Relationships Between Urinary Phthalate Metabolite and Bisphenol A Concentrations and Vitamin D Levels in U.S. Adults: National Health and Nutrition Examination Survey (NHANES), 2005–2010

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Context: Recent research suggests that environmental exposure to endocrine-disrupting chemicals may alter circulating 25-hydroxyvitamin D [25(OH)D] levels in humans. To date, no studies have assessed the associations between phthalates and bisphenol A (BPA) and total 25(OH)D in the U.S. general population.

Objective: To explore relationships between urinary concentrations of 11 phthalate metabolites and BPA and serum total 25(OH)D in a representative sample of U.S. adults.

Design: A cross-sectional study.

Setting: U.S. National Health and Nutrition Examination Survey, 2005–2010.

Patients or Other Participants: U.S. general adult population (aged ≥20 years).

Interventions: None

Main Outcome Measures: Serum total 25(OH)D measured by liquid chromatography-tandem mass spectrometry.

Results: Metabolites of di(2-ethylhexyl) phthalate (DEHP) were consistently inversely associated with total 25(OH)D in the overall study population and in gender-stratified models. In the overall population, we detected a significant inverse relationship for the molar sum of DEHP metabolites (ΣDEHP), where an interquartile range increase in ΣDEHP was associated with a 1.90% decrease (95% confidence interval [CI], −3.64, −0.17) in total 25(OH)D. A positive association was detected for monoethyl phthalate. For BPA, we found a statistically significant inverse relationship in women, but not in men. In women, an interquartile range increase in urinary BPA was associated with a 3.71% decrease (95% CI, −6.41, −1.02) in total 25(OH)D.

Conclusions: Overall, our results provide suggestive evidence that environmental exposure to phthalates and BPA may alter circulating levels of total 25(OH)D in adults. Future human and animal studies are required to resolve the direction, temporality, and impact of these relationships. **(*J Clin Endocrinol Metab* 101: 4062–4069, 2016)**

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Abbreviations: BMI, body mass index; BPA, bisphenol A; CI, confidence interval; DEHP, di(2-ethylhexyl) phthalate; DEP, diethyl phthalate; IQR, interquartile range; LC-MS/MS, liquid chromatography-tandem mass spectrometric; ln, natural logarithm; LOD, limit of detection; MBP, mono-n-butyl phthalate; MBzP, monobenzyl phthalate; MCNP, mono-(carboxynonyl) phthalate; MCOP, mono(carboxyoctyl) phthalate; MCPP, mono-(3-carboxypropyl) phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEHP, mono-(2-ethyl)-hexyl phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEP, monoethyl phthalate; MiBP, monoisobutyl phthalate; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

It is well-established that vitamin D, a fat-soluble prohormone, is essential in maintaining calcium homeostasis and bone health (1). Beyond its skeletal effects, vitamin D has a broad role in human health (2). Vitamin D receptors have since been found in many organs and tissues, including those of the cardiovascular, digestive, immune, and reproductive systems (1). Inadequate vitamin D has been implicated in cardiovascular disease, autoimmune disorders, cancer, diabetes, and adverse reproductive outcomes (3, 4).

Numerous factors influence vitamin D status in humans, including those related to sun exposure (eg, sunscreen use, latitude, season, etc) as well as race, age, obesity, and several chronic diseases (5). Recent epidemiological research suggests that environmental exposure to endocrine-disrupting chemicals may also play a role by altering levels of the circulating vitamin D metabolite, 25-hydroxyvitamin D [25(OH)D]. Data from available human health studies have shown inverse associations between levels of 25(OH)D, the biomarker of vitamin D status in humans, and exposure to certain persistent organic pollutants such as organochlorine pesticides (6) and polychlorinated biphenyls (7). Studies assessing the extent to which other widely used industrial chemicals, such as phthalates and bisphenol A (BPA), influence levels of 25(OH)D are scarce.

