

Urinary Phthalate Metabolites Are Associated With Decreased Serum Testosterone in Men, Women, and Children From NHANES 2011–2012

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Context: There is evidence of declining trends in T levels among men in recent decades, as well as trends in related conditions at multiple life stages and in both sexes. There is also animal and limited human evidence that exposure to phthalates, chemicals found in plastics and personal care products, is associated with reduced androgen levels and associated disorders.

Objective: To explore relationships between urinary concentrations of 13 phthalate metabolites and serum total T levels among men, women, and children when adjusting for important confounders and stratifying by sex and age (6–12, 12–20, 20–40, 40–60, and 60–80 y).

Design: A cross-sectional study.

Setting: US National Health and Nutrition Examination Survey, 2011–2012.

Patients or Other Participants: US general population.

Interventions: None

Main Outcome Measures: Serum total T measured by isotope dilution-liquid chromatography-tandem mass spectrometry.

Results: Multiple phthalates were associated with significantly reduced T in both sexes and in differing age groups. In females, the strongest and most consistent inverse relationships were found among women ages 40–60 years. In boys 6–12 years old, an interquartile range increase in metabolites of di-2-ethylhexyl phthalate was associated with a 29% (95% confidence interval, 6, 47) reduction in T. In adult men, the only significant or suggestive inverse associations between phthalates (metabolites of di-2-ethylhexyl phthalate and dibutyl phthalate) and T were observed among men ages 40–60 years.

Conclusions: Because T plays an important role in all life stages for both sexes, future efforts should focus on better defining these relationships and their broader impacts. (*J Clin Endocrinol Metab* 99: 4346–4352, 2014)

There have been recent reports of declining trends in T levels among men over the last half-century or so (1–4), as well as trends in related conditions encompassing multiple life stages such as reduced semen quality in men

(5–8), increased genital malformations in male newborns (9), and changes in the timing of puberty in both boys and girls (10, 11). Meanwhile, there is growing evidence that exposure to phthalates is associated with reduced T levels

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Abbreviations: BMI, body mass index; CI, confidence interval; DEHP, di-2-ethylhexyl phthalate; IQR, interquartile range; MBP, mono-n-butyl phthalate; MBzP, mono-benzyl phthalate; MCNP, mono-(carboxynonyl) phthalate; MCOP, mono-(carboxyoctyl) phthalate; MCP, 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, mono-ethyl phthalate; MiBP, mono-isobutyl phthalate; MiNP, mono-isononyl phthalate; MnMP, mono-n-methyl phthalate; PIR, poverty income ratio.

and associated disorders in human populations (12–14). Phthalates are a class of chemicals used in flexible polyvinyl chloride plastics, personal care products, and other applications; their widespread use results in ubiquitous population exposures, as demonstrated by 100% detection of urinary phthalate metabolites in population studies (15). Several phthalates are antiandrogenic in rats, but human studies remain limited (16). Biomarkers of exposure to di-2-ethylhexyl phthalate (DEHP) have been associated with decreased T levels among adult men in several studies (17–23). Preliminary studies of 8- to 14-year-old boys (24), pregnant women (25), and newborns with in utero exposure (26) have also shown evidence for these relationships.

In addition to being few in number, previous studies of the relationship between phthalate exposure and T have been limited by small sample sizes, and/or have typically been conducted among unique subpopulations. In the present study, we tested among a more general population whether urinary phthalate metabolites were associated with newly released data on serum T levels in the National Health and Nutrition Examination Survey (NHANES) from years 2011–2012. Because T has important functions at each life stage and in both males and females, we conducted our analysis among all NHANES participants who contributed data for phthalate biomarkers and T levels, stratified by age and sex.

Subjects and Methods

Study population

NHANES is an ongoing study that combines demographic, interview, examination, questionnaire, and laboratory data to assess health and nutrition status of the general US population. Subjects from minority groups are oversampled to enable accurate assessments of associations within these groups when data are properly analyzed with survey weights (27). In the 2011–2012 cycle, 13 431 subjects were screened, 9756 were interviewed (72.6% response rate), and 9338 were examined (69% response rate) (28). Response rates were relatively consistent across age and gender groups (approximately 60–80%) but were slightly lower in subjects 70–80 years of age in both males and females (approximately 45–55%) (28). A subset of participants, selected during screening based on age and gender, additionally provided biological specimens at a mobile examination center. Blood samples were taken from subjects over age 1, and urine samples were additionally collected from subjects over age 6 at the same visit. Testosterone was measured in all serum samples collected in 2011–2012, and phthalate metabolites were measured on a subset of urine samples selected strategically to avoid bias. Only participants with both T and phthalate metabolite levels were included in the present analysis ($n = 2208$). NHANES received approval from the National Center for Health Statistics Ethics Review Board, and informed consent was

obtained for all adult participants and from parents of participants under the age of 18.

