HHS Public Access

Author manuscript

Environ Int. Author manuscript; available in PMC 2022 November 01.

Published in final edited form as:

Environ Int. 2021 November ; 156: 106633. doi:10.1016/j.envint.2021.106633.

Urinary Phthalate Metabolite Concentrations and Serum Hormone Levels in Pre- and Perimenopausal Women from the Midlife Women's Health Study

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Abstract

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Author contributions

C.C.. and D.C.P. designed the analyses, interpreted the data, wrote the manuscript, and revised the manuscript in response to the co-authors and reviewers. R.S.S helped conduct the analyses, helped interpret the data, and helped write the manuscript. R.S., T.J-T., P.L.W., and R.H. helped design the analyses, provided input on the data interpretation, and helped edit the manuscript. D.D.M. processed the urine samples, provided input on the analyses, and edited the manuscript. Z.L. conducted the urinary phthalate measurements and provided input on the manuscript. J.A.F. designed the study, obtained funding for the study, recruited participants, provided input on the analyses, and edited the manuscript.

Declaration of interests

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

Background/Aims: Phthalate exposure is associated with altered reproductive function, but little is known about associations between phthalate and hormone levels in midlife women.

Methods: 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.

Results: 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).

Conclusions: 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.

INTRODUCTION

Phthalates are commonly used to impart strength and flexibility to a variety of plastic products (1, 2). Additionally, low molecular weight phthalates are often used in personal care products to stabilize scents and colors (1, 2). 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 (3, 4). 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 (2, 5, 6). Additionally, people undergoing medical procedures are exposed to phthalates directly via medical devices (7, 8). Further, humans are exposed through routes such as inhalation of house dust and air contaminated with phthalates (9). 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 (10, 11). In fact, studies often find that phthalate metabolites are detectable in 99–100% of samples submitted by women (12, 13), making women an especially vulnerable population.

Phthalate exposure is of concern because phthalates have been shown to have endocrine disrupting capabilities (14–18). Epidemiological studies have shown that phthalates are associated with altered hormone levels in both men and women (19–22). 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 (23–25). 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 (26). In a cycling woman, the ovary is the primary source of the sex steroid hormones estradiol, progesterone, and testosterone (27). 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 (28). 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 (29). Depletion of the ovarian reserve during aging leads to a loss of follicles that produce AMH, and subsequently, AMH levels decline (26). 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 (30).

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 (31–35).

METHODS

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 (34). 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/or 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.

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.

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 (36, 37). 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.

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 (38, 39). Urine samples were stored at −80 °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.

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 (Supplemental Figure 1). 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 status, 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 1. 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.

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 (40). 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) (17, 41, 42). All phthalate metabolites were also molar-covered 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^{β} – 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.

RESULTS

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 1). 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/m^2 (60%). Over half of women were never smokers (54%), 36% were former smokers, and only 10% were current smokers.

MWHS urinary phthalate metabolite concentrations

Median (25th, 75th percentile) urinary concentrations of phthalate metabolites are presented in Table 2. 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 25th and 75th percentiles overlapped in metabolite levels between the two studies.

MWHS plasma hormone concentrations

Plasma hormone concentrations from year 1 of the MWHS are presented in Figure 1. 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).

Overall associations of urinary phthalates 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 3). 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.

In analyses where phthalate metabolites were modeled in quartiles, phthalates were associated with all hormones, except for SHBG (Figure 2, Supplemental Table 1). 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 2a), 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 2c). 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 2b). 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 2f). 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 2e).

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 4), 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 4). Associations of phthalates with progesterone were observed in both pre- and perimenopausal women, but they differed depending on the phthalate metabolite (Table 4). ∑DEHP, ∑Plastics, and ∑AA were positively associated with progesterone in premenopausal women, whereas ∑Phthalates was positively associated with progesterone in perimenopausal women.

Associations between phthalate metabolites and hormones stratified by BMI

Associations of phthalate metabolites with estradiol were only observed in under-/normal weight women (Table 5). 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 5). 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 5). 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 5). 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.

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 6). 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 6). 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.

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 (11, 17, 21, 43, 44). 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.

Overall associations of phthalates 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 a study in U.S. pregnant women (45–47) and some 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, MBzP (46), and MBP and estradiol (48), 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 (17, 49). 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 (22).

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).

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 (50), 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 (51). 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 (48, 52). Lastly, we observed no associations between phthalates and SHBG. These results are consistent with studies in peripubertal girls and pregnant women (53–55). 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.

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 (56–58). 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.

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 (59). Additionally, the link between phthalates and obesity broadens the possibilities for the relationships that may exist between phthalates, adiposity, and hormone levels (60, 61). 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.

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 (62). 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 (63, 64). 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 (65, 66). 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.

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 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 (67, 68)(69, 70). 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.

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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This work was supported by the National Institute of Environmental Health Sciences (R01 ES026956 and T32 007326). The University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core is supported by the Eunice Kennedy Shriver NICHD/NIH (NCTRI) Grant P50-HD28934. The authors thank the MWHS participants for their time, information, and biological samples and the staff members at Johns Hopkins University and the University of Illinois at Urbana-Champaign who helped with this study. Thank you to Yonatan (Yoni) Segev and Tyler Beers for their help with aliquoting samples.

