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Clinical Research Article

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Urinary Phthalate Biomarkers and Bone Mineral Density in Postmenopausal Women

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Abbreviations: BMD, bone mineral density; BMI, body mass index; CDC, Centers for Disease Control and Prevention; CT, clinical trial; DBP, dibutyl phthalate; DEXA, dual-energy x-ray absorptiometry; FRAX, Fracture Risk Assessment Tool; GEE, generalized estimating equation; HT, hormone therapy; MBP, mono-n-butyl phthalate; MBzP, monobenzyl 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, monohydroxybutyl phthalate; MHiBP, monohydroxyisobutyl phthalate; MiBP, monoisobutyl phthalate; NHANES, National Health and Nutrition Examination Survey; OS, observational study; WHI, Women's Health Initiative.

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Abstract

Context: Phthalates are endocrine-disrupting chemicals that could disrupt normal physiologic function, triggering detrimental impacts on bone.

Objective: We evaluated associations between urinary phthalate biomarkers and BMD in postmenopausal women participating in the prospective Women's Health Initiative (WHI).

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Methods: We included WHI participants enrolled in the BMD substudy and selected for a nested case-control study of phthalates and breast cancer (N = 1255). We measured 13 phthalate biomarkers and creatinine in 2 to 3 urine samples per participant collected over 3 years, when all participants were cancer free. Total hip and femoral neck BMD were measured at baseline and year 3, concurrent with urine collection, via dual-energy x-ray absorptiometry. We fit multivariable generalized estimating equation models and linear mixed-effects models to estimate cross-sectional and longitudinal associations, respectively, with stratification on postmenopausal hormone therapy (HT) use.

Results: In cross-sectional analyses, mono-3-carboxypropyl phthalate and the sum of di-isobutyl phthalate metabolites were inversely associated with total hip BMD among HT nonusers, but not among HT users. Longitudinal analyses showed greater declines in total hip BMD among HT nonusers and with highest concentrations of mono-3-carboxyoctyl phthalate (–1.80%; 95% CI, –2.81% to –0.78%) or monocarboxynonyl phthalate (–1.84%; 95% CI, –2.80% to –0.89%); similar associations were observed with femoral neck BMD. Among HT users, phthalate biomarkers were not associated with total hip or femoral neck BMD change.

Conclusion: Certain phthalate biomarkers are associated with greater percentage decreases in total hip and femoral neck BMD. These findings suggest that phthalate exposure may have clinically important effects on BMD, and potentially fracture risk.

Key Words: phthalate, bone mineral density, postmenopausal, women, biomarkers

Phthalates are chemicals used in the production of many consumer products (eg, vinyl flooring, personal care products) and are increasingly linked to higher risk of numerous chronic health outcomes, including cancer, obesity, and diabetes (1-3). Among their known mechanisms of action, phthalates are well-established endocrine disruptors.

The potential effects of phthalates on bone homeostasis are less well understood. A recent study reported lower bone mineral density (BMD) in adult male mice prepubertally exposed to low-dose, but not high-dose, dibutyl phthalate (DBP). DBP may affect BMD through its antiandrogenic effects (4). Some animal studies observe adverse effects of prenatal exposure to certain phthalates on bone development, including di-n-hexyl phthalate exposure with skeletal malformations (5), diallyl phthalate with delayed ossification (6), and butyl benzyl phthalate and DBP with increased DNA damage and apoptosis of osteoblasts (7). Additionally, appropriate balance of estrogens and other hormones is critical to maintenance of bone health. Estrogenic activity has been demonstrated by various phthalates (8-10), and antiestrogenic actions of phthalates also have been reported (8).

Little is known about whether phthalates affect bone health in humans. Two small cross-sectional studies supported deleterious effects of phthalates on postmenopausal bone health. These studies observed significant inverse associations of the urinary metabolites of certain phthalates, namely mono-n-butyl phthalate (MBP), monobenzyl phthalate (MBzP), mono-3-carboxypropyl phthalate (MCPP), and monocarboxynonyl phthalate (MCNP) with BMD in the hip and femoral neck (11) and of monoethyl phthalate (MEP) with BMD in the spine (12). These results were surprising given the benefit of estrogen signaling in promoting bone health (13) and the demonstrated estrogenic activity of many of these phthalates, yet may relate to antiestrogenic effects observed from some phthalates (8).

While provocative, prior human studies were small and lacked prospective data, which are needed to establish temporality. Therefore, we evaluated associations between urinary phthalate biomarkers and BMD among a sample of participants in the prospective Women's Health Initiative (WHI) cohorts.

Materials and Methods

Study Population

As previously described, the WHI recruited 161 808 postmenopausal women from 40 clinical centers nationwide between October 1, 1993 and December 21, 1998 (14). All women were between ages 50 and 79 years at enrollment and participated either in 1 or more of 4 clinical trials (CT; N = 68 132) or an observational study (OS; N = 93 676). A bone density substudy, in which participants provided first-morning void urine samples and had BMD measurements at baseline, year 1, year 3, and year 6 clinic visits, included all participants at 3 clinical centers (N = 11 020).

The present analysis included WHI participants selected for a nested case-control study of phthalate biomarkers

and breast cancer (15). Briefly, eligible participants were enrolled in the bone density cohort, provided 2 or more urine samples available between baseline and the year 3 clinic visit, and had no prior cancer history (other than nonmelanoma skin cancer). Cases (N = 419) were diagnosed with breast cancer following their year 3 clinic visit, to ensure that phthalate biomarker concentrations were measured in prediagnostic urine samples. Controls (N = 838) were selected using incidence density sampling and were 2:1 matched to cases on enrollment date, length of follow-up, age at enrollment (within 3 years), and WHI study component (CT/OS). Because this is a highly selected population, we compared this analytic population to participants from the BMD substudy who were not selected. Overall, our sample was slightly younger (age 62.5 years vs 63.4 years; $P < .001$), and included more participants who self-identified as non-Hispanic White (82.3% vs 76.7%; $P < .001$) and were enrolled in the CT study (47% vs 43%; $P = .01$) but were of similar body mass index (BMI; 28.1 vs 28.2; $P = .30$) and total hip BMD (0.85 g/cm^2 vs 0.85 cm^2; $P = .30$).

