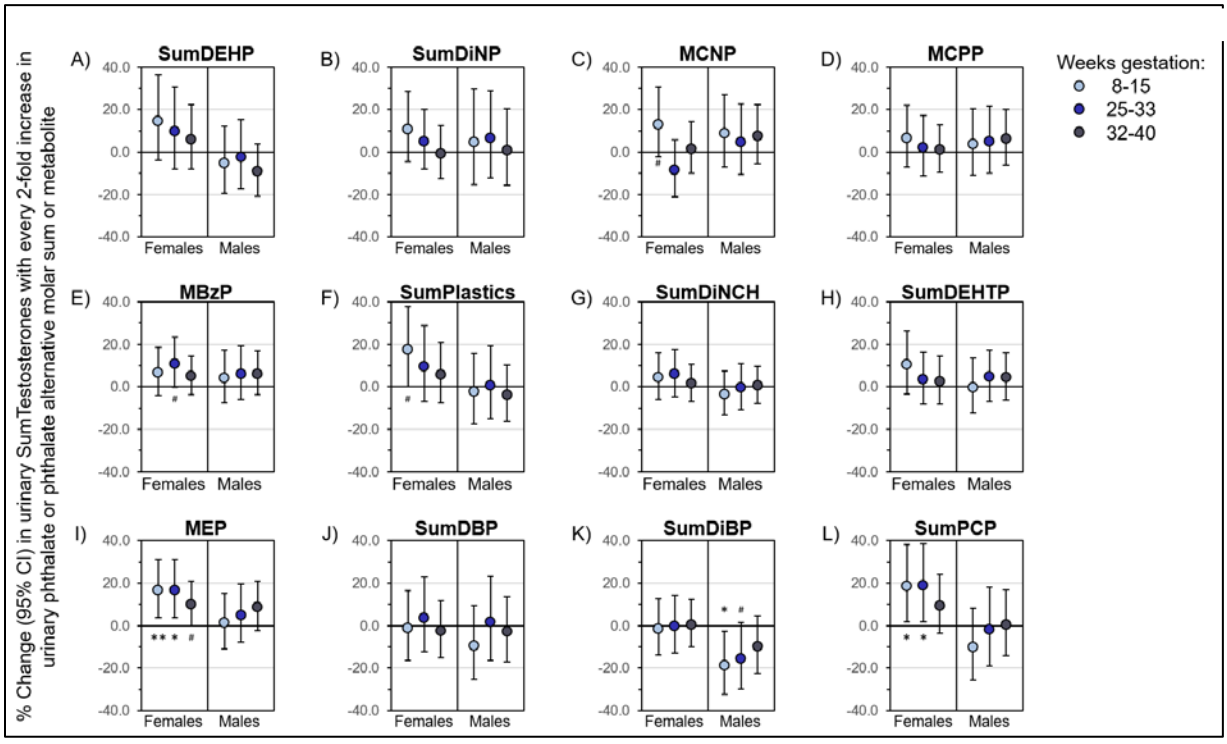

Figure 9. Gestational age- and fetal sex-specific associations of phthalate and phthalate alternative molar sums or metabolites with urinary SumTestosterones. Linear mixed models controlled for age, race/ethnicity, education, parity, smoking during 1st trimester, pre-pregnancy body mass index, diet quality, season of conception, fetal sex, and gestational age at hormone assessment. Models also included a three-way and all relevant two-way interactions between fetal sex, gestational age at hormone assessment, and chemicals. Data are presented as the percent change (filled circle) and 95% CI (solid lines) in urinary SumTestosterones with every 2-fold increase in urinary phthalate or phthalate alternative biomarker. CIs that do not cross the null are significant at #*P*<0.1, **P*<0.05, and ***P*<0.01. CI, confidence interval.

We also observed that some associations of phthalate (but not phthalate alternative) biomarkers with the Estrogen/Androgen ratio differed by gestational age and/or fetal sex (**Figure 10**). Negative associations of MBzP, MEP, and SumPCP with the Estrogen/Androgen ratio were strongest in women carrying female fetuses at 25-33 or 32-40 weeks gestation (**Figure 10E**, **I, L**). However, associations of SumDEHP with Estrogen/Androgen ratio were positive in women carrying male fetuses and strongest at 8-15 and 32-40 weeks gestation (**Figure 10A**), while associations of SumDiBP with Estrogen/Androgen ratio were also positive in women carrying male fetuses, but only at 8-15 weeks gestation (**Figure 10K**). In stratified analyses, associations of SumDiNP, MCNP, MCPP, SumPlastics, SumDiNCH, SumDEHTP, and SumDBP with Estrogen/Androgen ratio did not differ by gestational age or fetal sex (**Figures 10B**, **C**, **D**, **F**, **G**, **H**, **J**).

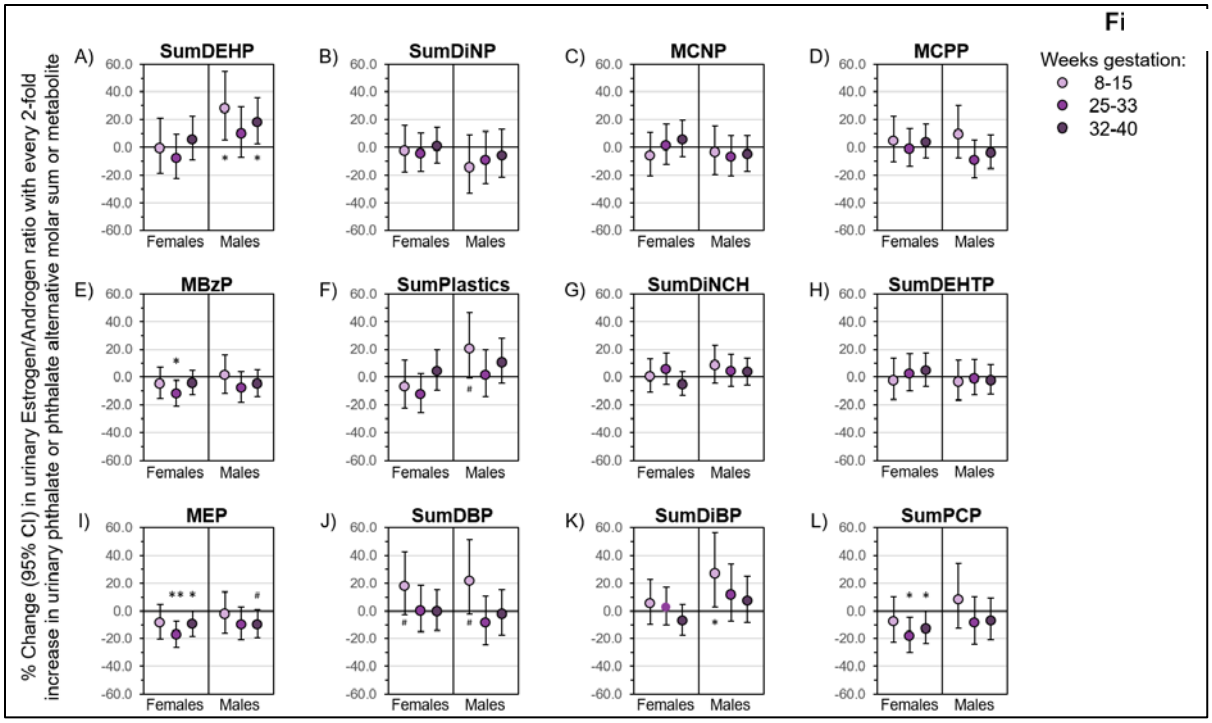

Figure 10. Gestational age- and fetal sex-specific associations of phthalate and phthalate alternative molar sums or metabolites with urinary Estrogen/Androgen ratio. Linear mixed models controlled for age, race/ethnicity, education, parity, smoking during the 1st trimester, pre-pregnancy body mass index, diet quality, season of conception, fetal sex, and gestational age at hormone assessment. Models also included a three-way and all relevant two-way interactions between fetal sex, gestational age at hormone assessment, and chemicals. Data are presented as the percent change (filled circle) and 95% CI (solid lines) in urinary Estrogen/Androgen ratio with every 2-fold increase in urinary phthalate or phthalate alternative biomarker. CIs that do not cross the null are significant at #*P*<0.1, **P*<0.05, and ***P*<0.01. CI, confidence interval.

3.6. DISCUSSION

3.6.1. Summary of major findings

Our study suggests that select phthalate concentrations in pregnancy are associated with higher maternal urinary SumEstrogens and SumTestosterones, and a lower Estrogen/Androgen ratio. Additionally, we found that two biomarkers of phthalate alternatives (SumDiNCH and SumDEHTP) were positively associated with SumEstrogens, but not with SumTestosterones or the Estrogen/Androgen ratio. Some associations of phthalate/alternative biomarkers with urinary hormones tended to be

linear, with the strongest relationships observed at higher quartiles of phthalate or phthalate alternative biomarker concentrations. Importantly, many associations of phthalate and phthalate alternative biomarkers with SumEstrogens tended to be strongest in early and mid-to-late gestation and in women carrying females, while gestational age- and fetal sex-specific associations of phthalate/alternative biomarkers with SumTestosterones and Estrogen/Androgen ratio were less consistent. These findings further confirm that phthalates may have endocrine disrupting properties in pregnant women, which may have important public health implications for maternal and child life-long health. Our findings also support the need for additional studies evaluating the potential endocrine disrupting capacity of newer phthalate alternatives.

3.6.2. Assessment of gestational estrogens and testosterones in urine

Our study is one of few that measured gestational hormone metabolite concentrations in urine. Validation studies in pre-menopausal women found that urinary estrogen metabolite concentrations have high within-person reproducibility (167). Other studies in pre-menopausal women also suggest that associations of certain reproductive and lifestyle factors, including age at first birth and smoking status, with urinary estrogens are consistent with those assessing plasma or serum estrogens (157, 158). However, findings from other studies suggest that evaluating urinary hormones may require a different interpretation compared to those evaluating plasma or serum hormone concentrations. For example, in pre-menopausal women, breast cancer risk was not associated with luteal plasma estrogens, was positively associated with follicular plasma estrogens, but was negatively associated with urinary estrogens (161). Additionally, a nested case-

control study of pregnant women found that women with pre-eclampsia had higher urinary estradiol concentrations than controls (160), which is inconsistent with studies evaluating estradiol in serum. Authors hypothesized that urinary estrogens may be markers of hormone metabolism rather than direct measurements of circulating concentrations (160), which further supports the use of summative measures of urinary estrogens in our study rather than individual urinary metabolites. This is evident in studies assessing concentrations of estrone, estradiol, and estriol in blood or urine showing that estradiol and estriol are the parent estrogens with the highest concentrations in blood and urine, respectively (4, 164). Urine and blood likely capture different hormone forms where unconjugated hormones are measured in plasma or serum, while conjugated hormones are generally measured in urine (161). Whether this is also the case in pregnant women (where the placenta is the major source of steroid hormones) will need to be confirmed in additional studies. However, urine may allow researchers to measure different types of hormone metabolites that cannot be measured in plasma or serum. Compared to blood sampling, urine may also provide opportunities for more extensive cross-pregnancy assessment of hormonal disruption in response to environmental exposures. Despite this, while our study findings suggest that phthalates may disrupt maternal hormones, some caution may be warranted when directly comparing the directionality/magnitude of our findings to studies where hormones were measured in maternal circulation.

3.6.3. Phthalate/alternatives are endocrine disruptors that alter gestational hormone levels

Our findings related to urinary SumEstrogens are consistent with in vitro studies showing

that phthalates are weakly estrogenic (19, 20), which is concerning since higher maternal third trimester circulating estrone and estradiol concentrations have been associated with higher risk of breast cancer in mothers years after pregnancy (168). Our findings that some phthalates are associated with higher urinary SumTestosterones may be concerning given that elevated second and third trimester maternal testosterone levels may be associated with higher risk of pre-eclampsia and gestational diabetes (8, 9). However, these findings in pregnant women are inconsistent with in vivo studies reporting anti-androgenic effects of maternal DEHP and DBP exposure in male offspring (21, 22) or reduced late pregnancy blood testosterone levels in pregnant dams with DEHP exposure (169). These inconsistencies may be because these studies evaluated testosterone levels in male offspring rather than mothers or because they exposed pregnant dams to phthalates at doses irrelevant to humans (i.e. 100 mg/kg/day). It is also possible that urinary testosterone levels are not directly comparable to plasma testosterone concentrations, thus our findings should be corroborated using repeated plasma sampling. The Estrogen/Androgen ratio may be a relevant indicator of placental P450 aromatase activity, which is required to convert testosterone to estradiol (170), and our findings evaluating this ratio in urine may align with experimental models demonstrating that some phthalates reduce aromatase activity during gestation (171). Most importantly, our study is one of the first to show that biomarkers of phthalate alternatives (SumDiNCH and SumDEHTP) may exert similar endocrine disrupting effects on gestational hormones as those of the phthalate parent compounds they replace (e.g. DEHP), which has not been shown in previous studies (152, 172). Given the importance of estrogens and testosterone for pregnancy outcomes, substantially more needs to be

understood about the impacts of phthalates and their replacements on these hormones in pregnant women and the consequences of these disruptions for maternal and child health.

3.6.4. Associations of phthalate/alternative biomarkers with maternal hormones differed by gestational age

The current study appears to be the first to evaluate associations of phthalate/alternative biomarkers with urinary markers of early, middle, and late gestation estrogen and testosterone concentrations. Associations of phthalate biomarkers with urinary estrogens at the three evaluated timepoints suggest that these exposures may be targeting placental hormonal pathways (4), which is consistent with experimental studies showing that phthalates modulate placental estrogen receptor activity and gene expression (173). However, our results are somewhat inconsistent with those from other prospective pregnancy cohort studies (77, 150-152, 155), which may be due to differences in assessing sex steroid concentrations in urine versus blood. One study of U.S. pregnant women found that urinary phthalate metabolites were positively associated with early pregnancy serum estrone or estradiol (consistent with our findings), but negatively associated with early pregnancy serum free testosterone (inconsistent with our findings) (150). However, a recent study in Michigan pregnant women found that urinary phthalate metabolites were not associated with first trimester plasma estrone, estradiol, estriol, or testosterone, but observed a negative association between MBP and maternal plasma estrone measured before delivery (155). Additionally, in a cohort of Puerto Rican pregnant women, phthalate/alternative biomarkers were not associated with maternal second

trimester serum estradiol or estriol, while MHBP and MEP were positively and negatively, respectively, associated with mid-pregnancy testosterone (77, 152). This same study also found that some associations of phthalate/alternative biomarkers with pregnancy serum estriol and testosterone differed by gestational age, such that positive and negative associations were observed in early and late second trimester, respectively (152). However, given that estrogens and testosterones have specific patterns of increases across all pregnancy trimesters, our study design may better represent hormone disruption at three key gestational timepoints. Additionally, unlike the previous studies described here, we assessed phthalate metabolites in a pooled urine sample across pregnancy, which may more accurately represent average gestational exposure to these chemicals. Nevertheless, to corroborate our findings, additional studies are needed that simultaneously assess (and compare) urinary and blood hormone concentrations at multiple key timepoints in pregnancy that correspond to important developmental windows.

3.6.5. Associations of phthalate/alternative biomarkers with maternal hormones differed by fetal sex

Associations of prenatal phthalate metabolite concentrations with hormonally-driven pregnancy and birth outcomes, including pre-eclampsia, gestational diabetes, birth weight, and pre-term birth, often differ by fetal sex (174). However, prior to our study, only two studies in U.S. pregnant women evaluated fetal sex-specific associations of phthalates with gestational hormones, and their findings were inconsistent with ours (151, 155). In the multi-center cohort of U.S. women, SumDEHP was negatively associated

with mid-to-late estradiol in women carrying females (151). Additionally, this study found that SumDEHP, MBzP, and MBP were negatively associated with mid-to-late free/total testosterone in women carrying females, but MEP was positively associated with free/total testosterone in women carrying males (151). However, a study of Michigan pregnant women found that associations of urinary phthalate metabolite concentrations with plasma estrone, estradiol, estriol, or testosterone measured during the first trimester or before delivery were not fetal sex-specific (155). In addition to differences in the biological medium used for hormone assessment, these inconsistencies may be related to exposure measurement, as these studies quantified phthalate metabolites from individual spot urine samples collected during pregnancy. Additionally, these other studies may have been underpowered (n=180 for the multicenter U.S. cohort and n=121 for the Michigan cohort) to detect sex-specific associations of various phthalate biomarkers with hormones (151, 155). Consequently, future studies are needed to corroborate fetal sex-specific findings from both previous studies as well as from the current study.

3.6.6. Strengths and limitations

First, using a pooled sample to assess pregnancy exposure to phthalates/alternatives means that there is uncertainty regarding the directionality of associations as some hormone measures were obtained prior to some exposure measures. However, using a pooled sample of five first morning urines for quantification of nonpersistent chemicals reduces exposure measurement error, provides a more stable measure of mean gestational exposure, and may, in fact, be a better reflection of exposure at any given

timepoint during pregnancy (162, 175). Second, while we did not validate urinary gestational sex-steroid hormones with those measured in serum or plasma, other studies in non-pregnant populations have established urine as a reliable biomarker for sex-steroid hormone assessment (156). Third, although urinary hormones and their metabolites may not directly reflect hormone levels in circulation, we only evaluated associations of phthalate/alternative biomarkers with the sum of estrogen and testosterone metabolites to characterize maternal hormonal disruption (161). Fourth, our findings may not be generalizable to more diverse population since our midwestern U.S. population of pregnant women is predominately white and highly educated. However, this rather homogenous population allows us to easily evaluate and propose biological pathways that can later be confirmed in more diverse populations and in appropriate experimental models. Fifth, while we accounted for urine dilution (i.e. hydration status) by specific gravity adjusting urinary analyte concentrations, specific gravity can vary by physiologic factors such body composition (176). However, specific gravity has low within-person variability, which makes it the more favorable marker of hydration status in pregnant populations relative to creatinine or osmolality (176). Sixth, while there may be residual or unmeasured confounding unaccounted for in our statistical models, we used a priori consideration and previous literature to make informed decisions about covariate selection. Seventh, there may be increased type I error because we did not adjust for multiple comparisons. However, our focus was on a qualitative interpretation of the findings, especially the stratified results, to identify trends in associations of phthalates/alternatives with hormones by gestational age at hormone assessment and fetal sex that will guide future research (166). Lastly, our study was limited to estrogens

and testosterones based on previous experimental studies, but studies in pregnant women suggest that phthalates can also alter maternal thyroid hormone, progesterone, and corticotropin-releasing hormone levels (77, 152). Given that phthalates may impact multiple hormonal pathways, future studies may be needed that consider a larger array of gestational hormones.

3.7. CONCLUSIONS

To our knowledge, this is the first study to evaluate associations of phthalate and phthalate alternative biomarkers with cross-pregnancy gestational sex-steroid hormones measured in urine. Our study suggests that phthalates and their replacements may have endocrine disrupting capacity during pregnancy, and that some of these associations differ by gestational age and fetal sex. Interestingly, our findings combined with results from previously published experimental and observational studies suggest that the direction of these associations remains inconsistent, likely because of numerous factors related to study design and data analysis. Given that pregnant women are exposed to multiple phthalates/alternatives, future studies should evaluate the combined or interactive effects of multiple phthalates/alternatives on gestational hormone concentrations. Most importantly, because altered gestational sex-steroid hormone levels are associated with numerous adverse pregnancy and fetal outcomes, pregnant women may benefit from limiting their use of phthalate and phthalate replacement-containing products during pregnancy.

CHAPTER 4: ASSOCIATIONS OF INDIVIDUAL AND CUMULATIVE URINARY PHTHALATE AND REPLACEMENT BIOMARKERS WITH GESTATIONAL WEIGHT GAIN THROUGH LATE PREGNANCY

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4.1. ABSTRACT

Phthalates and their replacements are endocrine/metabolic disruptors that may impact gestational weight gain (GWG) - a pregnancy health indicator. We investigated overall and fetal sex-specific associations of individual and cumulative phthalate/replacement biomarkers with GWG. Illinois women (n = 299) self-reported their weight pre-pregnancy and at their final obstetric appointment before delivery (median 38 weeks). We calculated pre-pregnancy body mass index and gestational age-specific GWG z-scores (GWGz). We quantified 19 phthalate/replacement metabolites (representing 10 parent compounds) in pools of up-to-five first-morning urine samples, collected approximately monthly between 8 and 40 weeks gestation. We used linear regression, quantile-based g-computation (QGComp), and weighted quantile sum regression (WQSR) to evaluate associations of ten biomarkers (individual metabolites or parent molar-sums) individually or as mixtures (in interquartile range intervals) with GWGz. We evaluated associations in all women and stratified by fetal sex. Individually, sums of metabolites of di(2-ethylhexyl)

phthalate (ΣDEHP), di(isononyl) cyclohexane-1,2-dicarboxylate (ΣDiNCH), and di(2-ethylhexyl) terephthalate (ΣDEHTP) had consistent inverse associations with GWGz, and some associations were fetal sex-specific. When evaluating phthalates/replacements as a mixture, QGComp identified ΣDEHP, ΣDEHTP, and mono-(3-carboxypropyl) phthalate, along with sum of di(isononyl) phthalate metabolites (ΣDiNP) and monobenzyl phthalate as notable contributors to lower and higher GWGz, respectively, resulting in a marginal inverse joint association in all women (β: -0.29; 95% CI: -0.70, 0.12). In women carrying females, ΣDEHP contributed to the marginal inverse joint association (β: -0.54; 95% CI: -1.09, 0.03). However, there was no overall association in women carrying males (β: 0.00; 95% CI: -0.60, 0.59), which was explained by approximately equal negative (driven by ΣDEHTP) and positive (driven by ΣDiNP) partial associations. WQSR analyses consistently replicated these QGComp findings. Biomarkers of phthalates/replacements were fetal sex-specifically associated with GWGz. Because ΣDEHTP contributed substantively to mixture associations, additional studies in pregnant women may be needed around this plasticizer replacement.

4.2. KEYWORDS

DEHTP; DiNCH; fetal sex; gestational weight gain; phthalates; pregnancy.

4.3. INTRODUCTION

Gestational weight gain (GWG) is an important, easily monitored indicator of maternal and fetal health. Deviations from the Institute of Medicine (IOM) GWG guidelines, including both excessive and inadequate GWG, may negatively impact health (3). For

example, compared to women with adequate GWG, those with inadequate GWG are at higher risk of delivering pre-term or small-for-gestational age newborns, whereas women with excessive GWG are at higher risk of developing gestational hypertension and having large-for-gestational age newborns (40). Long-term risks of inappropriate GWG include increased likelihood of maternal postpartum depression (43), excessive offspring adiposity (44), and greater maternal postpartum weight retention, all of which could lead to increased risk of later life-associated health complications (177, 178). Therefore, it is critical to identify modifiable risk factors associated with inappropriate GWG.

Phthalates are a class of chemicals found in a wide variety of consumer products, including (but not limited to) food packaging materials, coatings of medications and supplements, personal care products, and cosmetics, with ubiquitous exposure among pregnant women in the U.S. (14). This is particularly concerning given that certain phthalates have been implicated in pregnancy-related metabolic disorders, including gestational hypertension (25, 179, 180) and diabetes (181-183). Industry use of plasticizer replacements such as cyclohexane-1,2-dicarboxylic acid diisononyl ester (DiNCH, a non-phthalate alternative) and di(2-ethylhexyl) terephthalate (DEHTP) appear to be on the rise (65, 184, 185). These replacements may have similar hormonally-mediated adverse health impacts to the original phthalates (83, 132, 186-188). Pregnancy and fetal development, including GWG, are regulated by coordinated hormonal, inflammatory, and metabolic processes, which may be biological targets of phthalates and their replacements (189-191).

Current evidence evaluating associations of prenatal urinary concentrations of phthalate metabolites (used as biomarkers of phthalate exposure) with GWG is inconclusive (31, 32, 192-196). For example, studies from the U.S. and China observed positive associations of first, second, or third trimester monoethyl phthalate (MEP) concentrations with excessive total GWG (31, 32, 195) and first trimester GWG (193). However, other studies reported that elevated second trimester urinary levels of low molecular weight phthalate biomarkers (driven by MEP) were associated with inadequate total GWG (192). Additionally, a study from China reported lower first or second trimester MEP concentrations among women with inadequate compared to those with adequate total GWG (194).

It has become increasingly important to understand the cumulative impacts of phthalates/replacements and the role of each individual chemical within the context of the others. Of the few studies evaluating cumulative associations of phthalate biomarker mixtures with GWG, one study from China reported that a hazard index estimating exposures to di(2-ethylhexyl) phthalate (DEHP), diethyl phthalate (DEP), and di-n-butyl phthalate (DBP) across all pregnancy trimesters was associated with higher odds of excessive total GWG with most prominent associations observed for first trimester phthalate biomarkers (31). However, a Boston study observed no associations between sums of first, second, and third trimester phthalate metabolites of parents used in personal care products (MEP and monobutyl phthalate (MBP)), or phthalate metabolites shown to have anti-androgenic activity (MBP, monobenzyl phthalate (MBzP), mono-isobutyl phthalate (MiBP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-

ethyl-5-carboxypentyl) phthalate (MECPP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethylhexyl) phthalate (MEHP)) with total GWG (32). Finally, using Bayesian Kernel Machine Regression (BKMR), which can estimate the health effects of a complex mixture composed of highly correlated chemicals (197), a study of U.S. women observed that a mixture of select first trimester phthalate, bisphenol, and paraben biomarker concentrations was positively associated with total GWG, and this association was primarily driven by the molar sum of four DEHP metabolites (196).

Additional studies are needed to better understand the individual and cumulative associations of phthalate metabolite biomarkers with GWG. Furthermore, previous studies only evaluated a limited number of phthalates. Studies assessing a larger panel of phthalates to which pregnant women are exposed, as well as common plasticizer replacements (such as DiNCH and DEHTP), are needed. Therefore, our overall objective was to evaluate associations of phthalate/replacement biomarkers with GWG through late pregnancy by considering phthalate biomarkers individually and as a mixture. We hypothesized that individually and as a mixture, phthalates/replacements would be associated with higher GWG. GWG is a complex phenotype with both maternal and fetal contributions (198), and fetal sex appears to be an important determinant of GWG (41, 199-201). Therefore, for our secondary objective we hypothesized that individually and as a mixture, associations of phthalates/replacements would differ by fetal sex.

4.4. MATERIALS AND METHODS

4.4.1. Illinois Kids Development Study (I-KIDS) recruitment and enrollment

The current study includes pregnant women recruited from two local obstetric clinics in Champaign-Urbana, Illinois who were invited to participate in I-KIDS – a prospective pregnancy and birth cohort with the overarching aim of evaluating the impacts of prenatal chemical exposures on infant neurodevelopment. Recruitment and enrollment have been described in detail elsewhere (86). I-KIDS includes women who were≤ 15 weeks pregnant at enrollment, 18-40 years old, fluent in English, in a low-risk and singleton pregnancy, living within a 30-minute drive of the University of Illinois campus, and not planning to move out of the area before their child's first birthday. The current study includes a sample of 303 women who enrolled in I-KIDS between February 2015 and August 2018, remained in the study through the birth of their infant, and have measurable concentrations of at least one urinary phthalate/replacement metabolite and pre- and late-pregnancy weights to calculate GWG through late pregnancy. As we have previously described, women in the current analytic sample are representative of the full I-KIDS cohort (202). All women provided written informed consent, and the study was approved by the Institutional Review Board at the University of Illinois. The analysis of de-identified specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects' research.

4.4.2. Assessment of urinary phthalate/replacement biomarker concentrations

Women collected at least three and up to five first-morning urine samples in polypropylene urine cups at 8-15, 13-22, 19-28, 25-33, 32-40 weeks gestation (median

13, 17, 23, 28, and 34 weeks gestation, respectively), which corresponded to study home visits or routine prenatal care visits. Most of the 303 women contributed all five urine samples (94%), whereas 6% contributed three or four urine samples. Specifically, 99% of women provided a urine sample at 8-15 weeks gestation, 100% at 13-22 weeks gestation, 98% at 19-28 weeks gestation, 98% at 25-33 weeks gestation, and 98% at 32-40 weeks gestation. We have previously described urine collection, processing, and storage protocols in detail (86, 202). For the current study, we quantified phthalate/replacement metabolite concentrations and measured specific gravity from pooled samples of up to five individual first-morning urines collected from each woman. Pooled samples were shipped overnight to the CDC Division of Laboratory Sciences in two batches in chronological order of participant enrollment as follows: enrolled February 2015 - July 2016 and enrolled July 2016 - August 2018. Using previously published methods with rigorous quality assurance/quality control protocols and excellent long-term reproducibility (104-106), the following 19 phthalate/replacement metabolites were quantified: monocarboxynonyl phthalate (MCNP), monocarboxyoctyl phthalate (MCOP), monooxononyl phthalate (MONP), monoisononyl phthalate (MiNP), MEHP, MEHHP, MEOHP, MECPP, mono-(3-carboxypropyl) phthalate (MCPP), MBzP, MBP, mono-hydroxybutyl phthalate (MHBP), MiBP, mono-hydroxy-isobutyl phthalate (MHiBP), MEP, cyclohexane-1,2-dicarboxylic acid-mono(carboxyoctyl) ester (MCOCH), cyclohexane-1,2-dicarboxylic acid-monohydroxy isononyl ester (MHiNCH), mono(2-ethyl-5-hydroxyhexyl) terephthalate (MEHHTP), and mono(2-ethyl-5-carboxypentyl) terephthalate (MECPTP).

4.4.3. Collection and calculation of GWG z-scores through late pregnancy

Within 24 hours of delivery, women reported their measured weight (in pounds) and the date of their most recent obstetric appointment (median: 38, range: 28 – 41 weeks gestation). We subtracted pre-pregnancy weight (which was self-reported during a home visit at 8 – 15 weeks gestation) from the weight reported at the last obstetric appointment before delivery to calculate GWG through late pregnancy (in kg). In the few cases where weight at the last obstetric visit prior to delivery was not available (n = 17), we used the reported weight from an earlier obstetric visit (median: 34, range: 29 – 37 weeks gestation). We calculated pre-pregnancy BMI- and gestational age-specific GWG z-scores based on previously validated methods using a reference population of pregnant women from Europe, North America, and Oceania (203).

4.4.4. Collection of maternal sociodemographic, lifestyle, and health information

After enrollment (8 – 15 weeks gestation), study staff conducted home visits to interview women about their race/ethnicity, educational attainment, annual household income, parity, smoking in the first trimester, alcohol intake in the first trimester, as well as pre-pregnancy weight and height. Self-reported pre-pregnancy weight and height were used to calculate pre-pregnancy BMI (kg/m^2). To ascertain early pregnancy stress status, women completed the Perceived Stress Scale, a 10-item questionnaire that asks about thoughts and feelings during the past month (204, 205). At 8 – 15 and 32 – 40 weeks gestation, women also completed a semi-quantitative food frequency questionnaire (FFQ) that was adapted from the full length Block-98 FFQ (NutritionQuest, Berkeley, CA) and validated in a pregnant population (101, 206, 207). Reported dietary intakes

representing diet during the previous three months were used to calculate the mean of early and mid-to-late pregnancy Alternative Healthy Eating Index (AHEI-2010). The AHEI-2010 is an 11-component diet quality index (out of 110 total points) based on foods and nutrients shown to be predictive of chronic disease risk and mortality, where a higher score indicates better overall diet quality (102, 103).

4.4.5. Statistical analysis

Derivation of the analytic sample is presented in **Figure 11**. Out of 303 women with data on concentrations of all phthalate/replacement biomarkers and GWG z-scores, 299 (148 and 151 carrying males and females, respectively) were included in final covariate-adjusted single- and multi-pollutant analyses. Characteristics of the sample are presented as n (%) or median (range).

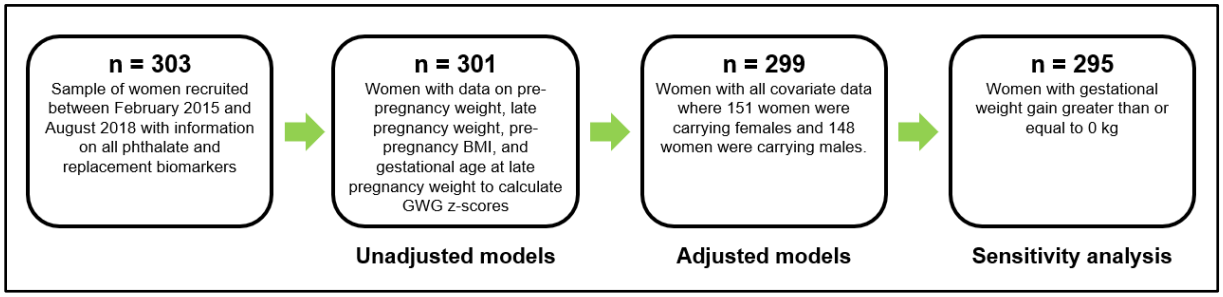

Figure 11. Derivation of analytic sample for evaluating associations of phthalate/replacement biomarkers with GWG z-scores. The chart presents sample sizes for sensitivity analyses and analyses evaluating unadjusted and adjusted associations of phthalate/replacement biomarkers with GWG z-scores using multiple linear regression models. Sample sizes for QGComp and WQSR models are those reported for adjusted models. GWG, gestational weight gain; QGComp, quantile-based g-computation; WQSR, weighted quantile sum regression.

We evaluated specific gravity-adjusted phthalate/replacement biomarkers as molar sums or individual metabolites, which reflect exposures to 10 phthalate/replacement parent

compounds (86, 202). We created phthalate/replacement molar sums (in nmol/mL) by summing metabolites from common precursors as follows: MEHP, MEHHP, MEOHP, and MECPP for the sum of DEHP metabolites (ΣDEHP); MCOP, MiNP, and MONP for the sum of metabolites of di-isononyl phthalate (ΣDiNP); MBP and MHBP for the sum of DBP metabolites (ΣDBP); MiBP and MHiBP for the sum of di-iso-butyl phthalate metabolites (ΣDiBP); MHiNCH and MCOCH for the sum of DiNCH metabolites (ΣDiNCH); and MEHHTP and MECPTP for the sum of DEHTP metabolites (ΣDEHTP). Specifics about equations are published elsewhere (86). Molar concentrations were back-converted to ng/mL by multiplying ΣDEHP, ΣDiNP, ΣDBP, ΣDiBP, ΣDiNCH, and ΣDEHTP by the molecular weights of MECPP, MCOP, MBP, MiBP, MHiNCH, and MECPTP, respectively (113, 186, 202). We estimated exposure to di-isodecyl phthalate, di-n-octyl phthalate, benzylbutyl phthalate (BBzP), and DEP using ng/mL concentrations of their corresponding urinary metabolites MCNP, MCPP, MBzP, and MEP, respectively.

Based on the previous literature, we considered an extensive number of potential covariates in statistical models evaluating associations of phthalate/replacement biomarkers with GWG z-scores (31, 32, 192-196). We generated a directed acyclic graph (DAG, **Figure 12**) (208), which included covariates that we and others found to be associated with both phthalate/replacement biomarkers and GWG z-scores. We used the DAG to guide the minimum sufficient adjustment set of covariates (208). We assessed correlations between covariates to test for potential multicollinearity, but all covariates were only weakly or moderately correlated ($r < 0.4$; **data not shown**). Therefore, all final covariate-adjusted single-pollutant and mixtures models accounted for race/ethnicity,

educational attainment, annual household income, smoking in the first trimester, pre-pregnancy BMI, and maternal diet quality. Annual household income, pre-pregnancy BMI, and maternal diet quality were included as continuous variables, whereas race/ethnicity, educational attainment, and smoking in the first trimester were categorized with the reference groups indicated in **Table 9**. We specified models that additionally accounted for perceived stress, parity, and alcohol intake in the first trimester, but our results were relatively unchanged with the inclusion of these variables (**data not shown**).

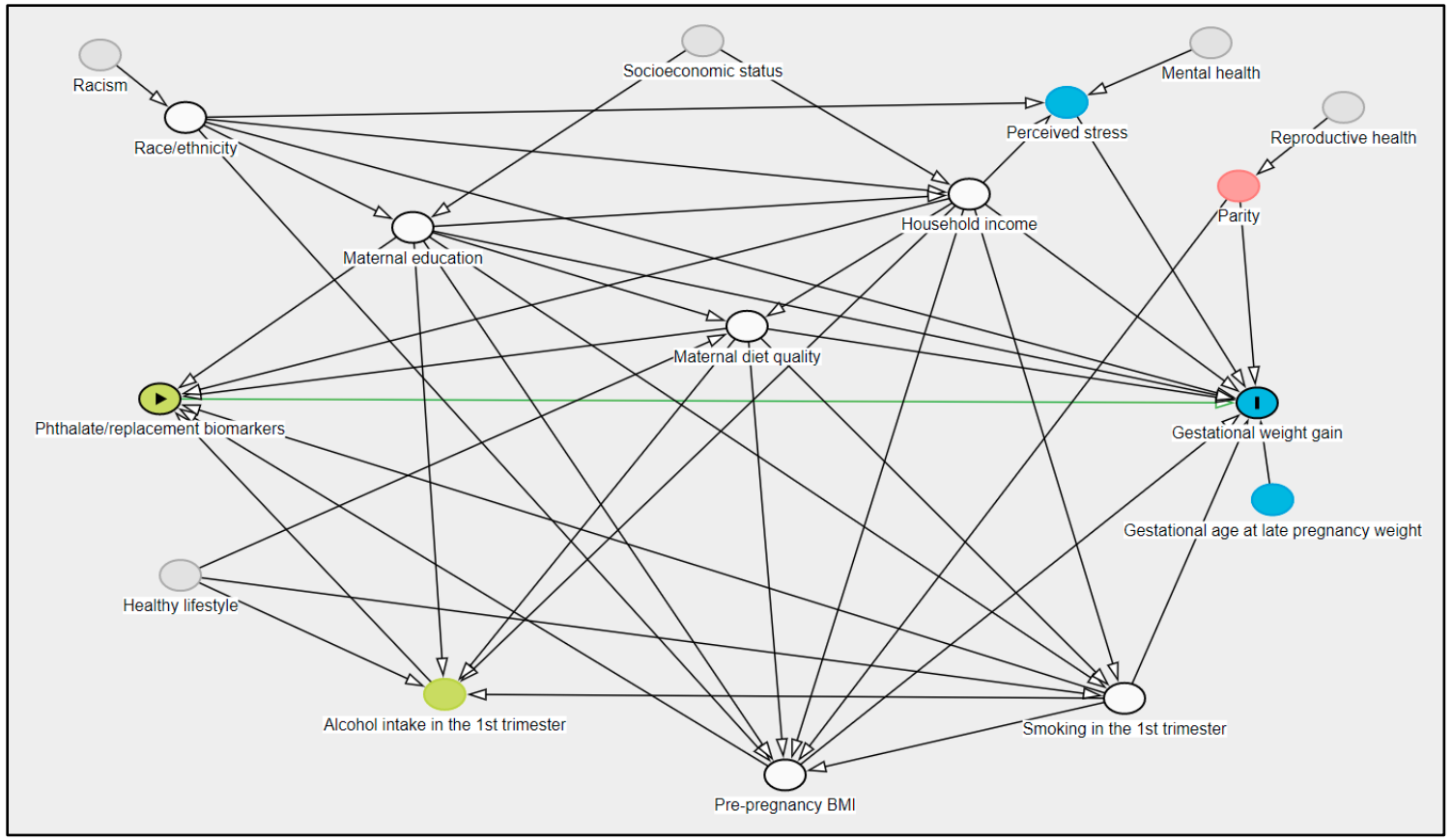

Figure 12. Directed acyclic graph for associations of phthalate/replacement biomarkers with GWG z-scores. Phthalate/replacement biomarkers are the exposures (green/black circle) and gestational weight gain is the outcome (blue/black circle). Green circles indicate variables associated with the exposure, blue circles indicate variables associated with the outcome, and red circles indicate variables associated with both the exposure and outcome. Gray circles represent latent variables, while white circles represent variables that were included in final covariate-adjusted analyses (minimum sufficient adjustment set). BMI, body mass index; GWG, gestational weight gain.

4.4.6. Primary and sensitivity single-pollutant analyses

To address our main objectives, we first specified multivariable linear regression models to evaluate single-pollutant associations of phthalate/replacement biomarkers with GWG z-scores. All phthalate/replacement biomarkers were ln-transformed because of their right-skewed distributions. For concentrations below the limit of detection (LOD) we used instrumental reading values to avoid bias associated with imputing values <LOD (107). Across the individual and molar sum biomarkers described above, only one woman had a concentration of zero for the two DiNCH metabolites MCOCH and MHiNCH. Therefore, we used the following equation [ln(ΣDiNCH + 1)] to avoid undefined estimates for ΣDiNCH. We did not transform GWG z-score because it was normally distributed. Regression diagnostics based on residuals were conducted to ensure all model assumptions were met. Because women with GWG below zero (n=4) could have unique characteristics that influence our results, we also conducted sensitivity analyses, using linear regression models, that excluded these women. For all linear regression analyses, the resulting β-estimates and 95% confidence intervals (CIs) represent the change in GWG z-scores for every interquartile range (IQR) increase in phthalate/replacement biomarker concentration.

4.4.7. Primary mixture analyses

To evaluate associations of phthalate/replacement biomarkers as a joint mixture with GWG z-scores, we first estimated quantile-based g-computation (QGComp) models (72). QGComp was adapted from weighted quantile sum regression (WQSR) (73) and relaxes the assumption that all chemical exposures are associated with the outcome in the same

direction. For our analyses, we included the following 10 individual or molar sum phthalate/replacement biomarkers in our mixture index: MCNP, ΣDiNP, ΣDEHP, MCPP, MBzP, ΣDBP, ΣDiBP, MEP, ΣDiNCH, and ΣDEHTP. These 10 components represent common phthalates or phthalate replacement compounds to which I-KIDS women are exposed. Then, QGComp was used to estimate the joint association of the mixture index with GWG z-scores (not transformed) via multiple linear regression, and determine the relative weights for each phthalate/replacement biomarker that indicate the contribution of each biomarker to the joint association. We generated results without bootstrapping to obtain the partial negative associations (i.e., scaled sum of negative biomarker weights) and partial positive associations (i.e., scaled sum of positive biomarker weights). As we transformed all phthalate/replacement biomarker concentrations into deciles, the resulting β-estimates and 95% CIs are interpreted as the change in GWG z-scores if concentrations of all phthalate/replacement biomarkers simultaneously changed by 10%. However, to make these results comparable to those from single-pollutant models, we multiplied the β-estimates and 95% CIs by 5.0 to represent the change in GWG z-scores for each IQR change in the mixture. The most prominent contributors to mixture associations were determined by the strength of the relative weights.

One limitation of QGComp is it assumes that all chemicals included in the mixture originate from a single source of exposure and does not allow exposures to vary independently. For this reason, we chose to additionally evaluate associations of phthalate/replacement biomarkers as a mixture with GWG z-scores using WQSR, as these chemicals have distinct sources and may have different mechanisms of action. We

specified WQSR models with the repeated holdout approach, which improves the stability of results by combining cross-validation and bootstrap resampling to implement WQSR in an iterative fashion (73, 209). Briefly, WQSR is a supervised mixture method that creates a unidirectional mixture index by transforming exposures into quantiles, and then evaluates the cumulative association of the mixture index with the outcome via multiple linear regression (73, 210). We included the same 10 phthalate/replacement biomarkers in the mixture index and converted concentrations into deciles. GWG z-scores were not transformed. We generated a distribution of results using 100 iterations (repeated holdouts), each with 100 bootstrap replications. Within each of the 100 iterations, data were randomly split 40/60% into training and validation datasets, respectively. WQSR weighs the relative variable importance of each individual mixture component, which can be used to determine the largest contributors to the cumulative association (73, 210). Biomarkers with mean weights above 0.10 (1/10 biomarkers included in the mixture) were identified as the largest contributors. Because WQSR models are specified to evaluate associations between all mixture components and the outcome in one direction at a time, the models assume all components are associated with the outcome in the same direction, and all component weights are non-negative and sum to one. Therefore, we explored WQSR models separately in the negative and positive directions.

Then, using findings from single-pollutant and total WQSR models, we determined which phthalate/replacement biomarkers tended to be positively and negatively associated with GWG z-scores. WQSR assumes that all biomarkers in the mixture are associated with the outcome in the same direction, which was not supported by our findings. Therefore,

after identifying the biomarkers that were positively associated with GWG z-scores, we multiplied their concentrations by (-1.0), thus reversing the order of their quantiles. Next, all 10 phthalate/replacement biomarkers were included in our mixture index using the updated scoring scheme, and we evaluated WQSR models in the negative direction using the same settings as described previously. From the 100 repeated holdout samples, we obtained the mean cumulative association of phthalate/replacement biomarker concentrations with GWG z-scores, as well as weights for each biomarker in the mixture that indicate its relative importance as a mixture component. The resulting β-estimate and 95% CIs (which we also multiplied by 5.0) can be interpreted as the change in GWG z-scores (not-transformed) if concentrations of all negative biomarkers increased by one IQR and those of all positive biomarker concentrations simultaneously decreased by one IQR. Phthalate/replacement biomarker weights were renormalized, so that the weights of all the positive biomarkers (i.e., partial positive association) and the weights all the negative biomarkers (i.e. partial negative association) separately summed to 1.0.

4.4.8. Secondary analyses

We performed several secondary analyses to evaluate fetal sex-specific associations. First, we conducted linear regression analyses to evaluate single-pollutant associations of ln-transformed phthalate/replacement biomarker concentrations with GWG z-scores stratified by fetal sex. GWG z-scores were operationalized as discussed in **section 4.4.6**. To obtain fetal sex-specific β-estimates and 95% CIs, we included a multiplicative interaction between the phthalate/replacement biomarker and fetal sex. We reported all results regardless of the significance of the interaction P-value ($P_{biomarker*sex}$) and

compared the direction, strength, and precision of associations to identify meaningful differences by fetal sex. Second, we evaluated fetal sex-specific associations of the phthalate/replacement biomarker mixture with GWG z-scores by specifying separate QGComp and WQSR models for women carrying females and males as we described previously.

When evaluating associations between risk factors and GWG, it is recommended that studies include pre-pregnancy BMI as an effect modifier (211). Therefore, as an additional sensitivity analysis, we first evaluated single-pollutant associations of phthalate/replacement biomarkers with GWG z-scores by pre-pregnancy BMI. To obtain the pre-pregnancy BMI-specific β-estimates and 95% CIs in linear regression analyses, we included a multiplicative interaction between the phthalate/replacement biomarker and pre-pregnancy BMI. Second, because we hypothesized that associations between phthalate/replacement biomarkers with GWG z-scores are fetal sex-specific, we also conducted linear regression analyses including a multiplicative three-way interaction (and all relevant two-way interactions) between the phthalate/replacement biomarker, pre-pregnancy BMI, and fetal sex. As discussed earlier, we reported all results regardless of the significance of the interaction P-value (P_{int}).

We performed linear regression analyses in SAS version 9.4 (SAS Institute Inc, Cary, NC) using PROC GLM. We performed QGComp and WQSR analyses in R Statistical Software (v4.1.1; R Core Team 2021) using R packages "qgcomp: Quantile G-Computation" (70) and "gWQS: Generalized Weighted Quantile Sum Regression" (71)

respectively. Rather than focusing on statistical significance, we focused on patterns (direction and magnitude) and precision of observed associations based on recommendations from the American Statistical Association and others (117, 118). Thus, in addition to results where the upper and lower limits do not cross the null (zero), we also considered results as potentially meaningful if either the upper or lower confidence limit crossed, but were close to the null, and if the confidence interval was narrow relative to other estimates. For our mixtures findings, our overall conclusions for notable phthalate/replacement biomarker contributors were based on identifying consistent findings from both QGComp and WQSR, at least in the qualitative sense. With regards to interpreting our findings, β-estimates and 95% CIs for a z-score difference in GWG can be converted (considering the gestational age at late pregnancy weight and pre-pregnancy BMI) to represent a difference in kg (211). As a reference, a 0.2 z-score difference in weight gain through 38 weeks gestation can be interpreted approximately as a 0.82 kg difference for underweight, 0.84 difference for normal weight, 1.09 kg difference for overweight, 1.18 kg difference for obese class I, 1.28 kg difference for obese class II, and 1.44 kg difference for obese class III pre-pregnancy BMI.

4.5. RESULTS

4.5.1. Characteristics of the I-KIDS population

Women had a median age of 31 years (**Table 9**). Most women were non-Hispanic white (82%), college-educated (83%), nulliparous (53%), and had annual household incomes > $60,000 (73%). Most women did not smoke (95%) or consume alcohol (58%) in the first trimester, around half had overweight or obesity before pregnancy (48%), and median

AHEI-2010 was 55.9 (min, max: 28.1, 80.2). Around two thirds of women reported having low perceived stress, whereas the rest reported having moderate or high perceived stress in the first trimester. Median GWG through median 38 weeks gestation was 15.0 kg (min, max: -10.9, 38.1).

Table 9. Characteristics of the study sample (n=303).

Category	n (%) or median (min - max)
Age, years	31.0 (18.0 - 40.0)
[1]Race/ethnicity	
Non-Hispanic white (ref)	247 (81.5)
[2]Others	56 (18.5)
[1]Educational attainment	
Some college or less	52 (17.2)
College grad or higher (ref)	251 (82.8)
[1]Annual household income	
< $60,000	82 (27.2)
$60,000 - $99,999	113 (37.5)
≥ $100,000	106 (35.2)
Parity	
0 children	161 (53.2)
1 child	94 (31.0)
≥ 2 children	48 (15.8)
[1]Smoking in the first trimester	
No (ref)	288 (95.1)
Yes	15 (4.9)
Alcohol intake in the first trimester	
None	176 (58.1)
Any alcohol consumed	127 (41.9)
[1]Pre-pregnancy BMI	
Under-/normal weight (< 25 kg/m^2)	158 (52.2)
Overweight (25 - 29.9 kg/m^2)	78 (25.7)
Obese (≥ 30 kg/m^2)	67 (22.1)
Perceived stress in the first trimester	
Low stress (0-13 pts)	188 (62.7)
Moderate stress (14-26 pts)	106 (35.3)
High stress (27-40 pts)	6 (2.0)
Fetal sex	
Female	153 (50.5)
Male	150 (49.5)
Birth weight (grams)	
Females	3360.8 (2353.0, 4507.6)
Males	3611.7 (2588.3, 4394.2)
AHEI-2010	55.9 (28.1 - 80.2)
GWG through late pregnancy (kg)	15.0 (-10.9 - 38.1)
GWG through late pregnancy (z-scores)	0.5 (-3.0 - 4.1)
Gestational age at pre-delivery weight report (weeks)	38.9 (28.9 - 41.9)

[1]Included in final statistical models as covariates. [2]Includes Hispanic, Black, Asian, American Indian, Multiracial, Other. Percentages may not add up to 100% due to missing. Subset sample (n missing): annual household income, GWG z-scores, gestational age at late pregnancy weight (2 missing); perceived stress (3 missing). AHEI-2010, Alternative Healthy Eating Index 2010 scored out of 110; GWG, gestational weight gain.

4.5.2. Urinary phthalate/replacement metabolite biomarker concentrations in I-KIDS

The distribution of urinary phthalate/replacement biomarker concentrations are presented in **Table 10**. Greater than 96% of women had detectable concentrations of at least one phthalate metabolite per parent compound (including DEHTP), and > 90% of women had detectable concentrations of at least one DiNCH metabolite. As we have shown previously, most phthalate/replacement biomarkers were weakly-to-moderately correlated with each other ($r < 0.4$), though we did observe a strong correlation between ΣDiNP and MCPP ($r > 0.8$) (202). As reported previously, median uncorrected urinary phthalate/DiNCH biomarker concentrations in I-KIDS were similar to those of same age women from the National Health and Nutrition Examination Survey (NHANES) during a similar time period (202). However, DEHTP metabolite concentrations in I-KIDS were 1.5 to 3 times higher than those from NHANES (202).

Table 10. Distribution of maternal urinary specific gravity-adjusted phthalate/ replacement biomarker concentrations (n = 303).

Parent	Biomarker	% detectable	25th percentile (ng/mL)	50th percentile (ng/mL)	75th percentile (ng/mL)
Di-isodecyl phthalate, DiDP	**MCNP***	100.0	1.33	1.81	2.58
Di-isononyl phthalate, DiNP	**ΣDiNP***	--	4.94	7.96	15.11
	MCOP	100.0	4.47	7.09	13.65
	MiNP	31.4	0.4	0.62	1.09
	MONP	100.0	1.71	2.64	4.63
Di(2-ethylhexyl) phthalate, DEHP	**ΣDEHP***	--	14.75	19.71	29.89
	MEHP	75.6	0.85	1.32	2.13
	MEHHP	100.0	3.62	5.40	8.32
	MEOHP	100.0	2.88	4.00	6.34
	MECPP	100.0	6.08	8.32	12.64
Di-n-octyl phthalate, DOP	**MCPP***	96.0	0.86	1.30	1.90
Benzylbutyl phthalate, BBzP	**MBzP***	99.3	2.54	5.16	10.00
Di-n-butyl phthalate, DBP	**ΣDBP***	--	9.36	14.05	19.22
	MBP	100.0	8.46	12.68	17.41
	MHBP	90.1	0.75	1.22	1.87
Di-iso-butyl phthalate, DiBP	**ΣDiBP***	--	7.86	11.56	19.59
	MiBP	99.7	5.74	8.53	14.40
	MHiBP	99.7	2.13	3.11	5.47
Diethyl phthalate, DEP	**MEP***	100.0	14.12	27.47	47.79
Diisononyl-cyclohexane-1,2-dicarboxylate, DiNCH	**ΣDiNCH***	--	1.16	1.88	3.43
	MHiNCH	90.7	0.69	1.14	2.26
	MCOCH	67.3	0.48	0.71	1.22
Di(2-ethylhexyl) terephthalate, DEHTP	**ΣDEHTP***	--	30.18	69.65	158.54
	MEHHTP	100.0	3.84	8.16	20.11
	MECPTP	100.0	25.69	58.88	135.53

All concentrations are presented in ng/mL. All metabolite concentrations were specific gravity-adjusted. Molar sums were converted back to ng/mL by multiplying each molar sum by the molecular weight of a corresponding major metabolite as discussed in the statistical analysis section. *Indicates the biomarkers of interest for the current study. MBP, monobutyl phthalate; MBzP, monobenzyl phthalate; MCNP, monocarboxynonyl phthalate; MCOCH, cyclohexane-1,2-dicarboxylic acid-mono(carboxyoctyl) ester; MCOP, monocarboxyoctyl phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MECPTP, mono(2-ethyl-5-carboxypentyl) terephthalate; MEHP, mono(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHHTP, mono(2-ethyl-5-hydroxyhexyl) terephthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEP, monoethyl phthalate; MHBP, mono-hydroxybutyl phthalate; MHiBP, mono-hydroxy-isobutyl phthalate; MHiNCH, cyclohexane-1,2-dicarboxylic acid-monohydroxy isononyl ester; MiBP, mono-isobutyl phthalate; MiNP, monoisononyl phthalate; MONP, monooxononyl phthalate; ΣDiNP, sum of di(isononyl) phthalate metabolites; ΣDEHP, sum of di(2-ethylhexyl) phthalate metabolites; ΣDBP, sum of di-n-butyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; ΣDEHTP, sum of di(2-ethylhexyl) terephthalate metabolites.

4.5.3. Associations of individual phthalate/replacement biomarkers with GWG z-scores

In single-pollutant linear regression models, ΣDEHP and ΣDEHTP were inversely associated with GWG z-scores (**Table 11**). For example, IQR increases in ΣDEHP and

ΣDEHTP were associated with -0.16 (95% CI: -0.29, -0.03) and -0.20 (95% CI: -0.38, -0.02) lower GWG z-scores, respectively. MCPP and ΣDiNCH were also meaningfully associated with GWG z-scores, where IQR increases in MCPP and ΣDiNCH were associated with -0.09 (95% CI: -0.21, 0.03) and -0.11 (95% CI: -0.22, 0.01) lower GWG z-scores, respectively. These results persisted in sensitivity analyses excluding the four women with GWG < 0 (**Supplemental Table 12**). We also observed suggestive differences in associations between some phthalate/replacement biomarkers and GWG z-scores by pre-pregnancy BMI (**Supplemental Table 13**). However, when additionally evaluating differences by pre-pregnancy BMI and fetal sex, no definitive patterns emerged and results were notably less precise (**Supplemental Table 14**).

Table 11. Single-pollutant associations of phthalate/replacement biomarkers with GWG z-scores.

	All women (n = 299)	**Women carrying males (n = 148)**	**Women carrying females (n = 151)**	
Biomarker	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	$P_{biomarker*sex}$
MCNP	0.00 (-0.12, 0.13)	0.03 (-0.16, 0.23)	0.00 (-0.16, 0.17)	0.80
ΣDiNP	0.01 (-0.13, 0.15)	0.25 (0.02, 0.49)	-0.08 (-0.25, 0.09)	0.02
ΣDEHP	-0.16 (-0.29, -0.03)	0.04 (-0.14, 0.22)	-0.35 (-0.53, -0.18)	0.002
MCPP	-0.09 (-0.21, 0.03)	-0.03 (-0.22, 0.17)	-0.11 (-0.27, 0.05)	0.49
MBzP	0.09 (-0.06, 0.25)	0.15 (-0.08, 0.37)	0.03 (-0.18, 0.25)	0.46
ΣDBP	-0.09 (-0.22, 0.05)	-0.08 (-0.28, 0.11)	-0.10 (-0.27, 0.07)	0.90
ΣDiBP	-0.03 (-0.16, 0.10)	0.01 (-0.20, 0.23)	-0.05 (-0.22, 0.12)	0.64
MEP	-0.08 (-0.24, 0.07)	0.00 (-0.22, 0.22)	-0.13 (-0.35, 0.09)	0.39
ΣDiNCH	-0.11 (-0.22, 0.01)	-0.17 (-0.35, 0.01)	-0.04 (-0.19, 0.11)	0.27
ΣDEHTP	-0.20 (-0.38, -0.02)	-0.26 (-0.50, -0.02)	-0.12 (-0.37, 0.13)	0.43

Data are presented as the change (95% CI) in GWG z-scores for every IQR increase in phthalate/replacement biomarker concentration. Models accounted for race/ethnicity, educational attainment, annual household income, smoking in the 1st trimester, pre-pregnancy BMI, and maternal diet quality. To obtain fetal sex-specific estimates and the interaction *P*-value ($P_{biomarker*sex}$), we additionally included a multiplicative interaction between phthalate/replacement biomarker and fetal sex. CI, confidence interval; GWG, gestational weight gain; MCNP, monocarboxynonyl phthalate; ΣDiNP, sum of di(isononyl) phthalate metabolites; ΣDEHP, sum of di(2-ethylhexyl) phthalate metabolites; MCPP, mono-(3-carboxypropyl) phthalate; MBzP, monobenzyl phthalate; ΣDBP, sum of di-n-butyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; MEP, monoethyl phthalate; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; ΣDEHTP, sum of di(2-ethylhexyl) terephthalate metabolites.

Table 12. Sensitivity analyses for single-pollutant associations of phthalate/replacement biomarkers with GWG z-scores.