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Highlights

- Phthalate metabolites were positively associated with estradiol levels in midlife women
- Phthalate metabolites were positively associated with testosterone levels in midlife women
- Phthalate metabolites were positively associated with progesterone levels in midlife women
- Phthalate metabolites were positively associated with anti-Müllerian hormone levels in midlife women
- Some demographic and lifestyle characteristics modify the associations between phthalate metabolites and hormones

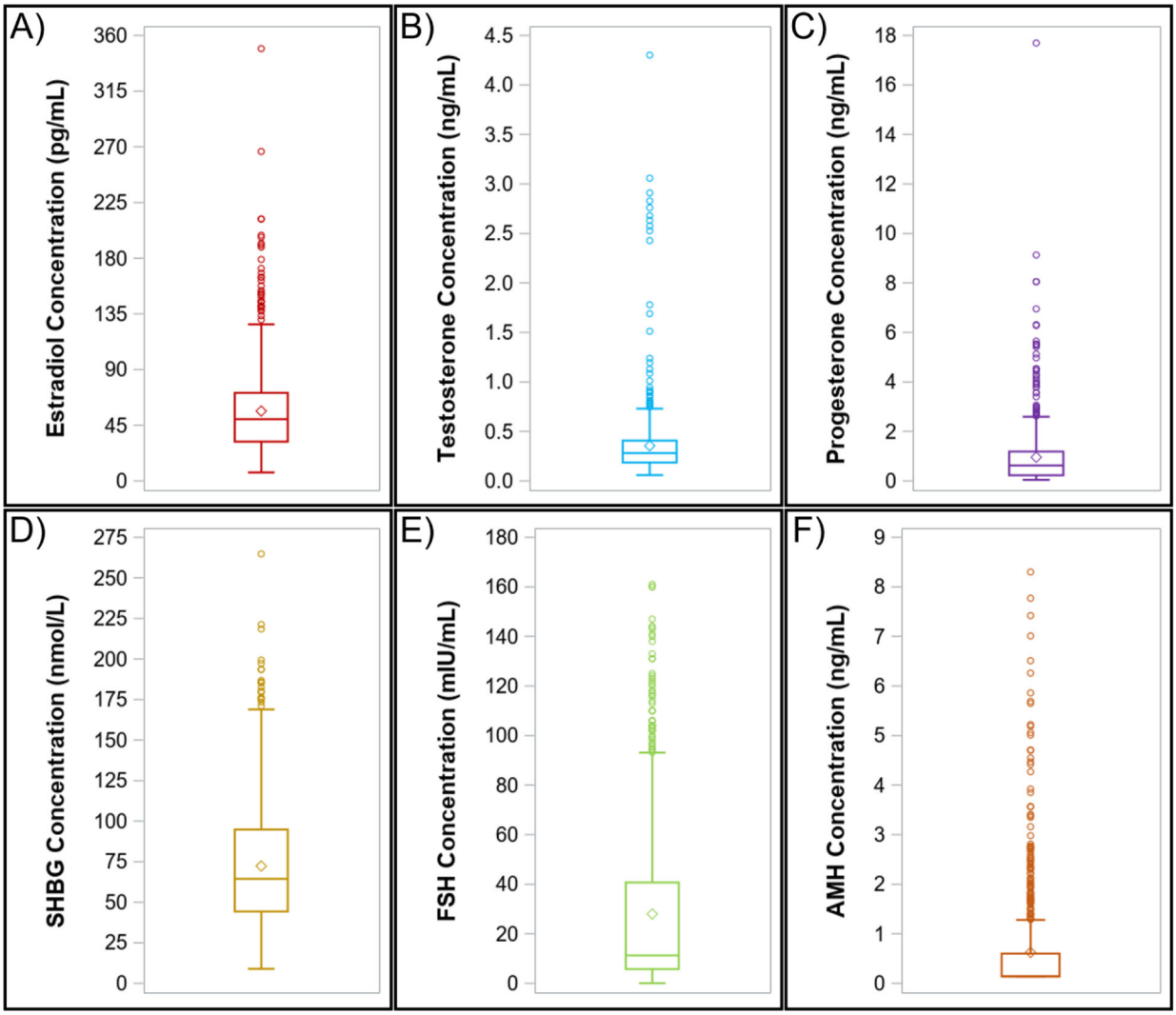

Figure 1. Hormone concentrations of women from MWHS (n=718).

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.