Phthalates and BPA are high-production chemicals found in numerous industrial and consumer products and have been detected extensively in the U.S. general population (8–10). Both agents are known endocrine disruptors, and they have been shown to alter sex steroid and thyroid hormone levels at multiple points along these hormonal axes, including at the nuclear receptors (11–14). Because the molecular structure of the active vitamin D metabolite (1,25-dihydroxyvitamin D [1,25$(OH)_2$D]) is similar to that of classic steroid hormones (1) and its nuclear receptor is within the same superfamily of proteins as steroid and thyroid hormone receptors (15), it is plausible that these environmental chemicals may also exert a broad range of actions on the vitamin D endocrine system (16). For example, whereas the specific biological mechanisms remain unknown, phthalates and BPA may influence 25(OH)D levels by acting directly on the vitamin D nuclear receptor or on the metabolic activity of enzymes involved in converting vitamin D to its active metabolite. Similar pathways have been suggested for the actions of these chemicals on other hormonal systems involved in the regulation of steroid and thyroid hormones (16–18). In the only study assessing these associations to date, an inverse correlation was observed between serum BPA and 25(OH)D in participants of a study designed to assess certain risk factors of obstructive sleep apnea syndrome (19). However, this investigation was limited by sample size, exposure assessment methods, and lack of statistical consideration for confounding variables in the association of interest. Similar studies on phthalates have not been conducted.

Currently, there are no published data on the relationships between phthalate and BPA exposure and circulating total 25(OH)D in the U.S. general population. Herein, we investigated these associations in a representative sample of U.S. adults participating in the National Health and Nutrition Examination Survey (NHANES) 2005–2010.

Subjects and Methods

We obtained publicly available data from three cycles of NHANES (2005–2006, 2007–2008, and 2009–2010), a cross-sectional study designed to collect health and nutritional data from a nationally representative sample of the resident civilian noninstitutionalized U.S. general population (20). All participants provided informed consent at the time of recruitment. Information regarding methods for survey planning and design are described in detail elsewhere (21). Of the 17 132 adult participants aged 20 years and older in cycles 2005–2010, we limited our study to 4724 men and women who had complete data on all of the following: urinary phthalate metabolites, urinary BPA, urinary creatinine, and serum 25(OH)D.

Urinary phthalate metabolites and BPA

Total urinary BPA (free plus conjugated species) and 15 urinary phthalate metabolites were measured in spot urine samples provided by a random subsample of participants. Samples were collected at mobile examination centers and were stored at −20°C until they were shipped to the National Center for Environmental Health, Center for Disease Control and Prevention (CDC), for analysis (22, 23). Urinary BPA concentrations were measured using solid phase extraction coupled to HPLC (22). Phthalate metabolites were analyzed using HPLC-electrospray ionization-tandem mass spectrometry as described in detail elsewhere (23). Concentrations below the limit of detection (LOD) were replaced with the LOD divided by the square root of 2 (24).

In addition to BPA, we included in our analyses 11 phthalate metabolites for which at least 50% of the study subjects had concentrations above the LOD: mono-(2-ethyl)-hexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP); mono-n-butyl phthalate (MBP); mono-isobutyl phthalate (MiBP); mono-ethyl phthalate (MEP); mono-benzyl phthalate (MBzP); mono-(3-carboxypropyl) phthalate (MCPP); mono(carboxynonyl) phthalate (MCNP); and mono(carboxyoctyl) phthalate (MCOP). In addition to analyzing individual metabolites in statistical analyses, we created a summary measure of the four metabolites that share DEHP as their parent compound (MEHP, MEHHP, MEOHP, MECPP) and thus have common sources of exposure. Specifically, we calculated the molar sum (nanomoles per milliliter) of these DEHP metabolites (ΣDEHP) by dividing each metabolite concentration by its molecular weight and then summing the individual concentrations.

Urinary creatinine was measured using a Jaffe rate reaction and a CX3 analyzer (Beckman Instruments) (25). In descriptive analyses, we corrected for urinary dilution by dividing urinary biomarker concentrations by urinary creatinine (μg/g creatinine). We used uncorrected biomarker concentrations in regression analyses and added urinary creatinine as a covariate because modeling corrected metabolite levels may introduce bias (26).