Written informed consent was obtained from all participants on their enrollment into WHI, and approval was received from institutional review boards at each WHI clinical center. The University of Massachusetts Amherst Institutional Review Board additionally approved the present research. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory in the analysis of samples did not constitute engagement in human participant research.

Quantification of Phthalate Metabolites and Creatinine

WHI followed a standard urine collection, processing, and storage protocol. First-morning void urine samples were collected at home, kept refrigerated, and then processed less than 30 minutes on clinic arrival. Urine samples were centrifuged for 5 minutes at 1330*g*; 1.8-mL aliquots were frozen and shipped to McKesson Bioservices packed in dry ice via overnight FedEx then stored at –80 °C.

Phthalate metabolites are used as biomarkers to ensure that measured concentrations relate to endogenous exposures, as opposed to contamination during collection and/or processing. The CDC quantified 13 phthalate metabolites in urine samples using baseline and year 3 urine samples (MBP, MBzP, MCNP, monocarboxyoctyl phthalate [MCOP], MCPP, mono-(2-ethyl-5-carboxypentyl) phthalate [MECPP], mono-(2-ethyl-5-hydroxyhexyl) phthalate [MEHHP], mono-(2-ethylhexyl) phthalate [MEHP], mono-(2-ethyl-5-oxohexyl) phthalate [MEOHP], monoethyl phthalate [MEP], monohydroxybutyl phthalate [MHBP], monohydroxyisobutyl phthalate [MHiBP], and monoisobutyl phthalate [MiBP]). The glucuronidated phthalate metabolites undergo enzymatic deconjugation followed by online solid-phase extraction and high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry. Samples were randomly distributed through the batches, with all replicates from cases and matched controls analyzed together. A blinded 10% quality control sample was included and used to estimate coefficients of variation: MBP 5.4%, MBzP 6.1%, MCNP 4.7%, MCOP 6.3%, MCPP 5.8%, MECPP 4.3%, MEHHP 5.4%, MEHP 19.5%, MEOHP 6.0%, MEP 3.1%, MHBP 9.0%, MHiBP 21.9%, and MiBP 10.3%. Laboratory staff were masked to the identity, disease status, and demographic and risk factor characteristics of the samples. Creatinine was measured using a Roche Modular P Chemistry Analyzer and an enzymatic assay. The limit of detection for creatinine was 1 mg/dL and the coefficient of variation was 2.5%.

Bone Mineral Density Measurement

BMD was measured in participants recruited at 3 of 40 clinical centers (Pittsburgh, Pennsylvania; Birmingham, Alabama; and Tucson/Phoenix, Arizona), chosen to provide maximum racial diversity. Participants of this WHI BMD cohort underwent dual-energy x-ray absorptiometry (DEXA) measurement using Hologic machines (QDR2000, 2000+, or 4500). Quality assurance methods included cross-clinic calibration phantoms and review of a random sample of scans. When the Hologic QDR 2000 machines were upgraded to QDR 4500 machines, in vivo cross-calibration procedures were performed and results were adjusted for these correction factors and for longitudinal changes in scanner performance (16). We used measurements of BMD at the total hip and femoral neck from DEXA scans performed on each participant at baseline and the years 3 and 6 clinic visits.

Covariate Data

Participants provided extensive data via self-report questionnaires and at clinic visits. We considered the following potential covariates all assessed at baseline, with updates at subsequent clinic visits for time-varying covariates: age (continuous), race and clinical site (state) (White/Pennsylvania, White/Alabama, White/Arizona, non-White/Pennsylvania, non-White/Alabama, non-White/Arizona), education (less than high school, high school/some college, college graduate, graduate degree), income (< $35 000, ≥ $35 000), current alcohol use (nondrinker, past drinker, < 1 drink per month,

< 1 drink per week, 1-< 7 drinks per week, 7+ drinks per week), smoking status (never, past, current), body mass index (BMI) (continuous), total physical activity metabolic equivalents (METs) per week (quartiles) thyroid medications (no, yes), psychotropic medications (no, yes), β-blocker medications (no, yes), statin medications (no, yes), supplemental vitamin D use (continuous), bisphosphonate use (no, yes), and calcium and vitamin D supplementation study arm (not randomly assigned to calcium and vitamin D, intervention, control). Probability of fracture within the next 10 years was estimated from baseline data using the FRAX model (FRAX Fracture Risk Assessment Tool). Hormone therapy (HT) use, including unopposed estrogen or estrogen + progesterone formulations, was derived from reported medication use at clinic visits and mailed questionnaires and, when applicable, HT trial intervention assignment. We defined use across the 3-year interval and considered women to be nonusers only if they did not report any use at baseline or at any follow-up during the 3-year interval. Nine women enrolled in the CT were classified as nonusers between baseline and year 3, but then reported initiating HT use between years 3 and 6 and were categorized as HT users for the latter interval.

Statistical Analyses

We calculated descriptive statistics of the analytic sample for characteristics at baseline and tested for differences by quartile of total hip BMD, using analysis of variance and chi-square tests as appropriate.

Phthalate biomarker concentrations were natural log-transformed to improve normality. Sum of metabolites of DBP (ΣDBP) was calculated as the molar sum of MBP and MHBP, sum of metabolites of di-isobutyl phthalate (ΣDiBP) was calculated as the molar sum of MiBP and MHiBP, and sum of metabolites of di(2-ethylhexyl) phthalate (ΣDEHP) was calculated as the molar sum of MEHP, MEHHP, MEOHP, and MECPP. We calculated adjusted means for the phthalate biomarkers using linear regression models with adjustment for age, creatinine, and race/site; natural log-transformed values were back transformed to calculate adjusted geometric means.