	Unadjusted associations in all women (n = 301)	Excluding women with GWG < 0 (n = 295)[1]
Biomarker	**β (95% CI)**	**β (95% CI)**
MCNP	0.00 (-0.12, 0.13)	0.03 (-0.09, 0.15)
ΣDiNP	0.01 (-0.12, 0.15)	0.05 (-0.09, 0.19)
ΣDEHP	-0.20 (-0.32, -0.07)	-0.14 (-0.27, -0.02)
MCPP	-0.08 (-0.20, 0.05)	-0.07 (-0.19, 0.05)
MBzP	0.04 (-0.11, 0.20)	0.08 (-0.07, 0.23)
ΣDBP	-0.11 (-0.24, 0.01)	-0.10 (-0.22, 0.03)
ΣDiBP	-0.05 (-0.18, 0.08)	-0.04 (-0.16, 0.09)
MEP	-0.14 (-0.29, 0.01)	-0.03 (-0.18, 0.12)
ΣDiNCH	-0.11 (-0.22, 0.01)	-0.09 (-0.20, 0.02)
ΣDEHTP	-0.22 (-0.39, -0.05)	-0.17 (-0.34, 0.00)

Data are presented as the change (95% CI) in GWG z-scores for every IQR increase in phthalate/replacement biomarker concentration. [1]Accounted for race/ethnicity, educational attainment, annual household income, smoking in the 1st trimester, pre-pregnancy BMI, and maternal diet quality. BMI, body mass index; CI, confidence interval; GWG, gestational weight gain; MCNP, monocarboxynonyl phthalate; ΣDiNP, sum of di(isononyl) phthalate metabolites; ΣDEHP, sum of di(2-ethylhexyl) phthalate metabolites; MCPP, mono-(3-carboxypropyl) phthalate; MBzP, monobenzyl phthalate; ΣDBP, sum of di-n-butyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; MEP, monoethyl phthalate; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; ΣDEHTP, sum of di(2-ethylhexyl) terephthalate metabolites.

Table 13. Pre-pregnancy BMI-specific associations of individual phthalate/replacement biomarkers with GWG z-scores.

	All women (n = 299)	Obese (n = 67)	Overweight (n = 77)	Under-/normal weight (n = 155)	
Biomarker	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	P_{int}
MCNP	0.00 (-0.12, 0.13)	-0.20 (-0.50, 0.10)	0.17 (-0.10, 0.44)	0.00 (-0.16, 0.16)	0.20
ΣDiNP	0.01 (-0.13, 0.15)	-0.10 (-0.36, 0.16)	0.13 (-0.17, 0.43)	0.02 (-0.18, 0.22)	0.50
ΣDEHP	-0.16 (-0.29, -0.03)	-0.30 (-0.55, -0.05)	-0.03 (-0.28, 0.22)	-0.16 (-0.35, 0.03)	0.31
MCPP	-0.09 (-0.21, 0.03)	-0.14 (-0.41, 0.12)	-0.04 (-0.31, 0.23)	-0.09 (-0.26, 0.07)	0.86
MBzP	0.09 (-0.06, 0.25)	0.03 (-0.31, 0.37)	0.28 (-0.01, 0.57)	0.02 (-0.20, 0.24)	0.33
ΣDBP	-0.09 (-0.22, 0.05)	0.11 (-0.19, 0.41)	-0.15 (-0.40, 0.10)	-0.12 (-0.29, 0.06)	0.37
ΣDiBP	-0.03 (-0.16, 0.10)	0.06 (-0.15, 0.27)	-0.04 (-0.32, 0.24)	-0.13 (-0.34, 0.08)	0.45
MEP	-0.08 (-0.24, 0.07)	-0.13 (-0.44, 0.17)	-0.22 (-0.54, 0.11)	0.00 (-0.22, 0.22)	0.52
ΣDiNCH	-0.11 (-0.22, 0.01)	-0.09 (-0.29, 0.10)	-0.24 (-0.47, 0.00)	-0.04 (-0.22, 0.14)	0.42
ΣDEHTP	-0.20 (-0.38, -0.02)	-0.29 (-0.66, 0.08)	-0.36 (-0.70, -0.03)	-0.08 (-0.33, 0.17)	0.36

Data are presented as the change (95% CI) in GWG z-scores for every IQR increase in phthalate/replacement biomarker concentration in all women and by the following pre-pregnancy BMI categories: BMI ≥ 30 kg/m^2 (obese), BMI 25-29.9 kg/m^2 (overweight), BMI < 25 kg/m^2 (under-/normal weight). All models accounted for race/ethnicity, educational attainment, annual household income, smoking in the 1st trimester, pre-pregnancy BMI, and maternal diet quality. Pre-pregnancy BMI-specific findings, including the interaction *P*-value (P_{int}), were obtained from models that also included a multiplicative interaction between biomarker and pre-pregnancy BMI. BMI, body mass index; CI, confidence interval; GWG, gestational weight gain; MCNP, monocarboxynonyl phthalate; ΣDiNP, sum of di(isononyl) phthalate metabolites; ΣDEHP, sum of di(2-ethylhexyl) phthalate metabolites; MCPP, mono-(3-carboxypropyl) phthalate; MBzP, monobenzyl phthalate; ΣDBP, sum of di-n-butyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; MEP, monoethyl phthalate; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; ΣDEHTP, sum of di(2-ethylhexyl) terephthalate metabolites.

Table 14. Pre-pregnancy BMI- and fetal sex-specific associations of individual phthalate/replacement biomarkers with GWG z-scores.

	Women carrying males			
	All (n = 148)	**Obese (n = 38)**	**Overweight (n = 39)**	**Under-/normal weight (n = 71)**
Biomarker	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
MCNP	0.03 (-0.16, 0.23)	-0.28 (-0.79, 0.23)	0.10 (-0.32, 0.53)	0.09 (-0.16, 0.34)
ΣDiNP	0.25 (0.02, 0.49)	0.07 (-0.32, 0.46)	0.28 (-0.16, 0.71)	0.42 (0.00, 0.84)
ΣDEHP	0.04 (-0.14, 0.22)	-0.14 (-0.43, 0.15)	0.08 (-0.24, 0.39)	0.22 (-0.11, 0.55)
MCPP	-0.03 (-0.22, 0.17)	0.11 (-0.28, 0.49)	-0.09 (-0.42, 0.25)	-0.07 (-0.38, 0.25)
MBzP	0.15 (-0.08, 0.37)	-0.01 (-0.46, 0.45)	0.22 (-0.17, 0.61)	0.20 (-0.13, 0.52)
ΣDBP	-0.08 (-0.28, 0.11)	0.31 (-0.09, 0.71)	-0.36 (-0.68, -0.04)	-0.06 (-0.36, 0.25)
ΣDiBP	0.01 (-0.20, 0.23)	0.08 (-0.32, 0.47)	0.09 (-0.50, 0.67)	-0.03 (-0.31, 0.26)
MEP	0.00 (-0.22, 0.22)	0.11 (-0.31, 0.52)	-0.13 (-0.62, 0.36)	0.00 (-0.31, 0.32)
ΣDiNCH	-0.17 (-0.35, 0.01)	-0.10 (-0.44, 0.25)	-0.23 (-0.55, 0.10)	-0.19 (-0.46, 0.09)
ΣDEHTP	-0.26 (-0.50, -0.02)	-0.10 (-0.58, 0.38)	-0.29 (-0.75, 0.18)	-0.36 (-0.71, -0.02)
	Women carrying females			
	All (n = 151)	**Obese (n = 29)**	**Overweight (n = 38)**	**Under-/normal weight (n = 84)**
Biomarker	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
MCNP	0.00 (-0.16, 0.17)	-0.12 (-0.50, 0.26)	0.27 (-0.09, 0.63)	-0.06 (-0.27, 0.16)
ΣDiNP	-0.08 (-0.25, 0.09)	-0.19 (-0.54, 0.15)	0.05 (-0.35, 0.46)	-0.08 (-0.31, 0.15)
ΣDEHP	-0.35 (-0.53, -0.18)	-0.79 (-1.25, -0.33)	-0.16 (-0.54, 0.22)	-0.31 (-0.53, -0.10)
MCPP	-0.11 (-0.27, 0.05)	-0.33 (-0.70, 0.05)	0.09 (-0.37, 0.55)	-0.10 (-0.29, 0.10)
MBzP	0.03 (-0.18, 0.25)	0.09 (-0.42, 0.61)	0.44 (0.01, 0.88)	-0.15 (-0.45, 0.14)
ΣDBP	-0.10 (-0.27, 0.07)	-0.21 (-0.68, 0.25)	0.19 (-0.21, 0.59)	-0.15 (-0.35, 0.06)
ΣDiBP	-0.05 (-0.22, 0.12)	0.10 (-0.16, 0.36)	0.00 (-0.34, 0.34)	-0.29 (-0.60, 0.02)
MEP	-0.13 (-0.35, 0.09)	-0.38 (-0.81, 0.05)	-0.18 (-0.64, 0.28)	0.02 (-0.29, 0.32)
ΣDiNCH	-0.04 (-0.19, 0.11)	-0.06 (-0.31, 0.19)	-0.22 (-0.55, 0.12)	0.07 (-0.16, 0.30)
ΣDEHTP	-0.12 (-0.37, 0.13)	-0.53 (-1.10, 0.04)	-0.45 (-0.92, 0.03)	0.22 (-0.12, 0.57)

Data are presented as the change (95% CI) in GWG z-scores for every IQR increase in phthalate/replacement biomarker concentration in all women and by pre-pregnancy BMI [BMI ≥ 30 kg/m^2 (obese), BMI 25-29.9 kg/m^2 (overweight), BMI < 25 kg/m^2 (under-/normal weight)] and fetal sex [males, females]. All models accounted for race/ethnicity, educational attainment, annual household income, smoking in the 1st trimester, pre-pregnancy BMI, and maternal diet quality. Pre-pregnancy BMI-specific findings, including the interaction *P*-value (P_{int}), were obtained from models that also included a multiplicative three-way interaction (and all relevant two-way interactions) between biomarker, pre-pregnancy BMI, and fetal sex. BMI, body mass index; CI, confidence interval; GWG, gestational weight gain; MCNP, monocarboxynonyl phthalate; ΣDiNP, sum of di(isononyl) phthalate metabolites; ΣDEHP, sum of di(2-ethylhexyl) phthalate metabolites; MCPP, mono-(3-carboxypropyl) phthalate; MBzP, monobenzyl phthalate; ΣDBP, sum of di-n-butyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; MEP, monoethyl phthalate; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; ΣDEHTP, sum of di(2-ethylhexyl) terephthalate metabolites.

4.5.4. Associations of phthalate/replacement biomarker mixtures with GWG z-scores

When using QGComp to evaluate the joint association of the phthalate/replacement biomarker mixture with GWG z-scores, we observed that simultaneous IQR increases in all biomarker concentrations in the mixture were marginally associated with lower GWG z-scores (β: -0.29; 95% CI: -0.70, 0.12) (**Figure 13** and **Table 15**), as the partial negative (β: -1.02) and positive (β: 0.73) associations were similar in strength. The largest phthalate/replacement biomarker contributors to partial negative associations were ΣDEHP (28%), ΣDEHTP (23%), and MCPP (18%), whereas the largest biomarker contributors to partial positive associations were ΣDiNP (64%) and MBzP (36%) (**Table 16**).

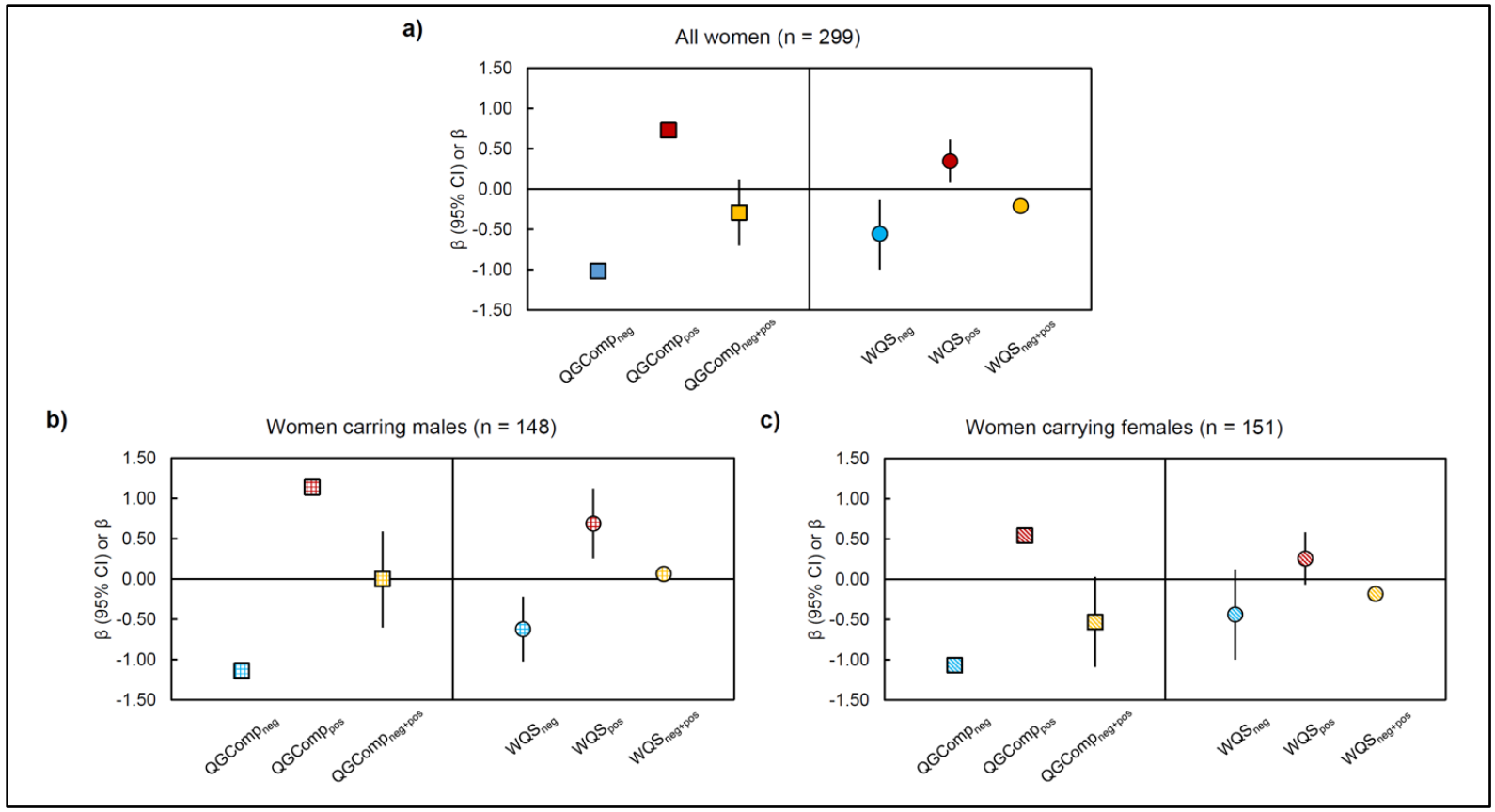

Figure 13. Joint associations of phthalate/replacement biomarkers with GWG z-scores in a) all women, b) women carrying males, and c) women carrying females. QGComp models and WQSR models using partial reverse scoring approach accounted for race/ethnicity, educational attainment, annual household income, smoking in the 1st trimester, pre-pregnancy BMI, and maternal diet quality. Data are presented as the change (square for QGComp, circle for WQSR models) and 95% CI (vertical black lines) in GWG z-scores for an IQR change in the mixture. Separate models were specified for all women (n = 299), women carrying males (n = 148), and women carrying females (n = 151). QGCompneg, scaled effect size in the negative direction; QGComppos, scaled effect size in the positive direction; QGCompneg+pos, joint association as the sum of QGCompneg and QGComppos. WQSneg and WQSpos, sum of negative and positive weights, respectively, calculated after renormalizing positive and negative weights to 1.0; WQSneg+pos, joint association as the sum of WQSneg and WQSpos. GWG, gestational weight gain; QGComp, quantile g-computation; WQSR, weighted quantile sum regression.

Table 15. Cumulative associations of phthalate/replacement mixture with GWG z-scores from QGComp and WQSR models.

	All women (n = 299)	Women carrying males (n = 148)	Women carrying females (n = 151)
QGComp[1]	**β (95% CI) or β**	**β (95% CI) or β**	**β (95% CI) or β**
$QGComp_{neg}$	-1.02	-1.14	-1.07
$QGComp_{pos}$	0.73	1.14	0.54
$QGComp_{neg+pos}$	-0.29 (-0.70, 0.12)	0.00 (-0.60, 0.59)	-0.54 (-1.09, 0.03)
WQSR Reverse Scoring Method[2]	**β (95% CI) or β**	**β (95% CI) or β**	**β (95% CI) or β**
WQS_{neg}	-0.56 (-1.00, -0.14)	-0.63 (-1.02, -0.23)	-0.44 (-1.00, 0.12)
WQS_{pos}	0.35 (0.08, 0.62)	0.69 (0.25, 1.12)	0.26 (-0.07, 0.58)
$WQS_{neg+pos}$	-0.21	0.06	-0.19
WQSR Standard Method[3]	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
WQS_{neg}	-0.30 (-0.65, 0.05)	-0.20 (-0.75, 0.30)	-0.50 (-0.90, -0.05)
WQS_{pos}	-0.02 (-0.35, 0.35)	0.35 (-0.05, 0.80)	-0.30 (-0.75, 0.15)

Data are presented as the change (95% CI) in GWG z-scores for an IQR change in the mixture. Separate models were specified for all women (n = 299), women carrying males (n=148), and women carrying females (n=151). [1]QGComp models. $QGComp_{neg}$, scaled effect size in the negative direction; $QGComp_{pos}$, scaled effect size in the positive direction; $QGComp_{neg+pos}$, cumulative associations as the sum of $QGComp_{neg}$ and $QGComp_{pos}$. [2]Negatively constrained WQSR models using the reverse scoring method where chemicals that were positively associated with GWG z-scores were reverse coded. WQS_{neg} and WQS_{pos}, sum of negative and positive weights, respectively, calculated after renormalizing positive and negative weights to 1.0; $WQS_{neg+pos}$, sum of WQS_{neg} and WQS_{pos}. [3]Standard WQSR models where separate negatively (WQS_{neg}) and positively (WQS_{pos}) constrained models were evaluated. GWG, gestational weight gain; QGComp, quantile g-computation; WQSR, weighted quantile sum regression.

Table 16. Relative weights from reverse scoring WQSR and QGComp models evaluating associations of the phthalate/replacement mixture with GWG z-scores.

	All women (n = 299)		Women carrying males (n = 148)		Women carrying females (n = 151)	
	Negative	**Positive**	**Negative**	**Positive**	**Negative**	**Positive**
Method	**Chemical (wt)**	**Chemical (wt)**	**Chemical (wt)**	**Chemical (wt)**	**Chemical (wt)**	**Chemical (wt)**
QGComp[1]	**ΣDEHP (0.284)** **ΣDEHTP (0.233)** **MCPP (0.175)** MCNP (0.117) MEP (0.094) ΣDiNCH (0.046) ΣDiBP (0.032) ΣDBP (0.020)	**ΣDiNP (0.639)** **MBzP (0.361)**	**ΣDBP (0.304)** **ΣDEHTP (0.292)** MCPP (0.151) MCNP (0.097) ΣDiNCH (0.087) MEP (0.036) ΣDEHP (0.034)	**ΣDiNP (0.518)** **MBzP (0.386)** **Σ**DiBP (0.096)	**ΣDEHP (0.553)** ΣDEHTP (0.122) MEP (0.118) MCNP (0.076) ΣDiBP (0.072) ΣDiNCH (0.038) MCPP (0.022)	ΣDiNP (0.372) MBzP (0.355) ΣDBP (0.273)
WQSR Reverse Scoring Method[2]	**ΣDEHP (0.250)** **ΣDEHTP (0.190)** **MCPP (0.175)** MEP (0.128) ΣDiNCH (0.113) ΣDiBP (0.075) ΣDBP (0.070)	**ΣDiNP (0.500)** **MBzP (0.427)** MCNP (0.073)	**ΣDBP (0.383)** **ΣDEHTP (0.255)** MCPP (0.203) ΣDiNCH (0.159)	**ΣDiNP (0.358)** **MBzP (0.249)** ΣDiBP (0.136) MCNP (0.094) MEP (0.092) ΣDEHP (0.073)	**ΣDEHP (0.372)** **MEP (0.177)** ΣDiBP (0.153) ΣDiNCH (0.139) MCPP (0.084) ΣDiNP (0.074)	**ΣDBP (0.342)** MBzP (0.265) ΣDEHTP (0.199) MCNP (0.194)

Separate models were specified for all women (n = 299), women carrying males (n=148), and women carrying females (n=151). Bolded chemicals are those that crossed the threshold (1/10 chemicals in the mixture). [1]Data are presented as the chemical (relative weight) from QGComp models. [2]Data are presented as the chemical (relative weight) from negatively constrained WQSR models using the reverse scoring method where chemicals that were positively associated with GWG z-scores (see positive column) were reverse coded; weights were renormalized to 1.0 within positive and negative columns. GWG, gestational weight gain; MCNP, monocarboxynonyl phthalate; ΣDiNP, sum of di(isononyl) phthalate metabolites; ΣDEHP, sum of di(2-ethylhexyl) phthalate metabolites; MCPP, mono-(3-carboxypropyl) phthalate; MBzP, monobenzyl phthalate; ΣDBP, sum of di-n-butyl phthalate metabolites; ΣDiBP, sum of di-iso-butyl phthalate metabolites; MEP, monoethyl phthalate; ΣDiNCH, sum of di(isononyl) cyclohexane-1,2-dicarboxylate metabolites; ΣDEHTP, sum of di(2-ethylhexyl) terephthalate metabolites; QGComp, quantile g-computation; WQSR, weighted quantile sum regression; Wt, weight.

Results from associations of all 10 phthalate/replacements as a cumulative mixture with GWG z-scores in all women using standard WQSR models are presented in **Supplemental Table 15**. When evaluating mixture effects via WQSR using the (partial) reverse scoring approach, so that all biomarkers are associated with the outcome in the same direction, we observed that the full mixture was associated with GWG z-scores (β: -0.90; 95% CI: -1.60, -0.20). Decomposing this overall association into contributions from originally negative and positive mixture components, we observed that IQR increases in the negative phthalate/replacement biomarkers were associated with 0.56 (95% CI: 0.14, 1.00) reductions in GWG z-scores (**Figure 13a**), with the largest contributors being ΣDEHP (25%), ΣDEHTP (19%), and MCPP (18%) (**Table 16**). Correspondingly, IQR increases in positive biomarkers were associated with 0.35 (95% CI: 0.08, 0.62) increases in GWG z-scores (**Figure 13a**), with the largest contributors being ΣDiNP (50%) and MBzP (43%) (**Table 16**).

4.5.5. Fetal sex-specific associations of phthalate/replacement biomarkers with GWG z-scores

When evaluating associations using individual phthalate/replacement biomarkers, the inverse associations of ΣDiNCH and ΣDEHTP with GWG z-scores observed in all women appeared to be more robust in women carrying males, whereas inverse associations in women carrying females were modest (**Table 11**). However, inverse associations of ΣDEHP with GWG z-scores in all women were driven by women carrying females (β: -0.35, 95% CI: -0.53, -0.18), with no association observed in those carrying males (β: 0.04, 95% CI: -0.14, 0.22). Additionally, we identified a positive association between ΣDiNP

and GWG z-scores in women carrying males (β: 0.25, 95% CI: 0.02, 0.49), with no association observed in those carrying females (β: -0.08, 95% CI: -0.25, 0.09).

Using QGComp, we observed that the phthalate/replacement biomarker mixture was marginally associated with lower GWG z-scores in women carrying females (β: -0.54; 95% CI; -1.09, 0.03) (**Figure 13c**), but not associated with GWG z-scores in women carrying males (β: 0.00; 95% CI: -0.60, 0.59) (**Figure 13b**). In women carrying females, ΣDEHP (55%) was identified as the largest biomarker contributor to the partial negative association (**Table 16**). However, in women carrying males, the magnitude of the partial negative (β: -1.14) and partial positive (β: 1.14) associations were equal in strength, resulting in no overall joint association of the mixture with GWG z-scores in this sub-sample (**Figure 13b** and **Table 15**). In women carrying males, ΣDBP (30%) and ΣDEHTP (29%) were the largest contributors to partial negative associations, while ΣDiNP (52%) and MBzP (39%) were the largest contributors to partial positive associations (**Table 16**).

Results evaluating associations of all 10 phthalate/replacements as a cumulative mixture with GWG z-scores separately in women carrying males and females using standard WQSR models are presented in **Table 15**. When evaluating associations of the phthalate/replacement biomarker mixture with GWG z-scores using WQSR with the reverse scoring method, we observed that the biomarker mixture was associated with GWG z-scores both in women carrying males and females, but the association was much more robust in women carrying males (β: -1.30; 95% CI: -2.15, -0.50) than in those carrying females (β: -0.70; 95% CI: -1.55, 0.20) (**Figure 13b,c** and **Table 15**). With

regards to the partial negative associations stratified by fetal sex, we observed that IQR increases in the negative biomarkers were associated with GWG z-scores decreases of 0.63 (95% CI: 0.23, 1.02) and 0.44 (95% CI: -0.12, 1.00) in women carrying males and females, respectively. In women carrying males, the strongest phthalate/replacement biomarker contributors to associations in the negative direction were ΣDBP (39%) and ΣDEHTP (26%), whereas in women carrying females, the strongest contributors were ΣDEHP (37%) and MEP (18%) (**Table 16**). When evaluating partial positive associations by fetal sex, we observed that IQR increases in positive biomarkers were associated with 0.69 (95% CI: 0.25, 1.12) higher GWG z-scores in women carrying males (**Figure 13b**), with ΣDiNP (36%) and MBzP (25%) identified as the strongest contributors to this association (**Table 16**). In women carrying females, IQR increases in positive biomarkers were weakly associated with 0.26 (95% CI: -0.07, 0.58) higher GWG z-scores (**Figure 13c**), with ΣDBP (34%) was identified as the strongest contributor to this association (**Table 16**).

4.6. DISCUSSION

We observed that urinary biomarker concentrations of phthalate plasticizers and their replacements were associated with GWG z-scores, which was generally consistent between single-pollutant and mixture modeling approaches, as well as between QGComp and WQSR. In secondary single- and multi-pollutant analyses, we observed that some associations of phthalates/replacement biomarkers with GWG z-scores were fetal-sex-specific, a finding which has not been reported previously. In mixtures models, while consistent marginal inverse associations were observed in women carrying females, in

those carrying males, we observed equal associations in both the negative and positive directions. These results contribute to the growing evidence that exposure to phthalates may alter GWG in pregnant women and provide novel information about the potential for widely used plasticizer replacements, DiNCH and DEHTP, to impact GWG.

4.6.1. Individual phthalate/replacement biomarkers are associated with GWG z-scores

Contrary to our hypothesis, in our study, select phthalate biomarkers (ΣDEHP and MCPP) and biomarkers of replacements (ΣDiNCH and ΣDEHTP) were generally inversely associated with GWG z-scores, and for some of these biomarkers, associations were either more prominent in women carrying females (for ΣDEHP) or males (for ΣDiNCH and ΣDEHTP). Interestingly, a positive association emerged for ΣDiNP with GWG z-scores in women carrying males. It is important to view GWG as a complex phenotype, with contributions from the fetus, placenta, amniotic fluid, maternal fluid (i.e., blood, extracellular fluid), protein and fat storage, as well as uterine and breast tissues (198). Our findings in women carrying females support the idea that some phthalates may target biological pathways that prevent weight gain in one or more of these storage depots (3). In pregnancy, exposures to certain phthalates are associated with altered metabolic processes required for appropriate GWG, such as glucose and lipid homeostasis (212, 213). Specifically, in experimental models, phthalates have been shown to interact with peroxisome proliferator-activated receptor gamma, liver X receptors, and retinoid X receptors, which are important regulators of glucose and lipid homeostasis (23). These metabolic processes are regulated by sex-steroid hormones (such as estrogen and

progesterone) and cytokines/adipokines produced by the maternal-fetal-placental unit (3), which are also potential biological targets of phthalates/replacements (86, 173, 174, 214-217). For example, phthalates have been shown to interfere with the regulation of the hypothalamic-pituitary-gonadal (HPG) axis by interacting with steroidogenic enzymes and by modulating the activity of hormone receptors (e.g. estrogen receptor, androgen receptor) (218). The hormone-mediated role of the placenta in GWG and the known sex differences in the placental response to phthalates may also explain our fetal sex-specific findings (173, 214), although further studies are needed to elucidate the biological basis for these observations.

Nevertheless, appropriate GWG is critical for maternal health and fetal development (3), and both insufficient and excessive GWG may have adverse implications for maternal and offspring health. For example, women with insufficient GWG may be more likely to experience postpartum depression (43), whereas those with excessive GWG are at higher risk of postpartum weight retention that could lead to cardiometabolic disease later in life (2). Babies of mothers with insufficient GWG have higher odds of being born pre-term and small-for-gestational age, whereas those of mothers with excessive total GWG have higher odds of macrosomia or being born large-for-gestational age (219). Importantly, these early birth phenotypes have been linked with the later development of respiratory problems, cognitive and/or behavioral deficits, and cardiometabolic disease in children (220-223). GWG modeled continuously has also been shown to be associated with these same adverse childhood outcomes. Specifically, one study showed that compared to a GWG z-score of 0.0, a GWG z-score of +0.5 was associated with 2.2 (95%

CI: 0.7, 3.7) excess cases of childhood overweight or obesity per 100 pregnancies (224). Another study found that compared to GWG z-scores between -1.0 and +1.0, children of women who had a GWG z-score over +1.0 spent 15.0 seconds (95% CI: 1.8, 28.0) longer completing a task measuring executive performance, suggesting that higher GWG is associated with poorer executive function performance in children (225). Phthalates are also sex-specifically associated with many of these same short- and long-term infant and child health outcomes (174), so it is possible that associations of certain phthalates with GWG could partially explain relationships of phthalates with child and maternal health, and this should be explored further.

The current literature evaluating associations of individual phthalates with GWG is mixed, but also limited with regards to phthalate replacements and evaluating differences by fetal sex. Consistent with our findings, one study from the Netherlands evaluated GWG through late pregnancy and reported that associations of mid-pregnancy MCPP with GWG trended in the negative direction (192). However, most other studies evaluated total GWG using the IOM categories or trimester-specific GWG and quantified phthalate metabolite biomarkers from individual spot urines collected in early, mid, and/or late pregnancy, which limits direct comparisons to our study. Of the studies evaluating total GWG, two (from Anhui Province in China and Salinas Valley, California in the U.S.) observed that higher urinary metabolite concentrations of DEHP, DBP, and DEP in early/mid pregnancy were associated with higher total GWG and higher odds of excessive GWG (31, 195). Additionally, in pregnant women from Boston, mean urinary MEP concentrations were associated with higher odds of excessive total GWG, although this

relation was non-monotonic (32). Conversely, in the previously discussed Netherlands cohort, higher early/mid gestation urinary low molecular weight phthalate biomarkers were associated with higher odds of insufficient total GWG (192), while in women from Hubei Province in China, MEP concentrations were lower among women with inadequate compared to adequate total GWG (33). Inconsistent findings among observational studies could also be explained by to covariates accounted for in statistical models and study population characteristics. For example, both our study and the Netherlands study adjusted for maternal diet, but this was not accounted for by other studies. Additionally, the majority of our women are non-Hispanic white, of higher socioeconomic status (SES), and almost half had overweight or obesity before pregnancy, which may also explain differences in findings from cohorts in China (where the majority of women were normal weight before pregnancy) and California (where most women were migrants from Mexico with lower SES). The experimental evidence related to maternal body weight gain is also mixed. For example, rodent studies evaluating the effects of prenatal DEP exposure observed decreasing, increasing, and no effects on maternal body weight in response to DEP (38). Another study observed that compared to controls, F0 generation dams exposed to DEHP (pre-conception to weaning), DiNP (mating to weaning, pre-conception to weaning), or DBP (pre-conception to weaning) gained more weight (39). Further research can assist in identifying what may be contributing to these conflicting findings within and between observational and experimental studies.

4.6.2. Phthalate/replacement biomarkers as a cumulative mixture are associated with GWG z-scores

Given that pregnant women are likely exposed to numerous phthalates/replacements, evaluating these chemicals as a cumulative mixture is important for understanding the aggregate association of many phthalates/replacement with pregnancy and fetal health (66). For the current study, we compared results from two widely used statistical mixtures methods, QGComp and WQSR (72, 210). To make our findings more comparable to those from QGComp, while simultaneously satisfying the WQSR assumption that all mixture components are associated with the outcome in the same direction, we utilized a (partial) reverse scoring approach for WQSR. Although QGComp and WQSR have distinct purposes and properties, we generally observed very consistent findings across the two methods – most notably, there was almost perfect agreement regarding the ordering and direction of the contribution of key phthalate/replacement biomarkers to joint mixture associations. Specifically, in all women, we observed that the partial negative and positive associations were similar in magnitude, with the overall biomarker mixture only marginally inversely associated with GWG z-scores through late pregnancy. ΣDEHP, ΣDEHTP, and MCPP were identified as the largest contributors to inverse associations and ΣDiNP and MBzP identified as the most prominent contributors to positive associations. When stratifying by fetal sex, we observed marginal inverse associations in women carrying females, a result that was largely driven by ΣDEHP. However, in women carrying males, we observed equal partial positive (driven by ΣDiNP) and negative (driven by ΣDEHTP) associations, which resulted in a negated joint association between the mixture and GWG z-scores in this sub-sample. Our results suggest that women carrying

males – more so than those carrying females – may be equally sensitive to chemicals that have opposing effects on GWG. Phthalates/replacements mainly exert their effects on the endocrine system by binding to hormone receptors, such as estrogen receptors alpha and beta that can be found and expressed in different quantities in multiple cell types, which could partially explain different and potentially opposing responses to endocrine disruption (226, 227). However, additional studies are needed to corroborate these fetal sex-specific mixture findings, as well as to understand what could explain these opposing chemical effects at the physiological level, especially in women carrying males.

Our mixture results in all women are somewhat consistent with those of the Netherlands cohort observing modest inverse associations of urinary phthalic acid (a proxy for total phthalate exposure) with GWG through late pregnancy (192). Conversely, using a different method to estimate exposure, the cohort from Anhui Province calculated a hazard index by summing estimated intakes of DBP, BBzP, and DEHP and observed the cumulative index was positively associated with total GWG and odds of excessive total GWG (31). Additionally, using BKMR, a study of pregnant women attending a fertility clinic in Boston identified that first trimester DEHP metabolites, MiBP, and propyl paraben contributed most to positive associations between a cumulative mixture of multiple non-persistent chemical classes and total GWG, with DEHP metabolites being the largest contributors (196). Lastly, the sum of phthalate metabolite biomarkers categorized as anti-androgenic was not associated with total GWG in the Boston women (32).

As discussed earlier, differences in study characteristics may explain discrepancies between our findings and those from other studies. However, each study, including ours, used different statistical approaches and different proxies of cumulative phthalate exposure or included additional classes of non-persistent chemicals in the mixture, which could also explain inconsistencies across studies. Each statistical mixtures method has its own unique limitations, but also strengths. While a limitation of WQSR is that it assumes that all chemicals in the mixture are associated with the outcome in the same direction, this method is quite appropriate for estimating the cumulative impact of chemicals from distinct exposure sources (i.e., phthalates/replacements) and identifying the most prominent chemical contributors to this association (210). In contrast, QGComp is best suited for chemicals from a common exposure source, as it assumes that all exposures are changing in the same direction, not independently; on the other hand, it is able to simultaneously consider chemicals that are associated with the outcome of interest in different directions (72). Overall, currently available statistical mixtures approaches have been developed to address a variety of questions that may arise in the field of environmental epidemiology. In the case of associations between phthalates/replacement and GWG, future studies can consider using the above-mentioned and other statistical mixtures methods, including BKMR, which is a robust approach when associations between the chemical mixture and outcome of interest are complex (i.e., interaction, non-linearity) (197).

4.6.3. Limitations and Strengths

This study has some limitations, but also several strengths. First, we were unable to

calculate total GWG, which limits our ability to compare our results to previous studies that considered total GWG or IOM clinical cut-offs. However, we used a previously validated method to calculate GWG z-scores that are standardized by pre-pregnancy BMI and gestational age at late pregnancy weight (203), which provides a valid assessment of gestational age- and BMI-specific GWG compared to raw measures alone when total GWG is not available. Second, our choice of using an international GWG reference chart to calculate GWG z-scores may have influenced our observed findings. However, we validated our findings by calculating GWG z-scores using a reference chart developed from a sample of Pittsburgh pregnant women (228, 229), and observed very consistent associations of phthalate/replacement biomarkers with GWG z-scores regardless of the reference chart (**data not shown**). Third, there are some limitations to using a pooled urine sample to quantify phthalates/replacement metabolite concentrations. We were unable to consider differences by the timing of exposure, which has been demonstrated in other studies (31, 192, 196). Also, we may have lost some temporality for evaluating associations of phthalates/replacements with GWG since our first urine sample was collected toward the end of the first trimester. However, given the non-persistent nature of phthalates/replacements in the body and relatively high within person variability in biomarker concentrations across pregnancy (162), pools of up to five first morning urine samples improved the stability of our exposure measure and better approximated total pregnancy exposure to phthalates/replacements compared to a single first morning sample. Fourth, given that I-KIDS is still ongoing, it will take some time to obtain information about the number of women who developed preeclampsia and gestational diabetes, which could influence observed associations. However, we expect very few

cases of pre-eclampsia and gestational diabetes since most women enrolled in I-KIDS did not have major preexisting conditions. Fifth, an important strength is that we evaluated phthalates/replacement biomarkers individually and in a cumulative mixture using both WQSR and QGComp – the latter two allowed us to estimate joint associations of multiple phthalates/replacement biomarkers with GWG z-scores and identify the relative biomarker contributors to these associations. Sixth, while we evaluated many individual-level maternal sociodemographic, lifestyle, and health factors, our results are subject to residual confounding. For example, we did not collect information pertaining to physical activity or sleep quantity/quality before or during pregnancy, which may be important determinants of GWG (230) and urinary phthalate biomarker concentrations (231, 232). There may also be concerns related to co-pollutant confounding. However, we conducted sensitivity analyses limiting our mixture to only include chemicals that crossed the threshold or only include chemicals with positive or negative β-estimates in single pollutant models and observed that all cumulative mixture associations with GWG z-scores remained consistent to what we reported (**data not shown**). Seventh, studies evaluating GWG typically consider pre-pregnancy BMI as an effect modifier (211), which was not a primary goal of our study because one of our objectives was to focus on fetal sex-specific associations, and we were therefore underpowered to examine associations stratified by fetal sex and pre-pregnancy BMI. However, this is the first study (to our knowledge) to propose and show fetal sex as an important moderator that should be considered in future studies. Lastly, most I-KIDS participants are non-Hispanic White women of relatively high SES, which limits the generalizability of our findings to other populations. However, urinary concentrations of most phthalate and replacement

metabolites were similar to those of same age women in the U.S. at similar time periods indicating that exposure in I-KIDS women is consistent with exposure in U.S. women.

4.7. CONCLUSION

In our relatively high-SES sample of U.S. pregnant women from the Midwest, a mixture of phthalates that included plasticizer replacements DiNCH and DEHTP was marginally associated with lower GWG through late pregnancy. However, our fetal sex-specific findings suggest that women carrying males may be more sensitive to phthalates that may be associated with GWG in opposing directions than those carrying females. Additionally, DEHTP was an important contributor to mixture associations with GWG, which, along with our studies showing that DEHTP is increasing in our sample (202) and is associated with maternal hormonal disruption (86), highlights a potential concern for regrettable chemical substitution. Therefore, further research related to this plasticizer replacement is needed in pregnant populations with particular consideration for fetal sex. Finally, experimental animal studies may help elucidate the biological mechanisms underlying the interaction of phthalates/replacement, fetal sex, and GWG.

CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

5.1. OVERALL CONCLUSIONS

In this dissertation, we evaluated maternal risk factors (**Chapter 2**), pregnancy hormonal targets (**Chapter 3**), and cardiometabolic consequences (**Chapter 4**) of exposure to phthalates and their replacements in pregnancy.

In **Chapter 2**, we characterized phthalate/replacement exposure and identified several sociodemographic, lifestyle, and reproductive characteristics, as well as seasonal and time trends predictive of a mixture of phthalate/replacement concentrations. Specifically, we identified two distinct clusters of women: those with low phthalate (including ∑DEHTP) and those with high phthalate (including ∑DEHTP) biomarker concentrations. We also identified four components representative of phthalate/replacement biomarker concentrations from common exposure sources or those that track with certain behaviors. We identified age, marital status, annual household income, parity, pre-pregnancy BMI, caffeine intake, conception season, and enrollment year as important predictors of *k*-means clusters and at least one PC. Additionally, race/ethnicity, education, employment, pregnancy intention, smoking and consuming alcohol in the first trimester, and first trimester diet quality were identified as important determinants of at least one PC. Our findings contribute additional information about predictors of phthalate/replacement mixtures that may be important confounding factors in studies evaluating associations of phthalates/replacements with pregnancy-related health outcomes, including pregnancy hormonal targets and cardiometabolic consequences. Furthermore, our results may inform future perinatal health recommendations by providing insights into both non-

modifiable and modifiable characteristics of pregnant women who are most likely to be exposed to these chemicals.

In **Chapter 3**, we observed that select phthalates and replacements individually were associated with altered maternal urinary estrogen concentrations, and these associations differed by fetal sex and by pregnancy timepoint. Specifically, we showed that select phthalate concentrations in pregnancy were associated with higher maternal urinary SumEstrogens and SumTestosterones, and a lower Estrogen/Androgen ratio. Additionally, two biomarkers of phthalate alternatives (SumDiNCH and SumDEHTP) were positively associated with SumEstrogens, but not with SumTestosterones or the Estrogen/Androgen ratio. Some associations of phthalate/alternative biomarkers with urinary hormones tended to be linear, with the strongest relationships observed at higher quartiles of phthalate or phthalate alternative biomarker concentrations. Importantly, many associations of phthalate and phthalate alternative biomarkers with SumEstrogens tended to be strongest in early and mid-to-late gestation and in women carrying females, while gestational age- and fetal sex-specific associations of phthalate/alternative biomarkers with SumTestosterones and Estrogen/Androgen ratio were less consistent. These findings further confirm that phthalates may have endocrine disrupting properties in pregnant women, which may have important public health implications for maternal and child life-long health. Our findings also support the need for additional studies evaluating the potential endocrine disrupting capacity of newer phthalate alternatives.

In **Chapter 4**, we reported that a mixture of phthalates that included plasticizer replacements DiNCH and DEHTP was marginally associated with lower GWG through late pregnancy, but these associations were also fetal sex-specific, a finding which has not been reported previously. Specifically, associations between urinary biomarker concentrations of phthalate plasticizers and their replacements and GWG z-scores were generally consistent between single-pollutant and mixture modeling approaches, as well as between QGComp and WQSR. In secondary single- and multi-pollutant analyses, we observed that some associations of phthalates/replacement biomarkers with GWG z-scores were fetal-sex-specific. In mixtures models, while consistent marginal inverse associations were observed in women carrying females, in those carrying males, we observed equal associations in both the negative and positive directions. Our fetal sex-specific findings suggest that women carrying males may be more sensitive to phthalates associated with GWG in opposing directions than those carrying females. These results contribute to the growing evidence that exposure to phthalates may alter GWG in pregnant women and provide novel information about the potential for widely used plasticizer replacements, DiNCH and DEHTP, to impact GWG.

Given the results presented in this dissertation, several notable findings warrant future research, which will be discussed in the next section.

5.2. FUTURE DIRECTIONS

5.2.1. Determine if associations between phthalates/replacements and GWG are mediated by urinary hormones

In **Chapters 2** and **3**, we demonstrated that maternal urinary phthalate/replacement biomarker concentrations were associated with urinary hormones and GWG z-scores. A natural next step for these studies would be to determine whether urinary hormones mediate associations of phthalates and GWG z-scores. Given that urinary hormone concentrations were assessed repeatedly across pregnancy, we would be able to utilize multiple mediator approaches under the causal framework to consider the joint mediation of urinary hormone concentrations at 8-15, 25-33, 32-40 weeks gestation (233). We could also simply evaluate mediation of association between phthalates/replacements and GWG z-scores by each hormone timepoint separately. However, we would first need to determine if urinary hormones are associated with GWG z-scores. Based on biological plausibility, we would hypothesize that hormones, particularly estrogens, would be directly associated with GWG, such that increases in estrogens would lead to higher GWG (3, 4). Unfortunately, we may not be able to justify conducting a formal mediation analysis given that we showed phthalates/replacements were associated with higher urinary estrogens, but lower GWG z-scores. We may not be able to directly interpret our findings assessing urinary hormones as we would if we measured hormones in blood (this is further discussed in the next section). This is supported by one study in pre-menopausal women finding breast cancer risk was not associated with luteal plasma estrogens, was positively associated with follicular plasma estrogens, but was negatively associated with urinary estrogens (161). Furthermore, a nested case-control study of pregnant women found that

women with pre-eclampsia had higher urinary estradiol concentrations than controls (160), which is inconsistent with studies evaluating estradiol in serum. Therefore, future studies in I-KIDS will need to determine not only how best to model the repeated urinary hormone measurements as mediators, but also carefully consider whether mediation is biologically plausible when assessing sex-steroid hormones in urine.

5.2.2. Corroborate associations of phthalates/replacements with urinary hormones using blood hormone levels

As discussed in **Chapter 3**, a major strength of quantifying hormones in urine was to provide a novel approach for assessing hormones repeatedly across pregnancy, which is often a major limitations of prior studies. Collecting repeated urine samples may also be easier and less invasive than collecting multiple blood samples, which would also ease participant burden. However, the literature assessing whether urinary hormones directly reflect circulating hormones suggests that urinary hormones may be markers of hormone metabolism rather than of circulating hormone concentrations (160). Urine and blood likely capture different hormone forms, where unconjugated hormones are measured in plasma or serum, whereas conjugated hormones are generally measured in urine (161). Unfortunately, in pregnancy, quantifying hormones in urine has not been extensively validated against the gold standard (blood), particularly in studies collecting both blood and urine consistently across gestation. In I-KIDS, blood was not collected at the three urine collection timepoints for hormone assessment (8-15, 25-33, 32-40 weeks gestation). Pregnant participants did provide a blood and urine sample at 13-22 weeks gestation. A future study could examine correlations between hormone concentrations quantified from

the 13-22 week urine and blood aliquots. While this would not directly help address the potential limitations of the study in **Chapter 3**, this would be one way to internally validate how well urine hormone levels reflect blood hormone levels. This is not to discount the utility of assessing urinary hormones in pregnancy since urine may allow researchers to measure different types of potentially biologically active hormone metabolites that cannot be measured in plasma or serum (164, 234). Additionally, compared to blood sampling, urine may also provide opportunities for more extensive cross-pregnancy assessment of hormonal disruption in response to environmental exposures. However, it will be important to further characterize urinary hormones, especially to determine what they reflect from a biological standpoint, which will aid in interpreting findings utilizing this approach. Future pregnancy cohort studies could also consider incorporating the collection of multiple blood and urine samples into their protocols to help address this.

5.2.3. Evaluate associations of a phthalate/replacement mixture with hormones

In **Chapter 3**, we only evaluated associations of individual phthalates/replacements with urinary hormones, but whether a mixture of these chemicals is also associated with hormones is also warranted. Assessing phthalates/replacements one at a time with health outcomes makes it challenging to know the true impact of real-life exposure to chemical mixtures (66). To better simulate real life exposures, evaluating cumulative or joint associations of phthalates/replacements with hormones will be necessary to further understand the endocrine disrupting potential of these chemicals. There are many statistical mixtures methods available to address this, but selecting the best mixtures method depends on the primary research question since each statistical mixtures method

has its own unique limitations, but also strengths. We used WQSR and QGComp to evaluate associations of a phthalate/replacement mixture with GWG z-scores in **Chapter 4**. While a limitation of WQSR is that it assumes that all chemicals in the mixture are associated with the outcome in the same direction, this method is quite appropriate for estimating the cumulative impact of chemicals from distinct exposure sources (i.e., phthalates/replacements) and identifying the most prominent chemical contributors to this association (Czarnota et al., 2015). In contrast, QGComp is best suited for chemicals from a common exposure source, as it assumes that all exposures are changing in the same direction, not independently; on the other hand, it is able to simultaneously consider chemicals that are associated with the outcome of interest in different directions (Keil et al., 2020). Another widely used approach that we have not discussed is BKMR, which is a robust approach when associations between the chemical mixture and outcome of interest are complex, including interactions between chemicals and non-linear associations (Bobb et al., 2015). In **Chapter 3**, we found that most phthalates/replacements were associated with urinary hormones in the same direction, and associations were generally linear. Therefore, a future I-KIDS study should first consider using WQSR to evaluate associations of a phthalate/replacement mixture with urinary hormones. However, it will also be important to verify results from WQSR models using other available mixtures approaches.

5.2.4. Examine associations of phthalates/replacements with total GWG and trimester-specific GWG

In **Chapter 4**, we evaluated GWG through late pregnancy as our outcome of interest, and we were able to take advantage of available reference charts to calculate GWG z-scores (203, 228, 229). We did not have access to medical records at the time of the study, so we relied on self-reported weights from the last obstetric visit before delivery or those from an earlier obstetric visit within the third trimester. Because of this, we were unable to assess GWG using the IOM categories due to potential for misclassification bias related to not having the final weight in pregnancy. The IOM categories are used by clinicians to provide women with weight gain recommendations based on their pre-pregnancy BMI to support maternal and child health and maximize favorable pregnancy and birth outcomes (3). Often, pregnancy cohort studies assess GWG as an outcome using IOM categories: insufficient, adequate, and excessive. The strength of assessing GWG using IOM categories is that studies evaluating risk factors of GWG can provide clinically-relevant interpretations of their findings. Therefore, evaluating associations between phthalates/replacements and total GWG (using IOM categories) would provide additional context for the results presented in **Chapter 4**. Future studies in I-KIDS will be able to utilize medical records to ascertain the final pregnancy weight and calculate total GWG.

Weight is not gained linearly across pregnancy – it is trimester-specific and follows a sigmoidal curve. Pregnancy is a time of positive energy balance, but energy needs are trimester-specific. First trimester energy needs are the same as before pregnancy leading to very little (if any) weight gained in this trimester (235). Around 95% of weight is gained

in the second and third trimesters of pregnancy, with a mean rate of 0.42 kg per week for women classified as normal weight before pregnancy (3, 235). Approaches that summarize GWG into a single measure reduce the ability to evaluate trimester-specific patterns of weight gain and the potential for identifying susceptible windows (211). In I-KIDS, women completed a questionnaire at baseline along with follow-up surveys five more times approximately monthly across gestation and right after delivery. In this questionnaire, women were asked to self-report their measured weight at their most recent obstetric visit. Future studies may consider using these serial weight gain data to calculate the rate of pregnancy weight gain within each trimester and consider associations of phthalates/replacements with GWG trajectories (211). Mixed modeling approaches can also be utilized to evaluate associations of phthalates/replacements with GWG across pregnancy, but also identify if associations are gestational timepoint-specific (211). Once these data are made available, there are many opportunities in I-KIDS to be able to further characterize GWG and understand how phthalates/replacements may impact GWG.

5.2.5. Identify potential metabolic targets of phthalates/replacements in pregnancy

As discussed throughout this dissertation, phthalates/replacements are also considered metabolism disrupting chemicals (23), which is important to note given that GWG is regulated by metabolic adaptations in glucose and lipid metabolism (3, 236). Therefore, the next step for understanding associations of phthalates/replacements with GWG may be to investigate whether phthalates/replacements are associated with altered maternal metabolic biomarker concentrations. We have already preliminarily evaluated these

associations in I-KIDS - published as an abstract for the International Society of Environmental Epidemiology 2022 Conference (237). Briefly, plasma aliquots of the 13-22 week fasting blood sample were analyzed for a large panel of maternal metabolic biomarkers, including glucose, insulin, connecting peptide, leptin, free fatty acids, total triglycerides, total cholesterol, and high-density lipoprotein (HDL) cholesterol. Low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels were calculated using published equations (238). To identify patterns among the metabolic biomarkers, we utilized PCA to reduce ten maternal metabolic factors into three uncorrelated PCs, which explained 73% of the variability in metabolic biomarker concentrations. PC 1 strongly loaded on total, HDL, and LDL cholesterols (cholesterol PC), PC 2 strongly loaded on glucose, insulin, C-peptide, and leptin (glucose homeostasis PC), and PC 3 strongly loaded on triglycerides, VLDL cholesterol, and free fatty acids (lipids PC). We defined strong loading as $r > 0.4$, and all correlations were positive. Using covariate-adjusted linear regression models, we evaluated associations of individual phthalates/replacements with each of the metabolic PCs. Overall, we observed that DiNP and DEHTP were positively associated with glucose homeostasis PC scores, while DEHP and MEP were positively and negatively associated with lipids PC scores, respectively (238). These findings suggest that concentrations of select phthalate biomarkers measured across pregnancy, including plasticizer replacement DEHTP, are associated with disrupted second-trimester maternal glucose and lipid homeostasis. These critical findings will need to be followed up in the full I-KIDS cohort and will incorporate several additional metabolic, but also inflammatory, biomarkers that are now available.

5.2.6. Assess dietary predictors of phthalates/replacements in pregnancy

Diet is one of the major exposure sources to phthalates/replacements, particularly plasticizers phthalates since they are used in food processing, and are also found in food packaging materials and the outer coating of medications and supplements (15). Therefore, characterizing dietary sources of phthalates/replacements is critical as recommendations are needed to minimize exposure while concurrently providing pregnant women with accessible and nutritious foods necessary to sustain a healthy pregnancy. In **Chapter 2**, we evaluated and identified AHEI-2010 – a diet quality index commonly used to characterize whole diets – as an important determinant of phthalate/replacement mixtures. Diet quality indices, such as the AHEI-2010, are excellent proxies for lifestyle behaviors that result in consuming foods with phthalates/replacements since generally unhealthy lifestyles are associated with higher exposure to these chemicals. However, understanding the components of diets that may lead to phthalate exposure, especially exposure to understudied phthalaste replacements DiNCH and DEHTP, is also necessary. This includes conducting additional food-monitoring studies to evaluate the safety of food and dietary patterns (239), but also using existing cohort studies to identify major dietary determinants of gestational urinary phthalate/replacement biomarker concentrations. We published a review of 10 pregnancy cohort studies evaluating dietary predictors of phthalate and bisphenol exposures in pregnancy (240), which is presented in **Appendix A**. In agreement with prior food-monitoring studies, the use of plastic containers was associated with higher urinary

phthalate metabolite concentrations, and increased consumption of canned foods was associated with higher urinary bisphenol A (BPA) concentrations – another prevalent endocrine disrupting chemical. Additionally, foods and dietary patterns associated with a healthier lifestyle, such as organic foods, grown/raised/caught foods, vegetarianism, and folic acid supplementation, as well as some other dietary patterns and foods, including soups and bouillon, spices, and grains, were generally associated with lower urinary phthalate metabolite and bisphenol concentrations in pregnant women. However, not all pregnancy cohort studies were able to reliably detect associations of specific foods/food groups with phthalates and BPA that have been identified as known sources of these chemicals in food-monitoring studies. Therefore, additional well-designed studies are warranted to address these limitations. In I-KIDS, women completed extensive semi-quantitative FFQs at 8-15 and 32-40 weeks gestation that collected information about dietary intakes throughout pregnancy. Future studies in this sample of women will need to verify already known dietary sources of phthalates, but also identify additional dietary determinants of these chemical along with their understudied replacements.

5.2.7. Examine phthalates/replacements as mediators for associations of diet with gestational weight gain and maternal gestational metabolic biomarkers

Maternal diet is an established modulator of pregnancy health, as well as fetal growth and development (241) by interacting with inflammatory and metabolic pathways (242-244). Diet is a known modulator of chronic inflammation and oxidative stress, and pregnant women with healthier dietary patterns consume more anti-inflammatory and antioxidant-rich foods that protect against adverse birth and child outcomes (244). Diet also has a

major influence on metabolic pathways that are closely linked with inflammation, especially glucose and lipid homeostasis. Pregnant women with healthier dietary patterns tend to have more favorable gestational weight gain, body fat distribution, and metabolic profiles (i.e., levels of insulin, total cholesterol), which are also important determinants of fetal growth and development. Unfortunately, while healthy diets support pregnancy and fetal health, diet is also an established major source of phthalates (discussed previously in **section 5.2.5**). As presented in **section 5.2.4**, our preliminary studies observed that select phthalates, particularly replacement DEHTP, were associated with worse mid-pregnancy glucose homeostasis (237). In a recent preliminary study, we also confirmed that higher AHEI-2010 scores, indicating better diet qualities, were associated with favorable mid-pregnancy glucose homeostasis and lower urinary DEHTP metabolite concentrations (**data not shown**). Therefore, we hypothesize that the favorable metabolic effects of better maternal diet quality may be explained by lower exposure to DEHTP. We aim to conduct a more formal study in the future to test this hypothesis, and also assess GWG as a critical metabolic endpoint in pregnancy. With this study, we hope to show that the maternal metabolic consequences of a poor diet is partly explained by the chemicals that travel with the food supply.

5.2.8. Assess the implications of phthalate/replacement exposure for birth outcomes

Our findings in **Chapter 4** that a mixture of phthalates/replacements was associated with lower GWG z-scores, which was driven by women carrying females, are interesting given that it is the opposite of what we hypothesized and what many other studies have

reported. This is because phthalates are generally considered to be obesogens (23). However, a few studies have corroborated our results, which warrants further investigation to explain these findings (54, 192). It is important to view GWG as a complex phenotype with contributions from the fetus, placenta, amniotic fluid, maternal fluid (i.e., blood, extracellular fluid), protein and fat storage, as well as uterine and breast tissues (198). Our finding that phthalates/replacements were associated with lower GWG, especially in women carrying females, supports the idea that some of these chemicals may target biological pathways that prevent weight gain in one or more of these storage depots (3). Prior experimental and human epidemiologic studies have shown that phthalates are associated with poor birth outcomes, particularly lower birth weight (26, 34-39, 245, 246). Therefore, we hypothesize that associations of phthalates/replacements with lower GWG z-scores in women carrying females may be explained by associations of these chemicals with birth weight. In I-KIDS, we published on associations of parabens, a different class of endocrine disrupting chemicals, with birth size measures (247), which is presented in **Appendix B**. We observed that maternal urinary methylparaben and propylparaben concentrations were negatively associated with birth weight, birth weight z-scores, body length, and weight/length ratio in female, but not male newborns; these results persisted even after additionally adjusting for gestational length. Given that parabens and phthalates have been shown to have similar biological targets, phthalates/replacements may also be associated with lower female birth size. Therefore, a future study is needed to investigate this to further explain our observed associations between phthalates/replacements and GWG z-scores, but also

begin to understand the impact of these chemicals on child health outcomes within the context of maternal health disruption.