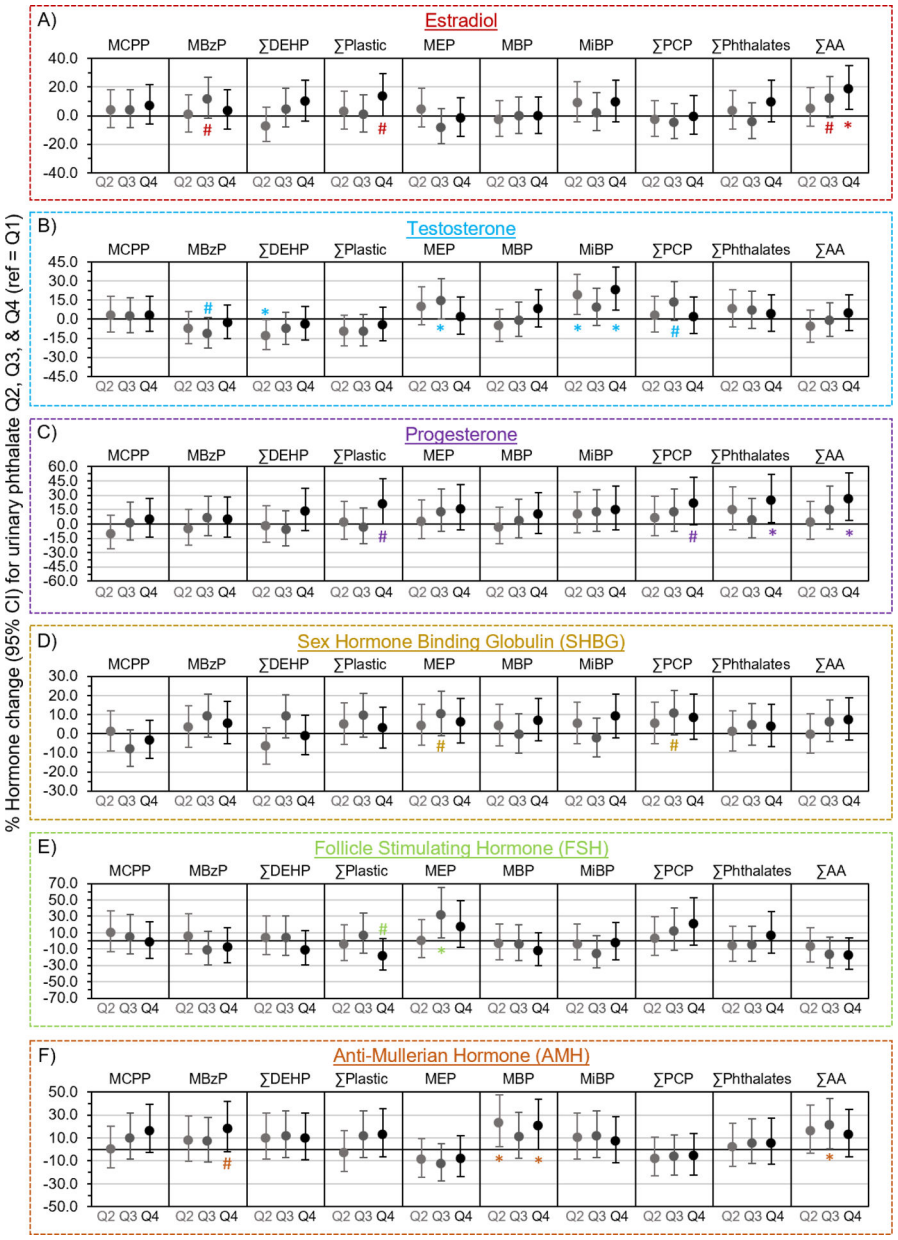

Figure 2. Associations of phthalate metabolites in quartiles with hormones (n=718).

Multivariable generalized linear 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). Data are presented as % change (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 $^{*}P<0.05$.

Table 1.

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})	

Demographic or Lifestyle Characteristic	n (%)
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.

Table 2.

Phthalate metabolite concentrations in MWHS and NHANES.

Name	Abbreviation	MWHS (2006–2015) n=718	NHANES (2005–2016) n=757[1]
Phthalate metabolitev		**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 (25 th, 75 th 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] Weighted phthalate 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. MWHS, Midlife Women's Health Study; NHANES, National Health and Nutrition Examination Survey.

[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.

Table 3.

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 (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.

Table 4.

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 hormones for every 2-fold increase in phthalate metabolite (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.

Table 5.

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)	

Environ Int. Author manuscript; available in PMC 2022 November 01.

BMI Category	Phthalate	Estradiol	P_{int}	Testosterone	P_{int}	Progesterone	P_{int}	SHBG	P_{int}	FSH	P_{int}	AMH	P_{int}
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 hormones for every 2-fold increase in phthalate metabolite (ng/mL or nmol/mL) in under-/normal weight and overweight/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 (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 = 288, 187, and 243 for under-/normal weight, overweight, and obese women, respectively.

Environ Int. Author manuscript; available in PMC 2022 November 01.

Table 6.

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

Race/Ethnicity	Phthalate	Estradiol	P_{int}	Testosterone	P_{int}	Progesterone	P_{int}	SHBG	P_{int}	FSH	P_{int}	AMH	P_{int}
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 hormones for every 2-fold increase in phthalate metabolite (ng/mL or nmol/mL) in 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 (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 = 241 and 477 for black/other and white women, respectively.