Cross-Sectional Analyses

Because HT use has known, positive effects on BMD (17, 18), we a priori decided to stratify analyses on HT use. For the cross-sectional analyses we stratified by whether or not women used HT from baseline through the year 3 visit. Generalized estimating equation (GEE) models were fit, using the identity link and Gaussian (normal) distribution, to estimate repeated cross-sectional associations between phthalate biomarkers and each BMD measure. This approach has the advantage of accommodating repeated measures, including updated covariate information at each time point. Initially, a single GEE model was fit for each phthalate biomarker concentration, including creatinine and a single covariate. In multivariable GEE model development, all variables in single predictor models that were significant at the *P* less than .05 level were included in our initial multiple predictor model. In subsequent model selection, age and creatinine were retained in all models regardless of statistical significance. In selecting our final multivariable models, we used multiple criteria, including significance of partial *F*-tests and changes in the magnitudes of estimated regression coefficients; this yielded the following covariates for inclusion in our final multivariable models: age, creatinine, race/site, smoking status, and BMI. We report estimated β, scaled by a factor of 1000 for interpretability, and 95% CI. We also specifically tested for effect modification by HT use through inclusion of a multiplicative interaction term between the phthalate biomarker and HT use.

Longitudinal Analyses

As with the cross-sectional analyses, we decided a priori to stratify on HT use. For the longitudinal analyses we stratified on whether or not women used HT during the 3-year interval. We fit multilevel mixed-effects models to estimate associations of phthalate biomarkers with percentage change in BMD over time (calculated as the difference between BMD at the beginning and at the end of the interval, divided by the BMD at the beginning of the interval and expressed as a percentage), Controls each contributed up to two 3-year intervals (baseline to year 3; year 3 to year 6), and cases contributed only one 3-year interval (baseline to year 3) to exclude potential effects of cancer treatment on BMD. We tested for association between the phthalate biomarker measurement at the beginning of the 3-year interval to the percentage change in total hip or femoral BMD during the 3-year interval. Percentage change in the BMD measure was calculated so that a negative value indicated a decrease in BMD and a positive value indicated an increase in BMD. Our final multivariable models included the following covariates: age, creatinine, calcium/vitamin D study arm, race/site, smoking status, and BMI. We also specifically tested for effect modification by HT use through inclusion of appropriate multiplicative interaction terms.

All analyses were performed using Stata version 16.0 (Stata Corporation LLC). Two-sided *P* values less than or equal to .05 were considered statistically significant.

Results

Characteristics of the study population at baseline, stratified by quartile of total hip BMD, are presented in Table 1. We observed that, compared to those in the fourth quartile, women in the first quartile of BMD were older ($P < .001$), more likely to be White ($P < .001$), more likely to be enrolled in the OS ($P = .005$), and had a lower BMI ($P < .001$). Also, women in the lowest quartile of BMD were more likely to report current alcohol intake ($P = .02$) and current smoking ($P = .03$) and reported higher physical activity levels ($P = .01$). Additionally, lower BMD was associated with reporting never using HT ($P < .001$), currently using bisphosphonates ($P < .001$), and a higher 10-year probability of fracture ($P < .001$).

Geometric mean concentrations of urinary phthalate biomarkers, adjusted for age, creatinine, and race/site are presented in Table 2. In general urinary phthalate biomarker concentrations were lower among women in the first quartile of total hip BMD, although the observed associations were statistically significant only for MCOP, MCPP, and DBP.

Estimates of multivariable-adjusted cross-sectional associations of phthalate biomarkers with BMD are reported in Table 3. MEP, MCOP, MCNP, DBP, and DEHP concentrations were not significantly associated with total hip or femoral neck BMD. Among nonusers of HT only concentrations of MCPP were negatively associated with total hip (P interaction = .03) and femoral neck ($P = .01$) BMD. For example, women with MCPP concentrations in the fourth quartile had 0.0069 g/cm^2 lower total hip BMD (P trend = .03) and 0.00737 g/cm^2 lower femoral neck BMD (P trend = .03) than women in the first quartile. We also observed significantly lower total hip BMD associated with increased urinary DiBP concentrations (eg, fourth vs first quartile $\beta = -11.09$ 1000 g/cm^2, P trend = .01) among non-HT users (P interaction = .01); similar associations were observed between DiBP and femoral neck BMD, although they did not reach statistical significance. Likewise, MBzP concentrations were nonstatistically significantly related to decreased total hip BMD in non-HT users (P trend = .07), although no significant effect modification by HT use status was observed (P interaction = .58). MBzP concentrations were not significantly associated with femoral neck BMD in either HT users or nonusers.

Table 4 reports the estimated associations between urinary phthalate biomarker concentrations and percentage change in BMD over a 3-year period. We observed statistically significant greater declines in total hip BMD among women not using HT during follow-up associated with higher concentrations of MCOP and MCNP. For example, compared to the first quartile, 3-year percentage change in total hip BMD was 1.8% lower among women with MCOP concentrations in the fourth quartile (P trend = .002) and 1.8% lower among women with MCNP concentrations in the fourth quartile (P trend = .0002). Similarly, higher concentrations of MCNP (P trend = .05) were associated with greater decreases in femoral neck BMD among non-HT users; MCOP showed suggestive, inverse associations with BMD, although associations were not statistically significant (P trend = .10). We also observed a statistically significant interaction between HT use and sum of DBP biomarkers on total hip BMD ($P = .007$); stratified analyses suggested a negative association between sum of DBP biomarkers among non-HT users and a positive association among HT users, although neither trend was statistically significant. Similar suggestive associations were observed between the sum of DBP biomarkers and femoral neck BMD. Other phthalate biomarkers (MEP, MBzP, MCPP, DiBP, and DEHP) were not associated with change in total hip or femoral neck BMD over 3 years.