Serum vitamin D measurements

Serum samples were collected from all participants at mobile examination centers and stored at −30°C until they were shipped to the CDC for analysis. In the 2005–2006 survey cycle, an RIA (DiaSorin) method was used to measure total 25(OH)D (sum of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3). The CDC changed its vitamin D laboratory method in cycles 2007–2010 to a more analytically accurate assay involving an ultra-high performance liquid chromatography-tandem mass spectrometric (LC-MS/MS) method (27). To facilitate comparisons of 25(OH)D across cycles, the CDC standardized concentrations of 25(OH)D measured from RIA in previous cycles (including 2005–2006) to predicted LC-MS/MS equivalents using regression equations described in detail elsewhere (27). Concentrations below the LOD were replaced with the LOD divided by the square root of 2 (24). No predicted total 25(OH)D LC-MS/MS equivalents were below the LOD in 2005–2006. For cycles 2007–2010, the CDC used imputed values in the calculation of total 25(OH)D when 25-hydroxyvitamin D_2 concentrations were below the LOD. No 25-hydroxyvitamin D_3 results were below the LOD.

Covariates

From in-home demographic questionnaire data, we examined the following as potential confounding variables: age, gender, race/ethnicity, education level, family income to poverty ratio, and 6-month sampling period (a proxy variable for season). From examination and laboratory data, we assessed body mass index (BMI) and serum cotinine, a marker of tobacco smoke exposure. We also examined past 30-day vitamin D supplement use based on participant responses to dietary supplement questionnaires. We used bivariate analyses to assess the individual relationships between these variables and total 25(OH)D and urinary biomarkers. Variables were included in final models based on ≥10% change in the main effect estimates when added in a forward stepwise approach.

Statistical methods

From the 4724 participants with exposure and vitamin D measurements, we excluded participants from multivariable regression analysis who were missing covariates (43 missing BMI, 12 missing serum cotinine, and three missing 30-day dietary supplement use; one participant was missing both BMI and dietary supplement information). A total of 4667 participants were included in final regression analyses.

We analyzed all data using SAS version 9.3 (SAS Institute, Inc) and R version 3.1.1 (The R Foundation for Statistical Computing). We used the R survey package to appropriately weight analyses for the complex, multistage sampling design of NHANES. We applied 6-year weights for the individual probability of selection into the urinary phenol and phthalate subsamples (28). We also included in our weighted analyses cluster and strata variables from demographic datasets to account for survey design (29). We considered associations statistically significant at the 5% level.

Before the analyses, we used the natural logarithm (ln) to transform all urinary biomarkers because the individual distributions were right-skewed. The distribution of total 25(OH)D approximated normality and remained untransformed in statistical analyses. In descriptive analyses, we examined differences in serum 25(OH)D levels by category of population characteristics using two-samples *t* tests and ANOVA. We calculated the geometric means and selected percentiles of weighted, creatinine-corrected urinary biomarkers to examine the distributions in the study population.

We used multivariable linear regression models with total 25(OH)D concentrations regressed on each individual urinary biomarker. Final models were adjusted for ln-transformed urinary creatinine (continuous), age (continuous), gender (male, female), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, other/mixed race), BMI (continuous), ln-transformed serum cotinine (continuous), season (winter months: participants sampled November 1 to April 30; summer months: participants sampled May 1 to October 31), 30-day vitamin D supplement use (yes: participant reported taking supplement(s) that contained vitamin D; no: participant reported no dietary supplement use or reported taking dietary supplement(s) that did not contain vitamin D), and survey cycle (2005–2006, 2007–2008, 2009–2010).

In addition to investigating associations in the overall population, we stratified multivariable regression models by gender because total 25(OH)D levels were significantly different between men and women, likely reflecting differences in reproductive function and/or lifestyle factors that influence sun exposure (4, 30). To enhance the interpretability, we expressed all regression coefficients and 95% confidence intervals (CIs) as the percentage change (%Δ) in 25(OH)D associated with an interquartile range (IQR) increase in urinary biomarker concentrations. In a separate analysis, we examined potential nonlinear associations between BPA and DEHP metabolites and total 25(OH)D using quintiles of creatinine-corrected urinary exposure biomarkers. We performed tests for trend by treating the quintiles as ordinal variables.