5.2.9. Determine if associations of phthalates/replacement with pregnancy endocrine and metabolic-related endpoints persist beyond pregnancy

As discussed throughout this dissertation, prenatal phthalate/replacement exposure is associated with adverse pregnancy/birth outcomes, long-term child health outcomes, but also may have long-term repercussions on maternal health (2). Several recent reviews suggest that various pregnancy pathologies, as well as the act of being pregnant, may be a “stress test” – in that pregnancy may serve as a first glance into potential long-term health outcomes in women (2). Additionally, in one of our published studies using data from the Midlife Women’s Health Study (248), we showed that pregnancy history, including age at first birth and parity, were important determinants of maternal midlife metabolic health (presented in **Appendix C**), further supporting pregnancy as a critical window for women’s later life health. In the same midlife cohort, we evaluated cross-sectional associations of midlife phthalate exposure with endocrine-related endpoints (presented in **Appendixes D, E, F**). We observed that higher concentrations of select urinary phthalate metabolites were associated with higher estradiol, testosterone, progesterone, and anti-Mullerian hormone concentrations (249), with higher odds of experiencing hot flashes in the past 30 days and experiencing daily/weekly hot flashes (250), and with higher risk of having a prior fibroid diagnosis (251). However, the relevant etiologic window of exposure to endocrine disrupting chemicals that may cause these midlife endpoints likely happens well before midlife, and potentially during pregnancy.

One likely mechanism behind this hypothesis is that exposure to phthalates/replacements in pregnancy alters maternal hormones (as we showed in **Chapter 3**), which impacts women's health long after pregnancy. For example, a prospective case-control study found that higher gestational estrogen concentrations were associated with increased risk of breast cancer in mothers after 38 years of follow-up (168). Changes in estrogens, as well as androgens, may also be implicated in cardiovascular disease and osteoporosis, which are prevalent in post-menopausal women (252). Experimental studies and those in pregnancy cohorts have begun trying to establish the link between gestational chemical exposures and women's health after pregnancy and have shown that maternal phthalate exposure during pregnancy is associated with postpartum weight retention that may persist six years after delivery (53, 54, 253). This will also be evaluated using data from I-KIDS women in a new study called the Illinois Metabolic Outcomes in Moms (I-MOMS) where at least 350 I-KIDS women will be recruited four-to-seven years after their I-KIDS pregnancy. This future study will provide critical findings about the long-term implications of association between phthalates/replacement and maternal lifelong cardiometabolic and endocrine health. The findings presented in this dissertation provided important evidence about the potential maternal health consequences of phthalate/replacement exposure in pregnancy, which is an important foundation for addressing these long-term goals.

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APPENDIX A: DIETARY PREDICTORS OF PHTHALATE AND BISPHENOL EXPOSURES IN PREGNANT WOMEN

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A.1. ABSTRACT

Endocrine disrupting chemicals (EDCs) can disrupt fetal developmental processes during pregnancy, leading to long-term adverse outcomes in humans. A major source of exposure to EDCs, such as phthalates and bisphenols, is the food supply, primarily due to contamination from processing and packaging. Therefore, this review aimed to 1) review food-monitoring sources of phthalates and bisphenols, and 2) evaluate methodologies and provide future directions needed to establish EDC-limiting dietary recommendations in pregnancy. Using PubMed, 10 peer-reviewed studies were found on dietary predictors of EDC exposure in pregnancy, and all were selected for review. Use of plastic containers in pregnancy was associated with higher urinary phthalate metabolites, whereas canned food consumption was associated with higher urinary bisphenol A (BPA) concentrations. Foods and dietary patterns associated with healthier food choices (e.g., organic/grown/raised/caught foods, folic acid supplements, vegetarianism) were generally associated with lower urinary phthalate metabolite and BPA concentrations. Despite the many food-monitoring studies reporting high BPA and phthalate concentrations in various foods, the designs of most studies described here

were not sufficiently robust to consistently detect associations of specific foods/food groups with phthalates and BPA. Given the limitations of currently available research, future studies should incorporate more valid questionnaires to accurately assess dietary EDC exposure, strive for concurrent diet and exposure assessment, and assess whether geographical and cultural differences modify associations of diet with gestational EDC exposures. Such progress will be critical for developing dietary recommendations that ensure the safety and health of pregnant women.

A.2. KEYWORDS

Pregnancy; phthalates; bisphenols; toxicology; diet.

A.3. INTRODUCTION

Many consumer products, including food contact materials, contain phthalates and bisphenols, resulting in widespread human exposure to these chemicals. Phthalates are diesters of phthalic acid (254, 255) that are classified into two categories based on their molecular weight: high molecular weight phthalates (HighMWPs) and low molecular weight phthalates (LowMWPs) (**Table 17**). HighMWPs are used as plasticizers in polyvinyl chloride products to make plastics flexible for building materials, medical devices, and food processing or packaging (153, 239, 256, 257), whereas LowMWPs are primarily used as solvents, fixatives, and adhesives in personal care products and cosmetics (153, 239, 256). Bisphenol A (BPA), and its replacements, bisphenol S (BPS) and bisphenol F (BPF), are used to manufacture polycarbonate plastics and epoxy resins for consumer and food product packaging, including canned foods (256, 258-261).

Due to their short half-lives (< 24 hours), exposures to phthalates and bisphenols are best characterized in urine (compared with blood) (262). Upon exposure, phthalates, specifically, are metabolized and excreted in urine, allowing for approximation of exposure by measuring urinary phthalate parent-specific metabolites (summarized in **Table 17**). For example, di(2-ethylhexyl) phthalate (DEHP) exposure is approximated by assessing the sum of its urinary metabolites (mono(2-ethylhexyl) phthalate, mono(2-ethyl-5-hydroxyhexyl) phthalate, mono(2-ethyl-5-carboxypentyl) phthalate, and mono(2-ethyl-5-oxohexyl) phthalate), whereas diethyl phthalate (DEP) exposure is approximated by measuring its major urinary metabolite [monoethyl phthalate (mEP)]. According to the 2013-2014 U.S. NHANES, most women of reproductive age (15-44 years of age) (263) have urinary concentrations of these chemicals that are above the laboratory levels of detection (phthalates: 88-100%, BPA: 96%, BPS: 88%, BPF: 66%) (264). This is concerning because phthalates and bisphenols are known endocrine disrupting chemicals (EDCs), associated with adverse health outcomes, especially in pregnancy (79). EDCs can alter, mimic, or disrupt the function of gestational hormones, such as thyroid hormone, estrogens, and androgens (75, 265, 266), making pregnancy especially sensitive to the actions of EDCs. Human epidemiological studies have shown that prenatal exposure to EDCs, specifically phthalates and bisphenols, is associated with adverse pregnancy (24, 28) and birth outcomes (29, 267), as well as childhood behavioral problems (46, 268), respiratory problems (49, 269), and obesity (270, 271). Diet is a ubiquitous source of chronic EDC exposure (272-274), because these chemicals have been shown to migrate from food contact materials (plastics, paper, metal, glass, and printing inks) that protect food from physical damage and microbial spoilage, thereby

affecting human health (275). Human exposure to EDCs from food can be attributed to various factors, including animal feeding practices, food production, processing, and packaging practices, as well as food storage conditions (276).

Characterizing dietary sources of EDCs requires accurate assessment of both EDC concentrations and dietary intake history, and this aim is especially challenging during pregnancy. This is due to the many anatomical, physiological (e.g., increased renal function), and metabolic changes (277, 278) that occur in pregnancy, as well as the numerous pregnancy-related changes in dietary patterns, including diet quality and quantity (279, 280). Despite these challenges, characterizing dietary sources of these chemicals during pregnancy is important, as recommendations are needed to minimize exposure, while providing pregnant women with accessible and nutritious foods necessary to sustain a healthy pregnancy. To address these pregnancy-specific challenges, the aims of this review are to (1) briefly review food-monitoring sources of phthalates and bisphenols in the general population and (2) evaluate methodologies and provide future directions to help establish EDC-limiting dietary recommendations for pregnant women.

Table 17. Summary of reviewed phthalate parent compounds/metabolites, bisphenols, and their proposed sources

Categorization	Parent Compound (Name; Abbreviation)	Metabolite (Name; Abbreviation)	Exposure Sources
High Molecular Weight Phthalate	Di(2-ethylhexyl) phthalate; DEHP	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	• PVC plastics • Food packaging & processing • Medical devices • Pharmaceutical coatings • Building materials
	Di-isononyl phthalate; DiNP	Mono-isononyl phthalate; mNP/miNP Monooxononyl phthalate; mONP Monocarboxyoctyl phthalate; mCOP	• PVC plastics • Food packaging • Building materials • Car interiors • Drinking straws
	Di-isodecyl phthalate; DiDP	Monocarboxynonyl phthalate; mCNP	• PVC plastics • Food packaging • Building materials • Car interiors • Swimming pools
	Di-n-octyl phthalate; DOP/DnOP	Mono(3-carboxypropyl) phthalate; mCPP	• PVC plastics • Food packaging • Building materials • Adhesives
	Benzylbutyl phthalate; BBzP	Monobenzyl phthalate; mBzP	• PVC plastics • Food packaging • Car care products • Some PCPs
Low Molecular Weight Phthalate	Diethyl phthalate; DEP	Monoethyl phthalate; mEP	• Fragrant PCPs: perfumes/colognes, deodorants, soaps, shampoos, lotions
	Di-n-butyl phthalate; DBP/DnBP	Mono-n-butyl phthalate; mBP/mnBP Mono-hydroxybutyl phthalate; mHBP	• PCPs: nail polish, cosmetics • Printing inks • Pharmaceutical coatings • Insecticides
	Di-iso-butyl phthalate; DiBP	Mono-isobutyl phthalate; miBP Mono-hydroxyl-isobutyl phthalate; mHiBP	
Bisphenol	Bisphenol A; BPA		• Polycarbonate plastics and epoxy resins • Food packaging: lining food cans, beverage containers • Plastic dinnerware • Dental sealants • Thermal receipts
	Bisphenol S; BPS		
	Bisphenol F; BPF		

References for phthalates (153, 239, 256), bisphenols (256, 258). PCPs: personal care products; PVC: polyvinyl chloride.

A.4. METHODS

PubMed was searched using combinations of various keywords including: diet(ary) + pregnant or pregnancy + predictor(s) or variability or determinants or distribution, + endocrine disruptors or EDCs or phthalate(s) or bisphenol(s) or BPA. Studies were included if they assessed associations of consumption of foods or dietary patterns with EDC exposures in pregnancy. Based on our literature search, only 10 pregnancy cohort studies have evaluated dietary predictors of EDC exposures (summarized in **Table 18** (90, 92, 93, 119, 137, 281-285)). Briefly, the 10 studies recruited ≥ 26 participants from 2003 to 2014; six cohorts were from the U.S./Puerto Rico, two from Spain, one from the Netherlands, and one from Australia. The following chemicals were assessed in these studies: HighMWPs [DEHP, di-isononyl phthalate (DiNP), di-isodecyl phthalate (DiDP), di-n-octyl phthalate (DOP), and benzylbutyl phthalate (BBzP)], LowMWPs [DEP, di-n-butyl phthalate (DBP), and di-iso-butyl phthalate (DiBP)], BPA, BPS, and BPF. One study investigated associations of foods with urinary paraben, benzophenone-3, triclosan, 2,4-dichlorophenol, and 2,5-dichlorophenol concentrations (119), but this review focuses on phthalates and bisphenols because diet is not a major source of exposure to these other chemicals (256, 258, 286, 287). The food categories in **Tables 19 and 20** were selected after abstracting all foods or dietary patterns from the 10 studies, and collapsing them across categories that were common to several studies (when possible). Additional subcategories were created when packaging or processing information was available for the same food item. For example, several studies reported on fish intake, but these studies assessed either general seafood intake, canned fish intake, or fish intake (unspecified type).

Table 18. Summary of studies assessing dietary predictors of phthalate and bisphenol exposures in pregnant women

Study Name (Reference)	Recruitment, Location, N	Chemicals Assessed	Urine Samples	Chemical Analysis	Foods Assessed	Dietary Assessment	Urinary Chemical Adjustment & Covariates
New Jersey Cohort **(137)**	• 2003-2004 • New Jersey • N = 150	**Phthalates:** • mCPP, mBzP • mEP, mBP, miBP	1 urine • Before delivery	• HPLC-MS/MS (CDC)	• Microwaved foods (plastic containers) • Plastic tableware • Plastic container storage	• Questionnaire (at delivery, about pregnancy)	• None
Generation R Study **(93)**	• 2004-2005 • Netherlands • N = 642	**Phthalates:** • Sum-HighMWPs = mECPP+mEHHP+mEOHP+mCMHP+ mCPP+mBzP+mHxP+mHpP • Sum-DEHP = mECPP+mEHHP+mEOHP+mCMHP • DOP: mCPP • Sum-LowMWPs = mMP+mEP+mBP+miBP • Phthalic acid **Phenols:** • BPA, BPS, BPF • Total bisphenols: BPA+BPS+BPF	1 urine • 1st trimester	• HPLC-ESI-MS/MS (The New York State Department of Health)	• Folic acid supplement • Vegetables • Grains • Fish/shellfish • Soft drinks • Soups & bouillon • Daily dietary caloric intake	• 3-month semi-quantitative FFQ (1st trimester) • Lifestyle questionnaire: supplements in pregnancy (1st trimester)	• Urinary creatinine (as covariate) • Maternal age • Ethnicity • Pre-pregnancy BMI • Education • Parity • Smoking status • Folic acid supplementation • Daily dietary caloric intake
Infancia y Medio Ambiente (INMA) Project **(90)**	• 2004-2006 • Spain • N = 391	**Phthalates:** • Sum-DEHP= mEHP+mEHHP+mEOHP+mECPP+mCMHP • mBzP • mEP, miBP, mnBP	2 urines • 1st trimester • 3rd trimester	• UPLC-MS/MS (Bioanalysis Research Group at the Hospital del Mar Medical Research Institute)	• Bottled water • Microwaved foods (plastic containers) • Organic food • Milk, yogurt, cheese • Packed meat (sausages, pates) • Canned fish • Potato chips • Canned beverages (soda, beer) • Other canned food (soups, sauces)	• 3-month FFQ (1st & 3rd trimesters) • Whole pregnancy questionnaire: organic food, plastics (3rd trimester)	• Creatinine adjusted chemical • Maternal age • Country of origin • Pre-pregnancy BMI • Education • Urine collection time
Healthy Start Pre-Birth Cohort **(119)**	• 2009-2014 • Colorado • N = 446	**Phthalates:** • Sum-LowMWPs = mMP+mEP+miBP+mBP+mHiBP+mHBP • Sum-DBP = mBP+miBP+mHBP+mHiBP • Sum-HighMWPs = mBzP+mEHP+mNP+mEOHP+mEHHP+ mECPP+mCOP+mCNP • Sum-DEHP = mEHP+mEOHP+mEHHP+mECPP **Phenols:** • BPA, BPS • Sum-parabens = methyl+ethyl+propyl+butyl • 2,4-dichlorophenol, 2,5-dichlorophenol • Triclosan, benzophenone-3	1 urine • 24-32 wks	• HPLC-MS/MS (CDC)	• Milk • Cheese • Yogurt • Ice cream • Soft drinks • Processed meat • Red meat • Seafood • Tofu • Fish oil supplements	• 3-month food propensity questionnaire (24-32 wks) • Questionnaire: fish oil supplement (24-32 wks)	• Creatinine adjusted chemical • Maternal age • Race • Pre-pregnancy BMI • Income • Education • Marital status • Employment Status
Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) **(92)**	• 2010-2012 • Northern Puerto Rico • N = 139	**Phthalates:** • mEHP, mEHHP, mEOHP, mECPP, mCOP, mCNP, mCPP, mBzP • mEP, mnBP, miBP	3 urines • 18 wks • 22 wks • 26 wks	• HPLC-MS/MS (CDC)	• Milk, cheese, ice cream • Meat, chicken, fish • Cold cuts, hot dog, sausage • Microwaved foods/drinks (plastic container) • Bottled water	• 48 hour questionnaires (18, 22, & 26 wks)	• Specific gravity adjusted chemical • Covariates that were associated with each specific chemical metabolite
Australian Maternal Exposure to Toxic Substances Study (AMETS) **(285)**	• 2008-2011 • Western Australia • N = 26	**Phenol:** BPA	1 urine • 38 wks	• HPLC-MS/MS • (The National Centre for Environmental Toxicology)	• Canned foods • Microwaved foods/drinks (plastic container) • Plastic container storage • Refillable bottles	• Pregnancy questionnaire • (38 wks)	• None

Table 18 (cont'd).

Study Name (Reference)	Recruitment, Location, N	Chemicals Assessed	Urine Samples	Chemical Analysis	Foods Assessed	Dietary Assessment	Urinary Chemical Adjustment & Covariates
Infant Development and Environmental Study (TIDES) **(282)**	• 2010-2012 • Minnesota, New York, Washington, California • N = 656 •	**Phthalates:** • Sum-DEHP = mEHP+mEHHP+mEOHP+mECPP • mBzP • mEP • mBP miBP	1 urine 1st trimester	• HPLC-MS/MS (CDC) • HPLC-ESI-MS/MS • (Environmental Health Laboratory at the University of Washington)	• Peanut butter • Beef, poultry • Other meats (pork, lamb) • Oils and fats (butter, lard), spices • Soy, dairy, fast food • Bottled beverages • Organic/chemical free food • Grown/raised/caught food • Unprocessed food • Canned fruit or vegetables • Frozen fruit or vegetables	• "Typical week" FFQ (1st trimester) • General pregnancy Behavior/Lifestyle questionnaire (1st trimester)	• Specific gravity adjusted chemical • Maternal age • BMI • Race • Study center • Education
Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) **(283)**	• 1999-2000 • California • N = 491	**Phenol:** • BPA	2 urines • 5-28 wks • 18-39 wks	• HPLC-MS/MS (CDC)	• Soda • Alcohol • Canned fruit • Bottled water • Pizza • Fish • Hamburgers	• Alcohol and soda consumption throughout pregnancy (5-28 & 18-39 wks) • Modified 3-month Block FFQ (18-39 wks)	• Specific gravity adjusted chemical • Maternal age • Years in U.S. • Pre-pregnancy BMI • Income/poverty ratio • Education • Marital status • Parity • Urine collection time • Smoke exposure • Alcohol and soda intake
Health Outcomes and Measures of the Environment (HOME) Study **(284)**	• 2003-2006 • Ohio • N = 389	**Phenol:** BPA	3 urines • 16 wks • 26 wks • Within 24 hrs of delivery	• HPLC-MS/MS (CDC)	• Fresh or Frozen fish (store) • Fresh fruit or vegetables (store) • Canned fruit or vegetables • Organic foods • Vegetarianism	• Frequency of consumption questionnaire (conception to 20 wks & 20 wks to birth)	• Creatinine adjusted chemical • Maternal age • Race • Income • Education • Marital Status
Sabadell Birth Cohort (INMA Project) **(281)**	• 2004-2006 • Spain • N = 479	**Phenol:** BPA	2 urines • 12 wks • 32 wks	• LC-MS (The Department of Analytical Chemistry Laboratory)	• Milk, yogurt • Packaged meat (sausages, pates) • Non-packaged meat (pork, chicken) • Canned fish • Non-canned fish (white fish, seafood) • Potato chips • Canned beverages (soda, beer) • Other canned foods (soups, sauces) • Fruits, Vegetables (fresh) • Bottled water (consumption & cooking) • Organic food • Microwaved foods/drinks (plastic container)	• 3-month semi-quantitative FFQ (12 & 32 wks) • Pregnancy questionnaire: water, organic foods, microwaving foods/drinks (32 wks)	• Creatinine adjusted chemical • Maternal age • Pre-pregnancy BMI • Social class • Education • Urine collection time • Smoking status • 2nd hand smoke exposure

BPA, bisphenol A; BPF, bisphenol F; BPS, bisphenol S; DEHP, di(2-ethylhexyl) phthalate; DOP, di-n-octyl phthalate; HPLC-EIS-MS/MS, HPLC-electrospray ionization-tandem MS; HPLC-MS/MS, HPLC-tandem MS; mBP, mono-n-butyl phthalate; mBzP, monobenzyl phthalate; mCMHP, mono(2-carboxymethyl)hexyl phthalate; mCNP, monocarboxynonyl phthalate; mCOP, monocarboxyoctyl phthalate; mCPP, mono(3-carboxypropyl) phthalate; mECPP, mono(2-ethyl-5-carboxypentyl) phthalate; mEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; mEHP, mono(2-ethylhexyl) phthalate; mEOHP, mono(2-ethyl-5-oxohexyl) phthalate; mEP, monoethyl phthalate; mHBP, mono-hydroxybutyl phthalate; mHiBP, mono-hydroxyl-isobutyl phthalate; mHpP, mono-2-heptylphthalate; mHxP, mono-hexylphthalate; miBP, mono-isobutyl phthalate; mMP, mono-methylphthalate; mnBP, mono-n-butyl phthalate; mNP, mono-isononyl phthalate; sum-DBPs, sum of di-n-butyl phthalate metabolites; sum-DEHPs, sum of di(2-ethylhexyl) phthalate metabolites; sum-highMWPs, sum of high-molecular-weight phthalate metabolites; sum-lowMWPs, sum of low-molecular-weight phthalate metabolites; UPLC-MS/MS, ultra-performance LC-tandem MS.

Table 19. Dietary predictors of phthalate exposure in pregnant women

Foods		DEHP	DiNP	DiDP	DOP	BBzP	HighMWPs	Phthalic acid	DEP	DBP	DiBP	LowMWPs
Meat	Packaged Meat (sausages)	NONE (90) ↓ (92)	NONE (92)	NONE (92)	NONE (92)	NONE (90) NONE (92)			NONE (90) NONE (92)	NONE (90) NONE (92)	NONE (90) NONE (92)	
	Processed Meat	NONE (119)					NONE (119)			NONE (119)		NONE (119)
	Red meat (beef, pork, lamb)	NONE (119) NONE (282)				NONE (282)	NONE (119)		NONE (282)	NONE (119) NONE (282)	NONE (282)	NONE (119)
	Poultry/Chicken	NONE (92) NONE (282)	↑ (92)	NONE (92)	NONE (92)	NONE (92) NONE (282)			NONE (92) NONE (282)	NONE (92) NONE (282)	NONE (92) NONE (282)	
	Unspecified Meat	NONE (92)	NONE (92)	NONE (92)	NONE (92)	NONE (92)			NONE (92)	NONE (92)	NONE (92)	
	Cold cuts, Hot Dog	NONE (92)	NONE (92)	NONE (92)	NONE (92)	NONE (92)			NONE (92)	NONE (92)	NONE (92)	
Seafood	Seafood	NONE (119) NONE (93) NONE (282)			NONE (93)	NONE (282)	NONE (119) NONE (93)	NONE (93)	NONE (282)	NONE (119) NONE (282)	NONE (282)	NONE (119) NONE (93)
	Canned Fish	↑ (90)				NONE (90)			NONE (90)	NONE (90)	NONE (90)	
	Unspecified Fish	NONE (92)	NONE (92)	NONE (92)	↓ (92)	NONE (92)			NONE (92)	↓ (92)	NONE (92)	
Fruits/ Vegetables	Unspecified Vegetables	↑ (93)			↑ (93)		↑ (93)	NONE (93)				NONE (93)
	Canned Fruit & Vegetables	NONE (282)				NONE (282)			NONE (282)	NONE (282)	NONE (282)	
	Frozen Fruit & Vegetables	NONE (282)				NONE (282)			NONE (282)	NONE (282)	NONE (282)	
Dairy Products	Milk	NONE (119) NONE (90) NONE (92)	NONE (92)	NONE (92)	NONE (92)	↑ (90) NONE (92)	NONE (119)		NONE (90) NONE (92)	↓ (119) ↑ (90) NONE (92)	NONE (90) NONE (92)	NONE (119)
	Yogurt	NONE (119) NONE (90)				NONE (90)	NONE (119)		NONE (90)	NONE (119) ↓ (90)	NONE (90)	NONE (119)
	Cheese	NONE (119) ↑ (90) NONE (92)	NONE (92)	NONE (92)	NONE (92)	NONE (90) ↓ (92)	NONE (119)		NONE (90) NONE (92)	NONE (119) NONE (90) NONE (92)	NONE (90) NONE (92)	NONE (119)
	Ice Cream	NONE (119) NONE (92)	NONE (92)	↑ (92)	↑ (92)	NONE (92)	NONE (119)		NONE (92)	NONE (119) NONE (92)	NONE (92)	NONE (119)
	Unspecified Dairy	NONE (282)				NONE (282)			↑ (282)	NONE (282)	↓ (282)	
	Oils, Butter, Lard, Shortenings	NONE (282)				NONE (282)			NONE (282)	NONE (282)	NONE (282)	
Fast Food	Fast Food	NONE (282)				NONE (282)			NONE (282)	NONE (282)	NONE (282)	
	Potato Chips	NONE (90)				NONE (90)			NONE (90)	NONE (90)	NONE (90)	

Table 19 (cont'd).

Foods		DEHP	DiNP	DiDP	DOP	BBzP	HighMWPs	Phthalic acid	DEP	DBP	DiBP	LowMWPs
Organic, Chemical-Free, and Environmentally Friendly Foods	Organic Food	NONE (90) NONE (282)				↓ (90) NONE (282)			NONE (90) ↓ (282)	NONE (90) NONE (282)	NONE (90) NONE (282)	
	"Marked" Organic Foods	↑ (282)				NONE (282)			NONE (282)	NONE (282)	NONE (282)	
	Grown/raised/caught food	NONE (282)				NONE (282)			↓ (282)	NONE (282)	↓ (282)	
	Unprocessed Food	NONE (282)				NONE (282)			NONE (282)	NONE (282)	NONE (282)	
Other Foods and Dietary Patterns	Soups & Bouillon	NONE (93)			NONE (93)		NONE (93)	NONE (93)				↓ (93)
	Spices	↓ (282)				↓ (282)			NONE (282)	↓ (282)	↓ (282)	
	Peanut Butter	NONE (282)				NONE (282)			NONE (282)	NONE (282)	NONE (282)	
	Grains	NONE (93)			NONE (93)		NONE (93)	↓ (93)				NONE (93)
	Daily Dietary Caloric Intake (< & > 2000-2399 kcal)	NONE (93)			NONE (93)		NONE (93)	NONE (93)				NONE (93)
Soy	Tofu	NONE (119)					NONE (119)			NONE (119)		NONE (119)
	Soy	NONE (282)				NONE (282)			NONE (282)	NONE (282)	NONE (282)	
Supplements	Fish Oil Supplements	NONE (119)					NONE (119)			NONE (119)		NONE (119)
	Folic Acid Supplements	↓ (93)			↓ (93)		↓ (93)	NONE (93)				NONE (93)
Plastic Containers and Tableware	Food/Water Stored in Plastic Containers	NONE (92)	NONE (92)	NONE (92)	NONE (92) ↑ (137)	NONE (92) ↑ (137)			NONE (92) NONE (137)	NONE (92) ↑ (137)	NONE (92) NONE (137)	
	Bottled Water/Other Drinks	NONE (90) NONE (92) NONE (282)	NONE (92)	NONE (92)	NONE (92)	NONE (90) NONE (92) NONE (282)			NONE (90) ↑ (92) NONE (282)	NONE (90) NONE (92) NONE (282)	NONE (90) NONE (92) NONE (282)	
	Foods/Drinks Microwaved in Plastic Containers	NONE (90) NONE (92)	↑ (92)	NONE (92)	NONE (92) ↑ (137)	NONE (90) NONE (92) ↑ (137)			NONE (90) NONE (92) NONE (137)	NONE (90) NONE (92) ↑ (137)	NONE (90) NONE (92) NONE (137)	
	Plastic Tableware				NONE (137)	↑ (137)			NONE (137)	NONE (137)	NONE (137)	
Canned Products	Canned Beverages (soft drinks, beer)	NONE (119) NONE (93) NONE (90)			NONE (93)	NONE (90)	NONE (119) NONE (93)	NONE (93)	NONE (90)	NONE (119) NONE (90)	NONE (90)	NONE (119) ↑ (93)
	Other Canned Food (soups, sauces)	NONE (90)				↑ (90)			NONE (90)	NONE (90)	NONE (90)	

References are provided in parentheses. ↑: positive association; ↓: negative association; NONE: no association; Empty fields: associations not assessed between food product and urinary phthalate metabolite concentrations. HighMWPs: high-molecular weight phthalates; LowMWPs: low-molecular weight phthalates. *Phthalate exposure was predicted from phthalate parent-specific metabolites in urine. Results for some parent phthalates (DEHP, DBP) represent either metabolite-specific or molar sum-metabolite associations.

Table 20. Dietary predictors of bisphenol exposure in pregnant women

Foods		Bisphenol A	Bisphenol S	Bisphenol F	Total Bisphenols
Meat	Packaged Meat (sausages)	NONE (281)			
	Non-Packaged Meat (chicken, pork)	NONE (281)			
	Processed Meat	NONE (119)	NONE (119)		
	Red Meat	NONE (119)	NONE (119)		
Seafood	Seafood	NONE (119) ↑ (93)	NONE (119) NONE (93)	NONE (93)	↑ (93)
	Fresh, Frozen, Un-canned Fish	NONE (281) NONE (284)			
	Canned Fish	↑ (281)			
	Unspecified Fish	NONE (283)			
Fruit and Vegetables	Fresh/Non-Canned Fruits	NONE (281) NONE (284)			
	Canned Fruits	NONE (283) NONE (284)			
	Fresh/Non-Canned Vegetables	NONE (281) NONE (284)			
	Canned Vegetables	↑ (284)			
	Unspecified Vegetables	NONE (93)	NONE (93)	NONE (93)	NONE (93)
	Organic Fruit and Vegetables	NONE (284)			
Dairy Products	Milk	NONE (119) NONE (281)	NONE (119)		
	Yogurt	NONE (119) NONE (281)	NONE (119)		
	Cheese	NONE (119)	NONE (119)		
	Ice Cream	NONE (119)	NONE (119)		
Fast Food	Hamburgers	↑ (283)			
	Pizza	NONE (283)			
	Potato Chips	NONE (281)			
Organic and Environmentally Friendly Foods	Organic Food	NONE (281) NONE (284)			
	Vegetarianism	↓ (284)			
Other Foods and Dietary Patterns	Alcohol	NONE (283)			
	Soups & Bouillon	NONE (93)	NONE (93)	NONE (93)	NONE (93)
	Tofu	NONE (119)	NONE (119)		
	Grains	NONE (93)	↓ (93)	NONE (93)	NONE (93)
	Daily Dietary Caloric Intake (< & > 2000-2399 kcal)	NONE (93)	↓ (93)	NONE (93)	NONE (93)
Supplements	Fish Oil Supplements	NONE (119)	NONE (119)		
	Folic Acid Supplements	↓ (93)	NONE (93)	NONE (93)	NONE (93)
Plastic Containers	Foods/Drinks Microwaved in Plastic Containers	NONE (281) NONE (285)			
	Bottled Water	NONE (281) NONE (283) NONE (285)			
	Foods Stored in Plastic Containers	NONE (285)			
Canned Products	Canned Beverages (soft drinks, beer)	NONE (119) NONE (93) NONE (281) ↑ (283)	NONE (119) NONE (93)	NONE (93)	NONE (93)
	Canned Foods	NONE (281) NONE (285)			

References are provided in parentheses. ↑: positive association; ↓: negative association; NONE: no association; Empty fields: associations not assessed between food product and urinary bisphenol concentrations. *Bisphenol exposure was predicted from their concentrations in urine. Total bisphenols include 3 bisphenols (BPA, BPS, and BPF) measured in urine.

A.5. CURRENT STATUS OF KNOWLEDGE

Food-monitoring studies are performed worldwide to evaluate the safety of foods and dietary patterns, including assessing exposures to environmental chemicals through certain dietary practices. These studies are performed by government agencies, as well as independent laboratories around the world. For example, the U.S. FDA has ongoing food-monitoring programs such as the Total Diet Study (TDS) and the Chemical Contaminants Monitoring Program to examine the safety of foods on the U.S. market (288). Through these programs, the FDA collects information about consumer food preparation and consumption practices (TDS), as well as the potential exposure to and risk of chemical contaminants found in the U.S. food supply (TDS and Chemical Contaminants Monitoring Program) (288). Similarly, the European Food Safety Authority carries out risk assessments and food-monitoring studies within the European Union to determine the safety of chemical contaminants in foods consumed by humans and animals (289). However, many of these government food-monitoring programs have not assessed concentrations of phthalates and bisphenols in foods. Therefore, the food-monitoring studies assessing exposure to phthalates and bisphenols from the food supply (reviewed below) were conducted by independent laboratories around the world. The goal of these food-monitoring studies was to evaluate the potential for human exposure to phthalates and bisphenols through food by capturing dietary habits from various countries, including the U.S., Canada, United Kingdom, France, Spain, Norway, Belgium, Tunisia, Israel, China, and Japan. There are extensive reviews in the literature summarizing food-monitoring studies that measured phthalate and BPA concentrations

in foods (239, 290), and some of the results from these reviews and additional food-monitoring studies are summarized below.

A.5.1. Food-monitoring studies

A.5.1.1. Meat

Foods of animal origin, including beef, pork, and poultry, are major sources of HighMWPs and BPA from processing and packaging (291), partially because HighMWPs, and to a lesser extent LowMWPs, are slightly lipophilic and can bioaccumulate in fat-containing foods (292). International food-monitoring studies consistently report high detectable concentrations of HighMWPs (especially DEHP) and BPA in meat and meat products (239, 293-298). Food-monitoring studies have also reported low, but detectable concentrations of LowMWPs (compared to HighMWPs (239)), in meat and meat products, suggesting that meats may also be important sources of DEP, DBP, and DiBP (293, 294).

A.5.1.2. Seafood

Numerous food-monitoring studies from the United Kingdom, Norway, Belgium, China, and the U.S. have reported detectable concentrations of phthalates and BPA in seafood products (293-295, 299). Similar to other foods packaged in plastics (257, 293) and cans (298, 300), these food-monitoring studies also suggest that food packaging materials contribute to phthalate and BPA concentrations detected in seafood products. A study in Spain found that 34.7% of Spanish pregnant women reported consuming canned fish > 1-3 times/week, making it the most frequently-consumed canned food in this population, and a major source of BPA during pregnancy (301).

A.5.1.3. Fruits and vegetables

Fruit and vegetable consumption is considered a measure of healthier lifestyles associated with lower EDC exposures (298, 302). Food-monitoring studies from Belgium, France, China, and the U.S. report low concentrations of both HighMWPs and LowMWPs in fruit and vegetable products, suggesting low likelihood for phthalate exposure from these foods (294-296, 303, 304). However, one study has shown that exposure to HighMWPs and LowMWPs in vegetables primarily comes from ready-to-eat vegetables (e.g. lettuce, arugula, parsley, carrot, and corn salad) packaged in plastic bags (305). BPA food-monitoring studies from Norway, Canada, and the U.S. suggest that canned fruits and vegetables, rather than fresh, are major exposure sources (298, 306), and that overall concentrations of BPA in noncanned fruits and vegetables are relatively low (293, 297, 307).

A.5.1.4. Dairy products

Milk, yogurt, cheese, ice cream, and butter can be high in fats, making it possible for phthalates to accumulate in these foods (292). Analyses of milk in Belgium found higher levels of HighMWPs (DEHP and BBzP) and LowMWPs (DBP and DiBP) in milk retail products compared to raw cow's milk, suggesting that phthalates can migrate into raw cow's milk from contaminated feed ingested by cows, during the mechanical milking process, and/or from milk packaging materials used at the dairy factory (308). Food-monitoring studies reported detectable concentrations of DEHP in select cheese samples from Canada (254), DEHP/DiNP/DOP in milk and cheese from Norway (293), DEHP/DOP/BBzP in milk and other dairy from the U.S. (295), DEHP in milk and dairy

products from Belgium (294), and BBzP in milk, butter/oil, and yogurt from Tunisia (309). In contrast, Norwegian and the U.S. food-monitoring studies reported low or undetectable concentrations of LowMWPs in milk and milk products (239, 293-295).

Similar to HighMWPs, BPA has also been detected in milk and dairy products. In Europe, higher BPA levels were found in canned milk and dairy products compared to un-canned products (European Food Safety Authority) (310). The BPA concentrations in European dairy are consistent with those from China (311), Canada (298), and Japan (312). Detectable BPA concentrations were also found in dairy products (milk, ultra-fresh dairy products, and cheese) consumed by French pregnant women (313), suggesting that these are important sources of BPA exposure in pregnancy.

A.5.1.5. Fast food

Food-monitoring and epidemiological studies suggest that fast food and/or foods served at restaurants are likely sources of phthalate and BPA exposures (239, 292). Although phthalates and bisphenols could leach into foods during processing, they have also been shown to migrate into foods from packaging, including pizza boxes or sandwich wrappers (314). A food-monitoring study in Canada reported detectable concentrations of BPA in some fast food products (French fries, hamburgers, and sandwiches), but not others (pizza and chicken nuggets) and concluded that hamburgers had the highest BPA concentrations of all fast foods assessed (298). An assessment of fast food intake in the general U.S. population (NHANES 2005-2014) found that compared with nonconsumers, both low and high consumers of food away from home had significantly higher

concentrations of urinary sum of di(2-ethylhexyl) phthalate (Sum-DEHP) and monocarboxyoctyl phthalate [but not monobenzyl phthalate (mBzP), mEP, mono-n-butyl phthalate (mBP), or mono-isobutyl phthalate (miBP) (315)]. A similar study further analyzed fast food consumption in the U.S. population by food group, and found that fast food grain, including pizza, intake was associated with urinary Sum-DEHP and sum of di-isononyl phthalate metabolites (Sum-DiNP), whereas potato chip and hamburger intakes were associated with higher urinary Sum-DEHP and Sum-DiNP (316).

A.5.1.6. Organic & environmentally-friendly food and dietary patterns

Organic, chemical-free and environmentally friendly foods are generally considered markers of healthier lifestyles, but associations of these dietary patterns with EDC exposures are not well-characterized. Although the literature is limited, a dietary intervention that focused on exclusive consumption of fresh and organic foods for three days found that urinary DEHP metabolite concentrations decreased by 53-56% and urinary BPA concentrations decreased by 66% lower from pre- to during-intervention. However, foods provided to and prepared by these participants were from plastic-free packaging and nonplastic containers (291), making it difficult to determine if the decreases in urinary chemical concentrations were due to the foods' organic status or their packaging/preparation materials. Similarly, residents of a rural vegetarian/vegan community in Israel had significantly lower urinary phthalate metabolite concentrations, but BPA concentrations in these individuals were not different from those in the general Israeli population (317). However, a U.S. dietary intervention with fresh and organic foods prepared without plastics found significantly higher urinary DEHP metabolites after the

intervention, which was due to increased intake of certain foods (e.g., spices and peanut butter) (318).

A.5.1.7. Plastic containers and tableware

Both phthalates and BPA are used to manufacture plastics for food storage and cooking containers (256). Data suggest that these are important sources of human EDC exposure, as these EDCs can migrate from plastic containers and tableware into foods and beverages, especially during heating and cooling (239, 301). For phthalates, migration levels of DBP were higher with prolonged plastic container use and longer heating time (319), and high levels of DEHP and DBP were found in plastic tableware at room temperature (320). BPA, however, migrates from reusable polycarbonate plastic water bottles into water at room and high temperatures (321), and the use of polycarbonate water bottles has been shown to increase urinary BPA concentrations (322), especially during the hot summer months (323).

A.5.1.8. Canned foods and beverages

BPA is used to manufacture polycarbonate and epoxy resins for metal can linings and is detectable in a variety of canned foods (293, 298, 324-326). A study characterizing dietary BPA exposure in the French population (including pregnant women) identified that canned products accounted for ~50% of total BPA exposure (313). Phthalates are generally found in plastic food packaging materials, so their concentrations in canned products are low, but detectable (293).

A.5.2. Dietary Predictors of Phthalates and Bisphenols in Pregnancy

Figure 14 summarizes potential sources of phthalates and bisphenols from food packaging materials and consumer food practices. As previously mentioned, both diet and physiology are greatly modified in pregnancy (277-280). Thus, despite the many food-monitoring studies assessing dietary sources of phthalates and BPA in the general public, studies specifically in pregnancy are important for establishing dietary recommendations for this vulnerable population. In the 10 studies reviewed here, urinary concentrations of phthalates and bisphenols in women from U.S. studies were comparable to those of women in the general U.S. population (using data from the 2013-2014 NHANES (327)). The studies performed outside the U.S. recruited cohorts to reflect their individual populations, suggesting that exposures described in these studies likely represent the general population of each country. Overall, in pregnancy, associations between use of plastic containers and increased urinary phthalate metabolite concentrations, and between consumption of canned foods and increased urinary BPA concentrations were consistent with previous food-monitoring studies. Foods and dietary patterns associated with a healthier lifestyle, such as organic foods, grown/raised/caught foods, vegetarianism, and folic acid supplementation, as well as some other dietary patterns and foods, including soups and bouillon, spices, and grains, were generally associated with lower urinary phthalate metabolite and bisphenol concentrations in pregnant women. However, despite the many food-monitoring studies reporting high phthalate and BPA concentrations in meats and dairy, the designs of most studies in pregnant women were unable to reliably detect associations of specific foods/food groups

with phthalates and BPA. Some of the differences and inconsistencies in study design are highlighted in **Table 18** and will be discussed in the following sections.

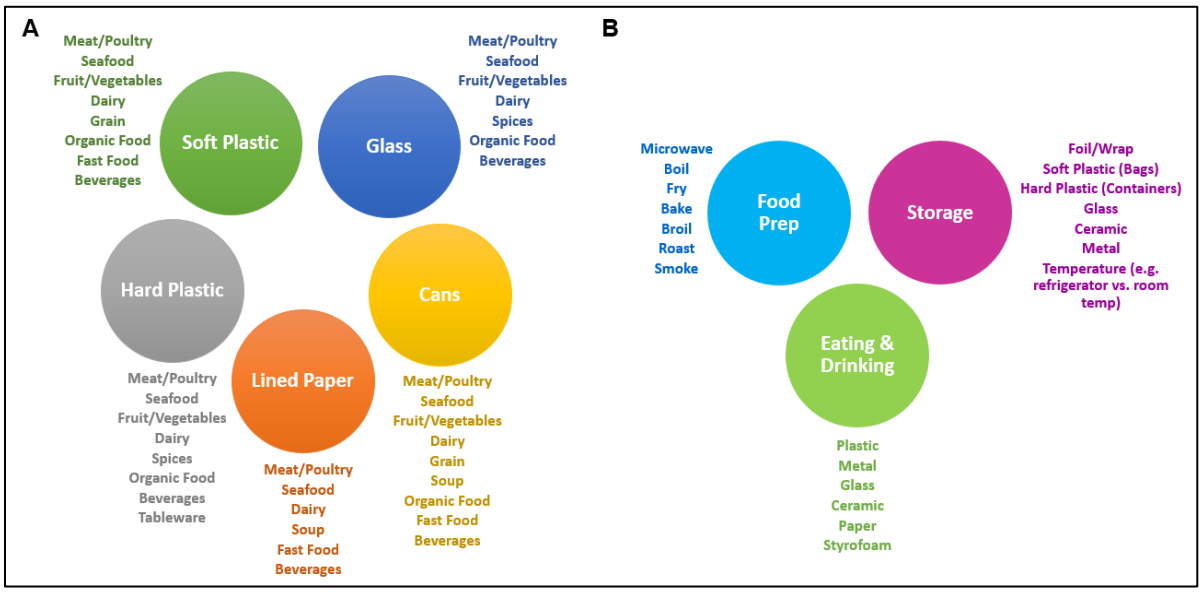

Figure 14. Summary of potential food packaging materials and consumer food practices. (A) Potential determinants of phthalate and BPA exposures through food packaging materials, including soft and hard plastics, cans, lined paper, and glass. (B) Consumer food practices, including food preparation, as well as storage and consumption materials that influence exposure to phthalates and BPA. BPA, bisphenol A.

A.5.2.1. Measuring urinary phthalates and bisphenols

Some technical/analytical factors may be important to consider when evaluating currently available studies in pregnant women. First, the analytical methodologies vary slightly among the 10 studies reviewed here (**Table 18**), and additional studies may be needed to determine whether unifying these methods would eliminate reported inconsistencies between studies. Another important technical consideration when using urine to assess chemical exposures is establishing appropriate methods to account for urine dilution across participants. In the studies reviewed here, two studies did not adjust for urinary dilution, whereas five adjusted for creatinine, and three adjusted for specific gravity (**Table**

18). Urinary density can vary greatly across pregnancy and depending on hydration status, therefore raw values of phthalate and bisphenol concentrations need to be adjusted for some measure of urine density. Furthermore, it has been suggested that creatinine in pregnancy is affected by pregnancy-related fluid dilution, making specific gravity adjustment a more accurate approach in pregnant women (328).

A.5.2.2. Evaluating exposure to phthalates and bisphenols

Most studies in pregnant women reviewed here included analyses of phthalates and bisphenols, but classification of these chemicals often varied across studies. For example, findings for associations between milk consumption and urinary mBP (metabolite of DBP) in pregnancy were conflicting. One study reported that the sum of di-n-butyl phthalate metabolite concentrations [composed of mBP, miBP, mono-hydroxybutyl phthalate (mHBP), and mono-hydroxyl-isobutyl phthalate (mHiBP)] was negatively associated with milk consumption (119), whereas other studies found no association (92) or a positive association (90) between milk consumption and urinary mBP, and no association with urinary miBP (90, 92). Similar examples were also observed for sum or individual measures of other phthalates and bisphenols (**Tables 18 – 20**), which suggests that classifying chemicals into their predicted exposure sources (parent chemicals) may yield differing results compared with assessment of individual breakdown metabolites.

Studies in pregnant women report low reproducibility and sensitivity of urinary phthalate metabolites and bisphenols across pregnancy (18, 108, 329-331). To overcome this

challenge, several of the studies reviewed here collected two (90, 281, 283) or even three (92, 284) urine samples across pregnancy to evaluate gestational exposure to bisphenols and phthalates. Assessing these relations at multiple times in pregnancy may be critical to account for some of the physiological and dietary shifts that occur in early compared with late pregnancy. However, aligning the timing of exposure assessment in relation to diet may be more important to accurately and consistently predict dietary sources of chemical exposure. Based on the current review of the literature, only one study accurately captured dietary sources of phthalates and bisphenols (92), as it assessed chemical exposure within 48 hours of the dietary report, whereas other studies used surveys that assessed much broader windows of dietary intakes. This appears to be a critical difference in study design, as many observations differed between this study and others. For example, in one study, milk consumption assessed by a 3-month FFQ was associated with increased urinary mBzP concentrations (90), whereas mBzP assessed within 48 hours of dietary assessment was not associated with milk consumption (92). Similarly, higher cheese consumption was associated with lower urinary mBzP (92) when assessed within 48 hours of the dietary survey, whereas no associations were reported with urinary mBzP in a study assessing diet using a 3-month dietary questionnaire (90). Given that the study assessing chemical exposure within 48 hours of the dietary report was limited to women in Northern Puerto Rico, future studies in other pregnant populations should expand on findings from this study by assessing chemical exposure at multiple times across pregnancy, and within 24-48 hours of dietary assessment.

A.5.2.3. Assessing diet in pregnancy

Dietary patterns are most-often assessed using FFQs, which are inexpensive, easy to use, and validated to reflect long-term dietary intake patterns in pregnant populations (332, 333). Because FFQs and other similar questionnaires are designed to assess dietary intake of nutrients, not chemicals in food, they may not accurately predict dietary sources of phthalate and BPA exposure. Especially problematic is that information collected from these questionnaires spans weeks or months, whereas the short half-lives of EDCs mean that urinary exposure assessment reflects their concentrations within (often) 24-hours of assessment. Based on the aforementioned examples, assessment of diet within ~24-hours of exposure assessment will be the most appropriate approach for quantifying dietary sources of phthalates, bisphenols, and other environmental chemicals that have relatively short half-lives.

The other critical factor to consider when evaluating currently available studies in pregnancy is the broad range of dietary surveys used in these studies (**Table 18**). First, many studies utilized exceedingly general categories of various food types. Examples of this include: using the vague category of “processed meat” (119), the term “seafood” (93, 119, 282) to refer to all types of fish or shellfish, the combined analysis of all “fruits and vegetables” (282), and the overly broad analysis of “fast food” (282) without specifying the food item or restaurant category. These general categories make it difficult to establish patterns of chemical exposures from specific foods, and to ultimately provide pregnant women with specific recommendations as they make food-purchasing decisions.

More importantly, many studies reviewed here acknowledged that their dietary surveys were unable to distinguish between various categories of food packaging, including fresh, frozen, or packaged foods. For example, in the Infancia y Medio Ambiente Project, first- and second-trimester consumption of canned fish was associated with higher urinary BPA concentrations (281), whereas two other studies found no associations between fresh, frozen, or un-canned fish consumption and urinary BPA (281, 284). BPA is used to manufacture polycarbonate and epoxy resins for metal can linings and is detectable in a variety of canned foods (293, 298, 324-326), so observations of higher BPA concentrations with consumption of canned (but not other) fish are consistent with food-monitoring studies. However, several other studies assessed seafood or fish intake with BPA, but the dietary questionnaires in these studies did not distinguish between fresh or canned fish, making it difficult to conclusively interpret these findings (93, 119, 283). Future studies should utilize dietary questionnaires that robustly assess both diet and the mode of food packaging/preparation by asking specifically about how each food item was packaged (e.g., fresh, canned, plastic, glass) and prepared (e.g., microwaved, steamed in plastic or glass).

A.5.2.4. Considering demographic and lifestyle factors

Another major challenge for establishing dietary correlates of EDC exposure are the cultural differences in both dietary intake and chemical production/food packaging. For example, associations of meat and dairy intake with phthalates and bisphenols differed greatly between pregnancy studies in the contiguous U.S., Puerto Rico, and Spain. These differences likely reflect variability in the types and amounts of meats/dairy foods

consumed in these cultures, and how these foods are processed/packaged. Although it is important to establish generalizable recommendations for pregnant women, these cultural differences may require culturally-sensitive recommendations.

Most studies reviewed here acknowledged that many of the unexpected associations observed between some particular EDCs and foods may be due to other concomitant lifestyle factors. Here, and in other pregnancy cohorts, these factors included maternal age, race/ethnicity, country of origin, pre-pregnancy BMI, marital status, education, employment status, annual income, and personal care/household product use (18, 90, 92, 93, 108, 119, 124, 281-284, 330, 331). For example, mEP concentrations were higher in pregnant women who used bottled water for cooking 48 hours before exposure assessment than in women who used the public water supply (92). Given that DEP (mEP's parent compound) is primarily found in personal care products, the authors of this study suggest that the positive association between bottled water use and mEP is unrelated to use of plastics, and could be explained by higher urinary mEP concentrations in women reporting use of perfume/cologne and colored cosmetics (92). In addition to carefully controlling for other important lifestyle factors in statistical models, if dietary questionnaires fail to capture cultural or geographic differences in food packaging or processing, future studies may need to investigate these factors as modifiers of food-exposure relations. For example, annual income or employment status may affect seafood choices. Therefore, although no overall associations may be observed between fish consumption and phthalate exposure, positive associations are possible in low-

income groups that tend to consume canned or packaged fish, but not in higher-income women who tend to eat fresh or unpackaged fish.

A.6. CONCLUSION

Maternal diet is an established determinant of a healthy pregnancy and fetal outcomes. Phthalates and BPA are known to affect pregnancy and fetal development, and the 10 studies reviewed here suggest that certain dietary components or patterns are important sources of BPA, HighMWPs, and LowMWPs in pregnancy. However, consistencies in observed associations between studies were limited to long-term lifestyle choices, including those related to home food preparation (use of plastics to store, prepare, and heat foods) and choices of food types (canned, organic). Several well-designed studies in non-pregnant populations do suggest that changing dietary behaviors can limit exposure to phthalates and bisphenols. For example, a dietary intervention of five families in the U.S. (n=20) found significant reductions in urinary BPA and DEHP metabolites after limited consumption of foods packaged and prepared in plastics and cans, and increased concentrations of these chemicals with resumption of packaged food consumption (291). Furthermore, a strict 48-hour fasting study of five individuals from Germany observed significant reductions in urinary HighMWPs from pre-fast to post-fast, whereas urinary LowMWP concentrations stayed consistent throughout the study (128). These findings suggest that addressing the inconsistencies in study designs among the pregnancy studies described here could provide valuable insight for establishing specific EDC-limiting dietary recommendations to improve pregnancy and fetal outcomes.

APPENDIX B: MATERNAL DIET QUALITY MODERATES ASSOCIATIONS BETWEEN PARABENS AND BIRTH OUTCOMES

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B.1. ABSTRACT

Maternal paraben exposure and diet quality are both independently associated with birth outcomes, but whether these interact is unknown. We assessed sex-specific associations of parabens with birth outcomes and differences by maternal diet quality. Illinois pregnant women (n = 458) provided five first-morning urines collected at 8–40 weeks gestation, which we pooled for quantification of ethylparaben, methylparaben, and propylparaben concentrations. We collected/measured gestational age at delivery, birth weight, body length, and head circumference within 24 h of birth, and calculated sex-specific birth weight-for-gestational-age z-scores and weight/length ratio. Women completed three-month food frequency questionnaires in early and mid-to-late pregnancy, which we used to calculate the Alternative Healthy Eating Index (AHEI)-2010. Linear regression models evaluated sex-specific associations of parabens with birth outcomes, and differences in associations by average pregnancy AHEI-2010. In this predominately non-Hispanic white, college-educated sample, maternal urinary paraben concentrations were only modestly inversely associated with head circumference and gestational length. However, methylparaben and propylparaben were inversely associated with birth weight, birth

weight z-scores, body length, and weight/length ratio in female, but not male newborns. For example, each 2-fold increase in methylparaben concentrations was associated with −46.61 g (95% CI: −74.70, −18.51) lower birth weight, −0.09 (95% CI: −0.15, −0.03) lower birth weight z-scores, −0.21 cm (95% CI: −0.34, −0.07) shorter body length, and −0.64 g/cm (95% CI: −1.10, −0.19) smaller weight/length ratio in females. These inverse associations were more prominent in females of mothers with poorer diets (AHEI-2010 < median), but attenuated in those with healthier diets (AHEI-2010 ≥ median). In newborn males of mothers with healthier diets, moderate inverse associations emerged for propylparaben with gestational length and head circumference. Maternal diet may moderate associations of parabens with birth size in a sex-specific manner. Additional studies may consider understanding the inflammatory and metabolic mechanisms underlying these findings.

B.2. KEYWORDS

Parabens; birth size; gestational length; head circumference; newborn sex; maternal diet.

B.3. INTRODUCTION

A newborn's gestational age at birth, birth size (weight and length), and head circumference are known predictors of neonatal morbidity and mortality (334), and are associated with maternal and child lifelong health. Babies born pre-term (birth before 37 weeks gestation) and early-term (birth after 37 weeks, but before 39 weeks gestation) have higher risks of developing metabolic disorders, respiratory issues, and cognitive

problems compared to full-term infants (birth after 39 weeks, but before 41 weeks) (335-338). Similarly, late-term birth (birth after 41 weeks gestation) is associated with higher risk of delivery complications for both mother and newborn, as well as childhood and adulthood obesity (221, 339, 340). After accounting for gestational age at birth, smaller body size at birth also predicts child cognitive problems and other adverse developmental outcomes that may persist through adolescence (220). Similarly, larger birth size is associated with long-term cardiometabolic consequences for children, including increased risk of type 2 diabetes and obesity (221), but also with delivery complications that can adversely impact both mother and newborn (341). Finally, head circumference measured at birth has been shown to be a clinical indicator of newborn brain volume (342), though the consequences of this are not fully understood. Therefore, it is important to understand risk factors in pregnancy that influence a baby's gestational age at birth, birth size, and head circumference.

Greater than 90% of reproductive-aged women in the United States (U.S.) have measurable urinary biomarker concentrations of parabens (alkyl esters of p-hydroxybenzoic acid) (343). Parabens are anti-microbial agents found in many personal care products and cosmetics, as well as some food products and medications (344). Studies in cells, animal models, and pregnant populations suggest that parabens target inflammatory (345-349), hormonal (265, 350-353), and metabolic (354-356) pathways, which are important pathways for fetal growth. Additionally, observational studies have detected parabens in cord blood (357), amniotic fluid (258), and placental tissue (358), suggesting that parabens can cross the placenta. Consequently, some studies evaluating

associations of parabens with gestational length, birth size, and head circumference observed that parabens are associated with unfavorable birth outcomes (359-364). However, a 2019 systematic review qualitatively summarized six previous studies evaluating associations of paraben biomarkers with birth weight, length, and head circumference, and concluded that the strength and direction of associations are inconsistent across studies (365). Similarly, a 2020 meta-analysis pooled six to eight studies (depending on the paraben) evaluating associations of paraben biomarkers with birth weight, but observed no significant pooled associations for any paraben biomarker (366). More recently, several studies suggest that associations of parabens with birth size may be sex-specific (134, 360-362, 367, 368), though results from these studies have also been inconsistent. Therefore, further research can help clarify sex-specific associations of parabens with birth outcomes.

Appropriate maternal diet is critical for optimal fetal growth and development, whereas poor maternal diet quality is associated with adverse birth outcomes, including shorter gestational length and smaller birth size (241, 369). Importantly, a growing body of literature suggests that healthy maternal diets may protect the developing fetus from the adverse effects of environmental exposures (370-372). With regards to birth size, some studies observed that adverse associations of maternal exposure to chemicals (e.g., per- and polyfluoroalkyl substances and metals) with birth weight were attenuated in newborns of mothers with better diets, as measured by consumption of select whole foods or specific individual macro- or micronutrients (373, 374). Studies suggest both parabens and diet may interact with similar inflammatory and metabolic pathways (244, 347, 375),

which are important mechanisms involved in regulating gestational length and fetal growth. However, no studies have evaluated diet as a moderator of associations between parabens and birth outcomes. Additionally, most prior studies evaluating diet as a moderator of associations between environmental chemicals and health outcomes only focused on individual nutrients or foods, while few considered assessing dietary patterns or diet quality (376). Utilizing diet indices that reflect diet quality accounts for the combined effect of many dietary behaviors and the interactive effects of foods and nutrients on health (102). For example, the Alternative Healthy Eating Index 2010 (AHEI-2010) focuses on foods predictive of chronic disease risk, and a higher AHEI-2010 score in pregnancy, specifically, has been associated with improved maternal and offspring outcomes (377-380). Additionally, higher AHEI-2010 scores are correlated with lower inflammation (381). Therefore, it may be important to consider holistic dietary patterns when evaluating whether diet can attenuate the negative impacts of environmental toxicant exposures, especially exposures to parabens.

Given the current status of the literature, our first objective was to evaluate sex-specific associations of maternal urinary paraben concentrations with gestational length, birth size (birth weight, birth weight z-scores, body length, and weight/length ratio), and head circumference. Our second objective was to determine if sex-specific associations of paraben biomarkers with birth outcomes differ by maternal diet quality, which we evaluated using the AHEI-2010.