Discussion

In this large sample of postmenopausal women, we observed that women not using HT experienced greater declines in total hip BMD and femoral neck BMD associated with increased urinary concentrations of MCOP and MCNP; no significant associations were observed among HT users. In cross-sectional analyses we observed significantly lower total hip BMD among women with the highest urinary concentrations of MCPP and ∑DiBP, although these associations were limited to women not using HT. Likewise, we observed inverse, cross-sectional associations between MCPP concentrations and femoral neck BMD among non-HT users.

Because data on effects of phthalates on bone are so limited, it is difficult to determine why these phthalate metabolites would be associated with BMD whereas others are not. Such associations may reflect common sources of exposure and/or similar mechanistic effects, such as antiestrogenic or antiandrogenic effects. Indeed, MCPP and DBP metabolites were negatively associated with circulating testosterone among US National Health and Nutrition Examination Survey (NHANES) participants (19).

HT use is known to increase BMD, as demonstrated within the WHI population (17, 18). Because of these strong effects, it is possible that smaller, negative associations between phthalates and BMD would be offset, and thus masked, by HT use. We therefore decided a priori to stratify our analyses on HT use. Indeed, the negative associations we observed were restricted to women not using HT.

Table 1. Descriptive characteristics, at baseline, of study group stratified by quartile of total hip bone mineral density, N = 1255

	Total hip BMD, g/cm^2				
Characteristic[a]	1st quartile ≤ 0.765 N = 314	2nd quartile 0.766-≤ 0.85 N = 311	3rd quartile 0.851-≤ 0.94 N = 309	4th quartile ≥ 0.95 N = 311	*P*
Age, mean (SD), y	65.90 (6.60)	62.50 (6.68)	61.96 (6.50)	59.62 (6.29)	< .001
Race/Clinical site; N (%)					< .001
White/PA	140 (44.6)	117 (37.6)	109 (35.3)	97 (31.2)	
White/AL	40 (12.7)	56 (18.0)	59 (19.1)	59 (19.0)	
White/AZ	108 (34.4)	96 (30.9)	89 (28.8)	65 (20.9)	
Non-White/PA	2 (0.6)	6 (1.9)	7 (2.3)	13 (4.2)	
Non-White/AL	7 (2.2)	19 (6.1)	24 (7.8)	52 (16.7)	
Non-White/AZ	17 (5.4)	17 (5.5)	21 (6.8)	25 (8.0)	
Education level, N (%)					.48
Less than high school	86 (27.5)	82 (26.5)	84 (27.3)	88 (28.5)	
Post-highschool/some college	119 (38.0)	105 (34.0)	108 (35.1)	118 (38.2)	
College degree	31 (9.9)	44 (14.2)	42 (13.6)	25 (8.1)	
Postgraduate/graduate degree	77 (24.6)	78 (25.2)	74 (24.0)	78 (25.2)	
OS participation, N (%)	189 (60.2)	170 (54.7)	157 (50.8)	144 (46.3)	.005
HT assignment, N (%)					.66
Not randomly assigned to HT	262 (83.4)	253 (81.4)	253 (81.9)	255 (82.0)	
E-alone intervention	9 (2.9)	11 (3.5)	9 (2.9)	13 (4.2)	
E-alone control	9 (2.9)	16 (5.1)	12 (3.9)	20 (6.4)	
E + P intervention	18 (5.7)	19 (6.1)	19 (6.1)	13 (4.2)	
E + P control	16 (5.1)	12 (3.9)	16 (5.2)	10 (3.2)	
BMI, mean (SD)	25.40 (4.37)	26.65 (4.31)	28.63 (5.30)	31.57 (6.47)	< .001
BMI category, N (%)					< .001
Underweight/normal, < 25	174 (55.6)	114 (36.8)	81 (26.7)	48 (15.4)	
Overweight, 25-< 30	91 (29.1)	136 (43.9)	121 (39.9)	99 (31.8)	
Obese, ≥ 30	48 (15.3)	60 (19.4)	101 (33.3)	164 (52.7)	
Alcohol intake; N (%)					.02
Nondrinker	39 (12.5)	40 (13.0)	47 (15.3)	46 (14.9)	
Past drinker	51 (16.3)	69 (22.4)	52 (16.9)	64 (20.7)	
< 1 drink per mo	48 (15.4)	27 (8.8)	45 (14.7)	41 (13.3)	
< 1 drink per wk	75 (24.0)	55 (17.9)	60 (19.5)	78 (25.2)	
1-< 7 drinks per wk	76 (24.4)	87 (28.2)	70 (22.8)	52 (16.8)	
≥ 7 drinks per wk	23 (7.4)	30 (9.7)	33 (10.7)	28 (9.1)	
Smoking status; N (%)					.03
Never smoked	175 (56.3)	164 (54.1)	174 (56.9)	179 (58.7)	
Past smoker	108 (34.7)	114 (37.6)	122 (39.9)	113 (37.0)	
Current smoker	28 (9.0)	25 (8.3)	10 (3.3)	13 (4.3)	

Table 1. Continued

Characteristic[a]	Total hip BMD, g/cm^2				
	1st quartile ≤ 0.765 N = 314	2nd quartile 0.766-≤ 0.85 N = 311	3rd quartile 0.851-≤ 0.94 N = 309	4th quartile ≥ 0.95 N = 311	*P*
Total physical activity, MET-h/wk, quartiles; N (%)					.01
≤ 1.87 METs/wk	56 (20.1)	78 (29.3)	78 (28.5)	86 (31.0)	
> 1.87-7.5 METs/wk	66 (23.7)	68 (25.6)	70 (25.5)	82 (29.6)	
> 7.5-17.5 METs/wk	81 (29.0)	61 (22.9)	65 (23.7)	46 (16.6)	
> 17.5 METs/week	76 (27.2)	59 (22.2)	61 (22.3)	63 (22.7)	
History of HT use; N (%)					.001
Never	170 (54.5)	136 (43.7)	136 (44.0)	134 (43.1)	
Past	54 (17.3)	51 (16.4)	39 (12.6)	40 (12.9)	
Current	88 (28.2)	124 (39.9)	134 (43.4)	137 (44.1)	
Current bisphosphonate use, N (%)	10 (3.3)	3 (1.0)	0 (0.0)	1 (0.3)	< .001
10-year probability of fracture (FRAX), mean (SD)	12.61 (7.33)	9.78 (5.62)	8.68 (5.26)	6.69 (4.24)	< .001

Abbreviations: AL, Alabama, AZ, Arizona; BMD, bone mineral density; BMI, body mass index; E, estrogen; FRAX, FRAX Fracture Risk Assessment Tool; HT, hormone therapy; MET, metabolic equivalent; OS, observational study; P, progesterone; PA, Pennsylvania.