Urinary phthalate metabolite analysis

Urine samples were analyzed for a panel of 13 urinary phthalate metabolites, including: 1) mono-(2-ethyl)-hexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-2-ethyl-5-carboxypentyl phthalate (MECPP), all metabolites of DEHP; 2) mono-benzyl phthalate (MBzP), a metabolite of benzylbutyl phthalate; mono-n-butyl phthalate (MBP) and mono-isobutyl phthalate (MiBP), metabolites of dibutyl phthalates; 3) mono-ethyl phthalate (MEP), a metabolite of di-ethyl phthalate; 4) mono-(3-carboxypropyl) phthalate (MCPP), a metabolite of di-n-octyl phthalate; 5) mono-(carboxynonyl) phthalate (MCNP), a metabolite of di-isodecyl phthalate; 6) mono-(carboxyoctyl) phthalate (MCOP) and mono-isononyl phthalate (MiNP), metabolites of di-isononyl phthalate; and 7) mono-n-methyl phthalate (MnMP), a metabolite of di-methyl phthalate (29). Analysis was performed by the National Center for Environmental Health, Centers for Disease Control and Prevention (CDC), with appropriate quality assurance/quality control procedures. Metabolites were measured in urine using on-line solid-phase extraction, coupled with isotope dilution-HPLC-electrospray ionization-tandem mass spectrometry, which has been described in detail previously (30). Detection limits were between 0.2 and 0.6 ng/mL, and concentrations below the detection limits were replaced with the limit of detection divided by the square root of 2 (31). To adjust for urinary dilution, creatinine concentrations were measured in all samples using an enzymatic assay. For examining population distributions, urinary phthalate metabolite levels were corrected for urinary creatinine by dividing urinary phthalate concentration by creatinine*0.01 for final units of micrograms per gram creatinine.

In addition to individual urinary phthalate metabolites, we examined associations with a summed measure of DEHP metabolites (Σ DEHP), including MEHP, MEHHP, MEOHP, and MECPP. The sum was created based on nanomolar concentrations of each metabolite using the following formula: Σ DEHP = (MEHP/278) + (MEHHP/294) + (MEOHP/292) + (MECPP/308). Final Σ DEHP concentrations were in micromoles per liter.

Serum T analysis

Total T levels were measured in serum using isotope dilution liquid chromatography tandem mass spectrometry by the National Center for Environmental Health, Centers for Disease Control and Prevention, as described elsewhere (32).

Statistical analysis

Data were analyzed using R version 3.1.0. Population distributions of categorical covariates and quantiles of continuous covariates were examined using the survey package to adjust for the complex NHANES sampling design (27, 33). Likewise, the survey package was used to calculate quantiles of creatinine-corrected urinary phthalate metabolite and serum T concentrations so that distributions would be nationally representative. Distributions were examined by gender and age categories (6–12, 12–20, 20–40, 40–60, and 60–80 y) because T levels differ distinctly between these groups. Linear regression analysis was performed to examine the associations between individual urinary phthalate metabolites and serum T within each age group

by sex. Subsetting into smaller groups reduced the number of strata in each analysis such that adjusting for survey methodology was not possible. However, because analyses are adjusted for variables considered in oversampling procedures, including age, race/ethnicity, and income, we expect our results to be generalizable. Crude regression models were created, adjusting for urinary creatinine only, and full models additionally included age (continuous), race/ethnicity (categorical), poverty income ratio (PIR), body mass index (BMI), and time of day of sample collection (morning, afternoon, or evening). In models for subjects ages 6–20, BMI was included as a categorical variable based on cutoffs established by CDC sex-specific 2000 BMI-for-age growth charts for the United States. In models for subjects >20 years old, BMI was included as a continuous variable. Beta estimates from logistic regression analysis were converted to percentage increase in T in association with an interquartile range (IQR) increase in urinary phthalate metabolite concentration for interpretability.