B.4. MATERIALS AND METHODS

B.4.1. Illinois Kids Development Study (I-KIDS) recruitment and enrollment

The current study includes pregnant women from I-KIDS, an ongoing prospective pregnancy/birth cohort designed to evaluate the impacts of prenatal environmental chemical exposures on infant neurodevelopment. I-KIDS recruitment and enrollment have been described elsewhere (86). Briefly, pregnant women were recruited at their first prenatal care appointment from two local obstetric clinics in Champaign-Urbana, IL. Women were eligible to participate if they were < 15 weeks pregnant, 18-40 years old, fluent in English, in a low-risk singleton pregnancy, living within a 30-minute drive of the University of Illinois campus, and not planning to move out of the area before their child's first birthday. A total of 482 pregnant women enrolled in I-KIDS between 2013 and 2018 and remained in the study through the birth of their infant. We excluded the limited number of women who delivered before 37 weeks gestation, and those who were missing information on important covariates. Therefore, the current analysis includes a total of 458 mothers-newborn pairs (233 females and 225 males). These women provided written informed consent, and the study was approved by the Institutional Review Board at the University of Illinois. The analysis of de-identified specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects' research.

B.4.2. Collection of maternal sociodemographic, lifestyle, and health information

Immediately after enrollment, study staff visited I-KIDS participants at home to obtain information about their sociodemographic, lifestyle, and health characteristics. We

collected information about the following characteristics from an interviewer-administered questionnaire: age, race/ethnicity, pre-pregnancy weight, height, and parity. Self-reported pre-pregnancy weight and height were used to calculate pre-pregnancy BMI (kg/m^2) (98-100). To calculate conception season, we used reported due dates based on the first day of the last menstrual period, which we confirmed after the first trimester ultrasound. To assess early pregnancy depression symptoms, participants also completed an adapted version of the Edinburgh Postnatal Depression Scale (EPDS), which excluded the question “The thought of harming myself has occurred to me” (382).

B.4.3. Assessment of urinary paraben biomarker concentrations

Women provided at least three and up to five first-morning urine samples at study home visits or routine prenatal care visits at the following gestational timepoints: 8-15, 13-22, 19-28, 25-33, 32-40 weeks gestation (median 13, 17, 23, 28, and 34 weeks gestation, respectively) as described previously (86). Greater than 95% of women contributed all five urine samples, and the remaining 5% contributed three or four urines. Urine samples were collected in polypropylene urine cups and refrigerated immediately. Within 24 hours of collection, we aliquoted urine samples for long-term storage (at -80 °C) or pooled from each timepoint. To create the pool sample, we added 900 µL of fresh urine from each timepoint to a 5 mL cryovial, and the sample was immediately stored at -80 °C between each gestational timepoint. After the first visit, we layered fresh urine onto frozen urine from previous gestational timepoints (frozen urine was not thawed). At the end of pregnancy, we thawed, vortexed, and measured specific gravity of pooled samples. We shipped frozen pooled samples on dry ice to the CDC Division of Laboratory Sciences in

three batches, in the chronological order of enrollment (batch one enrolled December 2013 - February 2015, batch two enrolled February 2015 - July 2016, and batch three enrolled July 2016 - August 2018). Pooled urine samples were analyzed for urinary concentrations of the following four commonly measured paraben biomarkers: butylparaben, propylparaben, ethylparaben, and methylparaben using automated on-line solid phase extraction-high performance liquid chromatography–isotope dilution tandem mass spectrometry based on previously published methods (383, 384). These methods have rigorous quality control/quality assurance protocols and excellent long-term reproducibility, and inter- and intra-day variability were < 9% indicating good precision for all measured paraben concentrations (384). The limits of detection were 0.1 ng/mL for butylparaben and propylparaben and 1.0 ng/mL for ethylparaben and methylparaben.

B.4.4. Collection of gestational length, birth size, and head circumference measures

Within 24 hours of birth, I-KIDS staff visited participants at the hospital to obtain delivery date, which we used to calculate gestational length, and to conduct newborn anthropometric measurements. Hospital staff measured birth weight (g) immediately after delivery, which we obtained from crib cards. Sex-specific birth weight for gestational age z-scores were calculated using a U.S. population-based reference according to published methods (385). I-KIDS staff measured body length (cm; Seca Light & Stable Measuring Board) and head circumference (cm; flexible retractable ruler) in triplicate, and the mean of multiple values was used in statistical analyses. In the few cases where we were unable to measure body length and head circumference at the hospital (n = 28 and 9,

respectively), we used body length and head circumference information obtained from hospital crib cards. We calculated newborn weight/length ratio (g/cm) by dividing birth weight by body length.

B.4.5. Collection of dietary intakes and calculation of Alternative Healthy Eating Index (AHEI-2010)

Participants completed semi-quantitative food frequency questionnaires (FFQs) at 8-15 and 32-40 weeks gestation. This FFQ was adapted for pregnant women from the full length Block-98 FFQ (NutritionQuest, Berkeley, CA) and has been validated in pregnant populations (206, 207). The FFQ asks women to report their diet during the previous three months (101). Therefore, dietary intakes at 8-15 and 32-40 weeks gestation reflect maternal diets in early and mid-to-late pregnancy, respectively. Data on dietary intakes at each timepoint were used to calculate early and mid-to-late pregnancy AHEI-2010, which is an 11 component diet quality measure (scored out of 110) based on foods and nutrients predictive of chronic disease risk and mortality (102, 103). The 11 food or nutrient components (six positive and five negative) include: vegetables, fruit, whole grain, nuts/legumes, omega-3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid), polyunsaturated fatty acids, sugar-sweetened beverages/fruit juice, red/processed meat, *trans* fat, sodium, and alcohol. Based on recommendations from the American College of Obstetrics and Gynecology that pregnant women should abstain from alcohol during pregnancy (386), we removed the alcohol component from the total AHEI-2010 score. Therefore, AHEI-2010 in this study is scored out of 100, and higher AHEI-2010 scores reflect better overall diet quality. The AHEI-2010 has been validated in other pregnancy

cohorts, reporting that AHEI-2010 is associated with gestational length and various measures of newborn size at birth (378-380, 387). We used the mean of early and mid-to-late pregnancy AHEI-2010 scores to reflect average pregnancy diet quality as we observed no meaningful differences in associations when considering the two timepoints separately (**data not shown**).

B.4.6. Statistical analysis

We used instrumental reading values for non-zero paraben biomarker concentrations below the LOD to avoid bias associated with imputing values for non-detectable concentrations (107). To account for urine dilution, we used the following formula to adjust all urinary paraben biomarker concentrations: $P_c = P[(1.016 - 1)/(SG - 1)]$, where P_c is the specific gravity-adjusted paraben biomarker concentration, P is the measured paraben concentration (ng/mL), 1.016 is the sample median specific gravity, and SG is the specific gravity of each woman's pooled urine sample (109). We evaluated ethylparaben, methylparaben, and propylparaben as continuous variables that were ln-transformed. Six women had ethylparaben concentrations of zero, so we added a constant (1.0) before ln-transformation to avoid undefined estimates (108). Due to the narrow distribution of urinary butylparaben concentrations that centered around the LOD (**Table 22**), and because only 44% of women had butylparaben concentrations above the LOD, we excluded butylparaben from further analyses.

We presented maternal characteristics by newborn sex and maternal average pregnancy diet quality as n (%) or median (range). Paraben biomarker concentrations from I-KIDS

and the National Health and Nutrition Examination Survey (NHANES) cycles 2013-2016 are presented as the median (25th, 75th percentiles) (110, 111) – for comparability, these concentrations were not adjusted for urine dilution. Birth outcomes are reported as the median (25^{th}, 75^{th} percentiles) by newborn sex and maternal diet quality. We tested for statistical differences in maternal characteristics and birth outcomes by newborn sex and diet quality using chi-squared or Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables.

We used linear regression models that included a multiplicative interaction between paraben biomarkers and newborn sex to evaluate sex-specific associations of maternal urinary paraben concentrations with continuous gestational length, birth size, and head circumference (objective 1). We were unable to evaluate newborn weight using clinical size-for-gestational-age categories (large, appropriate, or small-for-gestational age) because few newborns were born small-for-gestational age in this cohort. Gestational length, birth weight, birth weight z-scores, body length, weight/length ratio, and head circumference were normally distributed, and we checked regression models for non-constant residual variance to ensure model assumptions were met. We evaluated both unadjusted and adjusted sex-specific associations of paraben biomarkers with birth outcomes, and reported all results stratified by sex regardless of the significance of the interaction *P*-value (P_{int}) between parabens and newborn sex. We identified important confounders *a priori* and using previously published literature that informed a directed acyclic graph (**Figure 15**) (365). We evaluated correlations (Pearson for continuous and polychoric for categorical variables) between all covariates; however, none of the chosen

covariates were strongly correlated with each other ($r < 0.4$; **data not shown**). Therefore, adjusted linear regression models accounted for age, race/ethnicity, conception season, pre-pregnancy BMI, parity, depression status in early pregnancy, newborn sex, and average pregnancy diet quality. Pre-pregnancy BMI, EPDS scores, and diet quality were included as continuous variables, while the remaining variables were categorical, with the reference groups indicated in **Table 21**.

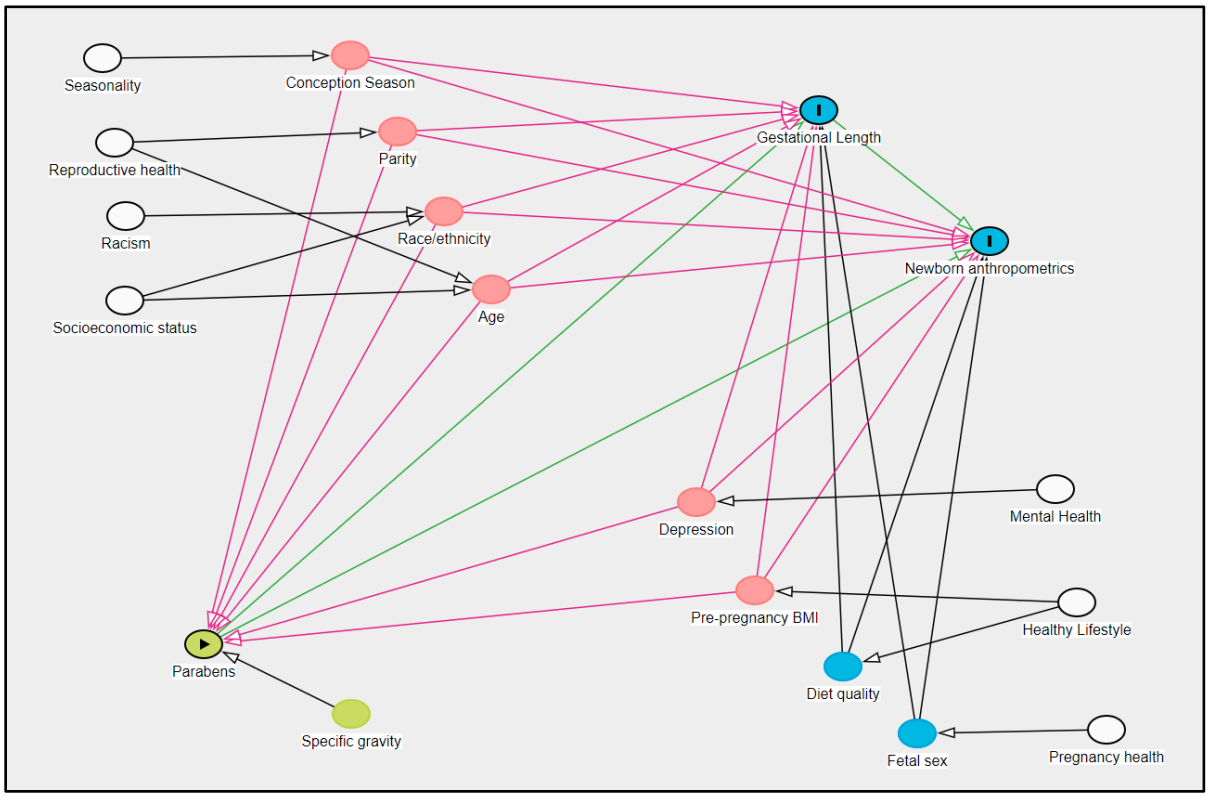

Figure 15. Directed acyclic graph for associations of paraben biomarkers with birth outcomes. The green circle with filled triangle represents the exposure (i.e. paraben biomarkers), while the blue circles with filled line represents the outcomes (i.e. gestational length and newborn anthropometrics). The white circles represent latent variables (socioeconomic status, racism, health lifestyle, seasonality, reproductive health, mental health, and pregnancy health), while the red and blue circles represent confounding or moderating variables, respectively, that were included in final adjusted models. Green circle represents variables that are ancestors of the exposure.

To evaluate if sex-specific associations of paraben biomarkers with gestational length, birth size, and head circumference differed by maternal diet quality (objective 2), we used linear regression models accounting for the previously mentioned covariates, a three-way interaction, and all relevant two-way interactions between parabens, sex, and AHEI-2010. We dichotomized mean pregnancy AHEI-2010 at the sample median (score of 55.9) to determine if sex-specific associations of parabens with birth outcomes differed in male or female newborns of mothers with poorer (AHEI-2010 < median) or better (AHEI-2010 ≥ median) average diet qualities. We reported all results regardless of the significance of the three-way interaction *P*-value (P_{int}) between parabens, sex, and diet quality.

We also conducted sensitivity analyses to determine the robustness of associations. First, gestational length could mediate associations of paraben biomarkers with birth weight, body length, weight/length ratio, and head circumference (388), so we also evaluated these relationships by additionally adjusting for gestational age at birth (**Table 24**). Second, we also conducted sensitivity analyses where we included preterm births (n = 21). Because we observed that associations of paraben biomarkers with birth outcomes did not change after inclusion of preterm births (**data not shown**), we excluded pre-term births from our analyses. Lastly, for a small number of newborns, we obtained body length and head circumference data from hospital crib cards (n = 28 and 9 measurements, respectively). Ultimately, we included these babies in final statistical models because associations of parabens with birth outcomes did not differ upon their exclusion (**data not shown**).

Because parabens were ln-transformed for all analyses, we back-transformed the resulting β-estimates and 95% confidence intervals (CIs) using the equation [β * ln(2.00)] to represent the change in birth outcome for every two-fold increase in paraben biomarker concentration. All analyses were conducted in SAS version 9.4 (SAS Institute Inc, Cary, NC) using PROC GLM. We focused on patterns of associations rather than statistical significance. We considered associations potentially meaningful if (1) the upper and lower confidence limits did not cross the null or (2) the upper or lower confidence limit did cross the null but the limit was close to zero (117, 118). Based on recommendations from others (166), we did not adjust for multiple comparisons.

B.5. RESULTS

B.5.1. Sociodemographic and lifestyle characteristics of the I-KIDS sample

In this sample, most mothers were older than 30 years (59%), non-Hispanic white (80%), college educated (81%), employed (85%), and did not smoke in the first trimester (87%), while 50% were nulliparous. Around half of mothers were normal weight, whereas the other half had overweight or obesity before pregnancy. Conception season was uniformly distributed over the four seasons, and most women (74%) had a vaginal delivery. Median (range) EPDS score was 4.0 (0.0 – 19.0), while maternal average pregnancy AHEI-2010 score was 51.9 (25.6 – 76.2). Characteristics of the I-KIDS sample by newborn sex and maternal average pregnancy diet quality are presented in **Table 21**. Out of 458 newborns included in this study, 121 and 112 were females of mothers with worse or better diet quality, respectively, while 108 and 117 were males of mothers with worse or better quality, respectively (**Table 21**). Maternal age, education, pre-pregnancy BMI, and AHEI-

2010 scores significantly differed by newborn sex and maternal diet quality (**Table 21**).

Table 21. Maternal and select delivery/newborn characteristics (n=458).

Characteristics	Mothers of female newborns		Mothers of male newborns		
	Worse diet quality	Better diet quality	Worse diet quality	Better diet quality	
	n = 121	n = 112	n = 108	n = 117	
	n (%)	n (%)	n (%)	n (%)	*P*[2]
Age					0.0002
< 30 years (ref)	64 (52.9)	40 (35.7)	53 (49.1)	32 (27.4)	
≥ 30 years	57 (47.1)	72 (64.3)	55 (50.9)	85 (72.7)	
Race/ethnicity					0.66
Non-Hispanic White (ref)	95 (78.5)	94 (83.9)	86 (79.6)	91 (77.8)	
Others[1]	26 (21.5)	18 (16.1)	22 (20.4)	26 (22.2)	
Education					<0.0001
Some college or less	30 (24.8)	8 (7.1)	36 (33.3)	13 (11.1)	
College grad or high	91 (75.2)	104 (92.9)	72 (66.7)	104 (88.9)	
Employment					0.43
Unemployed	19 (15.7)	11 (9.8)	18 (16.7)	19 (16.2)	
Employed	102 (84.3)	101 (90.2)	90 (83.3)	98 (83.8)	
Parity					0.84
Nulliparous (ref)	59 (48.8)	58 (51.8)	51 (47.2)	61 (52.1)	
Primiparous	37 (30.6)	39 (33.9)	35 (32.4)	38 (32.5)	
Multiparous	25 (20.6)	16 (14.3)	22 (20.4)	18 (15.4)	
Smoking in the first trimester					0.06
No	106 (87.6)	98 (87.5)	89 (82.4)	108 (92.3)	
Yes	7 (5.8)	2 (1.8)	11 (10.2)	4 (3.4)	
Unknown	8 (6.6)	12 (10.7)	8 (7.4)	5 (4.3)	
Conception season					0.13
Winter	30 (24.8)	25 (22.3)	37 (34.3)	22 (18.8)	
Spring (ref)	32 (26.4)	35 (31.3)	29 (26.9)	32 (27.4)	
Summer	29 (24.0)	22 (19.6)	17 (15.7)	37 (31.6)	
Fall	30 (24.8)	30 (26.8)	25 (23.1)	26 (22.2)	
Mode of delivery					0.51
Vaginal delivery	97 (80.8)	78 (70.8)	77 (73.3)	80 (69.0)	
Scheduled C-section	12 (10.0)	16 (14.6)	15 (14.3)	17 (14.6)	
Emergency C-section	11 (9.2)	16 (14.6)	13 (12.4)	19 (16.4)	
Size-for-gestational age					0.04
Small-for-gestational age (SGA)	5 (4.9)	8 (8.3)	0 (0.0)	7 (7.0)	
Appropriate-for-gestational age (AGA)	85 (82.5)	71 (74.0)	88 (88.0)	77 (77.0)	
Large-for-gestational age (LGA)	13 (12.6)	17 (17.7)	12 (12.0)	16 (16.0)	
	Median (min, max)	Median (min, max)	Median (min, max)	Median (min, max)	*P*[3]
Pre-pregnancy BMI (kg/m^2)	25.1 (17.5, 52.1)	23.2 (17.1, 41.0)	26.0 (18.2, 44.9)	24.0 (18.2, 48.8)	0.001
EPDS score	4.0 (0.0, 19.0)	4.0 (0.0, 15.0)	4.0 (0.0, 17.0)	4.0 (0.0, 19.0)	0.72
AHEI-2010 score	45.8 (25.6, 51.9)	59.7 (52.0, 75.5)	44.6 (30.7, 51.9)	59.6 (52.0, 76.2)	<0.0001

[1]Includes Hispanic white, non-Hispanic black, Asians, Native American or Alaska Natives, Native Hawaiians or Pacific Islanders, multiracial or others. [2]*P*-values from chi-squared or Fisher's Exact test. [3]*P*-values from Kruskal-Wallis test. Percentages may not add up to 100% due to missing. AHEI-2010, Alternative Healthy Eating Index 2010; BMI, body mass index; EPDS, Edinburgh Postnatal Depression Scale.

B.5.2. Maternal paraben biomarker concentrations

All women had detectable (above the LOD) urinary concentrations of methylparaben, while > 99% of women had detectable urinary levels of at least two paraben biomarkers

(**Table 22**). The order of median paraben biomarker concentrations was as follows: methylparaben > propylparaben > ethylparaben > butylparaben. Moderate-to-strong correlations were observed between methylparaben and propylparaben concentrations (*r* = 0.70) and between ethylparaben and methylparaben concentrations (*r* = 0.43; **Figure 16**). Median I-KIDS maternal urinary paraben biomarker concentrations were lower than those of same-aged women from NHANES during similar time periods (**Table 22**) (110, 111). Only maternal urinary ethylparaben concentrations significantly differed by newborn sex and maternal diet quality, where mothers who had females and better diet qualities had highest, while those who had males and worse diet qualities had lowest ethylparaben concentrations (**Table 23**).

Table 22. Urinary paraben biomarker concentrations.

	I-KIDS 2013-2018		NHANES 2013-2016
	Detectable	**Overall sample (n=458)**	**Women 18 – 40 years old (n=742)**
Biomarker	**% ≥ LOD**	**Median (25th, 75th percentile)**	**Median (25th, 75th percentile)**
Butylparaben	43.9	0.1 (<LOD, 0.3)	0.1 (0.1, 0.5)
Propylparaben	99.8	8.3 (2.2, 28.1)	15.4 (2.8, 68.7)
Ethylparaben	55.2	1.3 (<LOD, 7.5)	2.2 (0.7, 13.9)
Methylparaben	100.0	49.6 (18.2, 134.4)	86.2 (18.9, 263.7)

All reported concentrations in ng/mL are not corrected for specific gravity (I-KIDS) or creatinine (NHANES). I-KIDS, Illinois Kids Development Study; LOD, limit of detection; NHANES, National Health and Nutrition Examination Survey. LOD, Limit of detection (0.1 ng/mL for butylparaben and propylparaben, 1.0 ng/mL for ethylparaben and methylparaben).

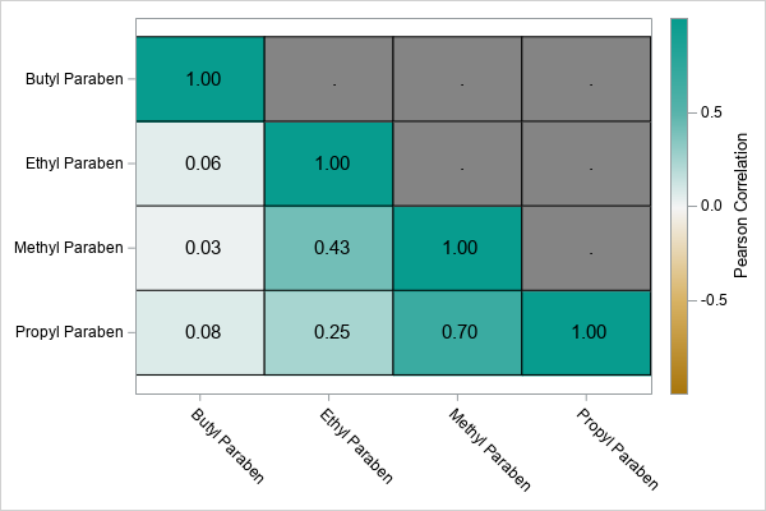

Figure 16. Pearson correlations between urinary specific gravity-adjusted paraben concentrations. Heat map presents Pearson correlation coefficients between butyl, ethyl, methyl, and propyl paraben concentrations. Yellow boxes represent negative correlations, while turquoise boxes represent positive correlations. Darker boxes represent stronger correlations (r closer to 1 or -1), while lighter boxes represent weaker correlations (r closer to 0).

Table 23. Maternal urinary specific-gravity adjusted paraben concentrations by newborn sex and maternal diet quality.

	Mothers of female newborns		Mothers of male newborns		
	Worse diet quality	**Better diet quality**	**Worse diet quality**	**Better diet quality**	***P*[1]**
Biomarker	**n = 121**	**n = 112**	**n = 108**	**n = 117**	
	Median (25th, 75th percentile), ng/mL				
Butyl paraben	0.1 (<LOD, 0.3)	0.1 (<LOD, 0.5)	0.1 (<LOD, 0.2)	0.1 (<LOD, 0.3)	0.05
Ethyl paraben	1.1 (<LOD, 5.6)	2.4 (<LOD, 13.8)	<LOD (<LOD, 3.6)	1.4 (<LOD, 8.5)	0.002
Methyl paraben	59.9 (16.2, 140.1)	51.1 (24.7, 148.6)	41.9 (16.5, 116.2)	49.0 (20.1, 131.6)	0.45
Propyl paraben	10.4 (1.6, 27.4)	10.4 (3.3, 39.5)	6.1 (2.2, 24.9)	9.9 (3.1, 24.6)	0.26

All reported concentrations are corrected for specific gravity. Limit of detection 0.1 ng/mL for butyl and propyl paraben, 1.0 ng/mL for ethyl and methyl paraben. [1]*P*-values from Kruskal Wallis test.

B.5.3. Distribution of gestational length, birth size, and head circumference

In the full sample, the distribution of gestational age at birth across clinical categories was as follows: 5% pre-term, 24% early term, 60% full term, and 11% late term. Around 5% of infants were born small-for-gestational age, 81% were born appropriate-for-gestational age, and 14% were born large-for-gestational age (**Table 21**). In the current analytic sample, women delivered at median 39 weeks gestation (min: 37, max: 42). Median (25th,

75th percentiles) birth weight, length, weight/length ratio, and head circumference were 3.5 kg (3.2, 3.8), 50.0 cm (48.3, 51.0), 70.2 g/cm (64.8, 75.3), and 34.9 cm (34.0, 35.8), respectively. Newborn females and males had similar mean gestational lengths, and newborns of mothers with worse diet qualities were born earlier (**Table 25**). Males appeared to have larger mean birth weight (before calculating z-scores), body length, weight/length ratio, and head circumference than females. Newborn females of mothers with worse diet qualities consistently had the smallest mean birth size and head circumference.

Table 25. Distribution of gestational length and newborn anthropometrics (n=458).

		Female newborns		Male newborns	
	n	**Mean (std)**	**n**	**Mean (std)**	***P*[1]**
Gestational length (wks)					0.02
Better diet quality	112	39.7 (1.0)	117	39.7 (1.1)	
Worse diet quality	121	39.5 (1.2)	108	39.2 (1.1)	
Birth weight (g)					0.004
Better diet quality	96	3452.7 (466.4)	100	3577.5 (430.0)	
Worse diet quality	103	3372.7 (402.2)	100	3569.7 (393.4)	
Birth weight z-score					0.48
Better diet quality	96	0.3 (1.1)	100	0.2 (0.9)	
Worse diet quality	103	0.1 (0.9)	100	0.3 (0.8)	
Body length (cm)					<0.0001
Better diet quality	101	49.4 (2.1)	110	50.6 (2.0)	
Worse diet quality	112	49.1 (1.9)	100	50.2 (2.1)	
Weight-to-length ratio (g/cm)					0.12
Better diet quality	95	69.9 (7.4)	100	70.7 (7.1)	
Worse diet quality	103	68.6 (6.5)	99	71.0 (6.5)	
Head circumference (cm)					<0.0001
Better diet quality	102	34.6 (1.3)	110	35.2 (1.3)	
Worse diet quality	112	34.5 (1.2)	101	35.2 (1.3)	

[1]*P*-value from Kruskal-Wallis test.

B.5.4. Sex-specific associations of paraben biomarkers with birth outcomes

Associations of maternal urinary paraben concentrations with birth outcomes in the full sample are presented in **Table 24**. When stratified by newborn sex and after adjusting for important confounders, associations of select parabens with birth weight, birth weight z-

scores, body length, and weight/length ratio appeared more prominent in females than males (**Table 26**). For example, in females, two-fold increases in maternal urinary methylparaben and propylparaben concentrations were associated with -46.61 g (95% CI: -74.70, -18.51) and -25.94 g (95% CI: -48.66, -3.21) lower birth weight, as well as -0.09 (95% CI: -0.15, -0.03) and -0.06 (95% CI: -0.11, -0.01) lower birth weight z-scores, respectively. Additionally, two-fold increases in maternal urinary methylparaben and propylparaben concentrations were associated with -0.21 cm (95% CI: -0.34, -0.07) and -0.15 cm (95% CI: -0.25, -0.04) shorter body length, respectively. We observed similar inverse association of methylparaben with weight/length ratio (β: -0.64 g/cm; 95% CI: -1.10, -0.19) and marginal inverse association of propylparaben with weight/length ratio (β: -0.33 g/cm; 95% CI: -0.70, 0.04) in females. In female, but not male newborns, we also observed a marginal inverse association between methylparaben and gestational length (β: -0.06 week; 95% CI: -0.13, 0.01), and a potentially meaningful inverse association between ethylparaben and birth weight (β: -20.96 g; 95% CI: -50.04, 8.12). After adjusting for gestational length, inverse associations of maternal urinary methylparaben and propylparaben with birth weight, body length, and weight/length ratio in female newborns remained, although the effect estimates were slightly reduced compared to models not accounting for gestational length (**Table 27**).

Table 24. Associations of urinary specific gravity-adjusted paraben concentrations with birth outcomes in the overall sample.

	Ethyl paraben	Methyl paraben	Propyl paraben
Birth outcome	**Δ (95% CI)**	**Δ (95% CI)**	**Δ (95% CI)**
Gestational length (wks)			
Unadjusted	-0.02 (-0.07, 0.03)	-0.03 (-0.08, 0.03)	0.00 (-0.04, 0.04)
Adjusted	-0.03 (-0.08, 0.02)	-0.03 (-0.09, 0.02)	-0.01 (-0.05, 0.03)
Birth weight (g)			
Unadjusted	**-19.83 (-41.22, 1.57)**	**-40.85 (-62.45, -19.24)**	**-27.43 (-44.28, -10.58)**
Adjusted	-7.69 (-28.14, 12.76)	**-27.80 (-48.70, -6.90)**	**-19.13 (-35.18, -3.08)**
Adjusted + gestational length	-5.47 (-24.65, 13.71)	**-22.23 (-41.92, -2.54)**	**-18.46 (-33.5, -3.43)**
Birth weight z-scores			
Unadjusted	**-0.04 (-0.09, 0.01)**	**-0.08 (-0.13, -0.04)**	**-0.06 (-0.10, -0.03)**
Adjusted	-0.02 (-0.06, 0.03)	**-0.06 (-0.10, -0.01)**	**-0.05 (-0.08, -0.01)**
Body length (cm)			
Unadjusted	-0.06 (-0.16, 0.04)	**-0.13 (-0.24, -0.03)**	**-0.11 (-0.19, -0.03)**
Adjusted	-0.03 (-0.13, 0.07)	**-0.09 (-0.19, 0.01)**	**-0.09 (-0.16, -0.01)**
Adjusted + gestational length	-0.01 (-0.11, 0.08)	-0.08 (-0.17, 0.02)	**-0.08 (-0.16, -0.01)**
Weight-to-length ratio (g/cm)			
Unadjusted	**-0.31 (-0.65, 0.04)**	-0.65 (-0.99, -0.30)	**-0.40 (-0.68, -0.13)**
Adjusted	-0.12 (-0.45, 0.21)	**-0.44 (-0.78, -0.10)**	**-0.27 (-0.53, -0.01)**
Adjusted + gestational length	-0.09 (-0.4, 0.23)	**-0.36 (-0.68, -0.04)**	**-0.26 (-0.51, -0.02)**
Head circumference (cm)			
Unadjusted	**-0.06 (-0.13, 0.01)**	**-0.07 (-0.14, -0.01)**	**-0.05 (-0.10, 0.00)**
Adjusted	-0.03 (-0.09, 0.03)	-0.03 (-0.09, 0.04)	-0.02 (-0.07, 0.03)
Adjusted + gestational length	-0.02 (-0.08, 0.04)	-0.02 (-0.08, 0.05)	-0.02 (-0.07, 0.03)

Data from linear regression models are presented as Δ (95% CI) in birth outcome for every 2-fold increase in urinary ethyl, methyl, and propyl paraben concentrations. Adjusted models include age (<30 (ref), ≥30 years), race/ethnicity (non-Hispanic white (ref), others), parity (nulliparous (ref), primiparous, multiparous), pre-pregnancy body mass index (continuous), conception season (winter, spring (ref), summer, fall), depression (continuous), fetal sex (male (ref), female), and average pregnancy diet quality (continuous). Bold indicate potentially meaningful findings. CI, confidence interval; EPDS, Edinburg Postnatal Depression Scale.

Table 26. Adjusted associations of urinary specific gravity-adjusted paraben concentrations with birth outcomes by newborn sex.

	Ethylparaben		
	Female newborn	**Male newborn**	P_{int}
	Δ (95% CI)	**Δ (95% CI)**	
Gestational length (wks)	-0.06 (-0.13, 0.01)	0.00 (-0.07, 0.08)	0.24
Birth weight (g)	**-20.96 (-50.04, 8.12)**	4.96 (-23.43, 33.35)	0.21
Birth weight z-scores	-0.05 (-0.11, 0.02)	0.01 (-0.05, 0.07)	0.20
Body length (cm)	-0.10 (-0.24, 0.04)	0.05 (-0.09, 0.18)	0.14
Weight/length ratio (g/cm)	-0.26 (-0.74, 0.21)	0.02 (-0.44, 0.48)	0.39
Head circumference (cm)	-0.03 (-0.12, 0.06)	-0.03 (-0.12, 0.06)	0.93
	Methylparaben		
	Female newborn	**Male newborn**	P_{int}
	Δ (95% CI)	**Δ (95% CI)**	
Gestational length (wks)	**-0.06 (-0.13, 0.01)**	0.00 (-0.08, 0.08)	0.30
Birth weight (g)	**-46.61 (-74.70, -18.51)**	-5.68 (-36.11, 24.75)	0.05
Birth weight z-scores	**-0.09 (-0.15, -0.03)**	-0.02 (-0.08, 0.05)	0.09
Body length (cm)	**-0.21 (-0.34, -0.07)**	0.04 (-0.1, 0.19)	0.01
Weight/length ratio (g/cm)	**-0.64 (-1.10, -0.19)**	-0.20 (-0.69, 0.30)	0.19
Head circumference (cm)	-0.03 (-0.11, 0.06)	-0.03 (-0.12, 0.07)	0.96
	Propylparaben		
	Female newborn	**Male newborn**	P_{int}
	Δ (95% CI)	**Δ (95% CI)**	
Gestational length (wks)	-0.01 (-0.07, 0.04)	0.00 (-0.06, 0.06)	0.80
Birth weight (g)	**-25.94 (-48.66, -3.21)**	-12.36 (-35.02, 10.3)	0.41
Birth weight z-scores	**-0.06 (-0.11, -0.01)**	-0.03 (-0.08, 0.02)	0.42
Body length (cm)	**-0.15 (-0.25, -0.04)**	-0.02 (-0.13, 0.09)	0.11
Weight/length ratio (g/cm)	**-0.33 (-0.70, 0.04)**	-0.22 (-0.58, 0.15)	0.68
Head circumference (cm)	-0.01 (-0.08, 0.06)	-0.03 (-0.10, 0.04)	0.70

Data from linear regression models are presented as Δ (95% CI) in birth outcome for every 2-fold increase in urinary ethylparaben, methylparaben, or propylparaben concentrations. Models account for age (<30 (ref), ≥30 years), race/ethnicity (non-Hispanic white (ref), others), parity (nulliparous (ref), primiparous, multiparous), pre-pregnancy body mass index (continuous), conception season (winter, spring (ref), summer, fall), depression (continuous), fetal sex (male (ref), female), the average pregnancy diet quality (continuous), and a multiplicative interaction between paraben and fetal sex (*P*-value for the interaction between parabens and newborn sex, P_{int}). Gestational length (female newborns n=233, male newborns n=225); birth weight and birth weight z-score (females newborns n=199, male newborns n=200); body length (female newborns n=213, male newborns n=210); weight/length ratio (female newborns n=198, male newborns n=199); head circumference (female newborns n=214, male newborns n=211). Bold indicate potentially meaningful findings. CI, confidence interval.

Table 27. Unadjusted and gestational length adjusted associations of urinary specific gravity-adjusted paraben concentrations with birth outcomes by newborn sex.

	Ethyl paraben		
	Δ (95% CI)	**Δ (95% CI)**	P_{int}
	Female newborn	**Male newborn**	
Gestational length (weeks)			
Unadjusted	-0.05 (-0.12, 0.03)	0.01 (-0.07, 0.08)	0.32
Birth weight (g)			
Unadjusted	**-31.29 (-61.45, -1.12)**	-2.18 (-31.75, 27.39)	0.18
Adjusted + gestational length	-16.49 (-43.79, 10.81)	5.01 (-21.62, 31.64)	0.27
Birth weight z-scores			
Unadjusted	**-0.07 (-0.14, 0.00)**	-0.01 (-0.07, 0.06)	0.17
Body length (cm)			
Unadjusted	-0.12 (-0.26, 0.02)	0.04 (-0.10, 0.18)	0.13
Adjusted + gestational length	-0.07 (-0.21, 0.06)	0.04 (-0.09, 0.18)	0.21
Weight/length ratio (g/cm)			
Unadjusted	**-0.46 (-0.95, 0.03)**	-0.10 (-0.58, 0.38)	0.30
Adjusted + gestational length	-0.2 (-0.65, 0.25)	0.02 (-0.42, 0.46)	0.49
Head circumference (cm)			
Unadjusted	-0.06 (-0.15, 0.03)	-0.04 (-0.13, 0.05)	0.83
Adjusted + gestational length	-0.02 (-0.10, 0.07)	-0.03 (-0.11, 0.06)	0.86
	Methyl paraben		
	Δ (95% CI)	**Δ (95% CI)**	P_{int}
	Female newborn	**Male newborn**	
Gestational length (weeks)			
Unadjusted	-0.05 (-0.12, 0.03)	-0.01 (-0.09, 0.07)	0.55
Birth weight (g)			
Unadjusted	**-54.61 (-83.92, -25.3)**	-20.87 (-51.77, 10.03)	0.12
Adjusted + gestational length	**-36.74 (-63.32, -10.16)**	-5.34 (-33.98, 23.30)	0.11
Birth weight z-scores			
Unadjusted	**-0.11 (-0.18, -0.05)**	-0.05 (-0.12, 0.02)	0.16
Body length (cm)			
Unadjusted	**-0.21 (-0.35, -0.08)**	0.00 (-0.15, 0.14)	0.03
Adjusted + gestational length	**-0.18 (-0.31, -0.05)**	0.04 (-0.1, 0.19)	0.02
Weight/length ratio (g/cm)			
Unadjusted	**-0.79 (-1.27, -0.32)**	**-0.44 (-0.94, 0.06)**	0.09
Adjusted + gestational length	**-0.5 (-0.94, -0.06)**	-0.19 (-0.66, 0.28)	0.34
Head circumference (cm)			
Unadjusted	-0.05 (-0.14, 0.04)	**-0.08 (-0.17, 0.02)**	0.64
Adjusted + gestational length	-0.01 (-0.09, 0.08)	-0.03 (-0.12, 0.06)	0.74

Table 27 (cont'd).

	Propyl Paraben		
	Δ (95% CI)	**Δ (95% CI)**	**P_{int}**
	Female newborn	**Male newborn**	
Gestational length (weeks)			
Unadjusted	0.00 (-0.06, 0.06)	0.00 (-0.06, 0.06)	0.97
Birth weight (g)			
Unadjusted	**-28.13 (-51.89, -4.37)**	**-22.87 (-46.19, 0.46)**	0.05
Adjusted + gestational length	**-24.83 (-46.11, -3.54)**	-12.14 (-33.36, 9.09)	0.41
Birth weight z-scores			
Unadjusted	**-0.07 (-0.12, -0.02)**	**-0.05 (-0.11, 0.00)**	0.71
Body length (cm)			
Unadjusted	**-0.14 (-0.25, -0.03)**	-0.04 (-0.15, 0.07)	0.22
Adjusted + gestational length	**-0.14 (-0.24, -0.04)**	-0.02 (-0.13, 0.08)	0.11
Weight/length ratio (g/cm)			
Unadjusted	**-0.38 (-0.77, 0.00)**	**-0.39 (-0.77, -0.01)**	0.98
Adjusted + gestational length	**-0.31 (-0.66, 0.04)**	-0.22 (-0.57, 0.13)	0.70
Head circumference (cm)			
Unadjusted	-0.02 (-0.09, 0.05)	**-0.06 (-0.13, 0.01)**	0.45
Adjusted + gestational length	-0.01 (-0.08, 0.06)	-0.03 (-0.10, 0.04)	0.66

Data from linear regression models are presented as Δ (95% CI) in birth outcome for every 2-fold increase in urinary ethyl, methyl, and propyl paraben concentrations. Adjusted models include age (<30 (ref), ≥30 years), race/ethnicity (non-Hispanic white (ref), others), parity (nulliparous (ref), primiparous, multiparous), pre-pregnancy body mass index (continuous), conception season (winter, spring (ref), summer, fall), depression (continuous), fetal sex (male (ref), female), average pregnancy diet quality (continuous), and a multiplicative interaction between paraben and fetal sex (*P*-value for the interaction, P_{int}). Bold indicate potentially meaningful findings. CI, confidence interval; EPDS, Edinburg Postnatal Depression Scale.

B.5.5. Differences in overall and sex-specific associations by early pregnancy maternal diet quality

Newborn sex-specific associations of paraben biomarkers with birth outcomes differed by maternal diet quality (**Figure 17**). Previously observed inverse associations of methylparaben and propylparaben with birth weight, birth weight z-scores, body length, and weight/length ratio in females were more prominent in females of women with poorer pregnancy diet (AHEI-2010 scores below the median) than in females of women with better pregnancy diet or male newborns. For example, in females of women with poorer diet quality, two-fold increases in methylparaben and propylparaben concentrations were associated with -60.96 g (95% CI: -96.97, -24.94) and -47.80 g (95% CI: -78.82, -16.78) lower birth weight (**Figure 17H, N**), as well as -0.12 (95% CI: -0.20, 0.04) and -0.10 (95%

CI: -0.17, -0.03) lower birth weight z-scores (**Figure 17I, O**), respectively. We observed similar, but marginal inverse associations of maternal urinary ethylparaben concentrations with birth weight (β: -33.26 g; 95% CI: -72.11, 5.60; **Figure 17B**) and birth weight z-scores (β: -0.07; 95% CI: -0.15, 0.02; **Figure 17C**) in females of mothers with worse diet quality. Additionally, in females of women with poorer diet quality, two-fold increases in maternal methylparaben and propylparaben concentrations were associated with -0.28 cm (95% CI: -0.45, -0.11) and -0.24 cm (95% CI: -0.38, -0.09) shorter body length (**Figure 17J, P**), respectively. We observed similar inverse associations of maternal urinary methylparaben (β: -0.84 g/cm; 95% CI: -1.42, -0.26) and propylparaben (β: -0.64 g/cm; 95% CI: -1.14, -0.13) with weight/length ratio in females of women with poorer diet quality (**Figure 17K, Q**). Additionally, in females, but not males, of mothers with worse diet quality, we observed marginal inverse associations of ethylparaben (β: -0.10 weeks; 95% CI: -0.20, 0.00) and methylparaben (β: -0.08 weeks; 95% CI: -0.17, 0.01) with gestational length (**Figure 17A, G**), as well as a marginal inverse association between propylparaben and head circumference (β: -0.08 cm; 95% CI: -0.17, 0.01; **Figure 17R**). Interestingly, in males of mothers with worse diet quality, two-fold increases in maternal urinary ethylparaben concentrations were marginally associated with -0.12 cm (95% CI: -0.25, 0.01) smaller head circumference (**Figure 17F**), whereas in male newborns of mothers with better diet quality, maternal urinary propylparaben concentrations were associated with -0.07 weeks (95% CI: -0.15, 0.01) shorter gestational length and -0.10 cm (95% CI: -0.19, 0.00) smaller head circumference (**Figure 17M, R**). After adjusting for gestational length, inverse associations of methylparaben and propylparaben with birth weight, body length, and weight/length ratio in females of

mothers with worse diet quality remained, but with slightly reduced effect estimates compared to models that did not account for gestational length (**Table 28**).

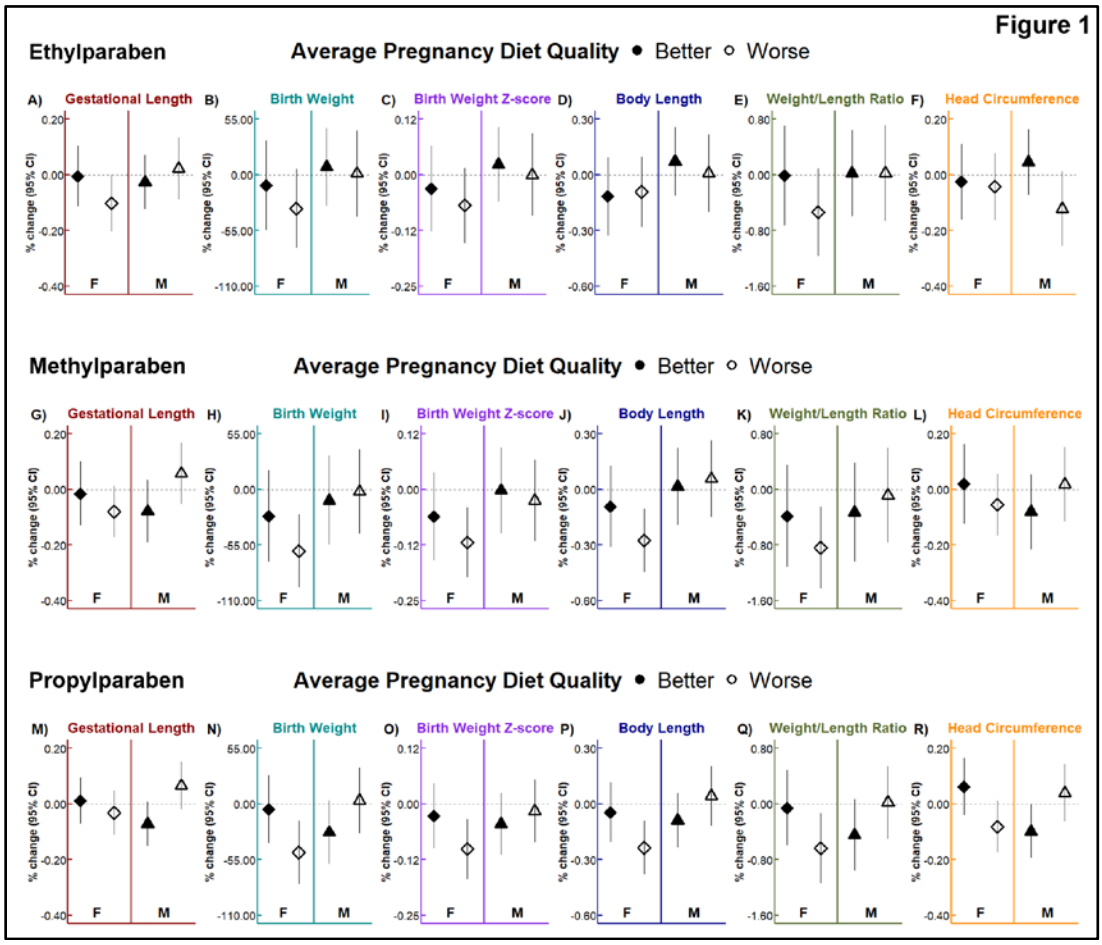

Figure 17. Adjusted sex-specific associations of urinary specific gravity-adjusted paraben concentrations with birth outcomes by maternal diet quality. Linear regression models accounted for age, race/ethnicity, parity, pre-pregnancy body mass index, conception season, depression, newborn sex, average pregnancy diet quality, and a three-way interaction (with all corresponding two-way interactions) between paraben, newborn sex, and diet quality. Worse and better diet quality are indicated by the empty and filled shapes, respectively. Data are presented as the change (shape) and 95% CI (vertical solid lines) in gestational length (wk), birth weight (g), birth weight z-score, body length (cm), weight/length ratio (g/cm), and head circumference (cm) for every 2-fold increase in maternal urinary ethylparaben (A, B, C, D, E, F), methylparaben (G, H, I, J, K, L), and propylparaben (M, N, O, P, Q, R) concentrations. Results are presented separately for female (F) and male (M) newborns. Sample sizes for each group can be found in Table 25. CI, confidence interval.

Table 28. Unadjusted and additionally adjusted newborn sex-specific associations of urinary specific gravity-adjusted paraben concentrations with birth outcomes by maternal diet quality.

	Ethyl paraben			Methyl paraben			Propyl Paraben		
	Δ (95% CI)	**Δ (95% CI)**	**Pint**	**Δ (95% CI)**	**Δ (95% CI)**	**Pint**	**Δ (95% CI)**	**Δ (95% CI)**	**Pint**
	Female newborn	**Male newborn**		**Female newborn**	**Male newborn**		**Female newborn**	**Male newborn**	
Gestational length (wks)									
Unadjusted			0.17			0.07			0.02
Better diet quality	-0.01 (-0.12, 0.10)	-0.03 (-0.13, 0.07)		-0.01 (-0.13, 0.11)	-0.09 (-0.21, 0.02)		0.02 (-0.06, 0.11)	**-0.08 (-0.16, 0.00)**	
Worse diet quality	**-0.10 (-0.20, 0.00)**	0.02 (-0.09, 0.14)		-0.07 (-0.16, 0.02)	0.05 (-0.06, 0.16)		-0.03 (-0.11, 0.05)	0.07 (-0.02, 0.16)	
Birth weight (g)									
Unadjusted			0.61			0.21			0.02
Better diet quality	-20.93 (-66.97, 25.12)	-2.02 (-42.14, 38.09)		-26.96 (-74.17, 20.24)	-26.20 (-71.34, 18.95)		-2.64 (-37.61, 32.33)	**-36.03 (-68.78, -3.29)**	
Worse diet quality	**-44.09 (-84.54, -3.63)**	-2.87 (-47.24, 41.5)		**-73.13 (-110.50, -35.75)**	-16.85 (-59.55, 25.84)		**-54.28 (-86.73, -21.83)**	-9.91 (-42.91, 23.08)	
Adjusted + gestational length			0.98			0.77			0.21
Better diet quality	-13.03 (-54.55, 28.49)	10.53 (-25.47, 46.52)		-24.07 (-66.69, 18.55)	1.57 (-39.98, 43.12)		-11.61 (-43.04, 19.81)	-16.19 (-46.12, 13.75)	
Worse diet quality	-24.08 (-60.64, 12.48)	0.44 (-39.52, 40.40)		**-46.94 (-81.11, -12.77)**	-9.70 (-49.26, 29.87)		**-41.1 (-70.37, -11.84)**	-6.51 (-36.98, 23.97)	
Birth weight z-scores									
Unadjusted			0.78			0.53			0.16
Better diet quality	-0.05 (-0.15, 0.05)	0.00 (-0.09, 0.09)		-0.06 (-0.16, 0.04)	-0.03 (-0.13, 0.07)		-0.02 (-0.10, 0.06)	-0.06 (-0.13, 0.01)	
Worse diet quality	**-0.09 (-0.18, 0.00)**	-0.01 (-0.11, 0.09)		**-0.15 (-0.23, -0.07)**	-0.06 (-0.15, 0.04)		**-0.12 (-0.19, -0.05)**	-0.05 (-0.12, 0.02)	
Body length (cm)									
Unadjusted			0.80			0.20			0.04
Better diet quality	-0.13 (-0.35, 0.08)	0.05 (-0.14, 0.24)		-0.08 (-0.30, 0.14)	-0.03 (-0.24, 0.18)		-0.03 (-0.19, 0.13)	-0.10 (-0.25, 0.05)	
Worse diet quality	-0.13 (-0.32, 0.06)	0.00 (-0.21, 0.21)		**-0.30 (-0.47, -0.13)**	0.01 (-0.19, 0.21)		**-0.25 (-0.40, -0.10)**	0.01 (-0.15, 0.16)	
Adjusted + gestational length			0.46			0.56			0.16
Better diet quality	-0.11 (-0.31, 0.09)	0.08 (-0.09, 0.26)		-0.08 (-0.29, 0.13)	0.06 (-0.14, 0.26)		-0.05 (-0.21, 0.10)	-0.04 (-0.19, 0.10)	
Worse diet quality	-0.05 (-0.23, 0.13)	0.00 (-0.20, 0.20)		**-0.24 (-0.40, -0.07)**	0.02 (-0.18, 0.22)		**-0.22 (-0.36, -0.08)**	0.00 (-0.15, 0.15)	
Weight/length ratio (g/cm)									
Unadjusted			0.41			0.22			0.05
Better diet quality	-0.22 (-0.97, 0.52)	-0.13 (-0.78, 0.52)		-0.42 (-1.19, 0.35)	-0.57 (-1.30, 0.17)		-0.04 (-0.61, 0.53)	**-0.58 (-1.11, -0.04)**	
Worse diet quality	**-0.72 (-1.37, -0.06)**	-0.04 (-0.76, 0.68)		**-1.05 (-1.65, -0.44)**	-0.32 (-1.01, 0.38)		**-0.75 (-1.27, -0.22)**	-0.19 (-0.73, 0.35)	
Adjusted + gestational length			0.65			0.71			0.30
Better diet quality	-0.05 (-0.73, 0.63)	0.06 (-0.53, 0.65)		-0.35 (-1.05, 0.35)	-0.15 (-0.83, 0.53)		-0.15 (-0.67, 0.37)	-0.27 (-0.76, 0.22)	
Worse diet quality	-0.40 (-1.00, 0.19)	0.01 (-0.65, 0.66)		**-0.64 (-1.20, -0.08)**	-0.19 (-0.84, 0.46)		**-0.54 (-1.02, -0.06)**	-0.13 (-0.63, 0.37)	
Head circumference (cm)									
Unadjusted			0.39			0.11			0.004
Better diet quality	-0.04 (-0.18, 0.09)	0.02 (-0.10, 0.14)		0.02 (-0.12, 0.17)	**-0.13 (-0.26, 0.01)**		0.07 (-0.03, 0.17)	**-0.11 (-0.21, -0.02)**	
Worse diet quality	-0.08 (-0.20, 0.05)	**-0.12 (-0.26, 0.01)**		-0.09 (-0.21, 0.02)	-0.03 (-0.17, 0.10)		**-0.11 (-0.20, -0.01)**	0.00 (-0.10, 0.10)	
Adjusted + gestational length			0.14			0.42			0.03
Better diet quality	-0.02 (-0.15, 0.11)	0.05 (-0.06, 0.17)		0.03 (-0.11, 0.17)	-0.05 (-0.18, 0.08)		0.06 (-0.04, 0.16)	-0.07 (-0.16, 0.02)	
Worse diet quality	-0.02 (-0.13, 0.10)	**-0.13 (-0.26, 0.00)**		-0.03 (-0.14, 0.08)	0.00 (-0.13, 0.12)		-0.07 (-0.16, 0.02)	0.01 (-0.09, 0.11)	

Data from linear regression models are presented as Δ (95% CI) in birth outcome for every 2-fold increase in maternal urinary ethyl, methyl, or propyl paraben concentrations. Adjusted models account for age (<30 (ref), ≥30 years), race/ethnicity (non-Hispanic white (ref), others), parity (nulliparous (ref), primiparous, multiparous), pre-pregnancy body mass index (continuous), conception season (winter, spring (ref), summer, fall), depression (continuous), fetal sex (male (ref), female), average pregnancy diet quality (continuous), and a three-way interaction (with all corresponding two-way interactions) between paraben, fetal sex, and diet quality (*P*-value for the interaction, P_{int}). Bold indicate potentially meaningful findings. CI, confidence interval; EPDS, Edinburgh Postnatal Depression Scale.

B.6. DISCUSSION

B.6.1. Summary of major findings

In this sample of pregnant women with lower paraben concentrations than U.S. reproductive-aged women, we observed that maternal urinary methylparaben and propylparaben concentrations were negatively associated with birth weight, birth weight z-scores, body length, and weight/length ratio in female, but not male newborns; these results persisted even after additionally adjusting for gestational length. Additionally, methylparaben was marginally negatively associated with gestational length in females, but not males, while parabens were not associated with head circumference in either sex. Furthermore, in females, negative associations of paraben biomarkers, especially methylparaben and propylparaben, with birth size measures and gestational length were more prominent in newborns of mothers with poorer diet quality. In males, some marginal inverse associations of propylparaben with gestational length and head circumference emerged if their mothers had a better diet quality. Overall, our results suggest that parabens are associated with birth size in a sex-specific manner and that diet quality could moderate this association. However, our diet- and sex-specific findings will need to be further corroborated in populations with higher prevalence of pre-term birth and small-for-gestational age newborns to determine whether intervening on maternal diet could be useful against paraben exposure in more at-risk populations.

B.6.2. Paraben biomarkers were associated with smaller birth size in female newborns

Birth weight, body length, and weight/length ratio are sex-specific outcomes that are

important determinants of child life-long health (389). Numerous studies suggest that newborn sex may influence susceptibility to environmental insults (390, 391), however studies evaluating sex-specific associations of paraben biomarkers with birth size have been inconsistent (365, 366). To our knowledge, no study has evaluated associations of parabens with weight/length ratio. Our results indicating that urinary concentrations of parabens, specifically methylparaben and propylparaben, were associated with lower birth weight and shorter body length in female newborns are consistent with some previous studies. For example, a study of 199 Taiwanese pregnant women showed that females of women with urinary methylparaben concentrations above the 75th percentile (compared to those with concentrations below the 75th percentile) had lower birth weight and shorter birth length (360). Two other studies from Iran (368) and China (359) observed that urinary maternal propylparaben and methylparaben concentrations, respectively, were negatively associated with body length in females. However, a study of 142 Belgian pregnant women observed that only ethylparaben concentrations measured in placental tissue were negatively associated with birth weight and body length in females (367). Additionally, some studies reported positive associations of paraben urinary biomarkers with birth weight in females (361, 392). Other studies only observed associations of paraben biomarkers with birth weight (134, 359, 362, 368, 393) or body length (361, 394) in males or reported null associations of paraben biomarkers with birth weight (394-400) and body length (392, 393, 395, 398) in both sexes. Although most studies approximated paraben exposure from maternal urine concentrations, the Belgian study quantified parabens from homogenized placental tissue, which could explain discrepancies in our findings. Parabens have been detected in cord blood (357), amniotic

fluid (258), and placental tissue (358), suggesting parabens can cross the placenta resulting in direct fetal exposure. However, in one study, maternal urinary paraben concentrations were only moderately positively correlated with concentrations in cord blood samples (401), and in another study, maternal urinary paraben concentrations were only weakly-to-moderately positively correlated with concentrations in amniotic fluid (402). Additionally, for other non-persistent chemicals, such as di(2-ethylhexyl) phthalate and bisphenol A, if urine samples are collected around delivery, the measured chemical concentrations could reflect exposure during the biospecimen collection procedure rather than throughout pregnancy (403). Therefore, it is important to consider the biological matrix and sample collection timing used for approximating paraben exposure. Discrepancies could also be due to differences in the number of urines collected for maternal urinary paraben biomarker assessment, the types of covariates included in statistical models, operationalization of birth size measures, and the study population. Overall, the current literature remains mixed with regards to sex-specific associations of parabens with birth outcomes.