[a]Missing data are as follows: education, n = 6; BMI, n = 8; alcohol use, n = 9; smoking status, n = 20; physical activity, n = 149; history of hormone use, n = 2; bisphosphonate use, n = 38.

These findings suggest that HT use may offer protection against deleterious effects of phthalate exposure, although future work will be needed to confirm these findings.

Our findings are consistent with prior cross-sectional analyses within the NHANES that reported inverse associations between MBzP, MBP (a major metabolite of DBP), and MCPP (a minor DBP metabolite) and total hip BMD (11). These prior findings adjusted for hormone use, but did not report results with stratification on hormone use. However, the majority of the NHANES participants included in the analysis were not using HT (55.5%). Together, these findings indicate that higher levels of urinary MCPP and MBzP are negatively associated with total hip BMD, although these associations may be limited to women not using HT. Importantly, a single BMD measurement can predict fracture risk up to 25 years later (20), with each 0.1-g/cm^2 decrease in total hip BMD conferring a 74% increased risk of hip fracture and 18% increased risk of nonvertebral fracture (21). Among women not using HT, those in the highest vs lowest quartiles of MCPP or ∑DiBP urinary concentrations had on average 0.004 to 0.011 g/cm^2 lower total hip BMD, which would translate to a 3.0% to 8.1% increase in hip fracture risk. These findings could suggest that women not using HT and with high urinary concentrations of these phthalate biomarkers may be a group at higher risk for future osteoporotic fracture.

In longitudinal analyses we observed negative associations between MCOP and MCNP that were restricted to women not using any HT during the 3-year interval. Importantly, use of HT appears to prevent such decreases in BMD associated with the higher urinary phthalate biomarker concentrations. BMD in older adults generally declines over time without pharmacological intervention; the observed declines associated with MCOP and MCNP are in addition to the declines experienced with normal aging. If confirmed in other populations, the finding that certain phthalates are associated with greater declines in BMD could have important implications for fracture risk in the population. MCNP is a metabolite of di-isodecyl phthalate, and MCOP is a metabolite of di-isononyl phthalate. Di-isodecyl phthalate and di-isononyl phthalate are both extensively used in food packaging, and we previously reported that urinary MCNP and MCOP levels were significantly, positively correlated in our study population ($r = 0.40$) (22). "Ultraprocessed" refers to food products that are typically packaged to be ready to eat; contain high amounts of fat, sugar, and/or salt; and are lacking in fiber, protein, and/or other micronutrients (eg, french fries, hot dogs, ice cream, prepackaged snack products) (23). A recent analysis of NHANES data by Buckley et al showed strong, positive associations between consumption of

Table 2. Adjusted means of phthalate biomarkers, at baseline, stratified by quartile of total hip bone mineral density, N = 1255

	Total hip BMD, g/cm^2				
	1st quartile ≤ 0.765 N = 314	2nd quartile 0.766-≤ 0.85 N = 311	3rd quartile 0.851-≤ 0.94 N = 309	4th quartile ≥ 0.95 N = 311	
Phthalate biomarker	Geometric mean (SD)[a]				*P*
MEP, ng/mL	72.20 (1.64)	83.94 (1.67)	87.31 (1.79)	99.92 (1.90)	.33
MBzP, ng/mL	10.72 (1.70)	12.45 (1.71)	12.32 (1.85)	13.69 (1.95)	.34
MCOP, ng/mL	3.27 (1.54)	3.85 (1.54)	4.02 (1.64)	4.55 (1.72)	.05
MCNP, ng/mL	2.53 (1.45)	2.84 (1.44)	2.91 (1.52)	3.15 (1.57)	.25
MCPP, ng/mL	3.12 (1.60)	3.44 (1.61)	3.32 (1.72)	3.52 (1.81)	.03
ΣDBP, µmol/L	0.12 (1.75)	0.13 (1.76)	0.13 (1.92)	0.14 (2.03)	.03
ΣDiBP, µmol/L	0.01 (1.71)	0.01 (1.72)	0.01 (1.87)	0.01 (1.98)	.76
ΣDEHP, µmol/L	0.16 (1.59)	0.18 (1.59)	0.19 (1.70)	0.21 (1.78)	.61

Abbreviations: BMD, bone mineral density; DBP, di-n-butyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DiBP, di-isobutyl phthalate; MBzP, monobenzyl phthalate; MCNP, mono carboxyisononyl phthalate; MCOP, monocarboxyoctyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MEP, monoethyl phthalate.
[a] Geometric means estimated from regression models adjusting for age, creatinine, and race/site.

ultraprocessed foods and urinary concentrations of MCNP and MCOP (24). Notably, individuals in the highest quartile of ultraprocessed food intake had urinary MCOP and MCNP concentrations that were 53.4% and 25% higher, respectively, than those in the lowest quartile. In the analysis by Buckley and colleagues, intake of ultraprocessed foods was high: The median percentage of total energy intake from ultraprocessed foods was 63.1% and the maximum was 100% (24). Given that the NHANES sample is selected to be representative of the US population, this indicates an alarming level of consumption of ultraprocessed foods, and potentially important implications for our findings that metabolites of food packaging components are associated with greater declines in BMD.