Results

Serum total 25(OH)D concentration significantly differed by population characteristics (Table 1). Mean total 25(OH)D concentrations were significantly higher in females than in males, in participants sampled in summer months compared to winter months, and in those who reported taking dietary supplements in the past month that contained vitamin D. All racial/ethnic subgroups had significantly lower total 25(OH)D compared to non-Hispanic whites (mean = 71.8 nmol/L), with the lowest mean concentration reported among non-Hispanic blacks (mean = 43.4 nmol/L). Mean concentrations of total 25(OH)D were also significantly lower in all BMI categories compared to those with a normal weight (BMI = 18.5–24.9 kg/m^2). The distributions of weighted, creatinine-adjusted urinary phthalate metabolites and BPA in

Table 1. Total Serum 25(OH)D Concentrations by Selected Population Characteristics of U.S. Adults (Age, ≥20 Years), NHANES 2005–2010

Population Characteristics	n	Unweighted, %	Weighted, %	Total 25(OH)D Levels, Weighted Mean (SE), nmol/L
Age, y				
20–39 [ref]	1646	34.8	36.7	64.5 (1.11)
40–59	1533	32.5	39.6	65.4 (0.80)
60+	1545	32.7	23.6	67.7 (1.15)[a]
Race				
Non-Hispanic white [ref]	2366	50.1	71.4	71.8 (0.84)
Non-Hispanic black	887	18.8	10.4	43.4 (0.96)[a]
Mexican American	879	18.6	8.28	53.8 (0.95)[a]
Other Hispanic	389	8.23	4.40	55.7 (2.06)[a]
Other race/multiracial	203	4.30	5.45	53.2 (1.77)[a]
Education level				
College or above [ref]	957	20.3	26.8	68.9 (1.07)
Some college	1270	26.9	30.1	65.6 (0.99)[a]
High school degree or the equivalent	1160	24.6	24.7	65.5 (1.29)[a]
Less than high school	1331	28.2	18.4	60.9 (1.18)[a]
Gender				
Male [ref]	2310	48.9	48.1	63.7 (0.74)
Female	2414	51.1	51.9	67.4 (0.98)[a]
BMI, kg/m^2				
Normal weight (18.5–24.9) [ref]	1269	27.1	29.9	71.8 (0.94)
Underweight (<18.5)	71	1.52	1.69	64.0 (4.35)[a]
Overweight (25–29.9)	1598	34.1	33.0	67.5 (0.99)[a]
Obese (30+)	1743	37.2	35.4	58.7 (0.96)[a]
Smoking status				
Nonsmoker [ref]	2499	52.9	52.8	63.8 (0.89)
Former	1161	24.6	24.7	70.3 (1.29)[a]
Current	1060	22.5	22.5	64.7 (1.12)
30-d Vitamin D supplement use				
Yes [ref]	712	15.1	16.1	77.5 (1.29)
No	4009	84.9	83.9	63.3 (0.79)[a]
Sampling season				
Winter months [ref]	2137	45.2	39.9	58.2 (1.03)
Summer months	2587	54.8	60.1	70.4 (0.86)[a]

[a] $P < .05$ for a significant difference in total 25(OH)D concentrations in the category compared to reference (first category listed) using weighted ANOVA or two-samples *t* tests.

the study population are presented in Table 2. All analytes were detected in at least 90% of the study population, with the exception of MEHP (69% > LOD).

DEHP metabolites were consistently inversely associated with total 25(OH)D in the overall study population and in gender-stratified models, although the results from the latter analyses were not statistically significant (Table 3). In the overall study population, we observed the strongest associations for MEHP and MEHHP, where an IQR increase in these metabolites was associated with a 2.07% (95% CI, −3.62, −0.52) and 2.09% (95% CI, −3.87, −0.32) decline in total 25(OH)D, respectively. We also detected a significant inverse relationship for ΣDEHP metabolites (%Δ, −1.90; 95% CI, −3.64, −0.17). These inverse associations observed between DEHP metabolites and 25(OH)D in the overall population persisted after stratifying analyses by race (eg, non-Hispanic white vs non-Hispanic black; data not shown). We also observed a significant positive association between urinary MEP and total 25(OH)D in both the overall population and in women alone. For BPA, we found a statistically significant inverse relationship in women, but not in men (Table 3). In women, an IQR increase in urinary BPA was associated with a 3.71% decrease (95% CI, −6.41, −1.02) in total 25(OH)D. We found no statistically significant associations for any analytes in male-stratified models.