Parabens, especially methylparaben and propylparaben, could be associated with reduced birth size through a variety of mechanisms, including hormonal, inflammatory, and metabolic pathways. In experimental animal and cell models, parabens disrupted hormonal pathways related to estrogens, androgens, and thyroid hormones by binding to hormone receptors (7). Similarly, studies in pregnant women reported that methylparaben and propylparaben were associated with altered testosterone and sex-hormone binding globulin concentrations (351, 404). *In vitro* and *in vivo* studies also observed that

parabens can disrupt inflammatory and oxidative stress pathways by altering mRNA levels of cytokines and disrupting immune cell activity (345, 348). Correspondingly, in pregnant women, methylparaben and propylparaben biomarker concentrations were found to be associated with altered concentrations of maternal blood cytokine and oxidative stress biomarker concentrations (346, 347). Furthermore, in experimental studies, parabens can disrupt adipocyte differentiation and metabolism (354), which can have critical implications for energy balance including glucose and lipid homeostasis (405). In pregnant women, parabens are associated with altered pregnancy glucose levels and gestational weight gain (375, 406). Sexually dimorphic responses of these pathways to paraben exposures could exist, since hormonal, inflammatory, and metabolic milieus differ in women carrying females versus males (407, 408). Given the sexual dimorphism of the placenta (409), it may also contribute to sex differences in the response to parabens. However, it remains unclear why for some studies (like ours) associations were primarily observed in female newborns, while in other studies, associations were only observed in male newborns.

B.6.3. Paraben biomarkers were not associated with gestational length or head circumference

Gestational age at delivery is an important determinant of neonatal morbidity and mortality (335, 410). In our study, we only observed a marginal negative association of maternal urinary methylparaben with gestational length in female newborns. However, some previous studies observed significant associations of paraben biomarkers with gestational length, many of which dichotomized gestational length at 37 weeks gestation to evaluate

associations of maternal urinary paraben concentrations with the odds of pre-term birth (363, 399, 411). These studies were conducted in populations with high pre-term birth prevalence (greater than 10%), while in our study, only 5% of newborns were born pre-term, making us unable to effectively evaluate associations of parabens with clinically-relevant markers of gestational length.

Head circumference is correlated with newborn brain volume (342). In our study, paraben biomarkers were not meaningfully associated with head circumference in female or male newborns, which was consistent with studies from the U.S. (395, 397) and France (393, 398). However, several other studies reported significant but inconsistent sex-specific associations of paraben biomarkers with head circumference (360, 361, 367, 368, 392). Results from these studies suggest that sex-specific associations of parabens with head circumference may be trimester-specific, which may explain why we did not observe associations by measuring paraben biomarkers in a pool of five first-morning urines collected across pregnancy. Most studies that observed significant associations of parabens with head circumference only collected one spot urine (generally in the first or third trimesters) or quantified parabens using another biological matrix (i.e., placental tissue), which may not accurately capture maternal paraben exposure during pregnancy (412). Therefore, findings for associations of parabens with head circumference should be interpreted with caution.

B.6.4. Maternal diet quality during pregnancy moderated associations of parabens with birth outcomes

Maternal diet quality is an important modulator of pregnancy health, as well as fetal growth and development (241). In our study, we consistently observed more prominent inverse associations of paraben biomarkers with birth size measures and gestational length in females of mothers with poorer diets. This suggests that better maternal diets may attenuate some associations of parabens with birth outcomes in females. Maternal diet influences birth outcomes by interacting with inflammatory and metabolic pathways (242-244) – the same pathways parabens could also target. Diet is a known modulator of chronic inflammation and oxidative stress, and pregnant women with healthier dietary patterns consume more anti-inflammatory and antioxidant-rich foods that protect against adverse birth and child outcomes, including unfavorable birth size or shorter gestational length (244). Diet also has a major influence on metabolic pathways that are closely linked with inflammation, especially glucose and lipid homeostasis. Pregnant women with healthier dietary patterns tend to have more favorable gestational weight gain, body fat distribution, and metabolic profiles (i.e., levels of insulin, total cholesterol), which are also important determinants of fetal growth and development. Therefore, our results suggest that diet may be a stronger modulator of inflammatory and metabolic pathways than parabens and could offset some paraben-related disruptions of these pathways in the mother and/or placenta (243). Interestingly, in males of women with better pregnancy diet qualities, some marginal inverse associations of propylparaben with gestational length and head circumference emerged. As a result, we cannot rule out chance findings due to confounding by other healthy lifestyle behaviors or socioeconomic factors unaccounted

for in our models that may explain our results. For example, we were unable to account for maternal physical activity in pregnancy, which has been shown to be associated with appropriate birth size (413), may predict maternal paraben biomarker concentrations (414), and has some of the same anti-inflammatory and favorable metabolic effects during pregnancy as diet (415, 416).

B.6.5. Strengths and limitations

Our study has several limitations. First, using urinary paraben concentrations from a pooled sample as a proxy for pregnancy exposure to parabens limited our ability to evaluate trimester-specific associations of parabens with birth outcomes. Second, the AHEI-2010 includes food/nutrients predictive of chronic disease risk in the Nurses' Health Study and Health Professional Follow-Up Study, which are cohorts of non-pregnant women and men, respectively. Third, given most women in this study are non-Hispanic white and of higher socioeconomic status, our findings may not be generalizable to other diverse populations or populations with high prevalence of clinically relevant adverse birth outcomes, including pre-term birth and small-for-gestational age. Fourth, there may be residual confounding unaccounted for in our statistical models. For example, previous studies accounted for nutritional supplementation and smoking status when evaluating associations of parabens with birth outcomes. However, all I-KIDS women regularly took prenatal vitamins throughout pregnancy and inclusion of smoking status in our statistical models did not influence observed associations, since very few women smoked in the first trimester. Fifth, we did not evaluate associations of a paraben mixture with birth outcomes, although our main objectives only included three parabens, and the general

recommendation in the field is to include more than three compounds for practical interpretation. Additionally, because there is limited data on the mechanisms of actions of each individual paraben on birth outcomes, it is important to first understand how each paraben is associated with birth outcomes. Lastly, we were likely underpowered to detect certain newborn sex- and diet-specific associations between parabens and birth outcomes.

Our study also has some important strengths. First, using a pooled sample of five first morning urines for quantification of nonpersistent chemicals reduces exposure measurement error, provides a more stable measure of mean gestational exposure, and may, in fact, be a better reflection of exposure at any given timepoint during pregnancy (175, 417). Second, using a diet quality index like the AHEI-2010 accounts for dietary patterns and the interactive effects of foods and nutrients, and the individual food and nutrient components of this index have been shown to be associated with birth size (377-380). Third, we used *a priori* consideration and previous literature to make informed decisions about covariate selection. Finally, our findings contribute information about environmental risk factors for birth outcomes in a sample with low pre-term birth and small-for-gestational age prevalence that is ubiquitously exposed to parabens but is heterogenous with regards to diet quality.

B.7. CONCLUSIONS

To our knowledge, this is the first study to evaluate whether associations between urinary paraben biomarkers and birth outcomes differ by maternal diet quality, as measured by a

diet quality index that accounts for the interactive effects of foods and nutrients. Our study suggests that parabens have sex-specific impacts on birth size independent of gestational length, and that maternal diet quality could attenuate these associations in a sex-specific manner. Our findings corroborate those from previous studies also showing that associations of paraben biomarkers with birth outcomes differ by newborn sex, although sex-specific associations differ across studies. Future studies in experimental models and pregnant women can help elucidate the sex-specific mechanisms and biological pathways by which parabens impact birth size, as well as the gestational windows most vulnerable to paraben exposure. Additionally, given that women are exposed to many environmental pollutants that may interact with parabens, future research could consider evaluating mixtures of chemicals, including parabens, with gestational length, birth size measures, and head circumference. Most importantly, even if women cannot reduce their exposure to parabens, our study informs how diet quality could be a potential modifiable factor that moderates the relationship between parabens and birth outcomes.

APPENDIX C: ASSOCIATIONS OF PREGNANCY HISTORY WITH BMI AND WEIGHT GAIN IN 45-54-YEAR-OLD WOMEN

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C.1. ABSTRACT

Midlife women have a higher risk of cardiometabolic disease than younger women, but the lifelong biological/lifestyle factors responsible for this increase are unclear. We investigated whether pregnancy history is a risk factor for midlife overweight/obesity and evaluated potential hormonal mechanisms. The Baltimore Midlife Women's Health Study, a prospective cohort, recruited 772 women aged 45–54 y. Women reported pregnancy characteristics via questionnaires, trained staff measured weight/height to calculate midlife BMI, and serum hormones were assessed by ELISA. Logistic regression models assessed associations of pregnancy history with risk of midlife overweight/obesity and BMI gain since age 18. We additionally explored whether associations differed by menopausal status, and whether midlife hormones mediated relationships of pregnancy history and midlife BMI. These premenopausal or perimenopausal women were 66% Caucasian/White and 30% African American/Black, with a median of 2 live births (range: 0–11) and median age at first birth of 27 y (range: 12–46 y). Women with 0 and ≥2 live births had lower odds of overweight/obesity than those with 1 birth (OR = 0.47; 95% CI: 0.23, 0.96; P = 0.04, and OR = 0.58; 95% CI: 0.35, 0.95; P = 0.03, respectively). Women

with ≥2 live births also had lower odds of BMI gain than those with 1 birth (OR = 0.66; 95% CI: 0.41, 1.06; P = 0.08). Furthermore, women who were older at their first birth had lower odds of overweight/obesity (OR = 0.96; 95% CI: 0.92, 1.00; P = 0.03) and BMI gain (OR = 0.97; 95% CI: 0.93, 1.00; P = 0.06). Number of pregnancies and age at last pregnancy were not associated with midlife overweight/obesity or BMI gain. Associations did not differ by menopausal status and were not explained by midlife hormones. Earlier childbirth and having 1 child increased women's risk of midlife overweight/obesity and BMI gain since age 18. Additional studies should focus on women's childbearing years as a critical determinant of midlife metabolic health.

C.2. KEYWORDS

Pregnancy; obesity; menopause; hormones; weight accumulation.

C.3. INTRODUCTION

In 2017, 72% of U.S. adults were overweight or obese, and 40% were obese (418), which exceeded the global 2015-2016 prevalence (419). Specifically, 44.7% of 40-59 year-old U.S. women were obese, which was higher than younger women and men (420). This is concerning because mid-life obesity in women is a major risk factor for many chronic diseases, including type-2 diabetes mellitus (421), stroke (422), depression (423), cardiovascular disease (CVD) (424), Alzheimer's Disease (425), and hormonally-driven cancers – including breast and endometrial cancers (426). Some of these deleterious health outcomes are likely directly caused by the menopausal transition (peri-menopause), which occurs naturally when women are 49-52 years of age (on average)

(427). Peri-menopause is characterized by the depletion of ovarian follicles and alterations in reproductive hormone concentrations (428-430), leading to rapid fat mass accumulation at central sites, which is an important predictor of cardiometabolic diseases (431, 432). For example, in a diverse cohort of U.S. pre- and peri-menopausal women, increased androgen (433, 434) and decreased estradiol concentrations (433) were associated with higher risk of obesity and other CVD-related risk factors. Given that obesity is an important predictor of various chronic diseases, it is likely that women who enter menopause being overweight or obese are at an even greater risk of menopause-driven cardiometabolic disruptions.

Another important consequence of being obese prior to and during menopause is that obese women appear to have more severe menopausal characteristics and symptoms. A large meta-analysis suggested that overweight/obese pre-menopausal women enter natural menopause at an older age than normal weight women (435). Interestingly, whereas this delay in menopause can protect against certain diseases, including CVD (436), delayed menopause is associated with increased risk of breast cancer (437). Compared with normal-weight women, obese women undergoing the menopausal transition are also more likely to experience exacerbated menopausal symptoms, including urogenital and vasomotor symptoms (438, 439), which we previously showed could be related to mid-life hormonal shifts in obese women (440). Overall, these results suggest that in addition to putting women at greater risk for cardiometabolic dysregulations, being overweight/obese can increase the severity of menopausal symptoms. Therefore, it is essential to understand factors associated with elevated

obesity risk in middle-aged women.

Several studies in women of different menopausal stages suggest that pregnancy and childbirth can additionally modify a woman's risk of mid-life obesity. In a representative sample of Korean post-menopausal women, younger age at first birth and higher parity (giving birth to more children) were associated with increased risk of general and abdominal obesity after menopause (441). Similarly, in a representative sample of retired U.S. women, higher parity was also associated with higher BMI (442). Although other studies have evaluated associations of pregnancy history with mid-life obesity in pre-, peri-, and post-menopausal women separately (442-447), it is unclear whether observed associations would differ by menopausal status. The menopausal transition is associated with hormonal and metabolic shifts, so our first aim was to assess relations between pregnancy history and mid-life overweight/obesity and determine whether these associations differ between pre- and peri-menopausal women. Because few studies have assessed whether pregnancy history influences life-long weight accumulation, our second aim was to assess associations of pregnancy history with change in obesity status since age 18. Lastly, pregnancy is also characterized by numerous hormonal and metabolic shifts that can persist into mid-life and influence mid-life obesity risk (448). Therefore, our third aim was to evaluate whether adulthood estradiol, testosterone, or progesterone mediate associations between pregnancy history and mid-life BMI.

C.4. METHODS

C.4.1. Participant recruitment and selection

This study consisted of a secondary data analysis of the Midlife Women's Health Study (MWHS), a prospective cohort in the Baltimore metropolitan area evaluating risk factors of hot flashes in 45-54 year-old women from 2006 until 2015. No part of the MWHS was a clinical trial, and study methodology is fully described elsewhere (449). Briefly, 45-54 year-old women were invited to participate in the study via mail, and interested women contacted the clinic to determine eligibility. Eligible women were 45-54 years-old, had intact ovaries and uteri, and were pre- or peri-menopausal. Pre-menopausal women were those who experienced their last menstrual period within the past three months and reported ≥11 menstrual periods within the past year. Women were characterized as peri-menopausal if they either 1) experienced their last menstrual period within the past year, but not within the past three months or 2) experienced their last menstrual period within the past three months, but reported ≤10 menstrual periods within the past year. Women were excluded if they were pregnant, taking hormone replacement therapy/hormonal contraceptives, had a history of ovarian or uterine cancer, or were postmenopausal (had not experienced a menstrual period within the past year). Ultimately, 772 pre- and peri-menopausal women enrolled into the study and provided written informed consent according to a procedure approved by the University of Illinois and Johns Hopkins University Institutional Review Boards.

C.4.2. Collection of demographic/lifestyle characteristics

Eligible MWHS participants came for a baseline visit at a Johns Hopkins clinical site to report important demographic/lifestyle characteristics, including those outlined in **Table 29**. Women's race/ethnicity was determined using the question: "What is your main ethnic/racial background? Mark only one; Answer = Caucasian/White, African American/Black, Hispanic/Latino, Asian, or Other". Women also reported the highest grade or year of schooling they completed, their current employment status, current marital status, and total household income during the year prior to study enrollment. To measure mid-life physical activity, women answered the question: "In comparison with others my own age, I think my physical activity leisure time is…; Answer = much more, more, as much, less, or much less". The question "In the last 12 months have you had at least 12 drinks of any kind of alcoholic beverage?; Answer = yes, no" evaluated a woman's alcohol consumption status, whereas smoking status was categorized as "never, former, or current smoker" using the questions "Have you ever smoked cigarettes?" and "Do you still smoke cigarettes?". Women also answered whether their mother smoked cigarettes while pregnant with them (Answer = yes, no, or don't know). Women reported the number of years they used oral contraceptives, and presence of fertility problems was assessed by asking "Did you ever seek medical consultation because of difficulty in getting pregnant?; Answer = yes, no". Self-reported weight at age 18 and mid-life height were used to calculate BMI at age 18.

Table 29. Demographic or lifestyle characteristics of women in the Mid-Life Women's Health Study (n=768).

Demographic & lifestyle characteristic	n (%)[#]
Age	
45-49 years	501 (65.2)
50-54 years	267 (34.8)
Menopausal status*	
Pre-menopausal	496 (64.6)
Peri-menopausal	272 (35.4)
Race/Ethnicity*	
Caucasian/White	503 (65.5)
African American/Black	233 (30.3)
Other	30 (3.9)
Annual household income	
< $40,000	176 (22.9)
$40,000 - $99,999	250 (32.6)
≥ $100,000	321 (41.8)
Education level	
Some college or less	275 (35.8)
College graduate or higher	490 (63.8)
Employment status	
Employed	608 (79.2)
Full-Time student or homemaker	72 (9.4)
Unemployed/retired/medical leave/disability	86 (11.2)
Marital status	
Single	140 (18.2)
Married/living with partner	499 (65.0)
Widowed/divorced/separated	126 (16.4)
Physical activity compared to others[#]	
Less than others	255 (33.2)
As much as others	240 (31.3)
More than others	264 (34.4)
Alcohol consumption during past year	
Yes	500 (65.1)
No	128 (16.7)
Participant smoking status	
Current smoker	78 (10.2)
Former smoker	269 (35.0)
Never smoked	420 (54.7)
Maternal smoking status during pregnancy*	
Yes	184 (24.0)
No	436 (56.8)
Don't know	137 (17.8)
Medical consultation for fertility problems*	
Yes	142 (18.5)
No	621 (80.9)
Oral contraceptive use*	
Never	113 (14.7)
<1 year	106 (13.8)
1-4 years	203 (26.4)
5-10 years	183 (23.8)
>10 years	159 (20.7)

Table 29 (cont'd).

Demographic & lifestyle characteristic	n (%)[#]
BMI at 18 years of age (kg/m^2)*	
Underweight (<18.5)	132 (17.2)
Normal weight (18.5-24.9)	531 (69.1)
Overweight (25-29.9)	65 (8.5)
Obese (≥30)	32 (4.2)
BMI at 45-54 years of age (kg/m^2)	
Underweight (<18.5)	11 (1.4)
Normal weight (18.5-24.9)	291 (37.9)
Overweight (25-29.9)	208 (27.1)
Obese (≥30)	258 (33.6)
Change in BMI from age 18 to 45-54	
Remained under/normal weight	294 (38.3)
Became overweight/obese	369 (48.1)
Became under/normal weight	5 (0.7)
Remained overweight/obese	92 (12.0)

*Variables selected as potential confounders in logistic and linear regression models based on a directed acyclic graph. BMI, body mass index. [#]May not add up to 100% due to missing data.

C.4.3. Collection of pregnancy history

At baseline, women also answered questions about gravidity (number of pregnancies), parity (number of live births), and ages at first birth and last pregnancy. Gravidity and parity were reported as counts and categorized as zero pregnancies/births, one pregnancy/birth, and two-or-more pregnancies/births. Ages at first birth and last pregnancy were reported in years and assessed as continuous variables.

C.4.4. Collection of anthropometrics

Weight and height were measured without shoes by trained clinic staff and values were rounded to the nearest 0.5 pound and 0.5 inch, respectively. We calculated BMI (kg/m^2) using weight (kilograms) and height (meters) and categorized BMI as under-/normal weight (BMI < 25) or overweight/obese (BMI ≥ 25) (450). We also categorized women by their BMI at ages 18 and 45-54 as follows: women who remained normal weight through age 45-54 (under-/normal weight at ages 18 and 45-54), women who became

overweight/obese by age 45-54 (under-/normal weight at age 18 but overweight/obese at age 45-54), women who became normal weight by age 45-54 (overweight/obese at age 18 but under-/normal weight at age 45-54), and those who remained overweight/obese through age 45-54 (overweight/obese at ages 18 and 45-54).

C.4.5. Assessment of mid-life hormones

Hormone analyses are described in detail elsewhere (449). Briefly, fasting morning blood samples were collected at baseline and once per week for four consecutive weeks to assess serum estradiol, testosterone, and progesterone concentrations at all phases of the menstrual cycle (averaged across the four visits). Participants were compensated after each clinic visit for their time and travel to the clinic (449). Hormone concentrations were assessed using commercially available and previously validated ELISA (DRG, Springfield, New Jersey, USA) according to manufacturers' instructions (440). Mean values of duplicates were used in analyses. Average intra- and inter-assay coefficients of variation were <5% (449, 451). Limits of detection (LOD) for each hormone were as follows: estradiol = 9.71 pg/mL; testosterone = 0.08 ng/mL; progesterone = 0.05 ng/mL (452). Values less than the LOD were assigned the LOD for that hormone.

C.4.6. Statistical analysis

Three women were excluded from the analysis because of missing information about pregnancy history, baseline weight/height, and/or progesterone concentrations. Another woman was excluded for having extreme testosterone concentrations across all visits. Therefore, 768 pre- and peri-menopausal women were available to assess associations

of pregnancy history with mid-life BMI. Binary logistic regression models assessed whether pregnancy history increased or decreased the probability of being overweight/obese compared with being under-/normal weight. To assess whether relations were linear across BMI, we also assessed the associations between pregnancy history and continuous mid-life BMI using linear regression. In linear regression models, mid-life BMI was natural log-transformed to meet normality assumptions.

To evaluate whether pregnancy history was associated with a "shift" in BMI status from age 18 to 45-54 (as described above), we aimed to assess whether pregnancy history was associated with changes in BMI across a woman's reproductive window (from under-/normal weight to overweight/obese or from overweight/obese to under-/normal weight). Only five women who were overweight/obese at age 18 became under/normal weight at age 45-54, so we ultimately only evaluated associations between pregnancy history and the risk of becoming overweight/obese at 45-54 years of age. We did not compare women who were overweight/obese at age 18 and remained overweight/obese at age 45-54 with other groups because these women were obese prior to being pregnant, which would not allow us to ask whether pregnancy history was the cause of their overweight/obesity. Furthermore, eight women did not report their weight at age 18, thus 663 women were available for assessing these associations in final binary logistic regression models. In sensitivity analyses, we examined associations between pregnancy history and BMI gain since age 18 but excluded women whose first birth occurred before age 18 in order to evaluate whether pregnancy history was the potential cause of weight gain/loss since age 18, specifically.

Data are presented before and after we controlled for potential confounders selected using previous literature that informed a directed acyclic graph (453), which was similar to the one published by Pirkle *et al*., 2014 (454). Final covariate-adjusted models included race, maternal smoking status, fertility problems, oral contraceptive use, menopausal status, and BMI at age 18 (except where we assessed BMI gain as our outcome). Associations of gravidity and parity with mid-life BMI or BMI gain were additionally adjusted for age at first birth (only in women who had ever given birth), because age at first birth could impact the number of pregnancies and births a woman could have. Similarly, associations of ages at first birth and last pregnancy with mid-life BMI or BMI gain since age 18 were additionally adjusted for parity (for age at first birth) and gravidity (for age at last pregnancy).

To understand whether hormones partially explained (mediated) the proposed relations between pregnancy history and mid-life BMI, we used a system of structural equations controlling for the same confounders mentioned above. Specifically, mediation analyses assessed the total effect (to evaluate the overall relationship between pregnancy history and mid-life overweight/obesity), the natural direct effect (to assess how much of the total relationship is explained by the direct relationship between pregnancy history → mid-life overweight/obesity) and natural indirect effect (to test how much of the total relationship is mediated through hormones: pregnancy history → hormone → mid-life overweight/obesity) (455). All hormone data were natural log-transformed to meet normality assumptions.

Because we hypothesized *a priori* that associations of pregnancy history with mid-life BMI or BMI gain since age 18 could differ between pre- and peri-menopausal women, we also assessed these associations separately by menopausal status. All associations were considered significant at $P<0.05$ using SAS 9.4 (version 14.3, SAS Institute), including PROC CAUSALMED to evaluate mediation.

C.5. RESULTS

C.5.1. Characteristics/pregnancy history of MWHS participants

At the baseline visit, the majority of women were 45-49 years old (65.2%), pre-menopausal (64.6%), Caucasian/White (65.5%), college graduates (63.8%), employed (79.2%), married or living with a partner (65.0%), and had annual household incomes of ≥$40,000 (74.4%) (**Table 29**). Physical activity "compared to others" was evenly distributed between "less than others" (33.2%), "as much as others" (31.3%), and "more than others" (34.4%). Most women (65.1%) reported consuming alcohol during the year before the study, whereas 54.7% reported never smoking, and 56.8% reported that their mother did not smoke while pregnant with them. Approximately 81% of women had never sought medical consultation for fertility problems, and 70.9% used oral contraceptives for at least one year (**Table 29**).

Most women had at least two pregnancies (76.8%) or live births (60.2%). The median (range) age at first birth was 27 (12-46) years, whereas the median (range) age at last pregnancy was 33 (14-53) years (**Table 30**). Although, 69.1% of women reported having a normal weight at age 18, the majority (60.7%) were overweight/obese at 45-54 years.

Around half of women did not change BMI categories since age 18, whereas 48.1% of women gained weight from age 18 to 45-54. Median (range) mid-life hormone concentrations were as follows: estradiol: 56.70 (9.71 - 434.52) pg/mL; testosterone: 0.29 (0.08 - 5.40) ng/mL; and progesterone: 1.60 (0.05 - 32.93) ng/mL.

Table 30. Pregnancy history of women in the Mid-Life Women's Health Study (n=768).

Pregnancy Characteristic	n (%) or Median (range)
Gravidity (number of pregnancies)	
Never pregnant	87 (11.3)
1 pregnancy	91 (11.9)
2 or more pregnancies	590 (76.8)
Parity (number of live births)	
Never pregnant	87 (11.3)
No live births	78 (10.2)
1 live birth	139 (18.1)
2 or more live births	462 (60.2)
Missing	2 (0.3)
Age at first birth (years)	27.0 (12.0 - 46.0)
Age at last pregnancy (years)	33.0 (14.0 - 53.0)
Data are presented as n (%) or median (range).	

C.5.2. Associations of pregnancy history with mid-life overweight/obesity

We first assessed whether pregnancy history was associated with BMI status at 45-54 years of age, where BMI was assessed in categories (logistic regression analysis) or linearly (linear regression analysis). We found that gravidity was not associated with mid-life overweight/obesity in unadjusted or covariate-adjusted models (**Figure 18A**). After additionally adjusting for age at first birth, women with two-or-more pregnancies had 62% lower odds of overweight/obesity than women with one pregnancy (OR=0.38; 95%CI: 0.17, 0.83; *P*=0.02). The linear relationship between gravidity and mid-life BMI was attenuated compared to results from logistic models, where women with two-or-more pregnancies had a non-significant 4.9% lower BMI compared to those with one pregnancy

after adjusting for age at last pregnancy (β=-0.05; 95%CI: -0.11, 0.007; *P*=0.09; **Table 31**).

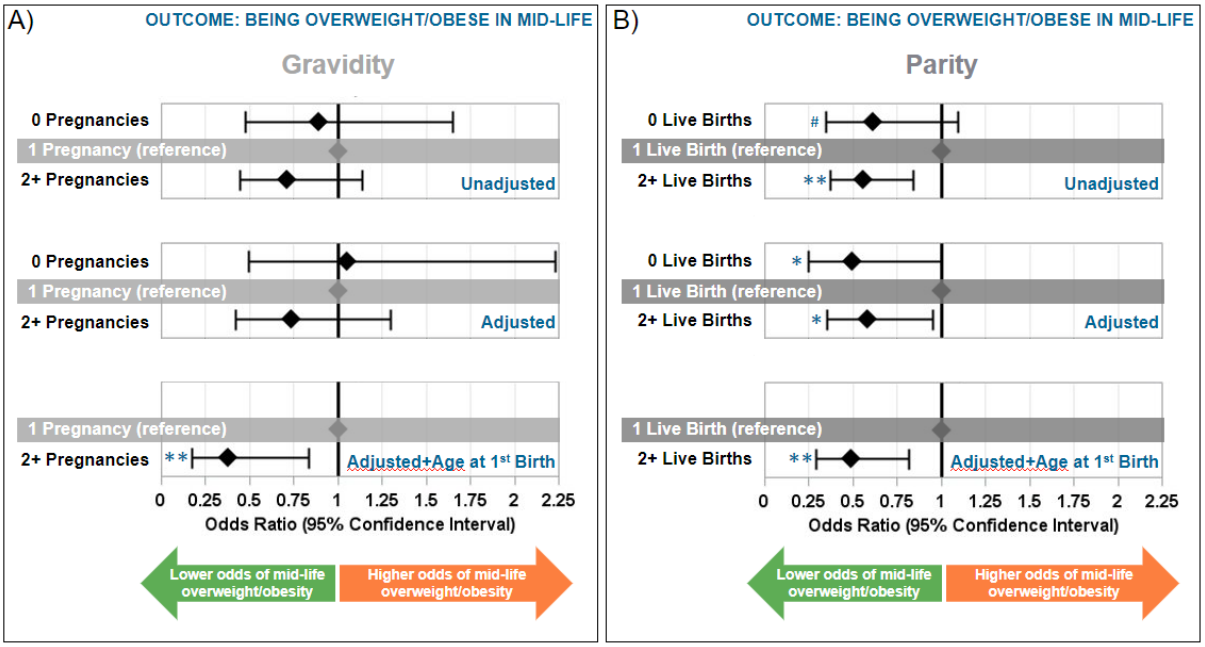

Figure 18. Associations of gravidity (number of pregnancies) and parity (number of live births) with mid-life overweight/obesity. Binary logistic regression models evaluated associations of A) gravidity and B) parity with the probability of being overweight/obese compared with under-/normal weight in mid-life. Results are expressed as odds ratio (filled diamond) and 95% confidence interval (solid lines) for the unadjusted, adjusted, and additionally adjusted models. Analyses control for race, maternal smoking status, fertility problems, oral contraceptive use, BMI at age 18, and menopausal status, and additionally control for age at first birth. Significant associations are those that do not cross the null (odds ratio=1.0) at $^{\#}P<0.10$, $^{*}P<0.05$, and $^{**}P<0.01$.

Compared with women who had one live birth, women who never gave birth and those who gave birth two-or-more times had lower odds of being overweight/obese by age 45-54 years (**Figure 18B**). In unadjusted models, women who gave birth two-or-more times had 44% lower odds of overweight/obesity than women who only gave birth once (OR=0.56; 95%CI: 0.37, 0.84; *P*=0.005), and women who had never given birth had 40% lower odds of overweight/obesity than women with one live birth, but this was marginally

non-significant (OR=0.60; 95%CI: 0.34, 1.07; *P*=0.09). After adjusting for potential confounders, women with zero or two-or-more live births had 53% (OR=0.47; 95%CI: 0.23, 0.96; *P*=0.04) and 42% (OR=0.58; 95%CI: 0.35, 0.95; *P*=0.03), respectively, lower odds of overweight/obesity than women who only gave birth once. Even after additionally adjusting for age at first birth, women with two-or-more live births had 51% (OR=0.49; 95%CI: 0.29, 0.82; *P*=0.007) lower odds of overweight/obesity than women with one birth. Linear regression models evaluating these associations differed somewhat from logistic regression analyses (**Table 31**). In the unadjusted linear model, women with two-or-more live births had 7.1% lower mid-life BMI compared with women with one birth (β=-0.07; 95%CI: -0.12, -0.03; *P*=0.002), but this was attenuated after adjusting for potential confounders and age at first birth. In linear regression models, mid-life BMI did not differ between women who never gave birth and those who gave birth once.

Table 31. Unadjusted and adjusted linear regression analyses evaluating associations between pregnancy history and natural log-transformed mid-life BMI.

	Model 1[a]		**Model 2[b]**			**Model 3[c]**	
	β (95% CI)	*P*-value	β (95% CI)	*P*-value	Modification by Menopause Status *P*-value[#]	β (95% CI)	*P*-value
Gravidity							
No pregnancies	-0.02 (-0.09, 0.05)	0.64	0.01 (-0.05, 0.07)	0.70	0.74		
1 pregnancy (reference)	**0**		**0**			**0**	
2+ pregnancies	-0.04 (-0.09, 0.01)	0.14	-0.02 (-0.06, 0.03)	0.47		-0.05 (-0.11, 0.007)	0.09
Parity							
No live births	-0.05 (-0.12, 0.01)	0.12	-0.04 (-0.09, 0.02)	0.19	0.62		
1 live birth (reference)	**0**		**0**			**0**	
2+ live births	-0.07 (-0.12, -0.03)	0.002	-0.03 (-0.06, 0.01)	0.15		-0.03 (-0.07, 0.004)	0.08
Age at first birth	-0.009 (-0.01, -0.005)	<0.0001	-0.002 (-0.005, 0.001)	0.21	0.33	-0.003 (-0.006, 0.000)	0.09
Age at last pregnancy	-0.004 (-0.007, -0.001)	0.01	-0.001 (-0.004, 0.002)	0.46	0.15	-0.001 (-0.003, 0.002)	0.70

β: beta-estimate; CI: confidence interval.
[a]Model 1: unadjusted model;
[b]Model 2: adjusted for race, maternal smoking status, fertility problems, oral contraceptive use, BMI at age 18, and menopause status;
[c]Model 3: model 2 additionally adjusted for age at first birth (gravidity and parity models), parity (age at first birth model), and gravidity (age at last pregnancy model).
[#]Model 2 assessed whether menopause status is an effect modifier of the association between pregnancy history and mid-life BMI.

The age at which women gave birth to their first child was associated with mid-life overweight/obesity in all logistic regression models (**Figure 19A**). In unadjusted models, each year increase in age at first birth was associated with 8% lower odds of mid-life overweight/obesity (OR=0.92; 95%CI: 0.90, 0.95; *P*<0.0001). After adjusting for potential confounders and additionally adjusting for parity, each year increase in age at first birth was associated with 4% (OR=0.96; 95%CI: 0.92, 1.00; *P*=0.03) and 6% (OR=0.94; 95%CI: 0.91, 0.98; *P*=0.005) respectively, lower odds of mid-life overweight/obesity. In unadjusted linear regression models (**Table 31**), each year increase in age at first birth was associated with a 0.9% decrease in mid-life BMI (β=-0.009; 95%CI: -0.01, -0.005; *P*<0.0001), which was attenuated after adjusting for confounders.

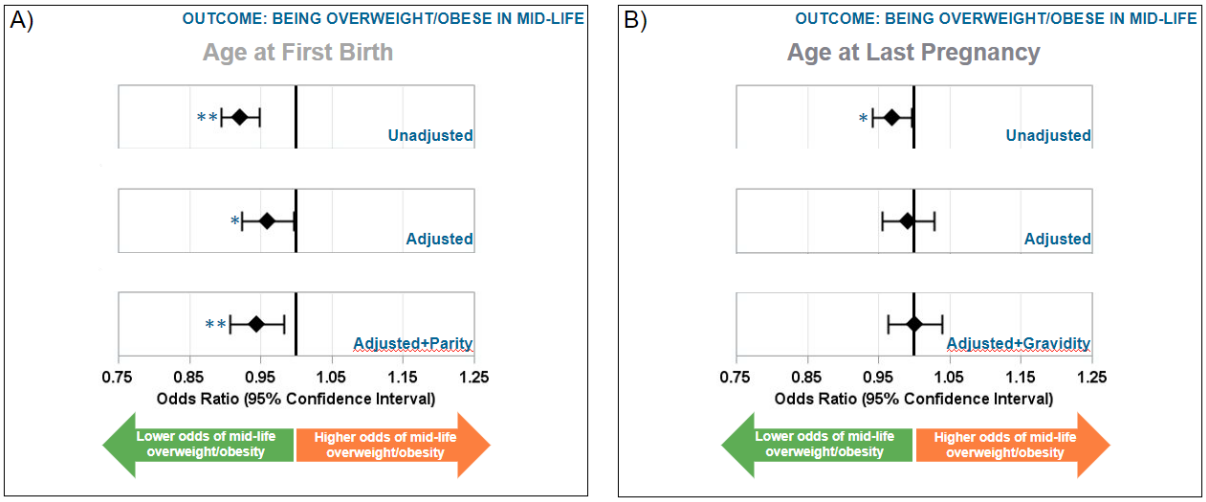

Figure 19. Associations of age at first birth and last pregnancy with mid-life overweight/obesity. Binary logistic regression models evaluated associations of age at A) first birth and B) last pregnancy with the probability of being overweight/obese compared to under/normal weight in mid-life. Results are expressed as odds ratio (filled diamond) and 95% confidence interval (solid lines) for the unadjusted, adjusted, and additionally adjusted models. Analyses control for race, maternal smoking status, fertility problems, oral contraceptive use, BMI at age 18, and menopausal status, and additionally control for parity (for age at first birth) or gravidity (for age at last pregnancy). Significant associations are those that do not cross the null (odds ratio=1.0) at $^{\#}P$<0.10, *P<0.05, and **P<0.01.

Age at last pregnancy was associated with mid-life overweight/obesity only in the unadjusted logistic regression model, such that every year increase in age at last pregnancy was associated with 3% lower odds of mid-life overweight/obesity (OR=0.97; 95%CI: 0.94, 1.00; *P*=0.03; **Figure 19B**). Similarly, only in the unadjusted linear regression model was age at last pregnancy associated with mid-life BMI, where every one year increase in age at last pregnancy was associated with 0.4% lower mid-life BMI (β=-0.004; 95%CI: -0.007, -0.001; *P*=0.01; **Table 31**). Associations between pregnancy history and mid-life overweight/obesity were not different by menopausal status (**data not shown**).

C.5.3. Associations of pregnancy history with BMI gain since age 18

Because we observed associations between pregnancy history and BMI status at age 45-54, we also wanted to ask whether pregnancy history was associated with BMI change (or more specifically, BMI gain) from age 18 to age 45-54. Our data suggest that parity, but not gravidity, was associated with BMI gain since age 18 (**Figure 20**). In the unadjusted model, compared with women who gave birth once, those with two-or-more births had 39% lower odds of becoming overweight/obese (OR=0.61; 95%CI: 0.40, 0.93; *P*=0.02; **Figure 20B**). This association was marginally non-significant after adjusting for confounders (OR=0.66; 95%CI: 0.41, 1.06; *P*=0.08). However, after additionally adjusting for age at first birth, women who gave birth two-or-more times had 43% lower odds of becoming overweight/obese in mid-life compared with women who only gave birth once (OR=0.57; 95%CI: 0.34, 0.94; *P*=0.03). BMI gain since age 18 in women who never gave birth was not significantly different from women who gave birth only once.

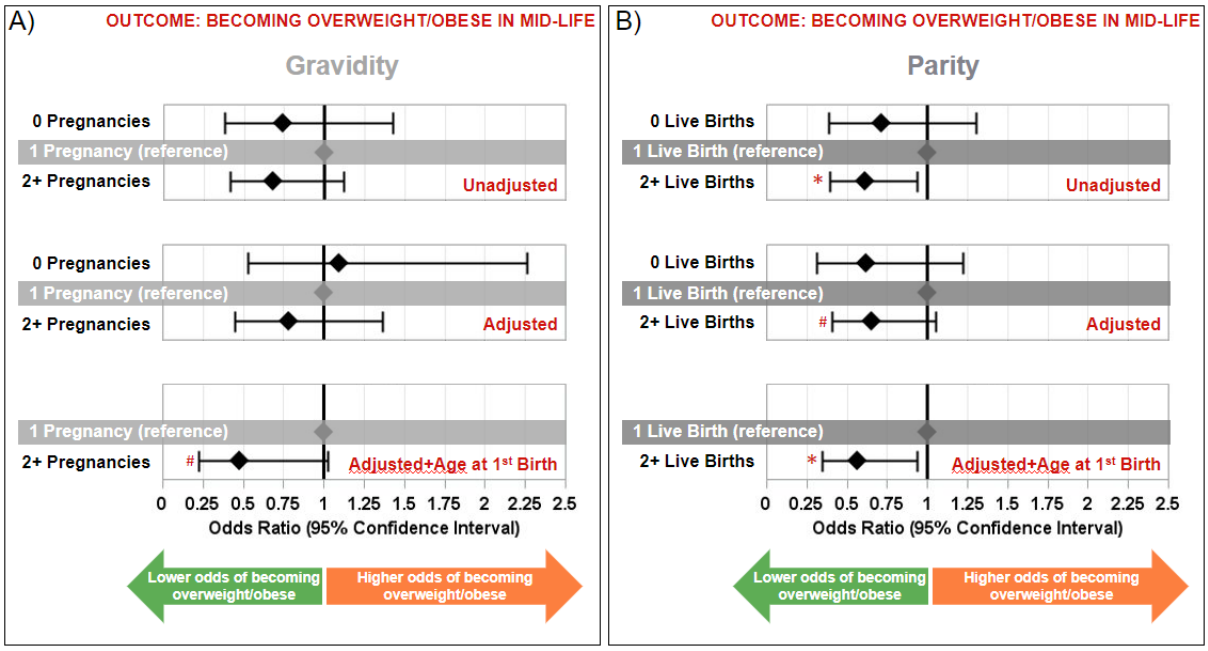

Figure 20. Associations of gravidity (number of pregnancies) and parity (number of live births) with becoming overweight/obese in mid-life. Binary logistic regression models evaluated associations of A) gravidity and B) parity with the probability of becoming overweight/obese compared with remaining under-/normal weight from age 18 to mid-life. Results are expressed as odds ratio (filled diamond) and 95% confidence interval (solid lines) for the unadjusted, adjusted, and additionally adjusted models. Models controlled for race, maternal smoking status, fertility problems, oral contraceptive use, BMI at age 18, and menopausal status, and additionally controlled for age at first birth. Significant associations are those that do not cross the null (odds ratio=1.0) at $^{\#}P<0.10$, $^{*}P<0.05$, and $^{**}P<0.01$.

Age at first birth was also associated with BMI gain since age 18 (**Figure 21A**). In unadjusted models, each year increase in age at first birth was associated with 8% lower odds of becoming overweight/obese in mid-life (OR=0.92; 95%CI: 0.89, 0.95; $P<0.0001$). After adjusting for confounders and additionally adjusting for parity, each year increase in age at first birth was associated with 3% (OR=0.97, 95%CI: 0.93, 1.00; $P=0.06$) and 5% (OR=0.95, 95%CI: 0.92, 0.99; $P=0.01$), respectively, lower odds of becoming overweight/obese in mid-life. Age at last pregnancy was only associated with BMI gain in the unadjusted model, where every year increase in age at last pregnancy was associated with 4% lower odds of becoming overweight/obese in mid-life (OR=0.96; 95%CI: 0.94,

0.99; *P*=0.02; **Figure 21B**). Associations between age at last pregnancy and BMI gain since age 18 were attenuated after adjusting for confounders and additionally adjusting for gravidity. Associations between pregnancy history and BMI gain since age 18 were not different by menopausal status (**data not shown**). All associations remained consistent in sensitivity analyses excluding women who gave birth before age 18 (**Table 32**).

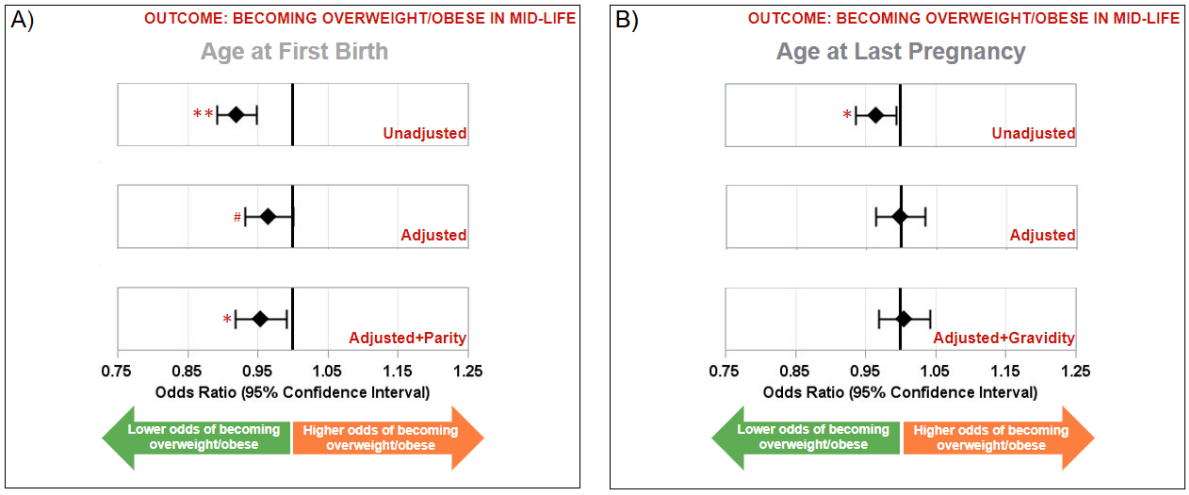

Figure 21. Associations of age at first birth and last pregnancy with becoming overweight/obesity in mid-life. Binary logistic regression models evaluated associations of age at A) first birth and B) last pregnancy with the probability of becoming overweight/obese compared with remaining under/normal weight from age 18 to mid-life. Results are expressed as odds ratio (filled diamond) and 95% confidence interval (solid lines) for the unadjusted, adjusted, and additionally adjusted models. Models controlled for race, maternal smoking status, fertility problems, oral contraceptive use, BMI at age 18, and menopausal status, and additionally controlled for parity (for age at first birth) or gravidity (for age at last pregnancy). Significant associations are those that do not cross the null (odds ratio=1.0) at $^{\#}P<0.10$, $^{*}P<0.05$, and $^{**}P<0.01$.

Table 32. Sensitivity analysis: unadjusted and adjusted logistic regression analyses modeling the probability of becoming overweight/obese (BMI≥25 kg/m^2) compared to remaining under/normal weight (BMI<25 kg/m^2) at 45-54 years of age (excluding women whose first birth occurred prior to age 18).

	Model 1[a]		Model 2[b]			Model 3[c]	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	Modification by Menopause Status *P*-value[#]	OR (95% CI)	*P*-value
Becoming overweight/obese versus remaining under/normal weight from age 18 to 45-54 years							
Gravidity							
No pregnancies	--	--	--	--	--	--	--
1 pregnancy (reference)	**1.00**		**1.00**			**1.00**	
2 or more pregnancies	0.55 (0.28, 1.10)	0.09	0.60 (0.28, 1.27)	0.18	0.37	0.56 (0.26, 1.19)	0.13
Parity							
No live births	--	--	--	--	--	--	--
1 live birth (reference)	**1.00**		**1.00**			**1.00**	
2 or more live births	0.60 (0.39, 0.92)	0.02	0.67 (0.41, 1.08)	0.10	0.99	0.59 (0.36, 0.96)	0.04
Age at first birth	0.93 (0.90, 0.96)	<0.0001	0.97 (0.93, 1.01)	0.09	0.52	0.96 (0.92, 1.00)	0.03
Age at last pregnancy	0.97 (0.94, 1.00)	0.04	1.01 (0.97, 1.04)	0.72	0.50	1.01 (0.98, 1.05)	0.53

OR: odds ratio; CI: confidence interval.
[a]Model 1: unadjusted model;
[b]Model 2: adjusted for race, maternal smoking status, fertility problems, oral contraceptive use, and menopause status;
[c]Model 3: model 2 additionally adjusted for age at first birth (gravidity and parity model), parity (age at first birth model), and gravidity (age at last pregnancy model).
[#]Model 2 assessed whether menopause status is an effect modifier of the association between pregnancy history and BMI gain since age 18.

C.5.4. Associations of pregnancy history and mid-life BMI mediated by mid-life reproductive hormones

We previously reported associations of mid-life hormones with mid-life BMI (440). Presently, we observed consistent associations of parity and age at first birth with both mid-life overweight/obesity and BMI gain since age 18. Therefore, we assessed whether mid-life estradiol, testosterone, or progesterone concentrations mediated these associations (**Figure 22**). While we observed that parity was significantly associated with mid-life overweight/obesity (natural direct effect), there was no mediation of the relationship between parity and mid-life overweight/obesity by estradiol, testosterone, or progesterone (natural indirect effect, **Figure 22A**). Similarly, age at first birth was also significantly associated with mid-life overweight/obesity (natural direct effect), but there was no mediation of the relationship between age at first birth and mid-life overweight/obesity by estradiol, testosterone, or progesterone (natural indirect effect, **Figure 22B**). Hormones also did not mediate these associations after concurrently adjusting for parity or age at first birth, and relationships in the mediation analyses were also not modified by menopausal status (**data not shown**).

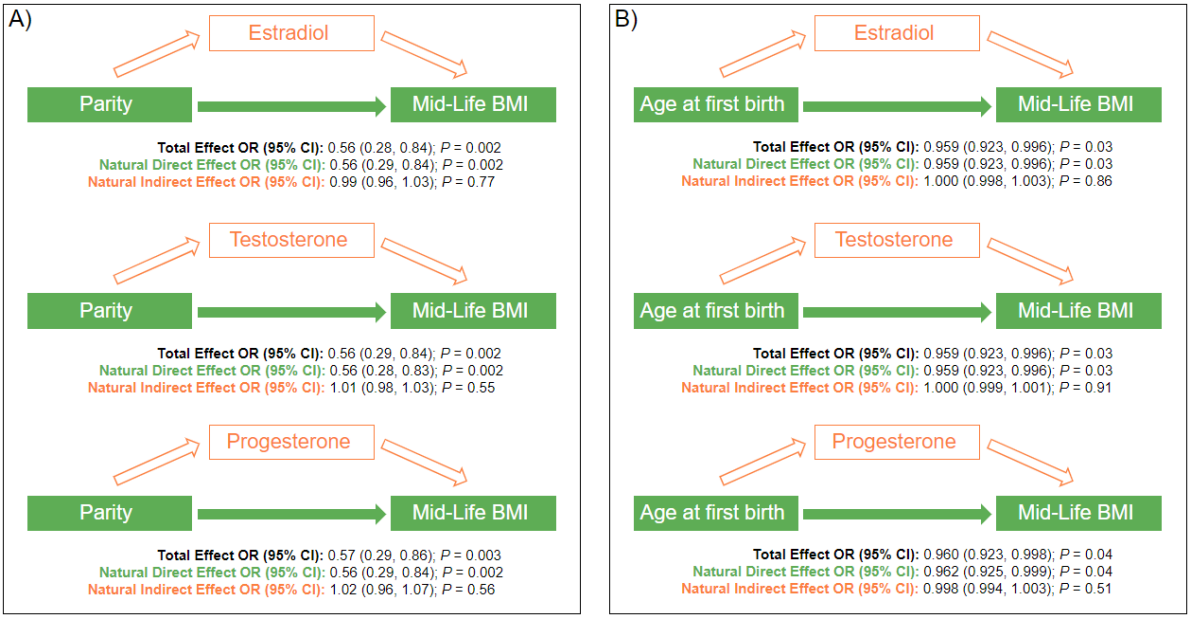

Figure 22. Associations of parity (number of live births) and age at first birth with mid-life BMI – mediation by mid-life hormones. A system of structural equations assessed the mediating effect of estradiol, testosterone, and progesterone on associations of parity and age at first birth with mid-life BMI. Models were adjusted for race, maternal smoking status, fertility problems, oral contraceptive use, BMI at age 18, and menopausal status. Results are expressed as OR (95%CI) for the total effect, natural direct effect, and natural indirect effect. Green filled arrows represent significant associations (P<0.05), whereas orange unfilled arrows represent non-significant (P>0.05) associations.

C.6. DISCUSSION

Results from this study suggest that parity and age at first birth are important predictors of overweight/obesity in pre- and peri-menopausal women. Specifically, women who gave birth only once and those who were younger at their first birth had higher odds of being overweight/obese in mid-life, even after controlling for important confounders. Additionally, parity and age at first birth were independently associated with weight gain from age 18 to age 45-54. These associations were consistent between pre- and peri-menopausal women, and associations of parity and age at first birth with mid-life BMI were not explained by mid-life hormones.

C.6.1. Parity, but not gravidity, was associated with mid-life overweight/obesity and BMI gain since age 18

Adaptations in maternal carbohydrate and lipid metabolism, especially in mid-to-late pregnancy, lead to gestation-related fat accumulation to central regions (236, 456). This could partially explain why we observed that parity, but not gravidity, was strongly associated with mid-life obesity and adult weight accumulation. Carrying a child to full term is associated with maternal metabolic changes and weight gain that persist after pregnancy. Furthermore, having children is accompanied by unique biological and lifestyle shifts, which put parous women at higher risk of obesity compared with nulliparous women (specifically those who become pregnant but do not give birth to a child).

Interestingly, our observation that having one child (being primiparous) puts women at greatest risk of mid-life overweight/obesity is consistent with studies showing that parous women gain the most weight during their first pregnancy/birth compared with subsequent ones (457, 458). This suggests that there is a higher likelihood of drastic and persistent body composition and metabolic changes after having one child. Although the exact causes of this “primiparous paradox” are not clear, studies suggest that multiparous women (compared with those who only have one child) are less likely to experience anxiety and depression after pregnancy (459), and are more motivated to make dietary changes and lose weight after subsequent pregnancies (460), which could put primiparous women at higher risk of obesity. Similar to our observations, a large prospective cohort of U.S. Caucasian/White and African American/Black pre-menopausal

women found that women with one live birth (but not two-or-more live births), had higher weight gain and waist-to-hip ratio compared with women with no live births over a five year follow-up (461). Similar findings were observed at the 10 year follow-up of the same cohort, where substantial weight gain occurred in women who had only one birth, whereas higher order births were not associated with excess weight gain (462). These findings are analogous to ours, showing that women who only give birth once have higher odds of mid-life overweight/obesity than women who never give birth or those who give birth two-or-more times.

Our results are somewhat inconsistent with several other studies in racially/ethnically diverse populations of only pre- (463, 464), post- (441, 465, 466), or a combination of pre-, peri-, and post-menopausal women (442-447), showing that having more live births is linearly associated with higher BMI or with weight gain since age 18 (444). Given that most women in our study were Caucasian/White or African American/Black and none were post-menopausal, these discrepant findings could be due to racial/ethnic and/or menopausal status differences, which will need to be further investigated. Overall, as discussed above, our findings are supported by previous research, and appear to suggest that women who give birth only once are more likely to experience weight gain during their reproductive years, but substantially more data are needed to understand the social and lifestyle factors that influence this observation.

C.6.2. Age at first birth, not age at last pregnancy, was associated with mid-life overweight/obesity and BMI gain since age 18

Pregnancy itself is associated with numerous metabolic changes to support fetal growth, including insulin resistance and fat accumulation (236, 467), and these metabolic changes can persist after pregnancy (468). Additionally, pregnancy and obstetric complications could impact physical activity and mobility well after delivery (469). These deleterious effects would emerge earlier in women who have children at a younger age, which could have major implications for lifelong health. Our finding that women who have their first child at a younger age are at elevated risk for mid-life overweight/obesity is consistent with previous studies in pre-, peri-, and post-menopausal women (441, 463, 470, 471). One study proposed that giving birth at a younger age provides women with more time to have children, leading to weight accumulation between pregnancies (470). The same study also found that among post-menopausal Chinese women, having more reproductive years was associated with higher mid-life BMI and waist circumference (470). In our study, younger age at first birth was associated with higher risk of BMI gain from age 18 to 45-54, and this was independent of parity. Because younger age at first birth has been associated with higher central adiposity (441, 470) and poorer physical performance during mid-life (469), earlier childbirth could be an independent risk factor for mid-life overweight/obesity.

Beyond biological factors, earlier childbirth is associated with numerous lifestyle shifts. For example, women who give birth in their teens or 20s might have to stall their education to care for their child (472). Younger mothers might also have lower incomes, leading to

poorer diet quality compared to older mothers (473). Although we did not record age at last birth, our findings were consistent with other studies showing no associations between age at last pregnancy and mid-life obesity or weight accumulation (441, 474), suggesting that age at first birth is a stronger predictor of lifetime obesity risk than the age at which a woman stops having children.

C.6.3. Hormones did not mediate associations of parity and age at first birth with mid-life overweight/obesity

We previously reported that as mid-life BMI increases, mid-life estradiol and progesterone concentrations decrease, whereas testosterone concentrations increase (440). In the current study, we hypothesized that pregnancy-related changes in hormones might persist into mid-life and partially explain observed associations of parity and age at first birth with mid-life overweight/obesity. However, we did not observe such a mediation. The relationship between reproductive hormones and obesity, especially during the menopausal transition, is complex. Studies in pre-, peri-, and post-menopausal women have shown that adipose tissue deposition can be influenced by reproductive hormones (434), but that the reverse can also be true (440, 475). Therefore, additional studies are needed to better understand the mechanisms connecting mid-life obesity and shifts in reproductive hormones.

C.6.4. Strengths and limitations

This study has several strengths and limitations. Although this cohort is not a true representative sample of U.S. women, we were able to contribute to the growing literature

supporting pregnancy history as a predictor of mid-life obesity in U.S. pre- and peri-menopausal women. However, because this study was designed to evaluate predictors of hot flashes in mid-life women, future studies should additionally obtain information about pre-pregnancy weight, gestational weight gain, and lifestyle before and during pregnancies. Although there is potential for bias from pregnancy history self-recall, there is good-to-excellent agreement between pregnancy history recall compared with medical records (476, 477). Additionally, each woman's mid-life height and weight were measured and recorded by trained clinical staff, which reduced the potential for bias and variability in our outcome. There is also potential for bias with self-reported weight at age 18; one validation study suggest that individuals have a tendency to under-report past body weight; however, the same study suggests that self-recall of past body weight is accurate at the population level (478). Although women in the study were predominately Caucasian/White and African American/Black (and not any other races/ethnicities), this relatively homogeneous population provided us with the power to assess potential hormonal mechanisms driving associations of pregnancy history with mid-life obesity. Lastly, blood samples were not drawn on specific days or phases of the menstrual cycle. Because women were experiencing irregular menses, the lack of standardized blood collection could have introduced variability in hormone measurements. Despite this limitation, four blood samples over four consecutive weeks were collected and averaged to provide better estimates of each woman's reproductive hormone status.

C.7. CONCLUSIONS

Findings from this study suggest that having one child or being younger at first childbirth

are important and persistent predictors of a woman's health before and during menopause. We observed that associations between pregnancy history and mid-life BMI are potentially unrelated to mid-life hormone concentrations, suggesting that other unmeasured modifiable/intervenable factors are involved in pregnancy-related mid-life obesity. Therefore, to reduce the prevalence and incidence of mid-life obesity and its associated morbidities in women, primiparous women and those who are younger at their first childbirth, specifically, might benefit from interventions that teach healthy lifestyle habits during their reproductive years.

APPENDIX D: URINARY PHTHALATE METABOLITE CONCENTRATIONS AND SERUM HORMONE LEVELS IN PRE- AND PERIMENOPAUSAL WOMEN FROM THE MIDLIFE WOMEN'S HEALTH STUDY

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D.1. ABSTRACT

Phthalate exposure is associated with altered reproductive function, but little is known about associations between phthalate and hormone levels in midlife women. This cross-sectional analysis includes 45–54-year-old pre- and perimenopausal women from Baltimore, MD and its surrounding counties enrolled in the Midlife Women's Health Study (n = 718). Serum and urine samples were collected from participants once a week for four consecutive weeks to span the menstrual cycle. Serum samples were assayed for estradiol, testosterone, progesterone, sex hormone binding globulin (SHBG), follicle-stimulating hormone (FSH), and anti-Müllerian hormone (AMH), and geometric means were calculated for each hormone across all four weeks. Urine samples were analyzed for nine phthalate metabolites from pools of one-to-four urine samples. Phthalate metabolite concentrations were specific gravity-adjusted and assessed as individual metabolites or as molar sums of metabolites from common parents (di(2-ethylhexyl)

phthalate metabolites, ∑DEHP), exposure sources (plastic, ∑Plastics; personal care products, ∑PCP), biological activity (anti-androgenic, ∑AA), and sum of all metabolites (∑Phthalates). We used linear regression models to assess overall associations of phthalate metabolites with hormones, controlling for important demographic, lifestyle, and health factors. We also explored whether associations differed by menopause status, body mass index (BMI), and race/ethnicity. Most participants were non-Hispanic white (67%) or black (29%), college-educated (65%), employed (80%), and had somewhat higher mean urinary phthalate metabolite concentrations than other U.S. women. Overall, the following positive associations were observed between phthalate metabolites and hormones: ∑DEHP (%Δ: 4.9; 95%CI: 0.5, 9.6), ∑Plastics (%Δ: 5.1; 95%CI: 0.3, 10.0), and ∑AA (%Δ: 7.8; 95%CI: 2.3, 13.6) with estradiol; MiBP (%Δ: 6.6; 95%CI: 1.5, 12.1) with testosterone; ∑DEHP (%Δ: 8.3; 95%CI: 1.5, 15.6), ∑Plastics (%Δ: 9.8; 95%CI: 2.4, 17.7), MEP (%Δ: 4.6; 95%CI: 0.1, 9.2), ∑PCP (%Δ: 6.0; 95%CI: 0.2, 12.2), ∑Phthalates (%Δ: 9.0; 95%CI: 2.1, 16.5), and ∑AA (%Δ: 12.9; 95%CI: 4.4, 22.1) with progesterone; and MBP (%Δ: 8.5; 95%CI: 1.2, 16.3) and ∑AA (%Δ: 9.0; 95%CI: 1.3, 17.4) with AMH. Associations of phthalate metabolites with hormones differed by menopause status (strongest in premenopausal women for estradiol, progesterone, and FSH), BMI (strongest in obese women for progesterone), and race/ethnicity (strongest in non-Hispanic white women for estradiol and AMH). We found that phthalate metabolites were positively associated with several hormones in midlife women, and that some demographic and lifestyle characteristics modified these associations. Future longitudinal studies are needed to corroborate these findings in more diverse midlife populations.