Interestingly, the urinary phthalate biomarkers that were negatively associated with BMD in cross-sectional analyses (MCPP, ΣDiBP) differed from those for which we observed negative associations in longitudinal analyses (MCOP, MCNP). The reasons for these inconsistencies are unclear. We note that MCPP was negatively associated with BMD in longitudinal analyses, although these associations did not reach statistical significance. Future prospective studies will be helpful in understanding which, if any, phthalates may have negative effects on BMD.

Our findings must be interpreted, however, in the context of relevant limitations. First, the statistically significant results may reflect a type I error, especially given that results were not adjusted for multiple comparisons. However, the consistency of our longitudinal findings for MCOP and MCNP indicates that these are not spurious associations. Second, it is possible that MCOP and MCNP are markers of poor diet or of some other, unmeasured, component of food packaging, as opposed to the causal agents themselves. Last, we measured urinary phthalate metabolites as biomarkers of exposure to parent phthalates. Phthalates are metabolized and the metabolites are excreted quickly following exposure, and moderate within-person variability in metabolite urinary concentrations is well known (25). Thus, a single measure of urinary phthalate biomarkers represents only short-term phthalate exposure. Accordingly, the resultant nondifferential misclassification of exposure may have attenuated true associations, especially if the effects were small. It is possible that other phthalate biomarkers are related to BMD but that we lacked the statistical power to detect such associations. Additionally, these results may not be generalizable to other populations of postmenopausal women, given the limited racial/ethnic diversity and higher socioeconomic status of WHI participants compared to the general population. However, the mechanisms linking phthalates to BMD are unlikely to vary by race/ethnicity. Finally, these results cannot demonstrate causality, only statistical associations.

Our study is strengthened by the availability of a large, well-characterized sample of women. Also, we quantified a broad panel of phthalate metabolites in first-morning void urine samples using an established analytic method with proven reliability and validity. The prospective, repeated measurements of key variables, including phthalate biomarkers, BMD, HT use, and body weight are an especially unique aspect of our study. Importantly, our BMD measurements were performed on calibrated machines with extensive quality control procedures to ensure comparability of measures between sites and over time. Additionally, we performed analyses with stratification on HT use, which allowed us to identify inverse associations between certain

Table 3. Cross-sectional associations between phthalate biomarkers and bone mineral density measures, stratified by hormone therapy use, N = 1229

Phthalate biomarker	Total hip BMD, 1000 × g/cm^2			Femoral neck BMD, 1000 × g/cm^2		
	No HT[a] use N = 457	HT[a] use N = 772		No HT[a] use N = 457	HT[a] use N = 772	
	β (95% CI)[b]	β (95% CI)[b]	*P* interaction	β (95% CI)[b]	β (95% CI)[b]	*P* interaction
MEP, ng/mL						
0.8-31.8	Ref	Ref	.71	Ref	Ref	.43
31.9-67.9	–4.58 (–10.44 to 1.28)	–0.55 (–5.30 to 4.19)		3.70 (–3.28 to 10.69)	1.21 (–4.74 to 7.16)	
68.0-163.0	–5.22 (–11.47 to 1.03)	–5.04 (–10.37 to 0.30)		–0.13 (–7.56 to 7.30)	1.63 (–5.03 to 8.30)	
164.0-26 000	–1.99 (–9.37 to 5.38)	–0.71 (–6.43 to 5.01)		1.80 (–6.94 to 10.54)	6.66 (–0.47 to 13.79)	
P trend	.63	.59		.98	.07	
MBzP, ng/mL						
0.2-5.9	Ref	Ref	.58	Ref	Ref	.90
6.0-11.8	–0.14 (–5.67 to 5.40)	4.84 (0.18 to 9.50)		4.33 (–2.31 to 10.96)	5.87 (0.03 to 11.71)	
11.9-22.0	–5.52 (–11.47 to 0.43)	–0.44 (–5.58 to 4.69)		–1.50 (–8.62 to 5.61)	1.80 (–4.62 to 8.22)	
22.1-3590.0	–4.94 (–12.42 to 2.54)	–1.13 (–7.21 to 4.95)		–0.53 (–9.45 to 8.38)	1.34 (–6.23 to 8.92)	
P trend	.07	0.42		.57	.96	
MCOP, ng/mL						
0.1-2.1	Ref	Ref	.75	Ref	Ref	.57
2.2-3.6	1.03 (–4.20 to 6.27)	2.73 (–2.10 to 7.55)		–1.52 (–7.79 to 4.76)	1.28 (–4.78 to 7.34)	
3.7-6.5	–2.19 (–7.97 to 3.59)	1.30 (–3.76 to 6.36)		–6.11 (–13.02 to 0.80)	–0.53 (–6.87 to 5.81)	
6.6-270.0	–0.68 (–7.42 to 6.06)	0.93 (–4.72 to 6.57)		–2.43 (–10.49 to 5.62)	–0.72 (–7.80 to 6.36)	
P trend	.61	0.90		.32	.72	
MCNP, ng/mL						
0.1-1.6	Ref	Ref	.40	Ref	Ref	.96
1.7-2.6	–0.11 (–5.49 to 5.26)	1.99 (–2.74 to 6.71)		2.22 (–4.30 to 8.74)	0.58 (–5.33 to 6.50)	
2.7-4.7	–2.94 (–8.95 to 3.07)	1.06 (–4.09 to 6.21)		0.30 (–6.97 to 7.57)	–1.45 (–7.89 to 4.99)	
4.8-222.0	4.12 (–2.52 to 10.75)	1.44 (–4.04 to 6.92)		1.52 (–6.50 to 9.54)	0.83 (–6.02 to 7.67)	
P trend	.38	.74		.85	.97	
MCPP, ng/mL						
0.1–1.7	Ref	Ref	.03	Ref	Ref	.01
1.8-3.0	–4.77 (–10.35 to 0.82)	3.87 (–0.79 to 8.53)		-4.71 (–11.35 to 1.93)	0.76 (–5.10 to 6.61)	
3.1-5.4	–8.22 (–14.19 to –2.24)	4.33 (–0.61 to 9.28)		–11.22 (–18.31 to –4.12)	4.34 (–1.87 to 10.56)	
5.5-124.0	–6.90 (–14.32 to 0.52)	1.10 (–4.88 to 7.09)		–7.37 (–16.16 to 1.42)	–0.63 (–8.12 to 6.86)	
P trend	.03	.58		.03	.77	
∑DBP, µmol/L						
0.002-0.056	Ref	Ref	.17	Ref	Ref	.60
0.06-0.1	3.69 (–1.70 to 9.07)	–1.46 (–6.31 to 3.39)		1.61 (–4.85 to 8.06)	–1.55 (–7.62 to 4.52)	
0.1-0.2	–1.71 (–7.58 to 4.15)	–1.12 (–6.41 to 4.16)		–1.53 (–8.56 to 5.49)	0.90 (–5.69 to 7.49)	
0.2-28.2	1.18 (–6.22 to 8.59)	–5.40 (–11.60 to 0.80)		–2.93 (–11.76 to 5.90)	–0.56 (–8.27 to 7.14)	
P trend	.85	.13		.45	.95	
∑DiBP, µmol/L						
0.002-0.006	Ref	Ref	.01	Ref	Ref	.12
0.006-0.01	–2.88 (–8.22 to 2.46)	5.37 (0.56 to 10.19)		–1.24 (–7.65 to 5.17)	–1.44 (–7.43 to 4.55)	