We observed similar patterns in our secondary analyses in which we investigated the relationships between quintiles of DEHP metabolites and BPA and total 25(OH)D (Figure 1). We detected a statistically significant inverse trend between quintiles of MEHHP and total 25(OH)D (p for trend = 0.02). We found suggestive inverse trends for quintiles of MEHP (p for trend = 0.06), MEOHP (p for trend = 0.10), and MECPP (p for trend = 0.08). A nonmonotonic trend was observed for BPA, where 25(OH)D levels were elevated in the third and fourth quintiles compared to the lowest. We did not stratify this secondary analysis by gender due to the small sample size within each quintile of exposure.

Table 2. Weighted, Creatinine-Corrected Urinary Phthalate Metabolite and BPA Concentrations (μg/g Creatinine), NHANES 2005–2010

Urinary Analyte	% > LOD	Geometric Mean	Selected Percentiles					
			25th	50th	75th	90th	95th	Maximum
MEHP	69.0	2.28	<LOD	2.05	4.22	10.0	20.4	890
MEHHP	99.5	18.3	8.67	15.6	31.8	81.0	167	6095
MEOHP	98.8	10.9	5.29	9.29	18.9	45.9	92.7	3973
MECPP	99.9	27.9	13.8	23.9	47.3	113	210	10 345
MBP	99.3	16.5	10.0	15.6	25.7	43.2	67.7	25 356
MiBP	98.2	6.13	3.75	6.25	10.4	16.5	22.4	8452
MEP	99.9	93.1	34.3	77.6	229	625	1095	66 594
MBzP	98.5	6.43	3.50	6.50	12.2	21.2	32.3	9599
MCPP	96.7	2.37	1.26	2.18	4.05	8.21	13.8	823
MCNP	92.6	2.62	1.40	2.37	4.39	8.67	13.7	702
MCOP	96.8	7.47	3.22	6.12	14.9	41.5	75.3	1044
BPA	91.3	1.89	1.09	1.77	3.08	5.43	8.25	1636

Discussion

In our analysis of data from NHANES 2005–2010, we report findings of inverse relationships between DEHP metabolites and total 25(OH)D. In stratified analyses, these associations were strongest in women. Urinary BPA was also inversely associated with 25(OH)D, albeit nonsignificantly, in the overall study population. When we limited the data to women alone, we detected a stronger and statistically significant inverse relationship. We did not find a similar association in men.

To date, no human health or animal studies have investigated the potential associations between environmental exposure to phthalates and total 25(OH)D. Currently, just one study has investigated this relationship with BPA in humans. Our findings are consistent with those reported by Erden and colleagues (19), who found a suggestive inverse unadjusted correlation between serum BPA and 25(OH)D in 128 participants (37 females, 91 males) of a study primarily aimed to investigate the associations between BPA and 25(OH)D and obstructive sleep apnea syndrome. However, these results are potentially limited by the study's small sample size, the lack of control for confounding factors in the relationship between BPA and 25(OH)D, and the exposure assessment method used to measure this polar, nonpersistent compound (ie, in blood vs urine).

In humans, there are two forms of vitamin D: vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol) (31). These prohormones are produced endogenously by the skin from sunlight exposure (vitamin D_3) and can also be

Table 3. Percentage Change (95% CI) in Total 25(OH)D Associated With an IQR Increase in Urinary Phthalate Metabolite and BPA Concentrations Among U.S. Adults (≥20 Years Old), NHANES 2005–2010