D.2. KEYWORDS

Phthalates; hormones; mid-life women.

D.3. INTRODUCTION

Phthalates are commonly used to impart strength and flexibility to a variety of plastic products (479, 480). Additionally, low molecular weight phthalates are often used in personal care products to stabilize scents and colors (479, 480). Phthalates are non-covalently bound to the products in which they are used, allowing them to leach from products over time and resulting in human exposure on a daily basis (481, 482). Phthalates used in food and consumer good production can lead to human exposure by ingestion of foods contaminated with phthalates through processing or packaging and dermal absorption through use of phthalate-containing personal care products and clothing (480, 483, 484). Additionally, people undergoing medical procedures are exposed to phthalates directly via medical devices (485, 486). Further, humans are exposed through routes such as inhalation of house dust and air contaminated with phthalates (487). Although phthalate exposure is ubiquitous in humans, exposure levels vary between populations and even sex. Women have higher exposure to phthalates than men, potentially due to their greater use of personal care products compared to men (488, 489). In fact, studies often find that phthalate metabolites are detectable in 99-100% of samples submitted by women (490, 491), making women an especially vulnerable population.

Phthalate exposure is of concern because phthalates have been shown to have

endocrine disrupting capabilities (492-496). Epidemiological studies have shown that phthalates are associated with altered hormone levels in both men and women (497-500). Although several epidemiological studies have focused on phthalate exposure in a variety of populations, few studies have investigated health outcomes associated with phthalate exposure in mid-life women. Some studies in older women have shown associations between phthalates and health outcomes such as bone mineral density, hot flash experience, and weight change (501-503). However, less is known about the relationship between phthalates and health outcomes during the menopausal transition (i.e. perimenopause) because most studies have thus far investigated women that classify as either pre- or postmenopausal.

The transition into the menopausal state is an event known for its hormonal fluctuations and discomforts. This transition begins when the ovaries undergo follicular exhaustion, which results in a shift in the hormonal milieu during the menopausal transition (504). In a cycling woman, the ovary is the primary source of the sex steroid hormones estradiol, progesterone, and testosterone (505). These sex steroid hormones interact with the hypothalamus and pituitary to affect the production of the gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), from the pituitary. As the ovary produces fewer sex steroid hormones with age, the negative feedback exerted by the ovarian hormones on the hypothalamus and pituitary is alleviated, leading to an increase in the release of FSH and LH (506). Additionally, in cycling women, anti-Müllerian hormone (AMH) is synthesized by cells within small, growing ovarian follicles, leading to high levels of AMH during prime reproductive years (507). Depletion of the ovarian

reserve during aging leads to a loss of follicles that produce AMH, and subsequently, AMH levels decline (504). Thus, the hormonal profile of the non-cycling woman (i.e. postmenopausal) can generally be characterized as having lower levels of sex steroid hormones and AMH and higher levels of gonadotropins (429).

The primary objective of this study was to address a gap in previous knowledge about the associations between phthalate levels and hormones that fluctuate during the menopause transition. To do so, we investigated the overall associations of common urinary phthalate metabolites with reproductive hormones including estradiol, testosterone, progesterone, sex hormone binding globulin (SHBG), AMH, and FSH in the Midlife Women's Health Study (MWHS). Because hormone levels may differ in women based on menopause status, midlife body mass index (BMI), and race/ethnicity, the secondary objective of this study was to evaluate differences in associations of phthalate metabolites with reproductive hormones by these characteristics (449, 508-511).

D.4. METHODS

D.4.1. Midlife Women's Health Study Cohort

The Midlife Women's Health Study Cohort *(*MWHS) is a longitudinal population-based study that recruited women from Baltimore, MD (USA) and its surrounding counties between the ages of 45 and 54 in 2006-2015. The full study protocol has been previously published (449). Briefly, women were eligible if they had 3 or more periods within the past 12 months (pre- or perimenopausal), had no history of oophorectomy or hysterectomy, were not on hormone therapy, were not taking botanical supplements to alleviate

menopausal symptoms, were not on oral contraceptives, were not pregnant, were not undergoing cancer treatment, and had no history of ovarian, breast, or endometrial cancer. The current study focused on year 1 of MWHS and included a total of 718 women who had information about reproductive hormones, urinary phthalate metabolite concentrations and specific gravity, and covariates (described in the statistical analysis section). All women gave written and informed consent according to procedures that were approved by the University of Illinois Review Board.

D.4.2. Demographic and lifestyle characteristics

At the baseline visit, participants filled out a detailed questionnaire about their demographic information, as well as lifestyle characteristics such as alcohol consumption, physical activity, and smoking status. Information on racial and ethnic background was obtained by asking women to choose their most representative race/ethnicity from the following options: Caucasian/white, African American/black, Hispanic, Asian, or other. Alcohol consumption was ascertained by asking women whether they consumed 12 alcoholic drinks in the past year (answer: yes, no). Women self-reported their leisure physical activity compared to others, and this was categorized as physically active much more or more than others, as much as others, or less or much less than others. Lifetime smoking status was self-reported as current, former, or never. Women who reported having at least 1 menstrual period within the last 3 months and at least 11 menstrual periods over the last year were considered premenopausal. Women were classified as perimenopausal if they experienced at least one menstrual period over the last year, but not the past 3 months, or if they experienced a menstrual period within the past 3 months,

but had experienced 10 or fewer menstrual periods over the last year. At clinic visits, women were asked to list medications (over the counter and prescription) that they were currently taking. Additionally, at the first clinic visit, women had their height and weight measured by clinic staff and values rounded to the nearest 0.5 pound and 0.5 inch. Body mass index (BMI) in kg/m^2 was calculated using the National Institutes of Health on-line BMI calculator.

D.4.3. Collection and measurement of hormones

Women visited the clinic once a week for up to four consecutive weeks for collection of serum samples. Visits to the clinic occurred in the morning to minimize fluctuation in hormones (512, 513). Levels of circulating hormones were measured in serum samples, which were stored at -20 °C prior to measurement. DRG® enzyme-linked immunosorbent assay (ELISA) kits were used to measure levels of estradiol, progesterone, testosterone, and SHBG. Lypocheck® from Bio-Rad Laboratories was used as a control with known values for estradiol, progesterone, testosterone, and SHBG for every assay of these hormones. All samples, controls, and standards were run in duplicates. The limits of detection (LODs) for estradiol, progesterone, testosterone, and SHBG were 9.714 pg/mL, 0.045 ng/mL, 0.083 ng/mL, and 0.77 nmol/L, respectively. The inter- and intra-assay %CVs were all ≤ 10.0, with the exception of estradiol which was ≤ 14.9.

Aliquots of serum from the first visit of each patient were submitted to the University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core for measurement of levels of AMH and FSH. AMH was assayed via ELISA, and FSH was

measured via radioimmunoassay (RIA). The LODs for AMH and FSH were 0.2 ng/mL and 0.1 mIU/mL, respectively. The intra- and inter-assay %CVs for AMH were 3.9 and 6.2, respectively. The intra- and inter-assay %CVs for FSH were 3.2% and 4.9%, respectively.

D.4.4. Collection and measurement of phthalate metabolites in urine

During the same visit in which women donated serum, spot urine samples were also collected. Each woman provided at least one and up to four urine samples (sample number was dependent on the number of clinic visits completed by each woman), which were pooled for each participant to measure phthalate metabolite concentrations. Due to the short half-lives of phthalates in the body and high within person variability of measured concentrations, previous studies have shown that a pooled sample better represents phthalate exposure compared to a single urine sample (162, 262). Urine samples were stored at -20 °C prior to measurement. Phthalates were measured in pooled urine samples via isotope dilution high-performance liquid chromatography negative-ion electrospray ionization-tandem mass spectrometry (HPLC-MS/MS) at the Metabolomics Center of the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana-Champaign. Phthalate metabolites measured included: 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), mono-(3-carboxypropyl) phthalate (MCPP), mono-benzyl phthalate (MBzP), monoethyl phthalate (MEP), monobutyl phthalate (MBP), and mono-isobutyl phthalate (MiBP). The limits of detection (LOD) for each phthalate metabolite were as follows: MEHP: 0.2 ng/mL; MEHHP: 0.05 ng/mL; MEOHP: 0.1 ng/mL; MECPP: 0.05 ng/mL; MCPP: 0.05 ng/mL;

MBzP: 0.05 ng/mL; MEP: 0.1 ng/mL; MBP: 0.05 ng/mL; and MiBP: 0.1 ng/mL. In addition, the intra-assay and inter-assay CVs were below 5%. Further, all standard curves had correlation coefficient values larger than 0.992 and all runs included internal standards.

D.4.5. Statistical analysis

To evaluate associations of midlife urinary phthalate metabolite concentrations with hormone concentrations, covariates were chosen *a priori* and based on previous literature that informed a directed acyclic graph (**Figure 23**). We assessed correlations among all covariates to evaluate potential multicollinearity issues and found that none of the covariates were strongly correlated with each other. Final statistical models evaluating overall associations of urinary phthalate metabolite concentrations with midlife hormones were adjusted for age, race/ethnicity, employment status, education, annual family income, marital status, menopausal status, alcohol consumption, smoking status, physical activity, midlife BMI, and current medication use. Age and income were included as continuous variables, while the other covariates were categorized with reference groups set as shown in **Table 33**. For our secondary objective, we *a priori* stratified our analyses as follows: pre- versus perimenopausal women; under-/normal weight (BMI < 25.0 kg/m^2), overweight (BMI ≥ 25.0 – 29.9 kg/m^2), versus obese (BMI ≥ 30.0 kg/m^2) women; and non-Hispanic white versus black/other women. All stratified models included the previously listed covariates.

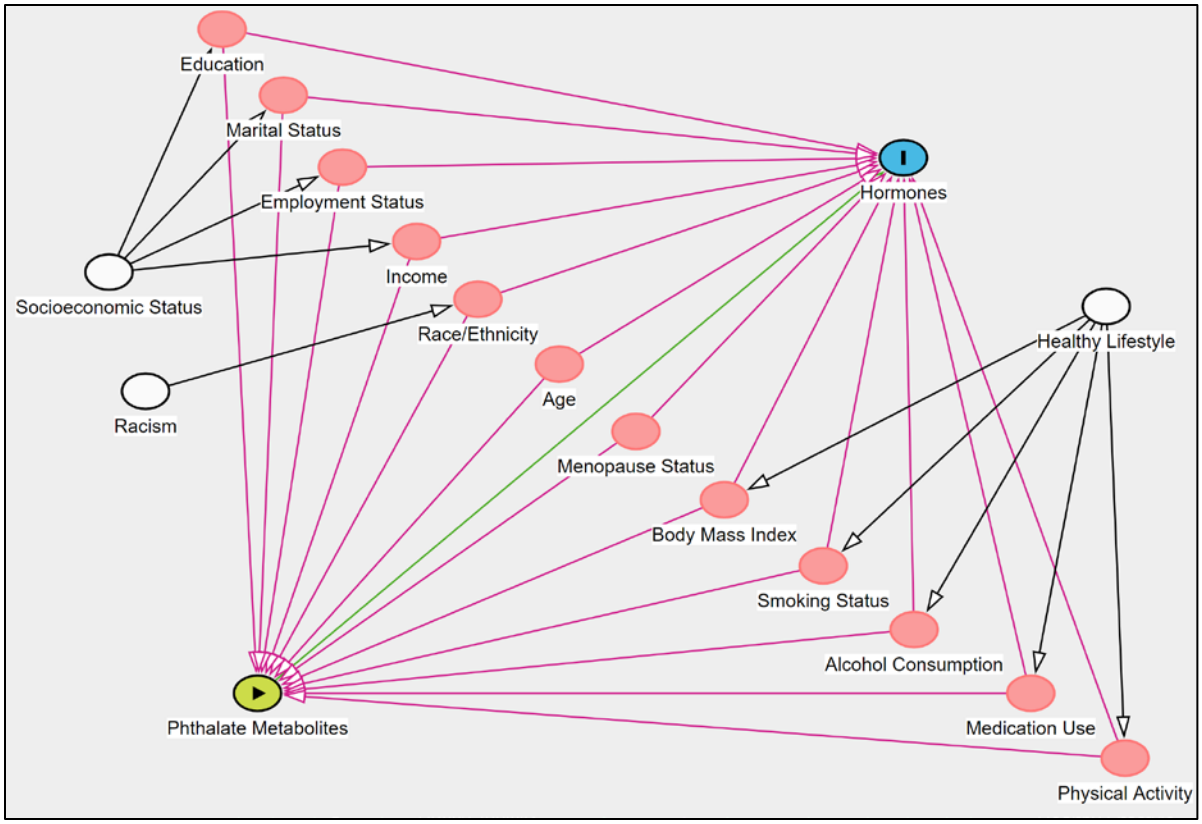

Figure 23. Directed acyclic graph for associations of phthalates with hormones. Green circle represents the exposure (i.e. phthalates), while the blue circle represents the outcome (i.e. hormones). White circles represent latent variables (i.e. socioeconomic status, racism, and healthy lifestyle), while red circles represent confounding variables that were measured in MWHS and included as covariates in final statistical analyses.

Urinary phthalate metabolite concentrations and serum hormone concentrations below the LOD were converted to the LOD/√(2). Because estradiol, testosterone, progesterone, and SHBG concentrations were assessed in multiple samples per participant, the geometric means of these hormones were calculated and used in statistical analyses. To account for differences in urine dilution, phthalate metabolite measurements were adjusted for specific gravity (SG) using the following formula: $P_c = P[(1.018 - 1)/(SG_i - 1)]$, where P_c is the specific gravity adjusted concentration, P is the measured concentration (ng/mL), 1.018 is the median specific gravity of the overall MWHS population included in this analysis, and SG_i is the specific gravity of each woman's

pooled urine sample (109). Specific gravity-adjusted phthalate metabolite concentrations were used to approximate exposure to common phthalate parent compounds. DEHP metabolites (MEHP, MEHHP, MEOHP, MECPP) were molar converted and summed (nmol/mL) to estimate exposure to DEHP (∑DEHP). The concentrations of the other major urinary phthalate metabolites (MCPP, MBzP, MEP, MBP, MiBP) were not molar converted (ng/mL). Additional phthalate sums (nmol/mL) were created to estimate phthalate exposure based on sources of exposure (personal care products, plastics) and potential biological mechanism (anti-androgenic). MEP, MBP, and MiBP were molar summed to estimate exposure to personal care product phthalates (∑PCP), while MCPP, MBzP, MEHP, MEHHP, MEOHP, MECPP were molar summed to estimate exposure to phthalates found in plastics (∑Plastics). Phthalate metabolites that were shown in *in vitro* and *in vivo* studies to have anti-androgenic properties (MBzP, MEHP, MEHHP, MEOHP, MECPP, MBP, MiBP) were molar summed to approximate exposure to anti-androgenic phthalates (∑AA) (22, 149, 495). All phthalate metabolites were also molar-converted and summed to estimate total phthalate metabolite concentrations (∑Phthalates).

We used linear regression models to assess overall and stratified associations of midlife urinary phthalate concentrations with midlife hormones. We first evaluated overall associations of continuous phthalates with hormones. Both phthalate and hormone concentrations were natural log-transformed to better approximate normality assumptions in these generalized linear regression models. Second, we evaluated dose-response relationships of phthalates with hormones by categorizing urinary phthalate concentrations into quartiles; hormones were transformed as previously described. For

our second objective, linear regression models were stratified by menopause status, midlife BMI, and race/ethnicity to evaluate differences in associations between phthalate metabolites and hormones by these factors.

All statistical analyses were conducted in SAS 9.4 (version 14.3, SAS Institute) using PROC GLM. In models where phthalates were assessed as continuous measures (objectives 1 and 2), β-estimates and 95% confidence intervals (CIs) were back-transformed using the equation $[((2.00)^{\beta} - 1)*100]$ to represent a percent change in hormones for each two-fold increase in phthalate concentration. For models where phthalates were categorized in quartiles, β-estimates and 95% CIs were back-transformed using the equation $[(e^{\beta} - 1)*100]$ to represent the percent change in hormones among women in quartiles two (Q2), three (Q3), and four (Q4) of urinary phthalate concentrations, compared to the lowest quartile (Q1). We tested for linear trends ($P_{linear\ trend}$) across quartiles by assessing separate linear regression models that treated the ordinal phthalate variables as continuous. For models evaluating stratified associations of phthalates with hormones, we formally tested for effect modification (P_{int}) in linear regression by including multiplicative interactions between phthalates and menopause status, phthalates and race/ethnicity, and phthalates and BMI. However, we reported all stratified associations regardless of P_{int} significance.

D.5. RESULTS

D.5.1. MWHS demographics and lifestyle characteristics

At the time of enrollment, all women were between the ages of 45 and 54 years, with 65% of women being 49 years or younger (**Table 33**). In terms of racial background, 66% were non-Hispanic white and 34% were black or of other racial/ethnic background. The majority of the women were employed (80%), had a college education or higher (65%), and were premenopausal (64%). Most women reported being at least occasional drinkers (66%) and over half had a midlife BMI ≥ 25 kg/m2 (60%). Over half of women were never smokers (54%), 36% were former smokers, and only 10% were current smokers.

Table 33. Demographic and lifestyle characteristics of women from MWHS (n=718).

Demographic or Lifestyle Characteristic	n (%)
Age (years)	
45 to 49	469 (65.3)
50 to 54	249 (34.7)
Race/ethnicity	
Non-Hispanic white (ref)	477 (66.4)
Black/Other[1]	241 (33.6)
Employment status	
Unemployed	143 (19.9)
Employed (ref)	575 (80.1)
Education	
Some college or less	251 (35.0)
College graduate or higher (ref)	467 (65.0)
Annual family income ($)	
<20,000	47 (6.5)
20,000 to 39,999	117 (16.3)
40,000 to 99,999	243 (33.8)
≥100,000	311 (43.3)
Marital status	
Single	129 (18.0)
Married/Living with Partner (ref)	466 (64.9)
Widowed/divorced/separated	123 (17.1)
Menopausal status	
Premenopausal (ref)	461 (64.2)
Perimenopausal	257 (35.8)
Alcohol consumption status	
No drinks or <12 drinks over past year	246 (34.3)
At least 12 drinks over past year (ref)	472 (65.7)
Smoking status	
Current	72 (10.0)
Former	258 (35.9)
Never (ref)	388 (54.0)
Leisure physical activity compared to others	
Much more/more (ref)	258 (35.9)
As much	223 (31.1)
Less/much less	237 (33.0)
Body mass index (kg/m^2)	
Under-/normal weight (<25.0) (ref)	288 (40.1)
Overweight (≥25.0-29.9)	187 (26.0)
Obese (≥30.0)	243 (33.8)
Current medication use	
None	306 (42.6)
Any (ref)	412 (57.4)

[1]Other includes Hispanic, Asian, or other race/ethnicity. MWHS, Midlife Women's Health Study.

D.5.2. MWHS urinary phthalate metabolite concentrations

Median (25^{th}, 75^{th} percentile) urinary concentrations of phthalate metabolites are presented in **Table 34**. Greater than 99% of women had detectable concentrations (≥ LOD) of all urinary phthalate metabolites (**data not shown**). Median phthalate metabolite concentrations from our study were generally higher than those in a nationally representative sample of 45-54-year-old women from the National Health and Nutrition Examination Survey (NHANES), likely due to different subpopulations of women in the MWHS and NHANES studies and measurements of urinary metabolites by different laboratories. However, it is important to note that the 25^{th} and 75^{th} percentiles overlapped in metabolite levels between the two studies.

Table 34. Phthalate metabolite concentrations in MWHS and NHANES.

Name	Abbreviation	MWHS (2006-2015) n=718	NHANES (2005-2016) n=757[1]
Phthalate metabolite		**Median (25th, 75th percentile) in ng/mL**	
Mono(2-ethylhexyl) phthalate	MEHP	4.5 (2.7, 9.4)	1.2 (0.6, 3.1)
Mono(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	33.7 (20.3, 58.1)	9.1 (3.4, 22.5)
Mono(2-ethyl-5-oxohexyl) phthalate	MEOHP	12 (7.3, 22.3)	5.6 (2.1, 13.2)
Mono(2-ethyl-5-carboxypentyl) phthalate	MECPP	25.8 (15.8, 48.1)	13.4 (5.6, 31.7)
Mono(3-carboxypropyl) phthalate	MCPP	2.5 (1.3, 5.4)	1.4 (0.6, 3.4)
Monobenzyl phthalate	MBzP	9.4 (5.4, 16)	4.1 (1.8, 10.4)
Monoethyl phthalate	MEP	95.4 (47.4, 192)	58.8 (20.0, 179.6)
Mono-n-butyl phthalate	MBP	19.7 (12.9, 32.6)	11.5 (5.4, 25.3)
Mono-isobutyl phthalate	MiBP	16.3 (9.8, 26.1)	5.7 (2.6, 13.1)
Phthalate molar-converted sum		**Median (25th, 75th percentile) in nmol/mL**	
Sum of di(2-ethylhexyl) phthalate metabolites	∑DEHP[2]	0.3 (0.2, 0.5)	0.1 (0.04, 0.2)
Sum of all plastic phthalate metabolites	∑Plastics[3]	0.3 (0.2, 0.6)	0.1 (0.1, 0.3)
Sum of all personal care product phthalate metabolites	∑PCP[4]	0.7 (0.4, 1.3)	0.4 (0.2, 1.2)
Sum of all phthalate metabolites	∑Phthalates[5]	1.1 (0.7, 2)	0.7 (0.3, 1.8)
Sum of anti-androgenic phthalate metabolites	∑AA[6]	0.5 (0.3, 0.8)	0.2 (0.1, 0.5)

[1]Phthalate metabolite concentrations for 45–54 year-old US women from combined NHANES cycles 2005–06, 2007–08, 2009–10, 2011–12, 2013–14, and 2015–16. [2]∑DEHP: MEHP, MEHHP, MEOHP, MECPP. [3]∑PCP: MEP, MBP, and MiBP. [4]∑Plastics): MCPP, MBzP, MEHP, MEHHP, MEOHP, MECPP. [5]∑Phthalates: all phthalate metabolites. [6]∑AA: MBzP, MEHP, MEHHP, MEOHP, MECPP, MBP, MiBP. MWHS, Midlife Women's Health Study; NHANES, National Health and Nutrition Examination Survey.

D.5.3. MWHS plasma hormone concentrations

Plasma hormone concentrations from year 1 of the MWHS are presented in **Figure 24**. Median (range) hormone concentrations were as follows: estradiol, 49.9 pg/mL (6.9 – 349.3); testosterone, 0.3 ng/mL (0.1 – 4.3); progesterone, 0.6 ng/mL (0.05 – 17.7); SHBG, 64.4 nmol/L (9.0 – 264.8); FSH, 11.3 mIU/mL (0.1 – 161.0); and AMH, 0.1 ng/mL (0.1 – 8.3).

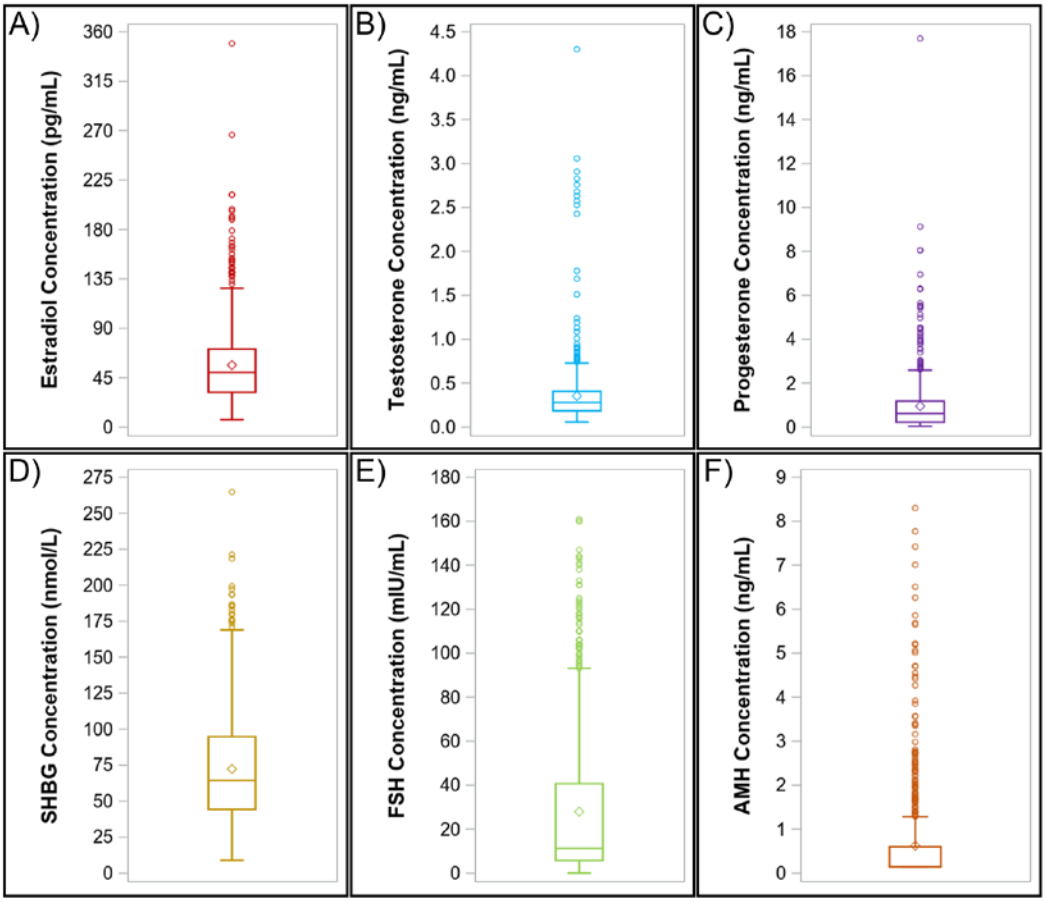

Figure 24. Hormone concentrations of women from MWHS (n=718). Mid-life **A)** estradiol, **B)** testosterone, **C)** progesterone, **D)** sex hormone binding globulin (SHBG), **E)** follicle stimulating hormone (FSH), and **F)** anti-Mullerian hormone (AMH) concentrations. Results are presented as 1.5 times the interquartile range below and above the 25th and 75th percentiles (lower and upper endpoints of whisker), the 25th and 75th percentiles (lower and upper edges of box), median (line inside box), and mean (diamond). MWHS, Midlife Women's Health Study.

D.5.4. Overall associations between phthalate metabolites with hormones

In linear regression models where phthalate metabolites were modeled continuously, select phthalates were positively associated with estradiol, testosterone, progesterone, and AMH, but not with SHBG or FSH (**Table 35**). Specifically, 2-fold increases in ∑DEHP, ∑Plastics, and ∑AA were associated with 4.9% (95%CI: 0.5, 9.6), 5.1% (95%CI: 0.3, 10.0), and 7.8% (95%CI: 2.3, 13.6) higher estradiol concentrations, respectively. Additionally, each 2-fold increase in MiBP was associated with 6.6% (95%CI: 1.5, 12.1) higher testosterone concentrations, whereas 2-fold increases in MBP and ∑AA were associated with 8.5% (95%CI: 1.2, 16.3) and 9.0% (95%CI: 1.3, 17.4) higher AMH concentrations, respectively. Lastly, 2-fold increases in ∑DEHP, ∑Plastics, MEP, ∑PCP, ∑Phthalates, and ∑AA were associated with 4.6 – 12.9% higher progesterone concentrations.

Table 35. Overall linear associations of phthalate metabolites with hormones (n=718).

Exposure	Estradiol	Testosterone	Progesterone	SHBG	FSH	AMH
	% change in hormones (95%CI)					
MCPP	1.2 (-1.7, 4.3)	0.1 (-3.0, 3.3)	2.4 (-2.1, 7.2)	-2.2 (-4.5, 0.2)	-1.9 (-6.9, 3.5)	4.2 (-0.1, 8.7)
MBzP	1.2 (-2.8, 5.4)	-1.3 (-5.4, 3.0)	3.5 (-2.6, 10.0)	1.6 (-1.7, 4.9)	-4.4 (-10.9, 2.7)	5.4 (-0.4, 11.6)
∑DEHP	**4.9 (0.5, 9.6)**	1.4 (-3.1, 6.1)	**8.3 (1.5, 15.6)**	0.9 (-2.5, 4.5)	-3.7 (-10.8, 4.0)	4.1 (-2.1, 10.7)
∑Plastics	**5.1 (0.3, 10.0)**	1.6 (-3.2, 6.6)	**9.8 (2.4, 17.7)**	0.9 (-2.8, 4.7)	-4.7 (-12.2, 3.4)	5.4 (-1.3, 12.5)
MEP	-0.2 (-3.1, 2.7)	0.0 (-3.0, 3.0)	**4.6 (0.1, 9.2)**	0.3 (-2.1, 2.6)	4.2 (-1.0, 9.7)	-1.8 (-5.7, 2.3)
MBP	2.0 (-3.0, 7.1)	2.9 (-2.3, 8.3)	6.2 (-1.4, 14.4)	1.1 (-2.8, 5.2)	-5.2 (-13.1, 3.4)	**8.5 (1.2, 16.3)**
MiBP	4.0 (-0.8, 9.0)	**6.6 (1.5, 12.1)**	5.3 (-2.0, 13.1)	1.5 (-2.3, 5.5)	-2.9 (-10.7, 5.6)	4.9 (-1.9, 12.1)
∑PCP	0.2 (-3.5, 4.1)	0.8 (-3.1, 4.9)	**6.0 (0.2, 12.2)**	0.9 (-2.1, 4.0)	3.7 (-2.9, 10.9)	-0.1 (-5.3, 5.4)
∑Phthalates	2.3 (-2.1, 6.9)	1.1 (-3.4, 5.9)	**9.0 (2.1, 16.5)**	1.0 (-2.5, 4.6)	2.1 (-5.5, 10.4)	2.0 (-4.2, 8.5)
∑AA	**7.8 (2.3, 13.6)**	3.5 (-2.0, 9.3)	**12.9 (4.4, 22.1)**	2.0 (-2.2, 6.4)	-6.9 (-15.1, 2.1)	**9.0 (1.3, 17.4)**

Data are presented as the % change in hormones for every 2-fold increase in phthalate metabolite concentration (ng/mL or nmol/mL). Linear regression models adjusted for age, race/ethnicity, employment status, education, income, marital status, alcohol consumption, smoking status, physical activity, medication use, menopausal status, and BMI. CI, confidence interval; AMH, anti-Mullerian hormone; BMI, body mass index; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin.

In analyses where phthalate metabolites were modeled in quartiles, phthalates were associated with all hormones, except for SHBG (**Figure 25**). Specifically, compared to those in Q1, estradiol concentrations were 18.7% (95%CI: 4.3, 35.0) higher in women at ∑AA Q4 ($P_{linear\ trend}$ = 0.07; **Figure 25a**), whereas progesterone concentrations were 24.4% (95%CI: 1.8, 51.9) and 26.1% (95%CI: 3.8, 53.2) higher, respectively, in women in the highest quartile of ∑Phthalates ($P_{linear\ trend}$ = 0.05) and ∑AA ($P_{linear\ trend}$ = 0.04; **Figure 25c**). Compared to those in the lowest quartile, testosterone concentrations were 14.7% (95%CI: 0.0, 31.6) higher in women at MEP Q3 ($P_{linear\ trend}$ = 0.81), as well as 18.5% (95%CI: 3.7, 35.5) and 23.0% (95%CI: 7.3, 41.0) higher, respectively, in women at MiBP Q2 and Q4 ($P_{linear\ trend}$ = 0.05; **Figure 25b**). However, testosterone concentrations were 13.1% (95%CI: 0.5, 24.0) lower in women at ∑DEHP Q2 ($P_{linear\ trend}$ = 0.28) compared to those in Q1. Compared to those in the lowest quartile, AMH concentrations were 23.1% (95%CI: 2.6, 47.7) and 19.9% (95%CI: 0.1, 43.7) higher in women at MBP Q2 and Q4 ($P_{linear\ trend}$ = 0.09), and 20.7% (95%CI: 0.7, 44.6) higher in women at ∑AA Q3 ($P_{linear\ trend}$ = 0.03; **Figure 25f**). Lastly, FSH concentrations were 31.3% higher (95%CI: 4.1, 65.5) higher in MEP Q3 compared to Q1 ($P_{linear\ trend}$ = 0.88; **Figure 25e**).

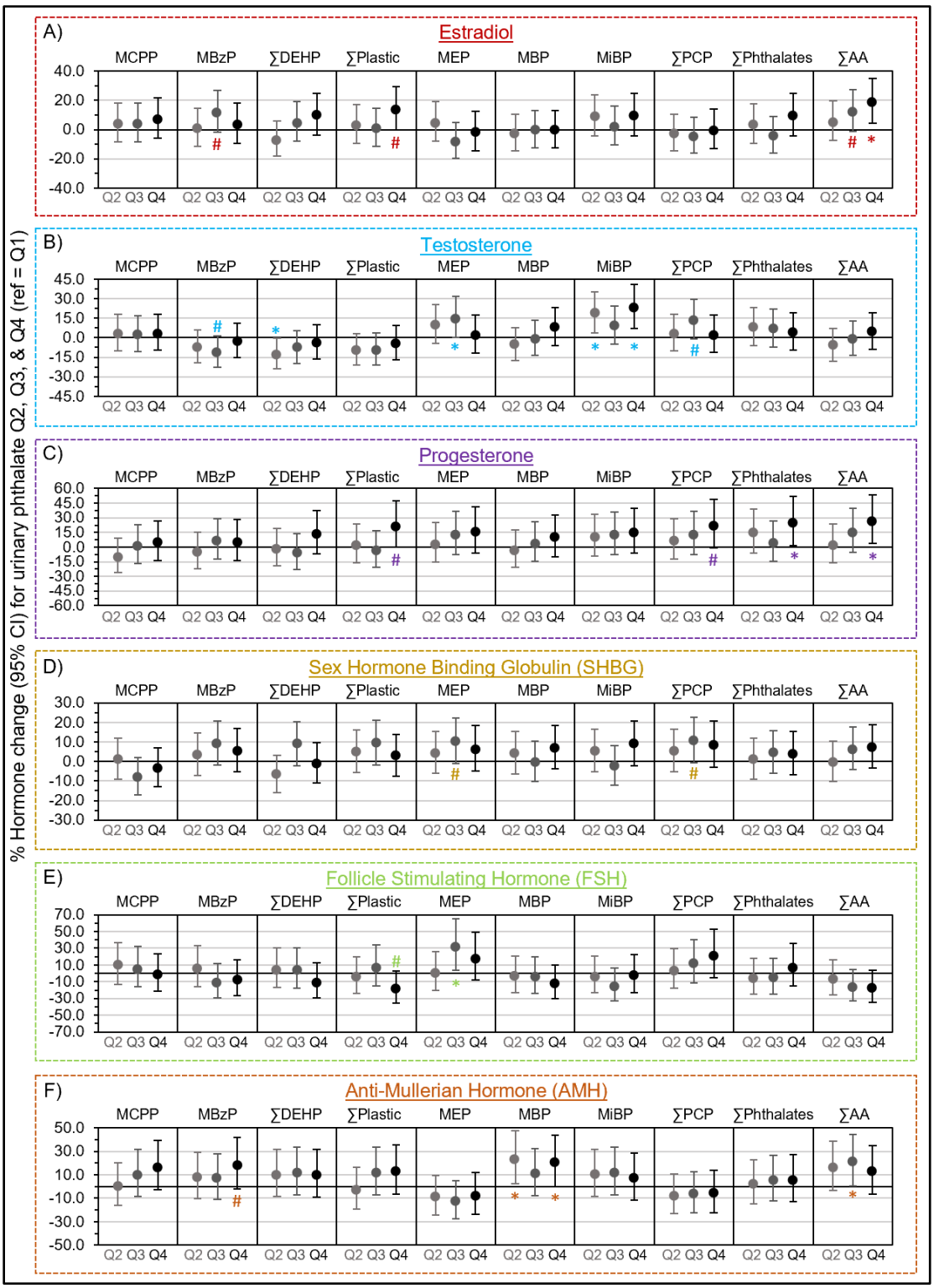

Figure 25. Associations of phthalate metabolites in quartiles with hormones (n=718). Multivariable linear regression models evaluated associations of urinary phthalate concentrations with **A)** estradiol, **B)** testosterone, **C)** progesterone, **D)** sex hormone binding globulin (SHBG), **E)** follicle stimulating hormone (FSH), and **F)** anti-Mullerian hormone (AMH).

Figure 25 (cont'd).
Data are presented as the difference in hormone concentration (filled circles) and 95% confidence interval (solid lines) comparing phthalate quartiles 2 (Q2), 3 (Q3), and 4 (Q4) to quartile 1 (Q1). Models were adjusted for age, race, employment status, education, income, marital status, alcohol consumption, smoking status, physical activity, medication use, menopausal status, and body mass index. Confidence intervals that do not cross the null are significantly different from quartile 1 at $^{\#}P<0.10$ and $^{*}P<0.05$.

D.5.5. Associations between phthalate metabolites and hormones stratified by menopause status

Associations of phthalates with estradiol, FSH, and AMH were only observed in premenopausal women (**Table 36**), in whom ∑DEHP, ∑Plastics, and ∑AA were positively associated with estradiol concentrations, MBzP, ∑Plastics, and ∑AA were negatively associated with FSH, while ∑AA was positively associated with AMH. Conversely, MiBP was positively associated with testosterone only in perimenopausal women (**Table 36**). Associations of phthalates with progesterone were observed in both pre- and perimenopausal women, but they differed depending on the phthalate metabolite (**Table 36**). ∑DEHP, ∑Plastics, and ∑AA were positively associated with progesterone in premenopausal women, whereas ∑Phthalates was positively associated with progesterone in perimenopausal women.

Table 36. Associations of phthalate metabolites with hormones stratified by menopause status.

Menopause Status	Phthalate	Estradiol	P_{int}	Testosterone	P_{int}	Progesterone	P_{int}	SHBG	P_{int}	FSH	P_{int}	AMH	P_{int}
% change in hormones for every 2-fold increase in phthalate concentrations													
Pre	MCPP	1.2 (-1.8, 4.2)	0.77	0.3 (-3.4, 4.2)	0.43	3.7 (-1.6, 9.3)	0.35	-2.5 (-5.2, 0.3)	0.70	-3.7 (-8.6, 1.5)	0.21	3.8 (-2.3, 10.2)	0.50
Peri		2.4 (-4.4, 9.7)		-1.7 (-7.4, 4.4)		1.3 (-7.4, 10.9)		-1.5 (-6.3, 3.5)		2.3 (-10.0, 16.3)		-0.4 (-3.9, 3.2)	
Pre	MBzP	1.8 (-2.2, 6.0)	0.96	0.4 (-4.5, 5.6)	0.26	4.9 (-2.2, 12.5)	0.65	1.0 (-2.7, 4.9)	0.59	**-9.0 (-15.0, -2.5)**	0.08	7.5 (-0.8, 16.5)	0.15
Peri		1.4 (-7.6, 11.3)		-4.7 (-12.1, 3.2)		0.0 (-11.4, 12.9)		3.8 (-2.9, 10.9)		7.3 (-9.7, 27.5)		1.9 (-2.8, 6.9)	
Pre	∑DEHP	**6.0 (1.5, 10.8)**	0.99	0.0 (-5.4, 5.7)	0.59	**9.4 (1.4, 18.2)**	0.83	1 (-3.1, 5.2)	0.76	-7.1 (-13.9, 0.3)	0.30	6.7 (-2.3, 16.5)	0.08
Peri		2.0 (-7.2, 12.1)		2.9 (-5.1, 11.6)		5.0 (-7.2, 18.8)		1.3 (-5.3, 8.4)		4.2 (-12.5, 24.1)		-1.4 (-6.1, 3.5)	
Pre	∑Plastics	**5.8 (1.0, 10.8)**	0.77	0.8 (-4.9, 6.8)	0.87	**10.8 (2.2, 20.1)**	0.89	0.7 (-3.5, 5.2)	0.69	**-8.8 (-15.8, -1.2)**	0.20	8.2 (-1.4, 18.8)	0.06
Peri		3.0 (-7.0, 14.2)		1.8 (-6.8, 11.3)		6.6 (-6.8, 21.9)		1.7 (-5.5, 9.5)		5.5 (-12.8, 27.7)		-1.1 (-6.2, 4.2)	
Pre	MEP	1.3 (-1.6, 4.3)	0.60	-1.2 (-4.8, 2.5)	0.13	4.2 (-1.1, 9.7)	0.41	0.5 (-2.2, 3.3)	0.71	1.7 (-3.4, 7.0)	0.37	-2.1 (-7.7, 3.9)	0.99
Peri		-1.4 (-7.4, 5.0)		2.4 (-3.0, 8.1)		7.8 (-0.6, 17)		0.4 (-4.0, 5.0)		7.0 (-4.7, 20.2)		-2.5 (-5.6, 0.7)	
Pre	MBP	3.5 (-1.7, 8.9)	0.68	1.4 (-4.9, 8.2)	0.69	8.4 (-0.9, 18.5)	0.45	-0.5 (-5.1, 4.4)	0.15	-7.5 (-15.3, 1.1)	0.43	10.2 (-0.5, 22.1)	0.32
Peri		1.8 (-8.3, 13.1)		3.8 (-5.2, 13.6)		1.6 (-11.4, 16.6)		5.8 (-1.8, 14.1)		-0.1 (-17.8, 21.4)		2.5 (-2.9, 8.2)	
Pre	MiBP	3.4 (-1.5, 8.6)	0.51	2.7 (-3.4, 9.1)	0.04	7.1 (-1.6, 16.5)	0.60	-0.2 (-4.6, 4.4)	0.14	-1.2 (-9.2, 7.5)	0.56	2.6 (-6.9, 13.1)	0.72
Peri		9.3 (-1.2, 20.9)		**14.8 (5.4, 25.2)**		4.3 (-8.6, 19.1)		7.3 (-0.2, 15.4)		-7.3 (-23.3, 11.9)		3.3 (-1.9, 8.9)	
Pre	∑PCP	1.8 (-2.3, 6.1)	0.70	-0.7 (-5.6, 4.6)	0.22	5.3 (-2.0, 13.1)	0.54	1.0 (-2.8, 4.9)	0.67	1.2 (-5.7, 8.7)	0.59	1.0 (-7.0, 9.7)	0.68
Peri		-0.1 (-7.2, 7.5)		3.0 (-3.3, 9.8)		9.3 (-0.7, 20.3)		1.3 (-3.9, 6.8)		5.0 (-8.4, 20.3)		-2.0 (-5.6, 1.8)	
Pre	∑Phthalates	4.2 (-0.6, 9.2)	0.64	-0.8 (-6.5, 5.3)	0.27	7.9 (-0.7, 17.1)	0.52	1.0 (-3.3, 5.5)	0.72	-1.9 (-9.6, 6.4)	0.34	5.4 (-4.1, 15.9)	0.20
Peri		0.7 (-7.8, 9.9)		3.7 (-3.9, 11.8)		**12.5 (0.4, 26.1)**		1.1 (-5.1, 7.7)		6.3 (-9.7, 25.1)		-2.4 (-6.7, 2.1)	
Pre	∑AA	**7.7 (2.2, 13.4)**	0.44	0.7 (-5.7, 7.5)	0.29	**12.6 (2.8, 23.3)**	0.81	0.4 (-4.3, 5.4)	0.15	**-9.8 (-17.6, -1.3)**	0.38	**12.3 (1.2, 24.7)**	0.07
Peri		9.2 (-2.9, 22.7)		7.7 (-2.7, 19.2)		12.2 (-3.8, 30.7)		6.8 (-1.8, 16.2)		0.8 (-18.9, 25.5)		0.3 (-5.6, 6.5)	

Data are presented as % change in hormone concentration for every 2-fold increase in phthalate metabolite concentration (ng/mL or nmol/mL) in pre- and peri-menopausal women from linear regression models adjusted for age, race/ethnicity, employment status, education, income, marital status, alcohol consumption, smoking status, physical activity, medication use, and BMI. In separate models, an interaction between phthalate and menopause status was included to formally test for effect modification by menopause status, and the resulting *P*-value (P_{int}) is provided in the table. CI, confidence interval; AMH, anti-Mullerian hormone; BMI, body mass index; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin. n = 461 and 257 for pre- and peri-menopausal women, respectively.

D.5.6. Associations between phthalate metabolites and hormones stratified by BMI

Associations of phthalate metabolites with estradiol were only observed in under-/normal weight women (**Table 37**). Specifically, ∑AA was positively associated with estradiol. Associations of phthalate metabolites with SHBG were only observed in overweight women, in whom MCPP was negatively associated with SHBG (**Table 37**). Associations of phthalates with progesterone were only observed in obese women, in whom ∑DEHP, ∑Plastics, MEP, ∑PCP, ∑Phthalates, and ∑AA were positively associated with progesterone (**Table 37**). Associations of phthalates with FSH and AMH were observed in both under-/normal weight and obese women, but they differed depending on the phthalate metabolite (**Table 37**). Specifically, ∑DEHP and ∑AA were negatively associated with FSH in obese women, but MBzP was negatively associated, while MEP and ∑PCP were positively associated with FSH in under-/normal weight women. Additionally, MBzP was positively associated with AMH in under-/normal weight women, while MBP was positively associated with AMH in obese women.

Table 37. Associations of phthalate metabolites with hormones stratified by mid-life BMI.

BMI Category	Phthalate	Estradiol	P_{int}	Testosterone	P_{int}	Progesterone	P_{int}	SHBG	P_{int}	FSH	P_{int}	AMH	P_{int}
% change in hormones for every 2-fold increase in phthalate concentrations													
Under/Normal	MCPP	0.8 (-3.5, 5.4)	0.90	-2.3 (-7.3, 2.9)	0.60	-1.9 (-8.8, 5.6)	0.27	-0.3 (-3.6, 3.1)	0.36	0.5 (-7.7, 9.4)	0.44	1.0 (-6.1, 8.7)	0.56
Overweight		1.6 (-4.6, 8.2)		-0.1 (-6.3, 6.6)		4.7 (-4.5, 14.7)		**-5.1 (-9.7, -0.3)**		-7.8 (-18.8, 4.7)		8.4 (-0.2, 17.7)	
Obese		1.1 (-4.4, 6.9)		3.0 (-2.2, 8.5)		5.9 (-2.2, 14.7)		-1.8 (-6.4, 3.1)		-1.7 (-9.3, 6.6)		5.7 (-1.5, 13.4)	
Under/Normal	MBzP	3.0 (-3.0, 9.3)	0.18	0.8 (-6.1, 8.1)	0.32	7.1 (-2.9, 18.2)	0.52	3.5 (-1.0, 8.3)	0.63	**-11.6 (-21.1, -1.0)**	0.20	**10.5 (0.2, 21.9)**	0.34
Overweight		5.9 (-3.4, 16.1)		0.7 (-8.4, 10.7)		4.9 (-8.3, 20.1)		-0.8 (-7.9, 6.8)		6.4 (-11.7, 28.2)		1.9 (-9.8, 15.2)	
Obese		-2.7 (-9.4, 4.5)		-5.2 (-11.3, 1.2)		-3.1 (-12.5, 7.4)		1.0 (-5.1, 7.4)		2.0 (-8.0, 13.0)		3.4 (-5.5, 13.1)	
Under/Normal	∑DEHP	5.7 (-1.3, 13.3)	0.95	0.8 (-7.0, 9.2)	0.52	4 (-7.1, 16.5)	0.11	2.5 (-2.7, 7.9)	0.86	-2.7 (-14.7, 11.0)	0.24	5.9 (-5.4, 18.7)	0.80
Overweight		5.1 (-3.3, 14.2)		4.5 (-4.1, 13.8)		4.3 (-7.7, 17.8)		0.2 (-6.3, 7.1)		2.2 (-13.7, 21.0)		1.9 (-8.8, 13.8)	
Obese		3.7 (-4.4, 12.6)		-1.6 (-8.8, 6.3)		**17.9 (5.1, 32.4)**		1.1 (-5.8, 8.6)		**-11.0 (-20.9, 0.0)**		5.4 (-5, 16.8)	
Under/Normal	∑Plastics	6.8 (-0.6, 14.7)	0.78	1.6 (-6.7, 10.5)	0.61	4.9 (-6.9, 18.1)	0.15	3.1 (-2.3, 8.9)	0.73	-5.0 (-17.2, 9.1)	0.45	7.5 (-4.6, 21)	0.82
Overweight		5.9 (-3.3, 15.9)		3.7 (-5.5, 13.8)		6.7 (-6.6, 21.8)		0.2 (-6.9, 7.7)		0.7 (-16.2, 21.1)		3.8 (-7.9, 17.1)	
Obese		2.6 (-6.0, 12.0)		-1.0 (-8.7, 7.4)		**19.0 (5.2, 34.6)**		0.6 (-6.8, 8.5)		-9.9 (-20.5, 2.1)		6.2 (-4.9, 18.5)	
Under/Normal	MEP	-4.2 (-9.1, 0.9)	0.21	1.6 (-4.5, 8)	0.81	1.0 (-7.4, 10.2)	0.21	0.8 (-3.2, 4.8)	0.01	**12.7 (2, 24.5)**	0.06	-4.2 (-12.2, 4.5)	0.11
Overweight		2.3 (-2.7, 7.6)		2.8 (-2.4, 8.2)		1.8 (-5.4, 9.6)		-2.7 (-6.5, 1.3)		3.4 (-6.6, 14.5)		-4.2 (-10.3, 2.4)	
Obese		1.2 (-3.7, 6.4)		-2.8 (-7.2, 1.9)		**9.7 (2.1, 17.7)**		2.6 (-1.7, 7.2)		-3.3 (-10.1, 3.9)		4.0 (-2.4, 10.8)	
Under/Normal	MBP	5.5 (-2.3, 13.9)	0.50	8.3 (-1.0, 18.5)	0.32	5.1 (-7.4, 19.3)	0.19	2.6 (-3.2, 8.7)	0.94	-8.7 (-21.1, 5.8)	0.25	**14.6 (1.1, 30.0)**	0.12
Overweight		-4.2 (-14.2, 6.9)		0.3 (-10.4, 12.4)		-5.3 (-19.4, 11.3)		-1.5 (-9.8, 7.6)		13.8 (-8.9, 42.2)		-6.1 (-18.8, 8.7)	
Obese		1.0 (-7.0, 9.7)		-1.6 (-8.9, 6.3)		12.3 (-0.2, 26.3)		0.8 (-6.2, 8.3)		-8.7 (-18.9, 2.8)		**12.8 (1.7, 25.1)**	
Under/Normal	MiBP	2.6 (-5.0, 10.7)	0.83	7.6 (-1.5, 17.6)	0.97	0.0 (-11.8, 13.4)	0.36	0.2 (-5.4, 6.1)	0.18	0.0 (-13.5, 15.7)	0.63	8.5 (-4.3, 22.9)	0.10
Overweight		6.4 (-2.8, 16.5)		7.3 (-2.2, 17.8)		9.9 (-3.7, 25.4)		0.5 (-6.5, 8.1)		-2.0 (-18.4, 17.9)		-6.0 (-16.6, 6.0)	
Obese		3.4 (-5.0, 12.5)		3.6 (-4.2, 12.1)		8.4 (-4.0, 22.3)		3.9 (-3.4, 11.8)		-5.9 (-16.7, 6.2)		9.1 (-1.9, 21.3)	
Under/Normal	∑PCP	-3.2 (-9.1, 3.0)	0.34	3 (-4.3, 10.8)	0.84	1.5 (-8.4, 12.6)	0.41	1.1 (-3.6, 6.0)	0.09	**12.6 (0, 26.9)**	0.12	-2.2 (-11.8, 8.5)	0.24
Overweight		2.9 (-3.9, 10.3)		4.1 (-3.0, 11.7)		5.2 (-4.9, 16.4)		-1.4 (-6.7, 4.3)		1.5 (-11.8, 16.8)		-2.5 (-11.1, 6.9)	
Obese		2.3 (-4.3, 9.4)		-2.6 (-8.4, 3.6)		**10.4 (0.4, 21.3)**		3.0 (-2.8, 9.1)		-4.7 (-13.4, 4.9)		6.2 (-2.4, 15.5)	
Under/Normal	∑Phthalates	0.7 (-6.5, 8.5)	0.78	2.5 (-6.1, 11.8)	0.80	3.0 (-8.9, 16.4)	0.18	2.7 (-2.9, 8.6)	0.38	9.9 (-4.6, 26.6)	0.14	0.7 (-10.9, 13.8)	0.55
Overweight		4.6 (-3.2, 13.0)		5.2 (-2.8, 13.9)		6.5 (-4.9, 19.3)		-0.4 (-6.5, 6.0)		1.0 (-13.7, 18.2)		1.1 (-8.8, 12.1)	
Obese		3.2 (-4.7, 11.9)		-2.7 (-9.7, 4.9)		**17.2 (4.6, 31.3)**		1.6 (-5.3, 8.9)		-7.3 (-17.4, 4.1)		6.8 (-3.5, 18.2)	
Under/Normal	∑AA	**11.0 (2.1, 20.6)**	0.83	6 (-3.9, 16.9)	0.42	8.5 (-5.5, 24.5)	0.25	4.4 (-2.0, 11.2)	0.84	-7.4 (-21.1, 8.7)	0.32	12.7 (-1.8, 29.3)	0.33
Overweight		7.0 (-3.4, 18.5)		5.2 (-5.3, 16.8)		9.4 (-5.7, 27.0)		1.9 (-6.1, 10.6)		2.1 (-17, 25.7)		1.0 (-11.9, 15.7)	
Obese		5.5 (-3.9, 15.8)		-1.0 (-9.2, 8.0)		**20.8 (5.9, 37.8)**		1.0 (-6.9, 9.5)		**-13.2 (-24.0, -0.8)**		**14.3 (1.7, 28.4)**	

Data are presented as the % change in hormone concentration for every 2-fold increase in phthalate metabolite concentration (ng/mL or nmol/mL) in under-/normal weight, overweight, and obese women from linear regression models adjusted for adjusted for age, race/ethnicity, employment status, education, income, marital status, alcohol consumption, smoking status, physical activity, medication use, and menopausal status. In separate models, an interaction between phthalate and BMI was included to formally test for effect modification by mid-life BMI, and the resulting P-value (Pint) is provided in the table. CI, confidence interval; AMH, anti-Mullerian hormone; BMI, body mass index; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin. n = 288, 187, and 243 for under-/normal weight, overweight, and obese women, respectively.

D.5.7. Associations between phthalate metabolites and hormones stratified by race/ethnicity

Associations of phthalate metabolites with estradiol, testosterone, and AMH were only observed in non-Hispanic white women (**Table 38**). Specifically, ∑DEHP, ∑Plastics, and ∑AA were positively associated with estradiol, MiBP was positively associated with testosterone, and MCPP and ∑AA were positively associated with AMH. However, associations of phthalate metabolites with progesterone were observed in both non-Hispanic white and black/other women (**Table 38**). Specifically, MCPP and ∑Plastics were positively associated with progesterone in black/other women, while ∑Phthalates was positively associated with progesterone in non-Hispanic white women.

Table 38. Associations of phthalate metabolites with hormones stratified by race/ethnicity.