Table 3. Continued

Phthalate biomarker	Total hip BMD, 1000 × g/cm^2			Femoral neck BMD, 1000 × g/cm^2		
	No HT[a] use N = 457	HT[a] use N = 772		No HT[a] use N = 457	HT[a] use N = 772	
	β (95% CI)[b]	β (95% CI)[b]	*P* interaction	β (95% CI)[b]	β (95% CI)[b]	*P* interaction
0.01-0.03	−3.57 (−9.62 to 2.48)	5.53 (0.09 to 10.97)		−2.83 (−10.06 to 4.41)	2.61 (−4.12 to 9.34)	
0.03-4.0	−11.09 (−18.69 to −3.49)	4.77 (−1.81 to 11.35)		−5.48 (−14.53 to 3.56)	5.19 (−2.91 to 13.30)	
P trend	.01	.15		.24	.16	
∑DEHP, µmol/L						
0.08-0.1	Ref	Ref	.49	Ref	Ref	.74
0.1-0.2	−4.83 (−10.56 to 0.90)	−0.68 (−5.42 to 4.06)		−2.88 (−9.77 to 4.02)	0.37 (−5.58 to 6.31)	
0.2-0.3	−0.52 (−6.64 to 5.59)	2.81 (−2.24 to 7.86)		−2.11 (−9.46 to 5.24)	2.13 (−4.20 to 8.47)	
0.3-68.5	0.10 (−6.58 to 6.78)	−1.80 (−7.59 to 3.98)		−0.47 (−8.50 to 7.55)	2.04 (−5.20 to 9.28)	
P trend	.70	.89		.98	.49	

Abbreviations: BMD, bone mineral density; DBP, di-n-butyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DiBP, di-isobutyl phthalate; HT, hormone therapy; MBzP, monobenzyl phthalate; MCNP, monocarboxyisononyl phthalate; MCOP, monocarboxyisooctyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MEP, monoethyl phthalate; Ref, reference.

[a]HT use includes both reported medication use as well as HT intervention assignment, where appropriate. "Never" is defined as no reported HT use at baseline and also no HT use at year 3; "ever" includes reported past or current HT use at baseline and/or year 3.

[b]Adjusted for creatinine, age, race/site, smoking status, and body mass index; scaled by a factor of 1000.

Table 4. Longitudinal associations between phthalate biomarkers and 3-year percentage change bone mineral density measures, stratified by hormone therapy use, N = 1154

Phthalate biomarker	Total hip BMD, g/cm^2			Femoral neck BMD, g/cm^2		
	No HT[a] use N = 422	HT[a] use N = 732		No HT[a] use N = 422	HT[a] use N = 732	
	β (95% CI)[b]	β (95% CI)[b]	*P* interaction	β (95% CI)[b]	β (95% CI)[b]	*P* interaction
MEP, ng/mL			.70			.11
0.8-31.8	Ref	Ref		Ref	Ref	
31.9-67.9	0.48 (−0.39 to 1.34)	−0.00 (−0.66 to 0.66)		−1.12 (−2.21 to −0.03)	−0.14 (−0.90 to 0.63)	
68.0-163.0	0.42 (−0.46 to 1.30)	0.45 (−0.26 to 1.17)		−0.38 (−1.49 to 0.74)	−0.33 (−1.16 to 0.50)	
164.0-26 000	0.42 (−0.54 to 1.39)	−0.10 (−0.83 to 0.64)		−0.19 (−1.41 to 1.03)	−0.94 (−1.78 to −0.09)	
P trend	.43	.94		.92	.03	
MBzP, ng/mL			.27			.89
0.2-5.9	Ref	Ref		Ref	Ref	
6.0-11.8	0.14 (−0.74 to 1.02)	−0.55 (−1.22 to 0.13)		−0.98 (−2.10 to 0.13)	−0.75 (−1.53 to 0.03)	
11.9-22.0	0.06 (−0.84 to 0.96)	0.38 (−0.32 to 1.08)		−0.14 (−1.28 to 1.01)	0.18 (−0.63 to 0.99)	
22.1-3590.0	0.28 (−0.74 to 1.31)	0.04 (−0.75 to 0.83)		0.03 (−1.27 to 1.33)	−0.32 (−1.24 to 0.59)	
P trend	.66	.39		.66	.99	
MCOP, ng/mL			.04			
0.1-2.1	Ref	Ref		Ref	Ref	.11
2.2-3.6	−1.33 (−2.16 to −0.49)	−0.25 (−0.93 to 0.43)		−1.53 (−2.60 to −0.46)	−0.22 (−1.00 to 0.57)	