Urinary Analyte	Overall Population (n = 4667 Observations)		Females (n = 2390 Observations)		Males (n = 2277 Observations)	
	%Δ (95% CI)	*P* Value	%Δ (95% CI)	*P* Value	%Δ (95% CI)	*P* Value
MEHP	−2.07 (−3.62, −0.52)	.01[a]	−2.65 (−5.41, 0.12)	.07	−1.46 (−3.14, 0.22)	.10
MEHHP	−2.09 (−3.87, −0.32)	.03[a]	−2.00 (−4.57, 0.58)	.14	−2.01 (−4.44, 0.41)	.11
MEOHP	−1.66 (−3.40, 0.09)	.07	−1.74 (−4.48, 0.99)	.22	−1.37 (−3.53, 0.80)	.22
MECPP	−1.88 (−3.77, 0.01)	.06	−2.28 (−5.18, 0.62)	.13	−1.33 (−3.56, 0.89)	.25
ΣDEHP	−1.90 (−3.64, −0.17)	.04[a]	−2.08 (−4.74, 0.59)	.14	−1.57 (−3.72, 0.58)	.16
MBP	−0.11 (−2.21, 1.99)	.92	0.54 (−2.70, 3.78)	.75	−0.72 (−3.37, 1.93)	.60
MiBP	−1.67 (−3.74, 0.41)	.13	−2.46 (−5.42, 0.50)	.11	−0.99 (−4.13, 2.14)	.54
MEP	2.29 (0.91, 3.66)	<.01[a]	3.72 (1.38, 6.06)	<.01[a]	0.98 (−0.68, 2.63)	.26
MBzP	0.71 (−2.12, 3.54)	.63	0.02 (−3.91, 3.94)	.99	1.45 (−1.78, 4.69)	.38
MCPP	0.82 (−1.39, 3.02)	.47	1.34 (−2.43, 5.12)	.50	0.28 (−1.99, 2.54)	.81
MCNP	0.95 (−1.48, 3.39)	.45	1.03 (−2.79, 4.84)	.60	0.92 (−1.19, 3.02)	.40
MCOP	−1.02 (−3.10, 1.06)	.34	−1.05 (−4.29, 2.19)	.53	−0.92 (−2.66, 0.82)	.31
BPA	−1.30 (−3.02, 0.43)	.15	−3.71 (−6.41, −1.02)	.01[a]	0.88 (−3.01, 0.37)	.35

All linear regression models are weighted for complex survey design and adjusted for ln-transformed urinary creatinine, age, race/ethnicity, BMI, ln-transformed serum cotinine, sampling season, 30-day vitamin D supplement use, and survey cycle. Overall population models are additionally adjusted for gender.

[a] $P < .05$.

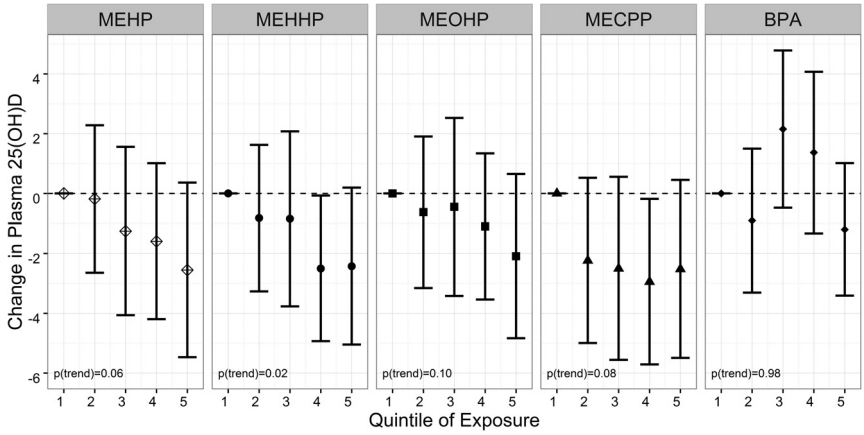

Figure 1. Adjusted regression coefficients for change in serum total 25(OH)D in relation to creatinine-corrected urinary DEHP metabolite and BPA quintiles. Values are weighted for complex survey design and adjusted for age, gender, race/ethnicity, BMI, ln-transformed serum cotinine, 30-day vitamin D supplement use, sampling season, and survey cycle.

obtained from the diet or supplementation (vitamins D_2 and D_3) (1, 4, 31). Both forms of vitamin D are inactive and are hydroxylated hepatically to 25(OH)D, the circulating biomarker of vitamin D status (1, 3, 31). A second hydroxylation occurs in the kidney, where 25(OH)D is converted to its active metabolite, $1,25(OH)_2D$ (33). Similar in structure and function to other steroid hormones (eg, estradiol, progesterone, and T), $1,25(OH)_2D$ is transported to target organs where it binds to its nuclear receptor to initiate biological responses (1, 15, 31).