Race/ Ethnicity	Phthalate	Estradiol	P_{int}	Testosterone	P_{int}	Progesterone	P_{int}	SHBG	P_{int}	FSH	P_{int}	AMH	P_{int}
% change in hormones for every 2-fold increase in phthalate concentrations													
Black/Other	MCPP	4.1 (-1.6, 10.1)	0.25	-0.1 (-5.1, 5.1)	0.89	**13.1 (4.1, 22.9)**	0.005	-2.2 (-6.4, 2.1)	0.97	-5.9 (-13.5, 2.5)	0.36	1.0 (-6.3, 9.0)	0.19
Non-Hispanic white		0.1 (-3.4, 3.8)		0.0 (-4.0, 4.1)		-2.6 (-7.8, 2.8)		-2.0 (-4.8, 1.0)		-0.2 (-6.8, 6.9)		**6.3 (0.9, 11.9)**	
Black/Other	MBzP	4.0 (-3.9, 12.6)	0.25	4.4 (-2.9, 12.2)	0.23	7.0 (-4.9, 20.5)	0.45	1.9 (-4.2, 8.2)	0.87	-1.6 (-12.7, 11.0)	0.96	4.8 (-5.7, 16.5)	0.57
Non-Hispanic white		-0.9 (-5.5, 3.9)		-3.9 (-8.9, 1.3)		1.3 (-5.7, 8.9)		1.0 (-2.8, 5.0)		-4.2 (-12.5, 4.8)		5.6 (-1.4, 13.1)	
Black/Other	∑DEHP	3.4 (-5.0, 12.6)	0.49	-3.5 (-10.7, 4.3)	0.08	12.1 (-1.2, 27.3)	0.56	-1.1 (-7.4, 5.6)	0.52	-1.2 (-13.1, 12.4)	0.80	2.3 (-8.7, 14.7)	0.49
Non-Hispanic white		**5.9 (0.7, 11.4)**		3.9 (-1.8, 9.9)		6.7 (-1.1, 15.1)		2.2 (-2.0, 6.5)		-4.5 (-13.3, 5.1)		5.2 (-2.2, 13.2)	
Black/Other	∑Plastics	4.6 (-4.5, 14.6)	0.75	-2.5 (-10.2, 6.0)	0.13	**17.1 (2.3, 34.1)**	0.29	-1.1 (-7.8, 6.1)	0.57	-2.3 (-14.9, 12.2)	0.90	2.2 (-9.6, 15.5)	0.31
Non-Hispanic white		**5.5 (0.0, 11.3)**		3.6 (-2.5, 10.0)		6.7 (-1.7, 15.7)		2.0 (-2.4, 6.6)		-5.2 (-14.4, 5.0)		7.2 (-0.8, 15.9)	
Black/Other	MEP	1.6 (-3.8, 7.4)	0.47	0.6 (-4.4, 5.8)	0.62	4.2 (-4.1, 13.2)	0.91	-0.4 (-4.5, 4.0)	0.85	1.5 (-6.7, 10.3)	0.58	0.7 (-6.5, 8.4)	0.45
Non-Hispanic white		-1.1 (-4.4, 2.3)		-0.4 (-4.1, 3.5)		4.9 (-0.4, 10.4)		0.6 (-2.2, 3.4)		5.2 (-1.4, 12.2)		-2.6 (-7.2, 2.4)	
Black/Other	MBP	0.5 (-9.0, 11.0)	0.92	0.6 (-8.1, 10.2)	0.86	6.1 (-8.6, 23.2)	0.83	-0.7 (-8.0, 7.2)	1.00	-5.5 (-18.7, 9.9)	0.71	8.3 (-5.2, 23.7)	0.96
Non-Hispanic white		1.6 (-4.1, 7.6)		2.3 (-4.1, 9.1)		4.8 (-3.9, 14.4)		1.0 (-3.6, 5.9)		-3.7 (-13.7, 7.5)		7.8 (-0.8, 17.1)	
Black/Other	MiBP	3.9 (-4.8, 13.4)	0.81	0.4 (-7.3, 8.8)	0.19	0.1 (-12.3, 14.3)	0.47	3.8 (-3.0, 11.1)	0.22	4.6 (-8.5, 19.5)	0.29	6.5 (-5.4, 19.8)	0.80
Non-Hispanic white		3.2 (-2.5, 9.2)		**9.0 (2.3, 16.1)**		7.2 (-1.7, 16.9)		0.1 (-4.5, 4.9)		-5.7 (-15.4, 5.2)		3.2 (-5.0, 12.1)	
Black/Other	∑PCP	2.5 (-3.8, 9.3)	0.43	0.5 (-5.2, 6.5)	0.92	5.0 (-4.6, 15.5)	0.76	-0.2 (-5.0, 4.8)	0.64	0.8 (-8.5, 11.0)	0.61	1.9 (-6.5, 11.0)	0.68
Non-Hispanic white		-1.2 (-5.8, 3.6)		0.8 (-4.5, 6.3)		6.9 (-0.5, 14.9)		1.8 (-2.1, 5.9)		5.3 (-3.8, 15.3)		-1.0 (-7.6, 6.1)	
Black/Other	∑Phthalates	3.9 (-3.5, 11.9)	0.74	-0.2 (-6.7, 6.8)	0.77	8.7 (-2.7, 21.4)	0.91	-1.2 (-6.7, 4.5)	0.38	0.7 (-10.0, 12.7)	0.86	2.6 (-7.1, 13.3)	0.85
Non-Hispanic white		1.4 (-4.2, 7.2)		1.7 (-4.5, 8.3)		**9.5 (0.6, 19.1)**		3.0 (-1.6, 7.9)		2.8 (-7.6, 14.4)		2.2 (-5.8, 10.8)	
Black/Other	∑AA	8.4 (-2.5, 20.6)	0.93	-1.1 (-10.3, 9.1)	0.26	**18.3 (0.9, 38.7)**	0.44	0.0 (-7.9, 8.6)	0.79	-3.7 (-18.1, 13.2)	0.90	8.9 (-5.6, 25.7)	0.75
Non-Hispanic white		**7.2 (1.0, 13.8)**		4.9 (-1.9, 12.1)		**10.3 (0.8, 20.8)**		2.9 (-2.1, 8.1)		-7.2 (-17.3, 4.0)		**9.1 (0.0, 19.0)**	

Data are presented as % change in hormone concentration for every 2-fold increase in phthalate metabolite concentrations (ng/mL or nmol/mL) in non-Hispanic white and Black/Other women from linear regression models adjusted for age, employment status, education, income, marital status, alcohol consumption, smoking status, physical activity, medication use, menopausal status, and BMI. In separate models, an interaction between phthalate and race/ethnicity was included to formally test for effect modification by race/ethnicity, and the resulting P-value (Pint) is provided in the table. CI, confidence interval; AMH, anti-Mullerian hormone; BMI, body mass index; FSH, follicle stimulating hormone; SHBG, sex hormone binding globulin. n = 241 and 477 for black/other and white women, respectively.

D.6. DISCUSSION

In the present study, we found that several phthalate metabolites were positively associated with both sex steroid and protein hormones. This particular trend was unexpected due to previous *in vitro* and *in vivo* studies, as well as observational studies suggesting that phthalates inhibit steroidogenesis (489, 495, 499, 514, 515). Previous observational studies evaluated these associations in men and women during their reproductive life, as well as in children, which may account for these discrepancies given that our study population is in midlife. We also found that some associations of phthalate metabolites with hormones differed by menopause status, midlife BMI, and race/ethnicity, which may provide critical information as to which midlife populations may be more susceptible to the endocrine disrupting effects of phthalates. Overall, our results suggest that phthalates may disrupt steroidogenesis through different mechanisms involving more than simple inhibition.

D.6.1. Overall associations of phthalate metabolites with hormones

We found that phthalates primarily found in plastic food packaging (i.e. ∑DEHP and ∑Plastics) and those shown to have anti-androgenic activity (i.e. ∑AA) share positive, linear associations with estradiol. ∑AA displayed positive relationships with estradiol in women in the third and fourth quartiles as well, further demonstrating the strength of this positive association. These results are consistent with studies conducted in pregnant women and women between the ages of 16 and 45 that have found positive associations between some phthalate metabolites such as MiBP and MBzP (516), as well as MBP (517) with estradiol, all of which are components of the ∑AA measurement used in our

study. However, some of our results are inconsistent with some experimental studies showing that phthalate exposure decreases estradiol levels in rodents (495, 518). Our results also differ from a study in Japanese pregnant women and a recent study in pre- and postmenopausal women from NHANES, which showed that DEHP was associated with lower serum estradiol concentrations (500).

We also observed associations of phthalates with testosterone and progesterone. While we did not observe an overall linear association between ∑DEHP and testosterone, our findings from quartile analyses showing negative associations between ∑DEHP and testosterone are consistent with experimental studies showing that DEHP has anti-androgenic properties (59, 63-65). However, we also found that MEP (quartile) and MiBP (linear and quartile) were positively associated with testosterone, which is not consistent with most studies (66-68). Although one observational study showed that prenatal MiBP exposure was associated with increased peripubertal testosterone in girls (69), a cross-sectional study using data from NHANES cycles 2013-2016 found that MEP, MiBP, and ∑DEHP were associated with reduced testosterone, and these associations were strongest in 40-60 year old females (22). Our study population acutely targeted women within a narrow age range to capture the menopausal transition, which may also account for discrepancies in our findings. Most notably, we found that ∑DEHP and MEP were positively associated with progesterone, and these were driving the associations observed for ∑Plastics, ∑PCP, ∑Phthalates, and ∑AA with progesterone. However, previous studies in animals and humans found equivocal results regarding these associations as those studies have reported positive and negative associations of

phthalates with progesterone (60, 70-72).

Overall associations of phthalates with non-steroid hormones (i.e. AMH, FSH, and SHBG) were less frequent. We found that MBP and ∑AA were positively associated with AMH in both linear and quartile analyses. Few studies have investigated associations between phthalates and AMH, but one research group found inverse associations between concentrations of MBP and AMH in follicular fluid (519), but also reported in an earlier study in the same group of women that MBP shared a positive association with serum AMH, similar to what we observed in our population (520). We also observed that MEP was positively associated with FSH in quartile analyses. However, two studies, one in healthy 16-45 year old women and the other in healthy 11-88 year old men found that some phthalate metabolites (but not MEP) were positively associated with FSH (517, 521). Lastly, we observed no associations between phthalates and SHBG. These results are consistent with studies in peripubertal girls and pregnant women (522-524). Overall, our results and those from previous studies further illustrate the complex relationships that phthalates can share with different hormones and that these associations may also differ across populations. However, additional studies, especially in midlife, are needed to corroborate our findings.

D.6.2. Differences in associations by menopause status

We found that associations of phthalates with estradiol, progesterone, and FSH were strongest in premenopausal women. Namely, ∑AA, ∑Plastics, and ∑DEHP were all positively associated with estradiol and progesterone in premenopausal women.

Coinciding with this finding is that ∑AA and ∑Plastics were also negatively associated with FSH in premenopausal women. Inverse relationships between estradiol and FSH are expected due to the negative feedback loop wherein FSH stimulates estradiol production and estradiol in turn suppresses FSH production. Studies have shown that phthalates are capable of modulating steroidogenic enzymes responsible for rate-limiting steps in the steroidogenesis pathway (525-527). Thus, it is possible that these effects may be due to direct phthalate-induced alterations of steroidogenic enzyme and/or activity. We speculate that these effects may be muted or not present in perimenopausal women because the entire hypothalamic-pituitary-gonadal (HPG) axis in perimenopausal women may be less sensitive to phthalate-induced changes or that the ovary itself is less sensitive to phthalate-induced changes due to the transition into menopause.

D.6.3. Differences in associations by midlife BMI

While we found that associations of phthalates with most hormones differed by midlife BMI, the most consistent associations were observed with progesterone. Most notably, positive associations of ∑DEHP, ∑Plastics, MEP, ∑PCP, ∑Phthalates, and ∑AA were positively associated with progesterone in obese women only. Adipose tissue is metabolically active with the capability to synthesize and metabolize sex steroid hormones (528). Additionally, the link between phthalates and obesity broadens the possibilities for the relationships that may exist between phthalates, adiposity, and hormone levels (529, 530). It is possible that phthalate-induced disruption in one steroidogenic organ (i.e., the ovary or the adipose tissue) can lead to compensatory action by the other. Alternatively, it is possible that subtle actions on both the ovary and

the adipose tissue in women with less adipose mass are less detectable than when in overweight and obese women, thus leading to relationships being observed in overweight and obese women only. The complex relationships that are likely to exist between phthalates, adipose tissue, and hormone levels merit further investigation.

D.6.4. Differences in associations by race/ethnicity

In race/ethnicity stratified analyses, positive associations of phthalate metabolites with estradiol and AMH were consistently strongest in non-Hispanic white women. Comparison to existing literature is difficult due to the lack of studies that investigate the interaction of hormones, race/ethnicity, and phthalates. However, one study that investigated the changes in hormones in different races found that African American women had a more rapid decline in estradiol concentrations during the menopausal transition than non-Hispanic white women (531). Although not a direct comparison, the study partially supports our findings in that we observed many different positive associations between phthalate measures and hormone levels, but we did not observe that black/other women had positive relationships between any phthalate measures and estradiol. However, this finding contrasts somewhat with other studies that have found that African American women have higher estradiol levels than non-Hispanic white women pre- and post-menopause (532, 533). Circulating hormone concentrations can be influenced by body composition and stress, which could also contribute to racial/ethnic differences in measured hormone levels, as well as result in differential impacts of phthalates on hormones in non-Hispanic white versus black women (440, 534). This highlights the need for further investigation into the complex relationships between

race/ethnicity, phthalate exposure, and hormones to fully appreciate the vulnerability of certain populations.

D.6.5. Strengths and limitations

Our study has limitations and strengths. Due to the cross-sectional nature of our analyses, we are unable to make conclusions about temporality of associations between phthalates and hormones. Further, it is possible that some women in our study experienced irregular menstrual cycles, which could impact hormone levels. However, to counterbalance the variability of menstrual cycles and timing during the cycle for collection of samples, we collected four blood samples that represent each week of a woman's menstrual cycle for hormone assessment and averaged these hormone concentrations for a more stable outcome measure. Further, the majority of the women in our study were either non-Hispanic white or black, leaving other races and ethnicities underrepresented in our study. While we *a priori* identified and adjusted for important confounders (i.e. sociodemographic characteristics, behavioral factors, and menopausal status), there may be unobserved or unmeasured confounding variables not accounted for in our statistical models, which could bias our observed associations. For example, diet is an important source of phthalate exposure and may also influence circulating hormone concentrations (535-538). Given that we were unable to control for diet, we may be overestimating associations between phthalates and hormones levels. Selection bias is also possible if participants with higher phthalate levels had certain characteristics that would impact their hormones. If selection bias exists, it could potentially lead to an under- or overestimation of the strength of our observed associations.

Major strengths of our study included the use of a pooled sample for assessing urinary phthalate metabolites, which is important given the short half-lives phthalates have in the body. Additionally, we were also powered enough to detect some differences in associations of phthalates metabolites with hormones by menopausal status, BMI, and race/ethnicity, revealing populations that are potentially more susceptible to the endocrine disrupting effects of phthalates. In addition, this was a multi-racial cohort of midlife women and one of the first studies to provide evidence of associations between urinary phthalate metabolites and hormone levels during a time period of rapid hormonal changes for women—midlife.

D.7. CONCLUSION

Our study found that some phthalates were associated with several critical hormones in midlife women. Specifically, the following positive associations were observed: ∑DEHP, ∑Plastics, and ∑AA with estradiol; MiBP with testosterone; ∑DEHP, ∑Plastics, MEP, ∑PCP, ∑Phthalates, and ∑AA with progesterone; MBP and ∑AA with AMH. Additionally, associations of phthalate metabolites differed by menopausal status, BMI, and race/ethnicity. Specifically, associations of phthalate metabolites with estradiol, progesterone, and FSH were strongest in premenopausal women, with progesterone were strongest in obese women, and with estradiol and AMH were strongest in non-Hispanic white women. Although some of our findings were corroborated by previous studies, many contrasted with the current literature. The variability in strength and direction of association between phthalates and reproductive hormones highlights the need for future studies to investigate a wide range of exposure windows and to elucidate

the mechanism(s) through which phthalates may act to disrupt the HPG-axis.

APPENDIX E: URINARY PHTHALATE METABOLITE CONCENTRATIONS AND HOT FLASHES IN WOMEN FROM AN URBAN CONVENIENCE SAMPLE OF MIDLIFE WOMEN

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E.1. ABSTRACT

Phthalate exposure is associated with altered reproductive function, but little is known about associations of phthalate exposure with risk of hot flashes. To investigate associations of urinary phthalate metabolite levels with four hot flash outcomes in midlife women. A cross-sectional study of the first year of a prospective cohort of midlife women, the Midlife Women's Health Study (2006–2015), a convenience sample from an urban setting. 728 multi-racial/ethnic pre- and perimenopausal women aged 45–54 years. Women completed questionnaires about hot flash experience and provided 1–4 urine samples over four consecutive weeks that were pooled for analysis. Phthalate metabolites were assessed individually and as molar sums representative of common compounds (all phthalates: ΣPhthalates; DEHP: ΣDEHP), exposure sources (plastics: ΣPlastic; personal care products: ΣPCP), and modes of action (anti-androgenic: ΣAA). Covariate-adjusted logistic regression models were used to assess associations of

continuous natural log-transformed phthalate metabolite concentrations with hot flash outcomes. Analyses were conducted to explore whether associations differed by menopause status, body mass index (BMI), race/ethnicity, and depressive symptoms. Overall, 45% of women reported a history of hot flashes. Compared to women who never experienced hot flashes, every two-fold increase in ΣPlastic was associated with 18% (OR: 1.18; 95%CI: 0.98, 1.43) and 38% (OR: 1.38; 95%CI: 1.11, 1.70) higher odds of experiencing hot flashes in the past 30 days and experiencing daily/weekly hot flashes, respectively. Some associations of phthalates with certain hot flash outcomes differed by menopause status, BMI, race/ethnicity, and depressive symptoms. This study suggests that phthalates are associated with hot flash experience and may impact hot flash risk in women who are susceptible to experiencing hot flashes.

E.2. KEYWORDS

Hot flashes; menopause; phthalates; women.

E.3. INTRODUCTION

Hot flashes are one of the most common symptoms of menopause, but little is known about the risk factors associated with increased risk of hot flashes. Hot flashes are characterized by sudden and transient periods of intense body heat accompanied by flushing, sweating, chills, and anxiety (539). Experiencing hot flashes can impact daily life for symptomatic women for years and results in estimated medical costs of $340 million in the U.S. each year and an additional $27 million in lost work (540, 541). Although the majority of peri- and postmenopausal women experience hot flashes, the dynamics of hot

flashes during menopause transition (such as age of onset, duration, intensity, and risk factors) are not well understood (540). Environmental factors (e.g. smoking), physiological factors (e.g. later stage of menopause), and decreasing estrogen levels are known to be associated with increased risk of hot flashes (542). Our own small cross-sectional analysis of a representative sample of 195 midlife women from the Midlife Women's Health Study (MWHS) indicated that exposure to phthalates may be associated with an increased odds of hot flashes in midlife women (543).

Phthalates are a class of synthetic chemicals composed of esters of ortho-phthalic acid with hydrocarbon side chains of varying lengths. Phthalates are used in a wide variety of consumer products, including food contact materials, medical equipment, car interiors, shower curtains, synthetic leather, and children's toys, as well as fragranced cleaning and personal care products (544). Humans are ubiquitously and unavoidably exposed to phthalates, with 99% of urine samples from the general U.S. population containing phthalate metabolites (255). Due to greater use of personal care products by women compared to men, women typically have higher concentrations of phthalates than men (545, 546).

Importantly, studies show that some phthalate metabolites exert toxicity in biological systems including the reproductive system (547-552). In both experimental and observational studies, phthalates have been shown to alter estradiol levels (495, 550, 553, 554). Animal studies have also shown that mixtures of phthalates can impact hormone levels and steroidogenesis in the ovary (495, 526, 555). This is concerning given

that midlife women are widely exposed to phthalates through diet and personal care products (495, 518, 548, 551). The molecular mechanisms of action of phthalates to cause hormone disruption are hypothesized to occur through activation of peroxisome proliferator-activated receptors (495, 550).

In general, factors that decrease estrogen levels in women are strongly associated with increased incidence of hot flashes (reviewed in (542)). Extensive literature suggests associations between estrogen levels and hot flashes in conditions that cause acute drops in estrogen levels, such as oophorectomy (556). As phthalates are associated with decreased estrogen levels in human and animals, we hypothesized that phthalate exposure may contribute to hot flash experience in women. To our knowledge, no studies have evaluated the impact of phthalate exposure on hot flash risk in women in detail. However, this study expands upon our previous pilot study (543) to include the entire cohort of the MWHS, which contains multiple racial/ethnic groups, and includes additional analyses based on participant characteristics. Therefore, the primary objective of this study was to assess associations of urinary phthalate metabolite levels with hot flash occurrence, frequency, and severity in midlife women enrolled in the first year of the MWHS. Because risk of hot flashes may differ in women based on menopausal status, midlife body mass index (BMI), race/ethnicity, and depression status (542, 557), the secondary objective of this study was to evaluate differences in associations of urinary phthalate metabolite levels with hot flash risk by these characteristics.

E.4. MATERIALS AND METHODS

E.4.1. Ethical approval

All participants gave written informed consent according to procedures approved by the University of Illinois and Johns Hopkins University Institutional Review Boards (file number: 06741).

E.4.2. Study population

This study was a cross-sectional analysis of data collected in the first year of the MWHS, a prospective cohort study with the overall goal of evaluating risk factors of hot flashes in midlife women. A detailed study protocol of the MWHS has been published previously (449). Briefly, participants were recruited from the city of Baltimore, MD (USA) and surrounding counties from 2006 to 2015. Women were eligible to participate in the study if they were 45-54 years old and pre- or perimenopausal with or without natural hot flashes. Women were excluded if they had a history of hysterectomy or oophorectomy, were currently pregnant, were taking hormone therapy or herbal/other agents for treatment of menopause symptoms, were taking oral contraceptives, were undergoing cancer treatment, or were postmenopausal. Menopausal status was defined using the Stages of Reproductive Aging Workshop + 10 (STRAW+10) criteria (558). Briefly, menopausal status was defined as follows: pre-menopausal women were those who experienced their last menstrual period within the past 3 months and reported 11 or more periods within the past year. Perimenopausal women were those who experienced their last menstrual period within the past year, but not within the past 3 months, or their last menstrual period within the past 3 months and experienced 10 or fewer periods within the

past year. Postmenopausal women were those who had not experienced a menstrual period within the past year. A total of 780 women enrolled in the study during year 1.

E.4.3. Collection of demographic and lifestyle characteristics

At the baseline clinic visit, women completed a detailed questionnaire and had anthropometrics measured by trained staff. Each woman's weight and height (without shoes) were measured by trained clinic staff, and values were rounded to the nearest 0.5 pound and 0.5 inch, respectively. The baseline questionnaire collected detailed information on demographics, reproductive history, menstrual cycle characteristics, menopausal symptoms, and medical history, as well as physical activity, smoking status, and alcohol use. Women self-reported their age in years and listed the types of prescription medications used. Each woman's racial/ethnic background was determined using the question "What is your main ethnic/racial background? (Answer, mark only one: (1) Caucasian/White, (2) African American/Black, (3) Hispanic/Latino, (4) Asian, (5) Other)". Women reported their highest completed grade or year of schooling using the following options: (1) elementary, (2) high school, (3) technical school, (4) college training, or (5) postgraduate. Smoking status was ascertained using the questions "Have you ever smoked cigarettes?" and "Do you still smoke cigarettes?" whereas the question "In the last 12 months have you had at least 12 drinks of any kind of alcoholic beverage?" was used to determine women's most recent alcohol consumption status. Leisure physical activity was assessed with the question: "In comparison with others my own age, I think my physical activity leisure time is" (choices: much more, more, as much, less, and much less). Women's depression status was assessed using the Centers for Epidemiologic

Studies Depression Scale (CESD) (559), which is a validated depression score that was calculated using 20 questions that asked about how the women were feeling during the past week.

E.4.4. Collection and assessment of hot flash outcomes

At baseline, a detailed history of hot flashes was collected using a series of validated questions that have been used in the MWHS for over 10 years (449, 557, 560-563). The current study evaluated four hot flash outcomes that were obtained from the following four questions: 1) whether the woman had ever experienced hot flashes, 2) whether she experienced hot flashes in the past 30 days, 3) the usual severity of her hot flashes, and 4) the usual frequency of her hot flashes. Women were first asked “Have you ever had hot flashes?” where hot flashes were defined as “a sudden feeling of heat in the face, neck, or upper part of the chest” with accompanied “reddening or flushing of the skin followed by sweating and chills.” Women who responded “no” to ever experiencing hot flashes were prompted to skip the more detailed hot flash questions and were categorized as “never experiencing hot flashes”. Those who responded “yes” to ever experiencing hot flashes answered whether they experienced hot flashes within the past 30 days (answer: no, yes). Additionally, women who had ever experienced hot flashes were asked (in general) to describe their hot flashes as: mild (sensation of heat without sweating), moderate (sensation of heat with sweating), or severe (sensation of heat with sweating that disrupts usual activity). We categorized severity of hot flashes as either mild or moderate/severe. Similarly, women who had ever experienced hot flashes were asked (in general) to describe their hot flashes as occurring: every hour, every 2–5 h, every 6–

11 h, every 12–23 h, 5–6 days per week, 1–2 days per week, 2–3 days per month, 1 day per month, less than 1 day per month, or never. We categorized frequency of hot flashes as either monthly or daily/weekly.

E.4.5. Assessment of urinary phthalate metabolites

Urinary phthalate metabolite concentrations are the preferred biomarkers of phthalate exposure (564). Humans are exposed to phthalate diesters (i.e. parent compounds), which are rapidly metabolized to monoester metabolites in the body (565). Therefore, epidemiological studies measuring human urinary phthalate metabolites often measure one or more metabolites for each parent phthalate and report as sums of metabolite concentrations based on parent compound, exposure source, or biological activity (29, 566-570).

Participants provided spot urine specimens at the initial baseline clinic visit and at visits during the next three consecutive weeks, which were used for urinary phthalate metabolite assessment. Each woman provided samples at 1–4 visits in the 4 week timeframe, which were pooled due to the short half-lives of phthalates in the body and the high daily and weekly intra-variability of measured concentrations (570). Pooled samples were analyzed for the following 9 phthalate metabolites: mono-2-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5 - carboxypentyl) phthalate (MECPP), mono-(2-ethyl- 5-oxohexyl)phthalate (MEOHP), mono-(3-carboxypropyl) phthalate (MCPP), monobenzyl phthalate (MBzP), monoethyl phthalate (MEP), monobutyl phthalate (MBP), and monoisobutyl phthalate (MiBP).

Analyses were performed using isotope dilution high-performance liquid chromatography negative-ion electrospray ionization-tandem mass spectrometry (HPLC–MS/MS) at the Metabolomics Lab of the Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign, using methods adapted from the Center for Disease Control and Prevention (571) and are described in the supporting information (572).

E.4.6. Statistical analysis

Out of 780 women enrolled during year 1 of the MWHS, 10 were missing information about hot flashes, an additional 10 were missing information about urinary phthalate metabolite concentrations and/or specific gravity, and an additional 32 women were missing information about covariates (which are described below). Therefore, the current study included a total of 728 women with information about baseline covariates, urinary phthalate metabolite concentrations, and hot flashes. Covariates for associations of midlife urinary phthalate metabolite concentrations with risk of hot flashes were chosen a priori and using previous literature that informed a directed acyclic graph (542, 557). To reduce potential for multicollinearity issues, we assessed correlations among all selected covariates (with none being strongly correlated). Therefore, final statistical models evaluating overall associations of urinary phthalate metabolite concentrations with four hot flash outcomes (objective 1) were adjusted for age, race, education, current drinking status, smoking status, medication use, menopause status, BMI, and CESD score. Age and CESD score were included as continuous variables, whereas the other variables were categorized with reference group set as shown in **Table 39**. Our second objective was to assess differences in associations of urinary phthalate metabolite concentrations

with 4 hot flash outcomes by menopausal status, BMI, race/ethnicity, and CESD score (objective 2) – factors that may influence the experience of hot flashes in midlife women (542). In addition to including the previously listed covariates in all stratified models, we *a priori* stratified our analyses as follows: pre- versus perimenopausal women, under-/normal weight (BMI<25kg/m2) versus overweight/obese (BMI≥25kg/m2) women, non-Hispanic white versus Black/other women, and women with fewer (CESD<16) versus more (CESD≥16) depressive symptoms (559).

Nine urinary phthalate metabolites were assessed from pooled urine samples. Urinary phthalate metabolite concentrations below the level of detection (LOD) were converted to the LOD/√(2). To account for urine dilution, we used the following formula to adjust all urinary phthalate metabolite concentrations: $P_c = P[(1.018 - 1)/(SG_i - 1)]$, where P_c is the specific gravity adjusted phthalate metabolite concentration, P is the measured phthalate metabolite concentration (ng/mL), 1.018 is the median specific gravity of MWHS population included in this analysis, and SG_i is the specific gravity of each woman's pooled urine sample (109). Specific gravity-adjusted urinary phthalate metabolite concentrations were used to approximate women's midlife exposure to phthalate parent compounds. Exposure to DEHP was approximated as the molar converted sum of 4 urinary metabolites using the following equation: ΣDEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308). Exposure to DOP, BzBP, DEP, DBP, and DiBP was approximated directly using the non-molar converted concentrations of their major urinary metabolites MCPP, MBzP, MEP, MBP, and MiBP, respectively. Additional phthalate sums were created based on primary sources of phthalate exposure, and sum of plasticizer

(ΣPlastic) and personal care product (ΣPCP) phthalate metabolites were estimated as follows: ΣPlastic = (MEHHP/294) + (MEHP/278) + (MEOHP/292) + (MECPP/308) + (MCPP/252) + (MBzP/256) and ΣPCP = (MEP/194) + (MBP/222) + (MiBP/222). Previous experimental and some epidemiological studies suggest that certain phthalate metabolites have anti-androgenic activity in the body (149, 495, 573). Therefore, the sum of anti-androgenic phthalate metabolites (ΣAA) was calculated as (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308) + (MBzP/256) + (MBP/222) + (MiBP/222). Lastly, all 9 urinary phthalate metabolites were molar converted and summed to approximate total midlife phthalate exposure (ΣPhthalates).

We used logistic regression models to evaluate overall and stratified associations of midlife urinary phthalate metabolite concentrations with the following 4 hot flash outcomes: 1) ever experiencing hot flashes, 2) experiencing hot flashes in the past 30 days, 3) experiencing daily/weekly or monthly hot flashes, and 4) experiencing moderate/severe or mild hot flashes (**Table 40**). All statistical analyses were conducted in SAS 9.4 (version 14.3, SAS Institute) using PROC LOGISTIC. Specifically, binary logistic regression models assessed overall and stratified associations of continuous midlife urinary phthalate levels with the odds of ever experiencing and experiencing hot flashes in the past 30 days compared to never experiencing hot flashes. Women who did experience hot flashes at some point, but not in the past 30 days (n=87) were excluded from binary logistic regression models comparing women who experienced hot flashes in the past 30 days to those who never experienced hot flashes. Multinomial logistic regression models assessed overall and stratified associations of continuous midlife

urinary phthalate levels with the odds of experiencing daily/weekly or monthly hot flashes and of experiencing moderate/severe or mild hot flashes compared to never experiencing hot flashes. Because all phthalate individual metabolites and molar sums were right-skewed, phthalates were natural log-transformed in all logistic regression models. All odds ratios (ORs) and 95% confidence intervals (CIs) were back transformed using the equation [$e^{\ln(OR)*\ln(2.00)}$] and data in **Table 42**, **Figures 26-29**, and the **Supplementary Tables** (included in (572)) are presented as the OR of experiencing these hot flash outcomes with every two-fold increase in phthalate metabolite or molar sum concentration with *a priori* alpha level of $P < 0.05$ (572). For logistic regression models evaluating stratified associations of phthalates with hot flashes, we provided formal test for effect modification in the **Supplementary Tables** (included in (572)), but reported on all relevant associations regardless of the interaction *P*-value.

E.5. RESULTS

E.5.1. Baseline MWHS population characteristics and hot flash prevalence

Baseline characteristics of 728 women in the MWHS are reported in **Table 39**. Overall, 64% of women were premenopausal, whereas 36% were perimenopausal. Most women were white (67%), whereas 33% were Black or of another race/ethnicity. Most women were employed (80%), college educated (65%), married or cohabiting (65%), and had an annual family income ≥$40,000 (75%). The prevalence of baseline healthy lifestyle characteristics in this study population were as follows: 35% did not regularly consume alcohol within the past year, 55% never smoked, 67% reported leisure time physical activity as more/much more than others, 43% were not taking any medications, and 80%

did not meet criteria for depression based on the CESD. Over one-third of the women were under- or normal weight (40%) and over half were overweight or obese (60%).

Table 39. Demographic and lifestyle characteristics of 45-54 year-old women from the Midlife Women's Health Study (n=728).

Demographic or Lifestyle Characteristic	n (%)[2]
Age (years)[1]	
45 to 49	477 (65.5)
50 to 54	251 (34.5)
Race[1]	
Non-Hispanic White (ref)	484 (66.5)
Black	215 (29.5)
Other	29 (4.0)
Employment status	
Unemployed	146 (20.1)
Employed	582 (79.9)
Education[1]	
Some college or less	255 (35.0)
College graduate or higher (ref)	473 (65.0)
Annual family income ($)	
<20,000	45 (6.2)
20,000 to 39,999	113 (15.5)
40,000 to 99,999	241 (33.1)
≥100,000	308 (42.3)
Marital status	
Single	133 (18.3)
Married/Living with Partner	476 (65.4)
Widowed/divorced/separated	118 (16.2)
Menopausal status[1]	
Premenopausal (ref)	468 (64.3)
Perimenopausal	260 (35.7)
Alcohol consumption status (>1 drink/month on average)[1]	
No	252 (34.6)
Yes (ref)	476 (65.4)
Smoking status[1]	
Current	69 (9.5)
Former	256 (35.2)
Never (ref)	403 (55.4)
Leisure physical activity compared to others	
Much more/more	257 (35.3)
As much	230 (31.6)
Less/much less	235 (32.3)
Body mass index (kg/m^2)[1]	
<25	290 (39.8)
≥25 (ref)	438 (60.2)

Table 39 (cont'd).

Demographic or Lifestyle Characteristic	n (%)[2]
Current medication use[1]	
No	311 (42.7)
Yes (ref)	417 (57.3)
CES depression score[1]	
Fewer depressive symptoms (<16)	581 (79.8)
More depressive symptoms (≥16)	147 (20.2)

[1]Variables included in logistic regression models. [2]Percentages may not add up to 100% due to missing values.

The self-reported baseline prevalence of hot flashes in the MWHS is presented in **Table 40**. Out of 728 total women in the current study, all women provided information about ever experiencing hot flashes, 722 had information about experiencing hot flashes within the past 30 days, 697 had information about hot flash frequency, and 720 had information about hot flash severity. Approximately 55% of women never experienced hot flashes, whereas 45% had experienced hot flashes and 32% had experienced hot flashes in the past 30 days. Overall, 22% had daily/weekly hot flashes and 19% had monthly hot flashes, whereas 29% had moderate/severe hot flashes and 15% had mild hot flashes.

Table 40. Prevalence of hot flashes self-reported by women from the Midlife Women's Health Study (n=728).

Hot Flashes	n (%)
History of hot flashes	
No	399 (54.8)
Yes	329 (45.2)
Hot flashes during past 30 days	
Never had hot flashes	399 (54.8)
Had hot flashes and experienced in past 30 days	236 (32.4)
Had hot flashes but did not experience in past 30 days	87 (12.0)
Missing	6 (0.8)
Frequency of hot flashes	
Never had hot flashes	399 (54.8)
Monthly hot flashes	139 (19.1)
Daily/weekly hot flashes	159 (21.8)
Missing	31 (4.3)
Severity of hot flashes	
Never had hot flashes	399 (54.8)
Mild hot flashes	108 (14.8)
Moderate/severe hot flashes	213 (29.3)
Missing	8 (1.1)

E.5.2. Baseline urinary phthalate metabolite concentrations

Table 41 presents median (25th, 75th percentiles) concentrations of individual urinary phthalate metabolites and phthalate molar sums during the first year of the MWHS. Concentrations of most urinary phthalate metabolites measured in the MWHS were ≥LOD in 100% of women, except for MEP, for which 99.7% of women had concentrations ≥LOD. Median urinary phthalate metabolite and molar sum concentrations in the MWHS were compared to those measured from a nationally representative sample of 45-54-year-old U.S. women from the 2005-2016 National Health and Nutrition Examination Survey (NHANES). Median metabolite levels were slightly higher in the MWHS than NHANES with overlapping 25–75th percentiles. Metabolite levels were similar to recently reported results from the Study of Environment, Lifestyle, and Fibroids (SELF) from non-Hispanic Black women (23–35 years old) (163). SELF also reports slightly higher DEHP metabolites than the corresponding NHANES samples (163).

Table 41. Concentrations of individual urinary phthalate metabolites and molar sums from 45-54-year-old women from the Midlife Women's Health Study and the National Health and Nutrition Examination Survey.

Name	Abbreviation	MWHS (2006-2015) n=728	NHANES (2005-2016) n=757[1]
	Phthalate metabolite	**Median (25th, 75th percentiles) in ng/mL**	
Mono(2-ethylhexyl) phthalate	MEHP	4.5 (2.7, 9.3)	1.2 (0.6, 3.1)
Mono(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	33.5 (20.5, 58.7)	9.1 (3.5, 22.6)
Mono(2-ethyl-5-oxohexyl) phthalate	MEOHP	12.0 (7.3, 22.4)	5.6 (2.1, 13.3)
Mono(2-ethyl-5-carboxypentyl) phthalate	MECPP	25.9 (15.9, 48.0)	13.4 (5.6, 31.7)
Mono(3-carboxypropyl) phthalate	MCPP	2.5 (1.3, 5.4)	1.5 (0.6, 3.4)
Monobenzyl phthalate	MBzP	9.4 (5.4, 16.1)	4.2 (1.8, 10.4)
Monoethyl phthalate	MEP	97.3 (48.2, 192.0)[2]	58.9 (20.0, 179.8)
Mono-n-butyl phthalate	MBP	19.8 (13.0, 32.8)	11.6 (5.4, 25.3)
Mono-isobutyl phthalate	MiBP	16.4 (10.0, 26.1)	5.8 (2.6, 13.2)
	Phthalate molar-converted sum[3]	**Median (25th, 75th percentiles) in nmol/mL**	
Di(2-ethylhexyl) phthalate	DEHP	0.3 (0.2, 0.5)	0.1 (0.04, 0.2)
Sum of all phthalate metabolites	ΣPhthalates	1.2 (0.7, 2.1)	0.7 (0.3, 1.8)
Sum of all personal care product phthalate metabolites	ΣPCP	0.7 (0.4, 1.3)	0.4 (0.2, 1.2)
Sum of all plastic phthalate metabolites	ΣPlastic	0.3 (0.2, 0.6)	0.1 (0.1, 0.3)
Sum of anti-androgenic phthalate metabolites	ΣAA	0.5 (0.3, 0.8)	0.2 (0.1, 0.5)

[1]Weighted phthalate metabolite concentrations for 45-54-year-old US women from combined NHANES survey years 2005-06, 2007-08, 2009-10, 2011-12, 2013-14, and 2015-16. [2]Two samples (0.3%) were < LOD for MEP.

E.5.3. Overall associations of phthalates with hot flashes

Midlife urinary phthalate metabolite concentrations were not associated with ever experiencing hot flashes or experiencing moderate/severe or mild hot flashes compared to never experiencing hot flashes (**Table 42**). However, phthalates were associated with experiencing hot flashes in the past 30 days. Specifically, women had 19% higher odds of the experiencing hot flashes in the past 30 days with every two-fold increase in MEHHP (OR: 1.19; 95%CI: 1.00, 1.43). Additionally, phthalates were associated with experiencing daily/weekly, but not monthly hot flashes. Specifically, women had 23–38% higher odds of experiencing daily/weekly hot flashes with every two-fold increase in the DEHP metabolites MEHHP (OR: 1.34, 95%CI: 1.09, 1.65), MEOHP (OR: 1.23, 95%CI: 1.03, 1.48), MECPP (OR: 1.28, 95%CI: 1.06, 1.54) and the summary measures ΣDEHP (OR:

1.32, 95%CI: 1.08, 1.61), ΣPlastic (OR: 1.38; 95%CI: 1.11, 1.71), ΣPhthalates (OR: 1.26; 95%CI: 1.03, 1.54), and ΣAA (OR: 1.36; 95%CI: 1.07, 1.73).

Table 42. Overall associations of urinary phthalate concentrations with hot flashes.

	Outcomes					
	Ever experiencing hot flashes[1]	**Experiencing hot flashes in the past 30 days[1]**	**Experiencing daily/weekly or monthly hot flashes[2]**		**Experiencing moderate/severe or mild hot flashes[2]**	
n	728	635	697		720	
Reference	Never experiencing hot flashes	Never experiencing hot flashes	Never experiencing hot flashes		Never experiencing hot flashes	
			Daily/weekly	**Monthly**	**Moderate/severe**	**Mild**
Exposure[3]	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
MEP	1.09 (0.98, 1.22)	1.05 (0.93, 1.18)	1.1 (0.96, 1.27)	1.1 (0.96, 1.25)	1.06 (0.94, 1.2)	1.12 (0.97, 1.3)
MBP	1.01 (0.85, 1.21)	1.1 (0.89, 1.35)	1.17 (0.93, 1.47)	0.92 (0.74, 1.16)	1 (0.81, 1.23)	0.98 (0.76, 1.25)
MiBP	0.98 (0.82, 1.16)	1.03 (0.85, 1.26)	1.03 (0.82, 1.3)	0.98 (0.79, 1.21)	1 (0.82, 1.22)	0.91 (0.72, 1.16)
ΣPCP	1.08 (0.94, 1.23)	1.04 (0.89, 1.22)	1.12 (0.94, 1.33)	1.07 (0.91, 1.27)	1.03 (0.88, 1.2)	1.12 (0.93, 1.34)
MCPP	0.92 (0.83, 1.03)	0.94 (0.82, 1.06)	1.02 (0.88, 1.18)	0.87 (0.76, 1)	0.92 (0.81, 1.05)	0.92 (0.79, 1.07)
MBzP	0.95 (0.82, 1.1)	0.93 (0.79, 1.11)	1.08 (0.89, 1.3)	0.94 (0.79, 1.12)	0.95 (0.81, 1.13)	0.94 (0.77, 1.15)
MEHP	1.05 (0.92, 1.19)	1.07 (0.92, 1.24)	1.18 (0.99, 1.4)	0.99 (0.85, 1.17)	1.05 (0.9, 1.22)	1.05 (0.88, 1.25)
MEHHP	1.14 (0.97, 1.33)	**1.19 (1, 1.43)**	**1.34 (1.09, 1.65)**	1.09 (0.9, 1.32)	1.17 (0.98, 1.39)	1.1 (0.88, 1.36)
MEOHP	1.07 (0.93, 1.23)	1.11 (0.95, 1.3)	**1.23 (1.03, 1.48)**	1.04 (0.87, 1.23)	1.07 (0.91, 1.25)	1.08 (0.9, 1.31)
MECPP	1.12 (0.97, 1.3)	1.17 (0.99, 1.38)	**1.28 (1.06, 1.54)**	1.09 (0.92, 1.3)	1.13 (0.96, 1.33)	1.12 (0.93, 1.37)
ΣDEHP	1.13 (0.96, 1.31)	1.18 (0.99, 1.42)	**1.32 (1.08, 1.61)**	1.08 (0.89, 1.3)	1.14 (0.96, 1.37)	1.1 (0.89, 1.36)
ΣPlastic	1.12 (0.95, 1.32)	1.18 (0.98, 1.43)	**1.38 (1.11, 1.71)**	1.05 (0.85, 1.29)	1.14 (0.95, 1.37)	1.1 (0.88, 1.38)
ΣPhthalates	1.13 (0.96, 1.32)	1.12 (0.93, 1.35)	**1.26 (1.03, 1.54)**	1.09 (0.89, 1.33)	1.07 (0.89, 1.29)	1.19 (0.97, 1.47)
ΣAA	1.08 (0.9, 1.31)	1.16 (0.93, 1.44)	**1.36 (1.07, 1.73)**	1 (0.79, 1.27)	1.09 (0.88, 1.35)	1.07 (0.83, 1.39)

[1]Binary logistic regression models evaluated associations of every 2-fold increase in urinary phthalate concentrations with the odds of ever experiencing and experiencing hot flashes in the last 30 days compared to never having hot flashes.

[2]Multinomial logistic regression models evaluated associations of every 2-fold increase in urinary phthalate concentrations with the odds of experiencing daily/weekly or monthly hot flashes and experiencing moderate/severe hot flashes compared to never experiencing hot flashes.

All logistic regression models were adjusted for age, race/ethnicity, education, alcohol consumption, smoking status, medication use, menopausal status, body mass index, and CESD score. CI, confidence interval; OR, odds ratio.

[3]ΣDEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308); ΣPhthalates = (MEP/194) + (MBP/222) + (MiBP/222) + (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308) + (MBzP/256) + (MCPP/252); ΣPCP = (MEP/194) + (MBP/222) + (MiBP/222); ΣPlastic = (MEHHP/294) + (MEHP/278) + (MEOHP/292) + (MECPP/308) + (MCPP/252) + (MBzP/256); ΣAA = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308) + (MBzP/256) + (MBP/222) + (MiBP/222)

E.5.4. Associations of phthalates with hot flashes stratified by menopause status

Associations of urinary phthalate metabolite concentrations with ever experiencing hot flashes, experiencing hot flashes in the past 30 days, or experiencing daily/weekly hot flashes did not significantly differ by menopause status (**Figure 26A**, **B, Supplementary Tables** (572)).

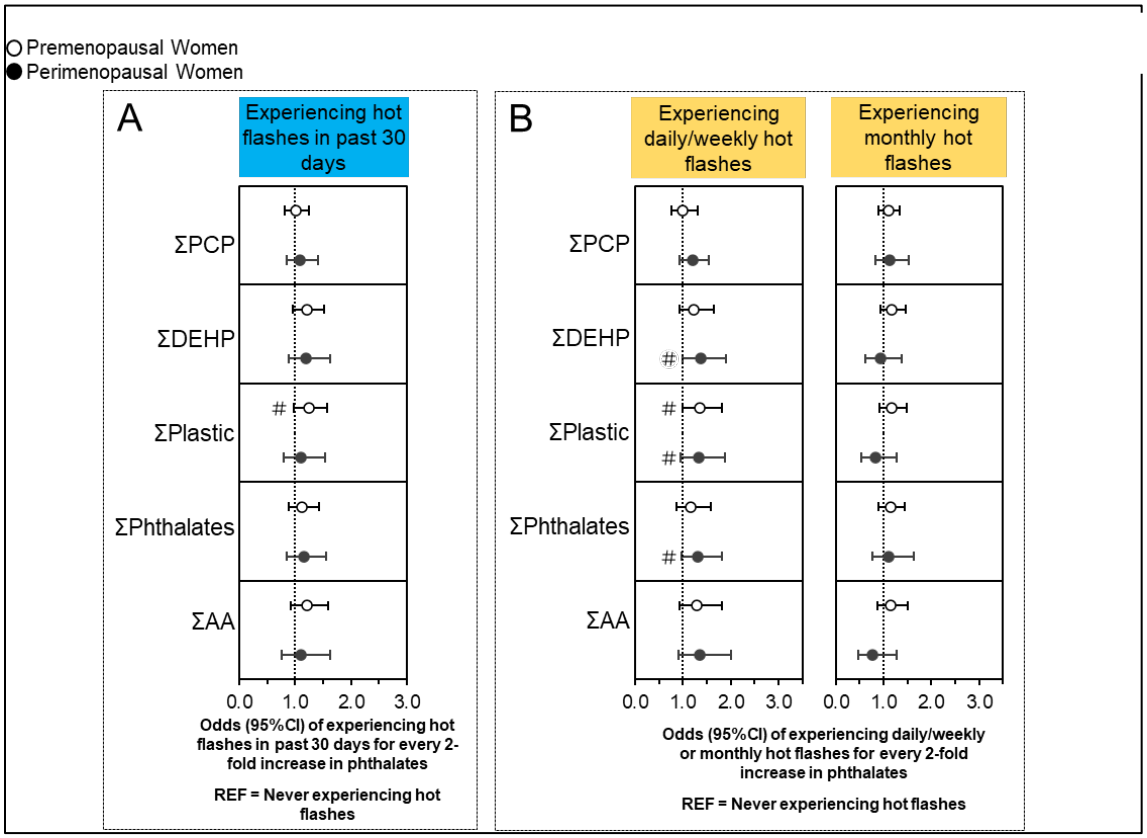

Figure 26. Associations of urinary phthalate concentrations with hot flashes stratified by menopause status. Binary logistic regression models evaluated associations of urinary phthalate concentrations with the odds experiencing hot flashes in the last 30 days compared to never having hot flashes (n=635), while multinomial logistic regression models evaluated associations of urinary phthalate concentrations with the odds of experiencing daily/weekly or monthly hot flashes compared to never experiencing hot flashes (n=697). All models were stratified by menopause status and were adjusted for age, race/ethnicity, education, alcohol consumption, smoking status, medication use, body mass index, and CESD score. Data are presented as odds ratio (filled circles) and 95% confidence interval (solid lines) for every two-fold increasing in urinary phthalate concentrations. Confidence intervals that do not cross the null are significant at $^{\#}P<0.10$ and $^{*}P<0.05$.

E.5.5. Associations of phthalates with hot flashes stratified by midlife BMI

Associations of urinary phthalate metabolite concentrations with ever experiencing hot flashes, experiencing hot flashes in the past 30 days, and experiencing moderate/severe or mild hot flashes were not different by midlife BMI (**Figure 27A**, **Supplementary Tables** (572)). However, some associations of phthalates with experiencing daily/weekly (but not monthly) hot flashes were only observed in under-/normal weight women (**Figure 27B**, **Supplementary Tables** (572)), who had 57–107% higher odds of experiencing daily/weekly hot flashes with every two-fold increase in MBP (OR: 1.57; 95%CI: 1.04, 2.38), MBzP (OR: 1.63; 95%CI: 1.18, 2.25), ΣDEHP (OR: 1.77; 95%CI: 1.24, 2.52), ΣPlastic (OR: 2.07; 95% CI: 1.40, 3.05), and ΣAA (OR: 2.05; 95%CI: 1.31, 3.19).

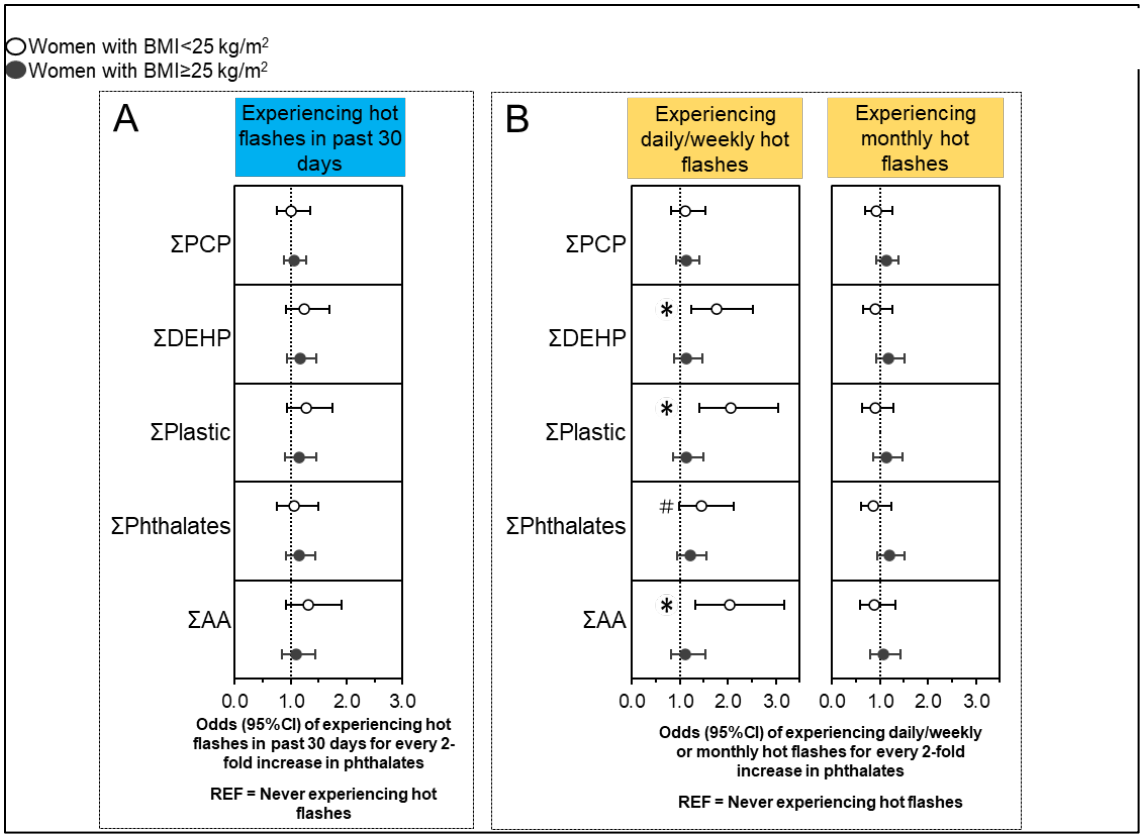

Figure 27. Associations of urinary phthalate concentrations with hot flashes stratified by midlife BMI. Binary logistic regression models evaluated associations of urinary phthalate concentrations with the odds of experiencing hot flashes in the last 30 days compared to never having hot flashes (n=635), while multinomial logistic regression models evaluated associations of urinary phthalate concentrations with the odds of experiencing daily/weekly or monthly hot flashes compared to never experiencing hot flashes (n=697). All models were stratified by midlife BMI and were adjusted for age, race/ethnicity, education, alcohol consumption, smoking status, medication use, menopause status, and CESD score. Data are presented as odds ratio (filled circles) and 95% confidence interval (solid lines) for every two-fold increasing in urinary phthalate concentrations. Confidence intervals that do not cross the null are significant at $^{\#}P<0.10$ and $^{*}P<0.05$. BMI, body mass index.

E.5.6. Associations of phthalates with hot flashes stratified by race/ethnicity

Some associations of urinary phthalate metabolite concentrations with ever experiencing hot flashes, experiencing hot flashes in the past 30 days, experiencing daily/weekly or monthly hot flashes, and experiencing mild (but not moderate/severe) hot flashes also differed by race/ethnicity (**Figure 28A**, **B**, **Supplementary Tables** (572)). Non-Hispanic

white women had 19-27% higher odds of ever experiencing hot flashes with every two-fold increase in MEP (OR: 1.19; 95%CI: 1.03, 1.38) and ΣPhthalates (OR: 1.27; 95%CI: 1.01, 1.58) (572). Additionally, non-Hispanic white women had 37-45% higher odds of experiencing daily/weekly hot flashes with every two-fold increase in ΣDEHP (OR: 1.37; 95%CI: 1.06, 1.76), ΣPlastic (OR: 1.45; 95%CI: 1.11, 1.90), ΣPhthalates (OR: 1.40; 95%CI: 1.06, 1.85), and ΣAA (OR: 1.40; 95%CI: 1.04, 1.89), as well as higher odds of experiencing monthly hot flashes with every two-fold increase in MEP (OR: 1.23; 95%CI: 1.03, 1.47) (**Figure 28B**, **Supplementary Tables** (572)). Conversely, Black/other women had 20% (OR: 0.80; 95%CI: 0.66, 0.98) and 28% (OR = 0.72, 95% CI 0.55, 0.95) lower odds of ever experiencing or experiencing mild hot flashes, respectively, with every two-fold increase in MCPP, but had 51% higher odds of experiencing hot flashes in the past 30 days with every two-fold increase in MEHHP (OR: 1.51; 95%CI: 1.08, 2.10) (572).

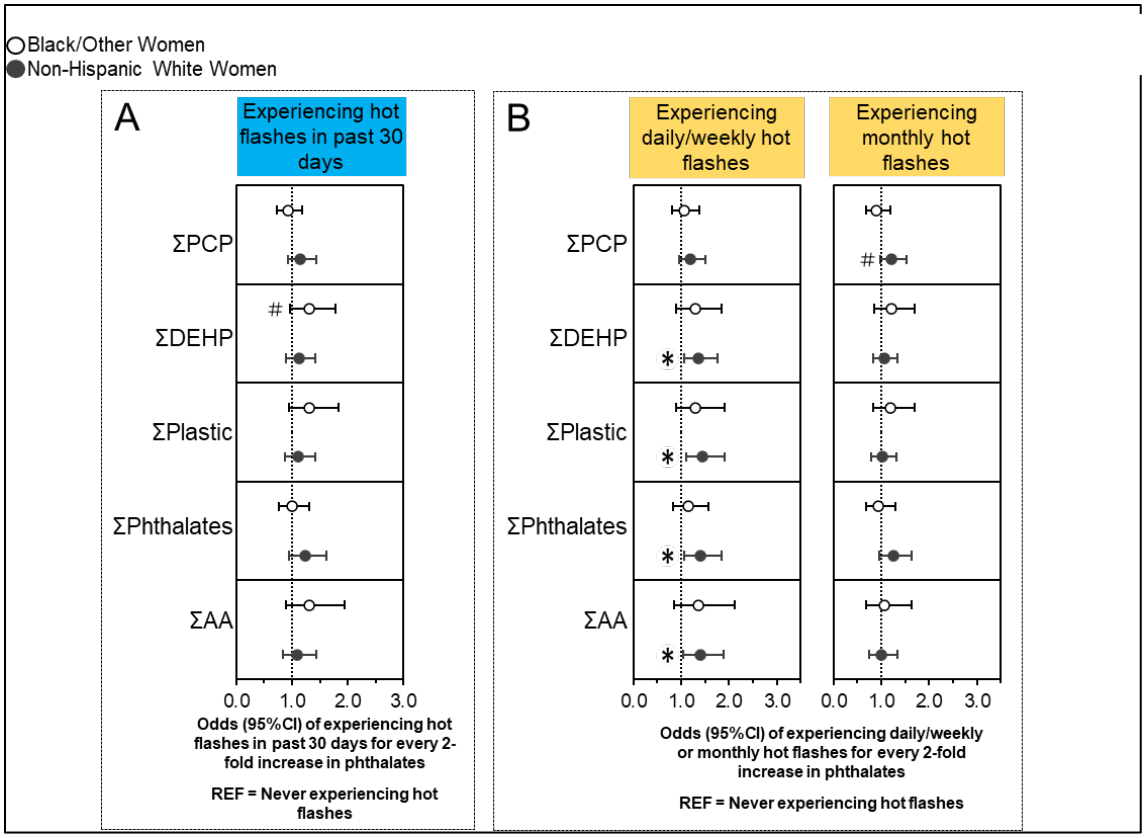

Figure 28. Associations of urinary phthalate concentrations with hot flashes stratified by race/ethnicity. Binary logistic regression models evaluated associations of urinary phthalate concentrations with the odds of experiencing hot flashes in the last 30 days compared to never having hot flashes (n=635), while multinomial logistic regression models evaluated associations of urinary phthalate concentrations with the odds of experiencing daily/weekly or monthly hot flashes and compared to never experiencing hot flashes (n=697). All models were stratified by race/ethnicity and were adjusted for age, education, alcohol consumption, smoking status, medication use, menopause status, body mass index, and CESD score. Data are presented as odds ratio (filled circles) and 95% confidence interval (solid lines) for every two-fold increasing in urinary phthalate concentrations. Confidence intervals that do not cross the null are significant at $^{\#}P<0.10$ and $^{*}P<0.05$.

E.5.7. Associations of phthalates with hot flashes stratified by CESD score

Some associations of urinary phthalate metabolite concentrations with ever experiencing hot flashes, experiencing hot flashes in the past 30 days, experiencing daily/weekly or monthly hot flashes, and experiencing mild (but not moderate/severe) hot flashes differed by CESD score (**Figure 29A**, **B**, **Supplementary Tables** (572)). For example, women

with fewer depressive symptoms (CESD<16) had 24% higher odds of experiencing hot flashes in the past 30 days with every two-fold increase in ΣDEHP (OR: 1.24; 95%CI: 1.02, 1.49) (572), and had 38-45% higher odds of experiencing daily/weekly hot flashes with every two-fold increase in ΣDEHP (OR: 1.38; 95%CI: 1.09, 1.73), ΣPlastic (OR: 1.45; 95%CI: 1.14, 1.86), and ΣAA (OR: 1.42; 95%CI 1.07, 1.88) (**Figure 29B**, **Supplementary Tables** (572)). Conversely, women with more depressive symptoms (CESD≥16) had 42-71% higher odds of experiencing hot flashes in the past 30 days with every two-fold increase in MEP (OR: 1.42; 95%CI: 1.02, 1.97), ΣPCP (OR: 1.50; 95%CI: 1.03, 2.19), and ΣPhthalates (OR: 1.71; 95%CI: 1.04, 2.79) (**Figure 29A**, **Supplementary Tables** (572)). Additionally, these women had 47-93% and 60-78% higher odds of experiencing daily/weekly or monthly hot flashes, respectively, for every two-fold increase in MEP (daily/weekly OR: 1.47; 95%CI: 1.03, 2.11; monthly OR: 1.60; 95%CI: 1.10, 2.33), ΣPCP (daily/weekly OR: 1.59; 95%CI: 1.06, 2.41; monthly OR: 1.60; 95%CI: 1.05, 2.46), and ΣPhthalates (daily/weekly OR: 1.93; 95%CI: 1.13, 3.28; monthly OR: 1.78; 95%CI: 1.04, 3.06), but also had 43% lower odds of experiencing monthly hot flashes with every two-fold increase in MBzP (OR: 0.57; 95%CI: 0.35, 0.94) (**Figure 29B**, **Supplementary Tables** (572)).