Table 4. Continued

Phthalate biomarker	Total hip BMD, g/cm^2			Femoral neck BMD, g/cm^2		
	No HT[a] use N = 422	HT[a] use N = 732		No HT[a] use N = 422	HT[a] use N = 732	
	β (95% CI)[b]	β (95% CI)[b]	*P* interaction	β (95% CI)[b]	β (95% CI)[b]	*P* interaction
3.7-6.5	–1.01 (–1.90 to –0.11)	0.10 (–0.60 to 0.81)		–0.63 (–1.77 to 0.51)	0.21 (–0.60 to 1.02)	
6.6-270.0	–1.80 (–2.81 to –0.78)	0.17 (–0.60 to 0.94)		–1.43 (–2.73 to –0.14)	0.32 (–0.57 to 1.21)	
P trend	.002	.51		.10	.35	
MCNP, ng/mL			.02			.05
0.1-1.6	Ref	Ref		Ref	Ref	
1.7-2.6	–0.38 (–1.24 to 0.48)	0.08 (–0.59 to 0.75)		–0.98 (–2.08 to 0.11)	0.22 (–0.55 to 0.99)	
2.7-4.7	–0.82 (–1.69 to 0.06)	0.21 (–0.50 to 0.92)		–0.23 (–1.35 to 0.89)	0.31 (–0.50 to 1.13)	
4.8-222.0	–1.84 (–2.80 to –0.89)	0.15 (–0.62 to 0.91)		–1.53 (–2.75 to –0.31)	0.41 (–0.48 to 1.29)	
P trend	0.0002	0.64		0.05	0.36	
MCPP, ng/mL			.38			.14
0.1-1.7	Ref	Ref		Ref	Ref	
1.8-3.0	–0.04 (–0.88 to 0.80)	–0.24 (–0.92 to 0.44)		–0.14 (–1.21 to 0.93)	–0.13 (–0.91 to 0.66)	
3.1-5.4	–0.22 (–1.12 to 0.68)	–0.34 (–1.05 to 0.36)		–0.15 (–1.30 to 0.99)	–0.70 (–1.52 to 0.11)	
5.5-124.0	–0.58 (–1.61 to 0.45)	0.11 (–0.71 to 0.93)		–1.06 (–2.36 to 0.25)	0.04 (–0.91 to 0.99)	
P trend	0.27	0.96		0.16	0.66	
∑DBP, µmol/L			.007			.08
0.002-0.056	Ref	Ref		Ref	Ref	
0.06-0.1	–0.72 (–1.57 to 0.12)	0.28 (–0.41 to 0.96)		–0.01 (–1.09 to 1.07)	0.46 (–0.33 to 1.24)	
0.1-0.2	0.40 (–0.48 to 1.28)	–0.07 (–0.78 to 0.65)		0.26 (–0.87 to 1.39)	–0.67 (–1.50 to 0.16)	
0.2-28.2	–0.43 (–1.47 to 0.61)	0.79 (0.01 to 1.58)		–0.18 (–1.50 to 1.15)	0.42 (–0.49 to 1.32)	
P trend	.98	.12		.97	.97	
∑DiBP, µmol/L			.59			.58
0.002-0.006	Ref	Ref		Ref	Ref	
0.006-0.01	0.16 (–0.68 to 1.01)	–0.54 (–1.22 to 0.13)		0.49 (–0.58 to 1.57)	–0.10 (–0.88 to 0.68)	
0.01-0.03	0.37 (–0.52 to 1.25)	–0.31 (–1.02 to 0.40)		0.92 (–0.20 to 2.05)	–0.22 (–1.04 to 0.60)	
0.03-4.0	–0.06 (–1.08 to 0.96)	v0.45 (–1.24 to 0.34)		0.33 (–0.96 to 1.62)	–0.61 (–1.52 to 0.30)	
P trend	.90	.37		.40	.20	
∑DEHP, µmol/L			.14			.21
0.08-0.1	Ref	Ref		Ref	Ref	
0.1-0.2	–0.73 (–1.59 to 0.13)	0.35 (–0.31 to 1.02)		–0.98 (–2.07 to 0.12)	0.28 (–0.49 to 1.05)	
0.2-0.3	–0.98 (–1.88 to –0.07)	0.07 (–0.64 to 0.79)		–0.98 (–2.13 to 0.17)	–0.18 (–1.01 to 0.64)	
0.3-68.5	–0.89 (–1.90 to 0.11)	0.31 (–0.48 to 1.11)		–0.50 (–1.78 to 0.78)	–0.19 (–1.11 to 0.72)	
P trend	.06	.61		.42	.50	

Abbreviations: BMD, bone mineral density; DBP, di-n-butyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DiBP, di-isobutyl phthalate; HT, hormone therapy; MBzP, monobenzyl phthalate; MCNP, monocarboxyisononyl phthalate; MCOP, monocarboxyisooctyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MEP, monoethyl phthalate; Ref, reference.

[a]HT use includes both reported medication use as well as HT intervention assignment, where appropriate, during the 3-year interval.

[b]Adjusted for creatinine, age, race/site, calcium and vitamin D trial assignment, smoking status, and body mass index; β represents percentage change in BMD measure comparing each quartile to first quartile.

phthalate biomarkers and BMD that are either masked or prevented by HT use.

To our knowledge, ours is the first prospective evaluation of urinary phthalate biomarkers in relation to BMD. Additional studies are warranted to either confirm or refute our findings.

If confirmed, reduction of phthalate exposure, through personal choices (perhaps especially reduced intake of ultraprocessed foods) and/or public health policy changes (including broader advocacy and legislative efforts), may be relevant for maintaining bone health.

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