There are various potential mechanisms, acting either directly or indirectly on the vitamin D endocrine system, that may explain the inverse relationships reported in our study between DEHP metabolites and BPA and total 25(OH)D. The first entails direct action on the metabolic pathways involved in the conversion of vitamin D to its active metabolite (7). The two hydroxylation steps in vitamin D metabolism are performed by enzymes in the cytochrome P450 family, including 1α-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24A1) (33). Both DEHP metabolites and BPA have been shown to induce or inhibit the activity of cytochrome P450 enzymes involved in steroid and/or thyroid hormone metabolism (17, 18, 34, 35). BPA also increased the mRNA expression of CYP27B1 in the kidneys of pregnant mice (36). Thus, phthalates and BPA may modulate levels of circulating 25(OH)D through their action on this group of enzymes involved in hormone metabolism. However, additional animal and in vitro studies are required to establish the specific actions of these compounds on the enzymes involved in vitamin D metabolic processes and to determine the extent to which phthalates and BPA alter the activity of other biological factors directly involved in the vitamin D endocrine system (eg, binding capacity of vitamin D transport proteins or actions at the nuclear receptor).

It is also possible that DEHP metabolites and BPA may influence circulating 25(OH)D levels indirectly through the disruption of calcium homeostasis, which is largely regulated by vitamin D and estrogen (37). The main action of the vitamin D steroid hormone, $1,25(OH)_2D$, is to stimulate the absorption of calcium from the gut (38). Levels of $1,25(OH)_2D$ are down-regulated by its own production as well as serum concentrations of calcium, whereas PTH stimulates the renal production of this active metabolite (1, 38). Several animal studies have shown that BPA may alter the mRNA expression of the calcium binding protein, calbindin-D9k (36, 39), as well as serum calcium levels (36). However, phthalates had no effect on the expression of calbindin-D9k in the uterus of immature rats (40), and no studies have investigated the effects of these compounds on calcium reabsorption.

We observed positive associations for urinary MEP, the primary metabolite of diethyl phthalate (DEP), in the overall study population and particularly in women, which was somewhat unexpected given previous data showing inverse associations between this phthalate metabolite and sex steroid hormones (41, 42). DEP is found in a wide range of personal care products, including perfumes, hairspray, deodorants, and lotions (43). It is possible that participants with high use of DEP-containing personal care products are also more likely to exhibit behaviors that increase their 25(OH)D levels, which may not have been captured in the variables of interest in the current study (ie, leading to residual confounding). Alternatively, it is possible that MEP may have actions on the vitamin D endocrine system that differ from those of DEHP metabolites and may subsequently result in elevated levels of 25(OH)D. Future research is required to determine the specific mechanisms by which phthalates may influence circulating levels of total 25(OH)D and how the actions of specific phthalate metabolites may differ.

This was the first study to investigate the associations between phthalates and BPA and total 25(OH)D in the U.S. general population. Strengths of our study included our large sample size and population-based data that allowed for the detection of subtle effects between our exposures and outcome of interest. Furthermore, total 25(OH)D concentrations were measured using a LC-MS/MS method developed by the CDC that is considered more analytically accurate than immunoassay methods

(27). Despite these strengths, our investigation was potentially limited by its cross-sectional design that precludes any conclusions regarding causation. Furthermore, unmeasured or residual confounding and reverse causation cannot be ruled out as potential explanations of our findings. Additionally, this study included single biomarker measurements of both the exposures and outcome of interest. NHANES vitamin D data are based on serum samples preferentially collected in northern states in the summer and southern states in the winter. Although we accounted for this 6-month sampling period in our analyses, seasonal variability in total 25(OH)D levels may not be fully captured in the present study. Moreover, our analyses are based on a single urinary measurement of each phthalate metabolite and BPA, which have short biological half-lives and are quickly excreted from the body; thus, urinary concentrations fluctuate within the individual over time and can lead to measurement error (44, 45). However, we expect this measurement error to be nondifferential with respect to total 25(OH)D concentrations, thereby biasing our results to the null. Finally, we performed a number of statistical comparisons, and there is the potential that some of the observed associations may have been due to chance.

Conclusions

Overall, our results provide suggestive evidence that environmental exposure to phthalates and BPA may alter circulating levels of total 25(OH)D in adults. Our stratified analyses showed that some of these associations may vary by gender. Future human health and animal studies are required to resolve the direction and temporality of these relationships, to elucidate the exact mechanisms through which these compounds may act to disrupt the vitamin D endocrine system, and to determine the implications of these findings to public health.

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