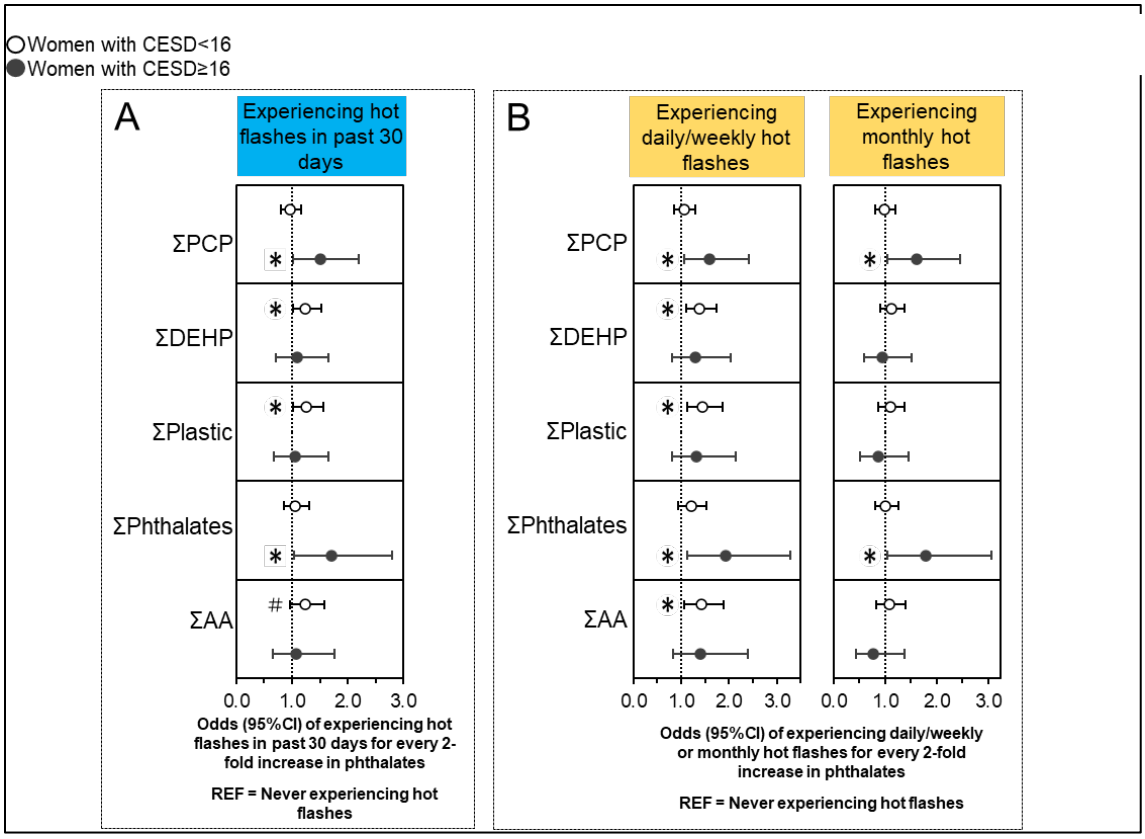

Figure 29. Associations of urinary phthalate concentrations with hot flashes stratified by CESD score. Binary logistic regression models evaluated associations of urinary phthalate concentrations with the odds of experiencing hot flashes in the last 30 days compared to never having hot flashes (n=635), while multinomial logistic regression models evaluated associations of urinary phthalate concentrations with the odds of experiencing daily/weekly or monthly hot flashes compared to never experiencing hot flashes (n=697). All models were stratified by CESD score and were adjusted for age, race/ethnicity, education, alcohol consumption, smoking status, medication use, menopause status, and body mass index. Data are presented as odds ratio (filled circles) and 95% confidence interval (solid lines) for every two-fold increasing in urinary phthalate concentrations. Confidence intervals that do not cross the null are significant at $^{\#}P<0.10$ and $^{*}P<0.05$.

E.6. DISCUSSION

In this cross-sectional analysis of year 1 data from the MWHS, a prospective cohort of pre- and perimenopausal women from Baltimore and its surrounding counties, urinary phthalate metabolite concentrations were associated with experiencing recent and experiencing more frequent hot flashes, but not associated with ever experiencing hot

flashes or hot flash severity. Generally, we found that phthalate metabolites of parent compounds found in plastics were associated with increased risk of experiencing hot flashes in the past 30 days and experiencing daily/weekly hot flashes. Some associations of phthalates with certain hot flash outcomes were different by menopause status, midlife BMI, race/ethnicity, and depressive symptoms. Most notably, we found that associations of personal care product phthalates with most hot flash outcomes were strongest in women with more depressive symptoms. Interestingly, MCPP (and to a lesser extent MBzP) was associated with lower risk of hot flashes, especially in perimenopausal and Black/other women.

Overall, these results are consistent with our previous findings that phthalate metabolites are associated with increased frequency of hot flashes in midlife women (543). In our pilot study of a representative sample of 195 women from the MWHS cohort, ΣPCP was associated with higher odds of ever experiencing hot flashes, experiencing hot flashes in the past 30 days, and experiencing daily/weekly hot flashes (543). In the current study, we found that associations of ΣPCP with risk of hot flashes only emerged when associations were stratified by CESD score. In the pilot study, ΣAA and ΣDEHP were not associated with hot flash frequency. However, in the current study, we found that ΣDEHP and ΣPlastic were consistently associated with experiencing hot flashes in the past 30 days and hot flash frequency, whereas ΣAA was only associated with hot flash frequency. The pilot study did not evaluate associations of individual phthalate metabolites, ΣPhthalates, or ΣPlastic with hot flash experience.

To our knowledge, no other observational studies have reported on the associations between phthalates and hot flashes in midlife women. However, phthalates are associated with disorders of the female reproductive system, including premature reproductive senescence and altered hormone levels, and these disorders may contribute to the risk of hot flashes (542, 557, 561, 574, 575). Studies in mice have shown premature reproductive senescence following adult exposure to DEHP and diisononyl phthalate (DiNP) (576, 577). Observational studies have identified associations between urinary phthalate metabolite levels and earlier onset of menopause (578) and premature ovarian failure (517, 579). Women who undergo earlier menopause report more frequent and severe hot flashes than premenopausal women (575, 580). In addition, higher urinary DEHP metabolite levels were associated with decreased levels of the hormones inhibin B and anti-Müllerian hormone, markers of ovarian reserve in women (581).

Hot flashes and declining estradiol levels occur simultaneously during menopause and other physiological transitions such as the postpartum period (542). Clinical trials have shown that estrogen therapy can alleviate hot flashes, suggesting a causal association, although the mechanism remains unknown (510, 582, 583). Numerous observational studies have also identified associations of altered estradiol and progesterone levels with risk of hot flashes (542, 557, 561, 574, 584). Using the MWHS cohort, the close relationship between hot flashes and estradiol was recently modeled using a Bayesian network (510). Animal studies have demonstrated altered estradiol and progesterone levels following phthalate exposure (495, 518, 548, 555, 585), and future studies from the MWHS will investigate the association between urinary phthalate metabolites and

hormone levels in midlife women. Other potential mechanisms through which phthalates could be linked to hot flashes include direct disruption of hypothalamic or thyroid function (542, 586, 587). However, as the etiology of hot flashes is not well understood, it is difficult to speculate on causal links between phthalates and hot flashes.

When we evaluated stratified associations of phthalates with these hot flashes outcomes by menopause status, BMI, and race/ethnicity, the strongest associations were observed in perimenopausal women, non-Hispanic white women, and under-/normal weight women. These sub-group associations were most consistent between DEHP metabolites and experiencing daily/weekly hot flashes. Black women generally have higher phthalate exposure than white women (588, 589), which may be due to the phthalate content in personal care products used by Black women (590). In the MWHS, Black women had higher urinary PCP phthalate concentrations than non-Hispanic white women (data not shown). However, we did not identify any consistent associations of ΣPCP with risk of hot flashes in Black/other women despite this being a high-risk exposure group, which may indicate that phthalates in personal care products do not contribute to hot flash experience in non-white women. Previously, we have identified high BMI as a risk factor for experiencing perimenopausal hot flashes (574). However, we found stronger associations between phthalates and risk of hot flashes in women with lower BMI. Interestingly, MBzP was associated with increased risk of hot flashes in normal weight women and trending towards decreased risk of ever experiencing hot flashes in overweight women. These differences suggest that women with higher body weight may be less susceptible to endocrine disruption by phthalates. One possible explanation is

that estrogen levels are already decreased in perimenopausal women with high BMI (574).

Previous studies have identified bidirectional associations of depression with risk of hot flashes, and suggest that they may be linked through sleep disruption (591). In addition, phthalates may be associated with depression in adults. MECPP, MBP, MiBP, and MBzP were associated with depression in adults from NHANES (592). In elderly populations (ages 59-93), DEHP metabolites, MCPP, and MBP have also been associated with depression (593, 594). When associations of phthalates with risk of hot flashes were stratified by CESD scores in the MWHS, we observed different associations of phthalate metabolites, with risk of hot flashes in women experiencing fewer and more depressive symptoms. DEHP metabolites and phthalates found in plastics were consistently associated with hot flashes in women experiencing fewer depressive symptoms, whereas MEP and phthalates associated with personal care products were associated with hot flashes in women with more depressive symptoms. The different observations in women in more vs. fewer depressive symptoms suggests that depression symptoms may be related to hormonal changes or that the physiology of depression may play a role, possibly hormonal, in phthalate mechanism of action. Additional studies are needed to investigate the role of depression in environmental chemical action. In addition, the interaction of each depression group with a different phthalate category may be evidence that the high molecular weight phthalates found in plastics act through different mechanisms than the low molecular weight phthalates found in personal care products.

Across multiple hot flashes measures, MCPP and to a lesser extent MBzP were associated with decreased risk of experiencing hot flashes. MCPP is a downstream oxidized metabolite and may be produced from multiple phthalates including MBP, BzBP, and phthalates with long *n*-hydrocarbon side chains (595). One hypothesis for the negative association between MCPP and hot flashes is that the presence of highly oxidized metabolites is a marker of the overall efficacy of metabolism and detoxification. Greater capacity to metabolize and excrete phthalates (and other environmental chemicals) could reduce their effects on multiple sensitive endpoints. MBzP, containing a benzyl group on its side chain, has a unique structure for a phthalate that is more similar to steroid hormones. As a result, MBzP may act through different mechanisms than other phthalate metabolites.

This study has several strengths. Of note, four urine samples taken over consecutive weeks at similar times of day were pooled for phthalate measurement. Within-subject pooling has been shown to decrease exposure misclassification for phthalates and improves the credibility of estimated exposure based on urine concentrations (162). Other strengths of this study include the large size of the population and the detailed information collected from each participant on hot flash experience using validated questionnaires that are accepted by the National Institute of Health to assess hot flashes (596).

This study also has several limitations. The MWHS cohort is composed of primarily white (67%) and Black (30%) midlife women. Few women of other races/ethnicities were enrolled in the study; therefore the results of this study are most applicable to non-

Hispanic white and Black women. Due to the cross-sectional analyses performed here, some outcomes may have occurred before phthalate exposure, obscuring temporality. The measure of “ever experiencing hot flashes” is the most imprecise and most likely to differ in temporal ordering with respect to phthalate exposure. Therefore, prospective studies are needed to confirm whether phthalates influence risk of experiencing hot flashes. We were unable to adjust for co-pollutants or diet, although we adjusted for a number of relevant and important confounders, including age, race, education, current drinking status, smoking status, medication use, menopause status, BMI, and CESD score. However, other environmental chemical exposures may be correlated with phthalates and hot flashes. Diet quality may be important given that we observed strong associations between plasticizing phthalates and hot flashes risk and exposure to plasticizing phthalates occurs primarily through diet. In addition, we did not evaluate non-linear associations.

E.7. CONCLUSION

In midlife women from the MWHS, some urinary phthalate metabolites were associated with higher risk of recently experiencing and experiencing frequent hot flashes, but not of ever experiencing or experiencing severe hot flashes. We observed that urinary phthalate metabolites of plasticizer parent compounds were associated with higher odds of experiencing hot flashes in the past 30 days and experiencing daily or weekly hot flashes. Additionally, we found that some associations of urinary phthalate metabolites with hot flashes were different by menopause status, midlife BMI, race/ethnicity, and CESD score. Although this is one of the first studies to assess the relationship between phthalate

exposure and risk of hot flashes, these results are consistent with previous studies showing that phthalates can interfere with normal female reproductive function (568). Our results suggest specific relationships between phthalates from common exposure sources and the evaluated hot flash outcomes. Future studies should investigate the mechanisms through which phthalates may be acting to facilitate the development of interventions to alleviate hot flashes in midlife women.

APPENDIX F: MIDLIFE URINARY PHTHALATE METABOLITE CONCENTRATIONS AND PRIOR UTERINE FIBROID DIAGNOSIS

This article/appendix has been published in *International Journal of Environmental Research and Public Health*; Volume 19 Issue 5; Pacyga DC, Ryva BA, Nowak RA, Bulun SE, Yin P, Li Z, Flaws JA, Strakovsky RS. *Midlife Urinary Phthalate Metabolite Concentrations and Prior Uterine Fibroid Diagnosis*. Copyright MDPI (2022); https://doi.org/10.3390/ijerph19052741.

F.1. ABSTRACT

Fibroid etiology is poorly understood but is likely hormonally mediated. Therefore, we evaluated associations between midlife phthalates (hormone-altering chemicals) and prior fibroid diagnosis, and considered differences by weight gain status. Women (ages: 45–54; n = 754) self-reported past fibroid diagnosis. We pooled 1–4 urines collected after fibroid diagnosis over the consecutive weeks to analyze nine phthalate metabolites and calculate relevant molar sums (e.g., di(2-ethylhexyl) phthalate, ΣDEHP; anti-androgenic phthalates, ΣAA; all metabolites, ΣPhthalates). Using Poisson regression, we evaluated associations between phthalate biomarkers and the risk of having fibroid diagnosis. We explored if associations differed by weight gain from age 18 to 45–54 or in women diagnosed with fibroids within 5 years of phthalate assessment. Our major finding was that women had a 13% (RR: 1.13; 95%CI: 1.02, 1.26) and 16% (RR: 1.16; 95% CI: 1.03, 1.31) greater risk of prior fibroid diagnosis for each two-fold increase in ΣDEHP or ΣAA, respectively. These associations were strongest in women who became overweight/obese from age 18 to 45–54 and in those diagnosed <5 years before phthalate

assessment. Based on these results, prospective studies should corroborate our findings related to associations between phthalates and fibroids, and may consider evaluating the role that weight gain may play in these associations.

F.2. KEYWORDS

Phthalates; endocrine disruptors; fibroids; leiomyoma; midlife; women.

F.3. INTRODUCTION

By midlife, most women will have uterine leiomyomata, commonly known as fibroids, which are non-cancerous tumors of uterine smooth muscle cells associated with adverse health outcomes, including abnormal uterine bleeding and miscarriage (597-599). Fibroids almost exclusively occur in reproductive-aged, pre- and perimenopausal women, with incidence increasing with age until women are post-menopausal (598, 600, 601). However, the exact prevalence of fibroids is difficult to determine because of the spectrum of clinical presentation. Many women with fibroids have a benign presentation, where fibroids are incidentally detected during imaging, whereas up to half of women with fibroids experience symptoms that are serious enough to impact quality of life, including excessive menstrual bleeding and pelvic pain (602, 603). With very severe symptoms, fibroids are the number one reason for hysterectomy in the United States (602). Given the detrimental impacts of fibroids on women’s quality of life, substantially more data are needed to identify modifiable risk factors that contribute to the development of fibroids.

Pre- and perimenopausal women are widely exposed to endocrine disrupting chemicals, including phthalates, which are found in many consumer products. For example, di(2-ethylhexyl) phthalate (DEHP) is a plasticizer used during food processing and in food contact materials, whereas diethyl phthalate (DEP) is used as a fragrance stabilizer in personal care products and cosmetics (240). Given that phthalates are metabolized and excreted within 24-48 hours of exposure, phthalate exposure is best approximated by measuring urinary concentrations of phthalate metabolites (262). Although most individuals in the U.S. general population are exposed to phthalates (604), women have higher measured urinary levels of phthalate metabolites than men, likely due to the use of personal care products and cosmetics (546). In women, studies have demonstrated that phthalates are associated with greater risk of hormone-mediated health outcomes, such as endometriosis (605), hot flashes (250), metabolic syndrome (606), breast cancer (607), and uterine fibroids (608). In cell and experimental animal models, phthalates can alter circulating sex-steroid hormone concentrations by binding to hormone receptors, including peroxisome proliferator-activated receptor alpha receptors and estrogen receptors (alpha and beta) (20, 21, 189, 609, 610). Similarly, in pregnant populations, as well as non-pregnant midlife women (ages 40 to 60), urinary phthalate biomarker concentrations were associated with altered serum and urinary sex-steroid hormone concentrations (86, 249). In addition to hormonal disruption, DEHP and its metabolites may disrupt other critical cellular processes, including cell viability and growth pathways, which may be responsible for the development of adverse health conditions in women, including uterine fibroids (611-613). For example, an *in vitro* study reported that cells isolated from human uterine fibroids and treated with DEHP had higher viability, lower

apoptosis, and increased expression of hypoxia inducible factor-1α and cyclooxygenase-2 (613). Additionally, a cross-sectional study of pre-menopausal women found that urinary concentrations of one DEHP metabolite, mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), were associated with altered micro-RNA levels (miR-10a-5p and miR-577), which are important for cell viability, survival, and apoptosis in fibroid tumors, suggesting that phthalates may play a role in fibroid pathogenesis by interacting with regulators of epigenetic machinery (611). Given this experimental evidence, additional studies in human populations are needed to determine whether these experimental findings recapitulate the real-life experience of women.

Many observational studies have investigated associations between phthalate metabolite biomarker concentrations and uterine fibroids (83, 187, 608, 614). A 2017 meta-analysis found no significant pooled associations between total urinary phthalate metabolite concentrations and uterine fibroids, whereas DEHP metabolites were associated with higher odds of fibroids (608). Additional recent studies have confirmed positive associations between urinary DEHP metabolites, especially mono-2-ethylhexyl phthalate (MEHP) and fibroids (83, 614). A major limitation of these studies is that urine samples for phthalate biomarker assessment were collected after fibroid diagnosis, which makes it difficult to establish temporality and causality. A recent prospective study of 23–35-year-old premenopausal black women in Detroit that evaluated the associations between phthalates and ultrasound-detected fibroids (n=301) women who did and 453 who did not develop fibroids) found a weak-to-moderate association between MEHP and higher risk of fibroids (187), supporting results from previous studies.

Similar to most previous studies, our current study did not prospectively evaluate associations between phthalate biomarker concentrations and fibroids. However, our goal was to contribute additional findings on the overall associations between phthalate biomarkers and fibroids in a large, diverse cohort of pre- and peri-menopausal midlife women, and also propose potential effect modification by adulthood weight gain, which (to our knowledge) has not been previously considered. Our first objective was to evaluate the overall associations between urinary phthalate biomarker concentrations and prior fibroid diagnosis. As weight change, specifically weight gain (615), is a risk factor for fibroid development, our second objective was to evaluate if associations between phthalate biomarkers and prior fibroid diagnosis differed in women who became overweight/obese from age 18 to midlife compared to women whose body mass index (BMI) remained stable (remained under-/normal weight or overweight/obese). To improve the window of our exposure measure relative to the outcome, our sensitivity analyses also considered whether associations between phthalate biomarkers and prior fibroid diagnosis differed based on the timing of diagnosis relative to phthalate assessment.

F.4. MATERIALS AND METHODS

The current study was a secondary analysis of baseline data collected as part of the Midlife Women's Health Study (MWHS). The MWHS is a prospective cohort that recruited midlife women living in and around Baltimore, Maryland, between 2006 and 2015, with the primary goal of assessing the risk factors of hot flashes. The study protocol has been described elsewhere (449). In brief, women were included in the study if they were between 45 and 54 years old and were pre- or peri-menopausal. Women were excluded

from the study if they had a history of hysterectomy or oophorectomy, were currently pregnant, were taking hormone therapy or herbal/other agents for menopause treatment, were taking oral contraceptives, were undergoing cancer treatment, or were postmenopausal. Menopause status was defined using the Stages of Reproductive Aging Workshop + 10 (STRAW+10) criteria as follows (558): premenopausal women were those who experienced their last menstrual period within the past three months and reported ≥ 11 periods within the past year; perimenopausal women were those who experienced their last menstrual period within the past year, but not within the past three months or experienced their last menstrual periods within the past three months and experienced ≤ 10 periods within the past year; and postmenopausal women were those who had not experienced a menstrual period within the past year. Overall, 754 pre- and peri-menopausal women with complete information about baseline midlife urinary phthalate metabolite concentrations and self-reported past uterine fibroid diagnosis were available for the study.

At baseline, women reported their race/ethnicity, annual household income, alcohol intake, weight at age 18, oral contraceptive use, age at menarche, fertility consultation, and parity via a self-administered questionnaire. Women reported their race/ethnicity by selecting one of the following options: Caucasian/White, African American/Black, Hispanic, Asian, or other. To determine women's most recent alcohol consumption status, women answered "yes" or "no" to the question, "In the last 12 months have you had at least 12 drinks of any kind of alcoholic beverage?". Women reported "yes" or "no" if they ever used oral contraceptive pills. If they marked yes, they also reported the duration of

use. To evaluate if women had reproductive problems, they answered “yes” or “no” to the question “Did you ever seek medical consultation because of difficulty in getting pregnant (infertility)?”. At the first baseline clinic visit, trained staff measured women’s height (inches) and weight (pounds), which we used to calculate midlife BMI (kg/m^2). We additionally used measured midlife height and self-reported weight at age 18 to calculate BMI at age 18 (kg/m^2).

Unfortunately, this study was not designed to assess fibroid incidence. Instead, the baseline questionnaire collected information about prior fibroid diagnosis. Women answered “yes” or “no” to the question “Have you ever been told by a doctor that you have uterine fibroids? If women reported “yes”, they indicated their age at diagnosis. We evaluated prior fibroid diagnosis as a binary variable comparing women who had a diagnosis to those without a diagnosis. We evaluated the timing of fibroid diagnosis as a three-level variable using the following categories: women who were diagnosed with fibroids within five years of the baseline visit, those diagnosed with fibroids five years or more before the baseline visit, and those never diagnosed with fibroids.

We were unable to ascertain women’s phthalate exposure at the time of their fibroid diagnosis. Instead, at the baseline clinic visit, women provided a urine sample and then provided up to three more urine samples over consecutive weeks. Staff physically pooled urine samples for each participant for analysis of phthalate metabolite biomarkers, which were used to approximate midlife exposure to these chemicals. Given the short half-lives of phthalates in the body and high within-person variability, pooling has been shown to

be an effective approach for reducing measurement error in phthalate biomarkers (162). MWHS staff sent one pooled urine sample per participant to the University of Illinois Urbana-Champaign Roy K. Carver Biotechnology Metabolomics Center for analysis. The metabolomics laboratory used isotope dilution high-performance liquid chromatography negative-ion electrospray ionization-tandem mass spectrometry (HPLC–MS/MS) and methods adapted from the Centers for Disease Control and Prevention (104) to analyze urine samples for concentrations (ng/mL) of the following nine phthalate metabolites: MEHP, MEHHP, mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(2-ethyl- 5-oxohexyl) phthalate (MEOHP), mono-(3-carboxypropyl) phthalate (MCPP), monobenzyl phthalate (MBzP), monoethyl phthalate (MEP), monobutyl phthalate (MBP), and monoisobutyl phthalate (MiBP).

We imputed phthalate metabolite concentrations below the limit of detection (LOD) using LOD/√2. All phthalate metabolite concentrations were specific gravity-adjusted to account for urine dilution using the following equation: $P_c = P[(1.018 - 1)/(SG - 1)]$, where P_c is the specific gravity-adjusted metabolite concentration, P is the measured metabolite concentration (ng/mL), 1.018 is the median specific gravity of the MWHS population included in this analysis, and SG is the specific gravity of each woman's pooled urine sample (109). We molar-converted and summed (mmol/mL) four DEHP metabolites (MEHP, MEHHP, MEOHP, and MECPP) to approximate exposure to DEHP (referred to as ΣDEHP). The remaining metabolites (MCPP, MBzP, MEP, MBP, and MiBP) were evaluated using non-molar converted concentrations (ng/mL). We created additional molar sums based on exposure sources, where metabolites MEHP, MEHHP, MEOHP,

MECPP, MCPP, and MBzP were molar summed to approximate exposure to plasticizer phthalates (ΣPlastics) and metabolites MEP, MBP, and MiBP were molar summed to approximate exposure to personal care product phthalates (ΣPCP). Some previous experimental studies in male pups suggest that metabolites MEHP, MEHHP, MEOHP, MECPP, MBzP, MBP, and MiBP have anti-androgenic activity in the body (149, 495, 616). Therefore, we molar summed these urinary biomarkers to approximate exposure to phthalates with anti-androgenic activity (ΣAA). Interestingly, in our previous study, we observed positive associations between ΣAA and estradiol (249), suggesting that classifying phthalate biomarkers based on their effects on male fetal rat testes may not be appropriate for evaluating reproductive endpoints in women. However, we evaluated associations between ΣAA and fibroids to corroborate findings from a previous study (614). Lastly, we molar summed all nine phthalate metabolites to approximate total midlife phthalate exposure (ΣPhthalates).

We used the chi-squared test to evaluate differences in sociodemographic, lifestyle, and health characteristics between women with and without prior fibroid diagnosis. We then used Poisson regression models with robust variance estimator to evaluate associations between phthalate biomarker concentrations (as individual metabolites or molar sums) and prior fibroid diagnosis (617). Due to skewed distributions, phthalate biomarker concentrations were natural log-transformed. To address our first and second objectives, we specified Poisson regression models to evaluate associations between phthalate biomarker concentrations and the risk of having a prior fibroid diagnosis compared to not having a prior fibroid diagnosis. To evaluate differences in associations between

phthalate biomarker concentrations and prior fibroid diagnosis by changes in BMI from age 18 to 45-54 (second objective), we first classified both midlife BMI and BMI at age 18 using the following clinical categories (618): under-/normal weight (< 25 kg/m^2) and overweight/obese (≥ 25 kg/m^2). Then, we categorized changes in BMI as follows: women who remained overweight/obese through age 45-54 (overweight/obese at ages 18 and 45-54), women who became overweight/obese by age 45-54 (under-/normal weight at age 18 but overweight/obese at age 45-54), women who remained under-/normal weight through age 45-54 (under-/normal at ages 18 and 45-54), and those who became under-/normal weight by age 45-54 (overweight/obese at age 18 but under-/normal weight at age 45-54) (248). In these models, we included a multiplicative interaction between phthalates and change in BMI to evaluate differences in associations in women who remained overweight/obese through age 45-54, who became overweight/obese by age 45-54, and who remained under-/normal weight. We excluded women who became under-/normal weight because this category only included five women. We reported results regardless of the significance of the interaction *P*-value.

We acknowledge that our study was not designed to prospectively evaluate the associations between urinary phthalate biomarker concentrations and fibroids diagnosis, and that midlife urinary phthalate biomarker concentrations likely do not represent concentrations at the time of prior fibroid diagnosis. However, in addition to our main analyses, we also conducted a sensitivity analysis to potentially provide a more relevant approximation of phthalate exposure in relation to prior fibroid diagnosis. Specifically, we wanted to assess whether our primary associations differed based on when women were

diagnosed with fibroids (timing) relative to when they provided their midlife urine samples for phthalate metabolite quantification. We used multinomial logistic regression models to evaluated the associations between urinary phthalate biomarker concentrations and the probability of being diagnosed with fibroids within five years of or more than five years before midlife urine collection compared to never being diagnosed with fibroids.

For our first objective, we assessed both unadjusted and adjusted models. In adjusted models, we *a priori* selected covariates associated with both our exposure and outcome. We evaluated correlations between all selected covariates to test for potential multicollinearity issues, but none were strongly correlated with each other ($r < 0.4$; **data not shown**). Therefore, final adjusted models included the following covariates: race/ethnicity, annual household income, alcohol intake, fertility consultation, midlife BMI, oral contraceptive use, age at menarche, and parity. These covariates are proxies of important latent constructs that we were unable to directly assess at the time of fibroid diagnosis, such as socioeconomic status (race/ethnicity, income), racism (race/ethnicity), lifestyle (midlife BMI, alcohol use), health (midlife BMI, alcohol use, age at menarche, fertility consultation), and reproductive history (oral contraceptive use, parity, age at menarche, fertility consultation). These covariates were also accounted for in sensitivity analyses. For our second objective, we included the previously listed covariates, except for midlife BMI due to multicollinearity issues with changes in BMI. The operationalization of covariates and reference groups are presented in **Table 43**.

We conducted all analyses in SAS 9.4 (SAS Institute Inc, Cary, NC, USA). We used PROC GENMOD for Poisson regression analyses with a robust variance estimator (main analyses) and specified an unstructured correlation matrix for the model's residuals. We back-transformed the resulting risk ratios (RR) and 95% confidence intervals (CI) using the equation [exp(ln(RR)*ln(2.00)] to interpret the results as risk of prior fibroid diagnosis with every two-fold increase in phthalate biomarker concentration. We used PROC LOGISTIC for multinomial logistic regression models (sensitivity analysis), and back-transformed the resulting odds ratios (OR) and 95% CIs using the equation [exp(ln(OR)*ln(2.00)] to interpret the results as odds of being diagnosed with fibroids within five years or more than five years before midlife urine collection with every two-fold increase in phthalate biomarker concentration. We considered associations as being meaningful at $P \leq 0.10$ and analyses were not adjusted for multiple comparisons (166).

F.5. RESULTS

F.5.1. Demographic and lifestyle characteristics of the MWHS population

The characteristics of the overall MWHS population have been described elsewhere (249, 250). The prevalence of prior fibroid diagnosis in MWHS was approximately 27% (**Table 43**), and median age at diagnosis was 40 years (range: 16 – 52; **data not shown**). Women with and without prior fibroid diagnosis differed significantly with regard to race/ethnicity, annual household income, alcohol intake, midlife BMI, age at menarche, oral contraceptive use, fertility consultation, and parity ($P < 0.05$; **Table 43**). Specifically, compared to women with no prior fibroid diagnosis, women with prior diagnosis were more likely to be black/other, have lower annual household incomes, have obesity during

midlife, start menarche earlier, have no or one live birth, use oral contraceptives for >10 years, consume ≤ 12 alcoholic drinks in the year before the first study visit, and not to have received fertility treatment when trying to become pregnant.

Table 43. Demographic and lifestyle characteristics of women with and without uterine fibroids.

	Fibroid diagnosis		
Participant characteristic	**Yes (n=207)**	**No (n=547)**	**P-value**
Age at baseline			0.49
45 – 49 years	131 (63.3)	361 (66.0)	
50 – 54 years	76 (36.7)	186 (34.0)	
Race/ethnicity			**< 0.0001**
Non-Hispanic white	88 (42.7)	407 (74.5)	
Black/other[1]	118 (57.3)	139 (25.5)	
Employment			0.94
Unemployed	41 (19.9)	110 (20.1)	
Employed	165 (80.1)	436 (79.9)	
Educational attainment			0.25
Some college or less	79 (38.5)	186 (34.1)	
College graduate or higher	126 (61.5)	360 (65.9)	
Annual household income			**0.01**
< $20,000	14 (7.0)	35 (6.6)	
$20,000 – 39,999	41 (20.4)	78 (14.7)	
$40,000 – 99,999	78 (38.8)	169 (31.8)	
≥ $100,000	68 (33.8)	249 (46.9)	
Marital status			0.21
Single	42 (20.5)	92 (16.8)	
Married/living with partner	124 (60.5)	368 (67.4)	
Widowed/divorced/separated	39 (19.0)	86 (15.8)	
Alcohol intake			**0.001**
No	91 (44.2)	169 (31.0)	
Yes	115 (55.8)	376 (69.0)	
Ever smoker			0.31
Yes	86 (41.7)	251 (45.9)	
No	120 (58.3)	296 (54.1)	
Menopause status			0.61
Premenopausal	137 (66.2)	351 (64.2)	
Perimenopausal	70 (33.8)	196 (35.8)	
Midlife BMI			**0.02**
< 25 kg/m^2	70 (33.8)	230 (42.0)	
25 – 29.9 kg/m^2	52 (25.1)	149 (27.2)	
≥ 30.0 kg/m^2	85 (41.1)	168 (30.7)	
Age at menarche			**0.001**
< 12 years	119 (58.0)	231 (42.5)	
13 – 14 years	64 (31.2)	238 (43.8)	
≥ 15 years	22 (10.7)	74 (13.6)	

Table 43 (cont'd).

	Fibroid diagnosis		
Participant characteristic	**Yes (n=207)**	**No (n=547)**	**P-value**
Oral contraceptive use			**0.04**
Never	24 (11.7)	86 (15.8)	
< 1 year	35 (17.1)	70 (12.8)	
1 – 4 years	51 (24.9)	146 (26.8)	
5 – 10 years	40 (19.5)	139 (25.5)	
> 10 years	55 (26.8)	104 (19.1)	
Fertility consultation			**0.03**
Yes	28 (13.7)	111 (20.4)	
No	177 (86.3)	433 (79.6)	
Parity			**0.03**
Never pregnant	20 (9.7)	66 (12.1)	
No live births	25 (12.1)	50 (9.2)	
1 live birth	49 (23.8)	86 (15.8)	
≥ 2 live births	112 (54.4)	344 (63.0)	
Change in BMI from age 18 to 45-54			0.29
Remained under-/normal weight	68 (33.7)	224 (41.2)	
Became overweight/obese	107 (53.0)	253 (46.5)	
Became under-/normal weight	1.0 (0.5)	4 (0.7)	
Remained overweight/obese	26 (12.9)	63 (11.6)	

[1]Women of other race/ethnicity represent less than 4% of the analytic sample. *P*-value from chi-squared test. Missing information (n) for women with prior fibroid diagnosis: race/ethnicity, employment, alcohol intake, ever smoker, parity (n=1); education, marital status, age at menarche, oral contraceptive use, fertility consultation (n=2); change in BMI from age 18 to 45-54 (n=5); income (n=6). Missing information (n) for women without prior fibroid diagnosis: race/ethnicity, employment, education, marital status, parity (n=1); alcohol intake, oral contraceptive use (n=2); fertility consultation, change in BMI from age 18 to 45-54 (n=3); age at menarche (n=4); income (n=16). BMI, body mass index.

F.5.2. Urinary phthalate metabolite biomarker concentrations

More than 99% of women had urinary concentrations of all measured phthalate metabolites above the LOD (**data not shown**). MWHS women had somewhat higher median phthalate metabolite concentrations compared to midlife women from the National Health and Nutrition Examination Survey (NHANES) for the years 2005-2016, but with overlapping 25th and 75th percentiles (249, 250) (**Figure 30**).

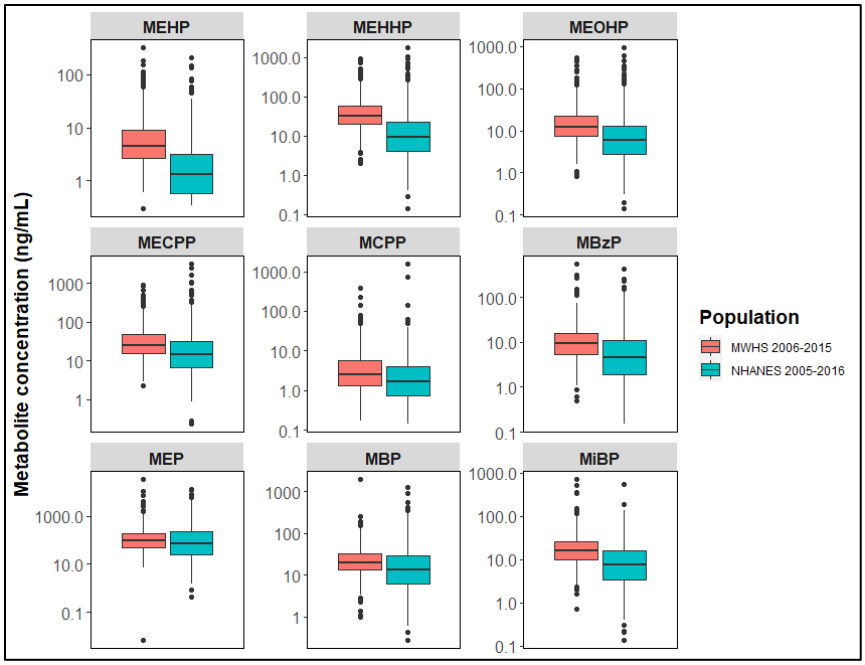

Figure 30. Midlife urinary phthalate metabolite concentrations. Box plots display urinary phthalate metabolite concentrations (ng/mL) of women in MWHS (2006-2015, n=754) and women ages 45-54 from 6 NHANES survey cycles (2005-2016, n=902). Concentrations were not adjusted for urine dilution. Box plots include the median (center line in box), the 25th percentile (lower line of box), and the 75th percentile (upper line in box). Numeric values for phthalate metabolite concentrations have been published elsewhere (249, 250). MEHP (mono-2-ethylhexyl phthalate); MEHHP (mono-(2-ethyl-5-hydroxyhexyl) phthalate); mono-(2-ethyl- 5-oxohexyl) phthalate (MEOHP); mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP); mono-(3-carboxypropyl) phthalate (MCPP); monobenzyl phthalate (MBzP); monoethyl phthalate (MEP); monobutyl phthalate (MBP); and monoisobutyl phthalate (MiBP); MWHS, Midlife Women's Health Study; NHANES, National Health and Nutrition Examination Survey.

F.5.3. Associations of phthalate biomarker concentrations with prior fibroid diagnosis

Overall, higher concentrations of some phthalate biomarkers were associated with higher risk of prior fibroid diagnosis (**Table 44**). In unadjusted models, women had 10% - 19% higher risk of prior fibroid diagnosis for every two-fold increase in ΣDEHP (RR: 1.10; 95% CI: 1.00, 1.22), MEP (RR: 1.11; 95% CI: 1.02, 1.20), MiBP (RR: 1.18; 95% CI: 1.06, 1.31), ΣPCP (RR: 1.16; 95% CI: 1.08, 1.25), ΣPhthalates (RR: 1.19; 95% CI: 1.10, 1.29), and ΣAA (RR: 1.15; 95% CI: 1.02, 1.29). Interestingly, in unadjusted models, we also

observed a marginal inverse association of MBzP with prior fibroid diagnosis (RR: 0.92; 95% CI: 0.84, 1.02). After accounting for important confounders, only associations of ΣDEHP, ΣPhthalates, and ΣAA with fibroids remained. Women had a 9-16% higher risk of prior fibroid diagnosis, respectively, for every two-fold increase in ΣDEHP (RR: 1.13; 95% CI: 1.02, 1.26), ΣPhthalates (RR: 1.09; 95% CI: 1.00, 1.19), or ΣAA (RR: 1.16; 95% CI: 1.03, 1.31). In adjusted models, we also observed a marginal association with ΣPlastics, where women had a 12% (RR: 1.12; 95% CI: 1.00, 1.25) higher risk of prior fibroid diagnosis for every two-fold increase in ΣPlastics.

Table 44. Associations of phthalate biomarker concentrations with diagnosis of uterine fibroids.

	Unadjusted (n=754)	**Adjusted (n=712)**
Phthalate biomarker	**RR (95% CI)**	**RR (95% CI)**
∑DEHP[1]	**1.10 (1.00, 1.22)[#]**	**1.13 (1.02, 1.26)***
MCPP	0.99 (0.92, 1.07)	0.99 (0.91, 1.07)
MBzP	**0.92 (0.84, 1.02)[#]**	0.97 (0.88, 1.07)
∑Plastics[2]	1.08 (0.97, 1.20)	**1.12 (1.00, 1.25)[#]**
MEP	**1.11 (1.02, 1.20)***	1.01 (0.94, 1.09)
MBP	1.06 (0.94, 1.20)	1.05 (0.94, 1.19)
MiBP	**1.18 (1.06, 1.31)***	1.09 (0.98, 1.22)
∑PCP[3]	**1.16 (1.08, 1.25)***	1.05 (0.97, 1.14)
∑Phthalates[4]	**1.19 (1.10, 1.29)***	**1.09 (1.00, 1.19)***
∑AA[5]	**1.15 (1.02, 1.29)***	**1.16 (1.03, 1.31)***

Poisson regression models with robust variance estimator evaluating the risk of being diagnosed with fibroids (unadjusted model n=207, adjusted model n=193) compared to never being diagnosed with fibroids (unadjusted model n=547, adjusted model n=519) for every 2-fold increase in phthalate biomarker concentration. Adjusted models account for race/ethnicity, income, age at menarche, oral contraceptive use, parity, fertility consultation, and midlife BMI. [1]∑DEHP = MEHP/278 + MEHHP/294 + MEOHP/292 + MECPP/308; [2]∑Plastics = MEHP/278 + MEHHP/294 + MEOHP/292 + MECPP/308 + MCPP/252 + MBzP/256; [3]∑PCP = MEP/194 + MBP/222 + MiBP/222; [4]∑Phthalates = MEHP/278 + MEHHP/294 + MEOHP/292 + MECPP/308 + MCPP/252 + MBzP/256 + MEP/194 + MBP/222 + MiBP/222; [5]∑AA = MEHP/222 + MEHHP/294 + MEOHP/292 + MECPP/308 + MBzP/256 + MBP/222 + MiBP/222. BMI, body mass index. CI, confidence interval; RR, risk ratio. [#]$P \leq 0.10$ and *$P < 0.05$.

F.5.4. Differences in associations by change in BMI from 18 years of age to midlife

Associations of phthalate biomarker concentrations with prior fibroid diagnosis were strongest in women who became overweight or obese (**Figure 31**). Specifically, women who became overweight or obese had a 17 - 21% higher risk of having a prior fibroid diagnosis for every two-fold increase in ΣDEHP (RR: 1.21; 95% CI: 1.06, 1.38), ΣPlastics (RR: 1.17; 95% CI: 1.01, 1.35), and ΣAA (RR: 1.20; 95% CI: 1.04, 1.39). Additionally, we observed a marginal association between ΣPhthalates and fibroids, where women who became overweight or obese had a 10% (RR: 1.10; 95% CI: 1.00, 1.22) higher risk of having a prior fibroid diagnosis for every two-fold increase in ΣPhthalates. Lastly, we observed a marginal association between ΣAA and fibroids, where women who remained under-/normal weight had a 22% (RR: 1.22; 95% CI: 0.96, 1.55) higher risk of having a prior fibroid diagnosis for every two-fold increase in ΣAA.

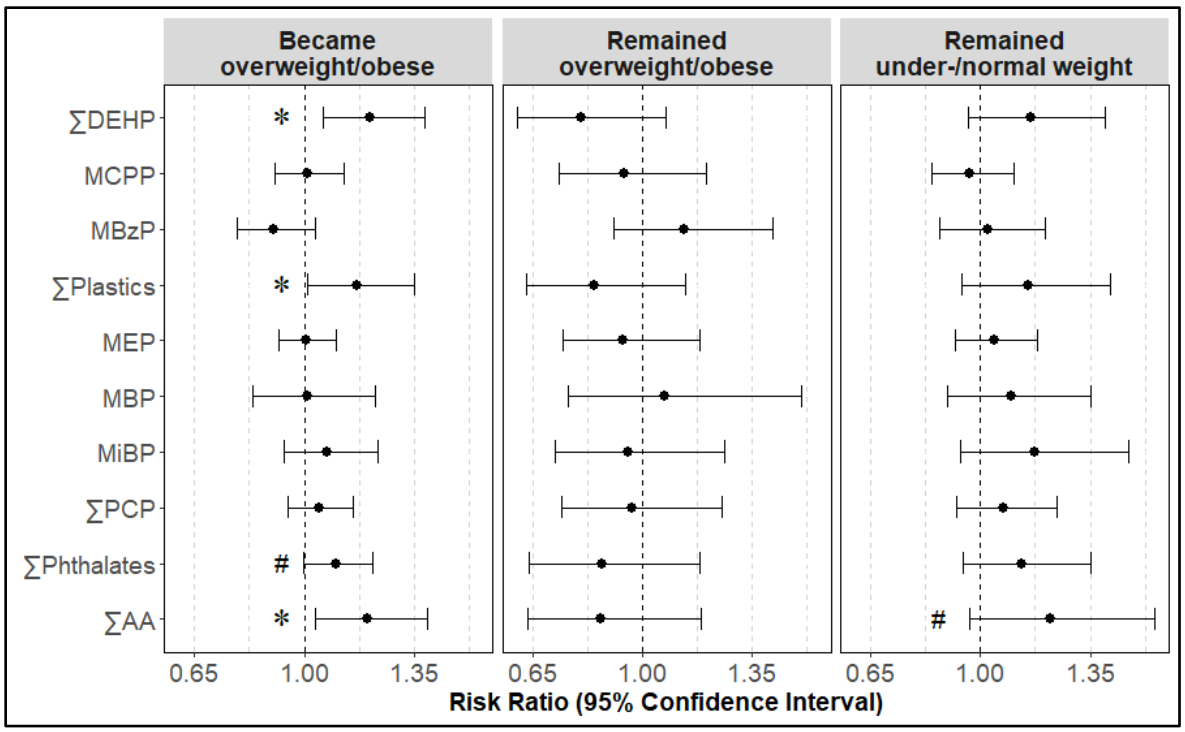

Figure 31. Differences in associations of phthalate biomarker concentrations with uterine fibroids by change in BMI from age 18 to 45-54. Forest plots display risk ratios (filled circle) and 95% confidence intervals (horizonal lines) for the risk of prior fibroid diagnosis for every 2-fold increase in phthalate biomarker concentrations among women who remained overweight/obese (n=223 no prior fibroids diagnosis, n=66 yes prior fibroids diagnosis), became overweight/obese (n=135 no prior fibroids diagnosis, n=47 yes prior fibroids diagnosis), and remained under-/normal weight (n=161 no prior fibroids diagnosis, n=80 yes prior fibroids diagnosis) from age 18 to 45-54. Models account for race/ethnicity, income, age at menarche, oral contraceptive use, parity, fertility consultation, change in BMI from age 18 to 45-54, as well as a multiplicative interaction between phthalate biomarker and change in BMI. Confidence intervals that do not cross the null (dashed vertical line) are significant at $^{\#}P \leq 0.10$ or $^{*}P < 0.05$. ∑DEHP = MEHP/278 + MEHHP/294 + MEOHP/292 + MECPP/308; ∑Plastics = MEHP/278 + MEHHP/294 + MEOHP/292 + MECPP/308 + MCPP/252 + MBzP/256; ∑PCP = MEP/194 + MBP/222 + MiBP/222; ∑Phthalates = MEHP/278 + MEHHP/294 + MEOHP/292 + MECPP/308 + MCPP/252 + MBzP/256 + MEP/194 + MBP/222 + MiBP/222; ∑AA = MEHP/222 + MEHHP/294 + MEOHP/292 + MECPP/308 + MBzP/256 + MBP/222 + MiBP/222.

F.5.5. Associations of phthalate biomarker concentrations in women with more recent diagnosis

Among women who had a prior fibroids diagnosis, median time of diagnosis relative to midlife urine collection for phthalate biomarker assessment was 8 years (range: 0 – 33

years). In sensitivity analyses, the associations between some phthalate biomarkers and fibroid diagnosis differed according to the recency of diagnosis relative to urine collection for phthalate biomarker assessment (**Table 45**). Overall associations between ΣDEHP, ΣPlastics, ΣPhthalates, ΣPCP, and ΣAA and prior fibroid diagnosis were more robust in women diagnosed within five years of midlife urine collection. Specifically, women diagnosed within five years of midlife urine collection generally had 19% - 35% higher odds of prior fibroid diagnosis for every two-fold increase in ΣDEHP (OR: 1.29; 95% CI: 1.03, 1.61), ΣPlastics (OR: 1.23; 95% CI: 0.96, 1.56), ΣPCP (OR: 1.30; 95% CI: 0.99, 1.43), ΣPhthalates (OR: 1.30; 95% CI: 1.05, 1.61), and ΣAA (OR: 1.35; 95% CI: 1.04, 1.76).

Table 45. Associations of urinary phthalate biomarker concentrations with timing of uterine fibroid diagnosis.

	Fibroid diagnosis ≥ 5 years before midlife urine collection (n=111)	**Fibroid diagnosis < 5 years before midlife urine collection (n=82)**
Phthalate biomarker	**OR (95% CI)**	**OR (95% CI)**
∑DEHP[1]	1.18 (0.95, 1.45)	**1.29 (1.03, 1.61)***
MCPP	0.96 (0.83, 1.11)	1.01 (0.86, 1.18)
MBzP	1.06 (0.87, 1.28)	0.84 (0.68, 1.05)
∑Plastics[2]	1.17 (0.93, 1.46)	**1.23 (0.96, 1.56)[#]**
MEP	0.98 (0.86, 1.11)	1.08 (0.93, 1.26)
MBP	1.02 (0.80, 1.31)	1.16 (0.91, 1.49)
MiBP	1.14 (0.91, 1.44)	1.16 (0.91, 1.49)
∑PCP[3]	1.00 (0.84, 1.20)	**1.19 (0.99, 1.43)[#]**
∑Phthalates[4]	1.06 (0.86, 1.33)	**1.30 (1.05, 1.61)***
∑AA[5]	1.20 (0.93, 1.55)	**1.35 (1.04, 1.76)***

Multinomial logistic regression models evaluated the odds of being diagnosed with fibroids ≥ 5 years (n=111) or < 5 years (n=82) before baseline compared to never being diagnosed with fibroids (n=519) for every 2-fold increase in phthalate biomarker concentration. Models account for race/ethnicity, income, age at menarche, oral contraceptive use, parity, fertility consultation, and midlife body mass index. [1]∑DEHP = MEHP/278 + MEHHP/294 + MEOHP/292 + MECPP/308; [2]∑Plastics = MEHP/278 + MEHHP/294 + MEOHP/292 + MECPP/308 + MCPP/252 + MBzP/256; [3]∑PCP = MEP/194 + MBP/222 + MiBP/222; [4]∑Phthalates = MEHP/278 + MEHHP/294 + MEOHP/292 + MECPP/308 + MCPP/252 + MBzP/256 + MEP/194 + MBP/222 + MiBP/222; [5]∑AA = MEHP/222 + MEHHP/294 + MEOHP/292 + MECPP/308 + MBzP/256 + MBP/222 + MiBP/222. CI, confidence interval; OR, odds ratio. [#]$P \leq 0.10$ and *$P < 0.05$.

F.6. DISCUSSION

Overall, we observed that ΣDEHP was associated with a higher risk of prior fibroid diagnosis, which was the main contributor to the associations between ΣPlastics, ΣPhthalates, and ΣAA and prior fibroid diagnosis. These associations were strongest in women who became overweight/obese from ages 18 to 45-54. The associations between phthalate biomarker concentrations and prior fibroid diagnosis were also stronger in women diagnosed with fibroids within five years of midlife urine collection for phthalate biomarker assessment. Our overall results related to ΣDEHP with prior fibroid diagnosis corroborate those from previous studies. However, additional large-scale prospective studies in diverse populations are needed.

Our findings that ΣDEHP and related phthalate molar sums are associated with higher odds of prior fibroid diagnosis are consistent with previous experimental and observational studies. Specifically, experimental studies observed that human fibroid cells treated with DEHP metabolites had disrupted cell viability, apoptotic, and growth pathways (611-613). A 2017 meta-analysis pooled five observational studies conducted between 1999 and 2015 in populations from the U.S., Korea, China, and Taiwan, and observed that DEHP metabolites were associated with higher odds of fibroids (608). However, this meta-analysis also reported that MBP and MiBP were also marginally significantly associated with higher odds of fibroids. These differences in findings may be related to the study population, since women in the meta-analysis were predominately non-Hispanic white or Asian, while most women in our population were non-Hispanic white or black. Additionally, our study population included women between the ages of

45 and 54, while the meta-analysis included a wider age range of women. Our findings are also consistent with a recent U.S. cross-sectional study of predominately black 26 - 54-year-old women (recruited 2014 - 2017) undergoing hysterectomy or myomectomy that reported positive associations between ΣDEHP and ΣAA and fibroid volume (614). Similarly, a case-control study of 20 - 40-year-old Korean women (recruited 2015 - 2016) reported that the odds of fibroids was higher in women in quartiles 2, 3, and/or 4 (compared to quartile 1) of urinary concentrations of ΣDEHP and its metabolites (83). These two more recent studies collected spot urine samples for phthalate biomarker assessment and ascertained fibroid status using imaging technology (i.e., magnetic resonance imaging (MRI), ultrasound) and/or pathology reports (83, 614). Despite the differences in study population, as well as phthalate biomarker and fibroid assessment, in these studies compared to ours, associations between DEHP and its metabolites and fibroids appear to be consistent.

The causal interpretability of results from most studies evaluating the associations of phthalate metabolite biomarkers with fibroids is limited because urine collection for phthalate biomarker assessment often occurred after women had already been diagnosed with fibroids. To our knowledge, only one study of Detroit-area 23 – 35-year-old black women (recruited 2010 - 2012) prospectively evaluated associations between phthalate biomarkers and incidence of fibroids (187). Although we were also unable to prospectively evaluate these associations prospectively, we conducted sensitivity analyses to address this limitation. In sensitivity analyses assessing the timing of fibroid diagnosis relative to midlife urine collection for phthalate biomarker assessment, we found

that associations of ΣDEHP and related phthalate molar sums with higher likelihood of prior fibroid diagnosis were stronger in women diagnosed within five years of urine collection. This five-year cutoff makes it more likely that women's urinary phthalate biomarker concentrations at the time of fibroid diagnosis were similar to those during the study. However, even with these sensitivity analyses, we cannot rule out the potential of reverse causation. Women diagnosed with fibroids may have developed certain lifestyles or behaviors over the years leading up to their enrollment into the MWHS that may have influenced their midlife exposure to phthalates. Additionally, given the high temporal variability in urinary phthalate biomarker concentrations (619), we are likely not capturing the correct exposure window, which may result in the underestimation of associations between phthalate biomarkers and risk of fibroids. Therefore, additional prospective studies are needed to elucidate the directionality of associations between phthalate biomarkers and fibroid development, and to consider the sensitive window of exposure framework to establish the temporal relationship between phthalate exposure and fibroid development. Nevertheless, the findings from this study should be interpreted with caution.

To our knowledge, our study is the first to show that associations between ΣDEHP and related phthalate molar sums and prior fibroid diagnosis were driven by women who became overweight or obese from age 18 to midlife. Obesity and especially weight gain are important risk factors for the development of fibroids. A recent meta-analysis that pooled 22 studies observed that higher body weight and adiposity (measured by BMI, waist/hip ratio, and waist circumference), as well as weight gain since age 18 were

associated with higher odds of fibroids (620). Given that both phthalates and gaining weight since age 18 are determinants of fibroid development, our findings suggest that women who undergo major changes in weight may be more susceptible to the impact of phthalates than those whose weights remain stable from age 18 until midlife. These results could be due to the metabolic disruptions that occur with weight change. For example, changes in weight/fat distribution and reproductive hormones (i.e., estrogens) are linked, with obesity influencing hormone concentrations (440, 475), as well as reproductive hormones influencing changes in weight and fat deposition (434). Given this relationship between body composition/adiposity and hormones, the interaction between endocrine disrupting chemicals (i.e., phthalates) and adipose tissue may contribute to the increased risk of phthalate-induced fibroids in women who gained weight. While most individuals experience gradual weight gain across the lifespan (621), because we did not have information about women's weight at fibroid diagnosis, it is possible that some weight gain may have occurred after diagnosis. Additionally, women who gained weight may have engaged in certain behaviors or have lifestyles resulting in the use of products associated with increased exposure to DEHP, which may explain why associations between ΣDEHP and related phthalate molar sums and prior fibroid diagnosis were stronger in these women. For example, unhealthy dietary behaviors, such as consumption of processed and fast foods, are major determinants of phthalate exposure (240). A study using data from NHANES found that individuals who consumed more fast foods had higher urinary ΣDEHP concentrations than non-consumers (316). Future studies that consider diet are needed to corroborate our findings, and to address

biologically plausible pathways connecting phthalate exposure and weight change with the development of fibroids.

This current study has important strengths, but also some limitations in addition to the potential for reverse causation. First, we may be underpowered to detect certain associations of phthalate biomarkers with prior fibroid diagnosis, especially in sensitivity analyses and in analyses evaluating differences by changes in BMI from age 18 to midlife. However, we evaluated these cross-sectional associations in a large cohort of pre- and peri-menopausal midlife women with a relatively high prevalence of fibroids. Second, prior fibroid diagnosis was based on self-reports and women with a history of hysterectomy or oophorectomy were excluded, which could lead to the misclassification of fibroid status and underestimation of the number of women with fibroids (622). However, our findings that race/ethnicity, BMI, and age at menarche are important predictors of self-reported fibroid diagnosis are consistent with the prior literature (623). Additionally, our results related to the associations between ΣDEHP and prior fibroid diagnosis are also consistent with previous studies that determined fibroid status using imaging technology (i.e., MRI, ultrasound) and/or pathology reports (83, 614). Third, we used self-reported weight at age 18 to calculate changes in BMI from age 18 to midlife, which may also be subject to recall bias. However, at the population level, self-reported past body weight is reliable for predicting measured past body weight (478). Fourth, we are unable to causally interpret our results given that urine for midlife phthalate biomarker assessment was collected after women's fibroid diagnosis. However, we conducted sensitivity analyses and observed that associations between phthalate biomarkers and fibroids are stronger when the time

between midlife urine collection and prior fibroid diagnosis is reduced. Additionally, phthalate metabolite concentrations were quantified from pools of up to four urine samples, which provides a more stable measure of our exposure and may better represent midlife urinary concentrations (162, 412). Lastly, as previously discussed, there may be unmeasured confounding factors (i.e., by diet), which were unaccounted for in our statistical models evaluating associations between phthalate biomarkers and prior fibroid diagnosis. However, we selected covariates *a priori* using the previous literature, and included covariates that are proxies for important latent constructs that we were unable to directly assess at the time of fibroid diagnosis. Overall, this secondary data analysis leverages data collected as part of the MWHS to contribute additional information pertaining to associations between phthalate biomarkers and prior fibroid diagnosis in midlife women.

F.7. CONCLUSION

In this population of mostly non-Hispanic white or black pre- and peri-menopausal midlife women, we observed that ΣDEHP and related phthalate molar sums were associated with higher risk of prior fibroid diagnosis, and these associations were stronger in women who became overweight or obese from age 18 to midlife. Our findings corroborate results from previous experimental and observational studies and suggest that interventions targeting the lifestyle behaviors associated with phthalate exposure are needed. However, as with most previous studies, we were unable to prospectively evaluate these associations, which makes it challenging to causally interpret our results, and our findings should be interpreted with caution. Therefore, future longitudinal, prospective cohort

studies are needed to corroborate these findings and further elucidate the independent and interactive contribution of phthalates and weight gain to the development of uterine fibroids.