Results

In the 2011–2012 NHANES cycle, urinary phthalate metabolites and serum T were measured in 2208 participants. Population characteristics of this subset are presented in Table 1. All urinary phthalate metabolites were detected in >99% of samples measured, except for MEHP (76.3%),

Table 1. Population Characteristics in NHANES Participants With Phthalate and T Data 2011–2012 (n = 2208)

	n (Weighted %)	Weighted Median (25th, 75th Percentile)
Age group, y		41 (23, 56)
6–12	293 (6.63)	
12 to <20	351 (12.0)	
20 to <40	572 (29.5)	
40 to <60	507 (31.6)	
60–80	485 (20.2)	
Sex		
Female	1098 (51.3)	
Male	1110 (48.7)	
Race/ethnicity		
Mexican American	277 (8.82)	
Other Hispanic	232 (7.14)	
Non-Hispanic white	742 (64.6)	
Non-Hispanic black	573 (12.2)	
Other race/multiracial	384 (7.20)	
PIR continuous (no units)		2.72 (1.26, 4.76)
BMI continuous, kg/m ²		26.5 (22.8, 31)
Child/adolescent BMI categories ^a		
Underweight	18 (2.86)	
Normal weight	370 (57.8)	
Overweight	109 (17.7)	
Obese	141 (21.6)	

^a BMI categories for children/adolescents only based on CDC sex-specific 2000 BMI-for-age growth charts for the United States.

MBP (94.3%), MiNP (59.1%), and MnMP (63.5%). Distributions of urinary phthalate metabolites and serum T by sex and age categories are presented in Table 2. In males, all metabolites except for MEP and MiNP were highest in children (ages 6–12), but we observed few differences across other age groups. In females, MEHHP, MEOHP, MECPP, MBzP, MBP, and MiBP were higher in children, but otherwise were rather consistent across age groups. As expected, serum T concentrations were much higher in males compared to females and in males >12 years old compared to male children. Distributions of all urinary phthalate metabolites and serum T were log-normally distributed and ln-transformed for regression analyses. For metabolites of DEHP, we focused on the results from models of Σ DEHP; results for the individual DEHP metabolites are available in Supplemental Tables 1 and 2.

In females, most of the urinary phthalate metabolites were associated with decreased serum T concentrations in at least one age group. Some effect estimates were stronger, particularly among children, in models adjusted for urinary creatinine only (Supplemental Table 3) compared to models additionally adjusting for age, race/ethnicity, PIR, BMI, and session of sample collection (Table 3). Associations were generally strongest in females aged 40–60. Among this age group, IQR increases in all phthalate metabolites (except for MCNP and MCOP) were associated with 10.8 to 24.0% decreases in serum T concentrations. For example, an IQR increase in Σ DEHP was associated with a 20.1% decrease in T. In children (ages 6–12), associations were generally null except for a significant inverse relationship with MCNP and MCOP (percentage change with IQR increase of MCNP = -34.9 ; 95% confidence interval [CI], $-47.1, -19.8$). Further age stratification in an attempt to separate prepubertal and pubertal girls showed consistent negative effect estimates between groups for these phthalates (Supplemental Table 4). In females ages 12–20, associations were negative for all metabolites but only statistically significant for MBzP and MCPP (Table 3).

In males, the associations between urinary phthalate metabolites and serum T concentrations were also mostly inverse. As with models in females, effect estimates from full models (Table 4) were somewhat attenuated compared to those observed in crude models (Supplemental Table 5). In boys (age, 6–12 y), IQR increases in DEHP metabolites were significantly associated with 24.0 to 34.1% decreases in serum T concentrations. MBzP, MBP, MiBP, MCNP, MCOP, and MiNP were also inversely associated with T, but effect estimates were imprecise ($P > .05$). Further age stratification suggested that negative effect estimates were further from the null among boys 10–12 years of age compared to boys 6–9 years old for

Table 2. Creatinine Corrected Urinary Phthalate Metabolite (ng/mL) and Serum T (ng/dL) Medians (25th, 75th percentiles) by Age and Sex Categories in Weighted NHANES Data 2011–2012

	MEHP	MEHHP	MEOHP	MCCPP	MBzP	MBP	MiBP
Male							
6 to <12	2.10 (1.16, 2.93)	14.0 (9.06, 24.4)	9.80 (6.45, 15.3)	24.6 (17.9, 34.8)	11.9 (6.49, 19.2)	16.3 (8.17, 24.3)	11.1 (5.92, 16.7)
12 to <20	1.50 (0.73, 2.79)	7.81 (4.83, 11.9)	4.92 (3.04, 7.41)	12.2 (7.97, 21.6)	5.78 (3.55, 11.3)	8.88 (4.95, 14.3)	7.28 (4.26, 11.4)
20 to <40	1.81 (0.97, 3.08)	7.19 (4.85, 11.3)	4.15 (2.77, 7.06)	11.1 (6.95, 17.7)	3.67 (2.20, 6.74)	6.96 (3.92, 11.2)	5.12 (3.46, 9.13)
40 to <60	1.22 (0.68, 1.94)	7.03 (4.58, 11.4)	4.33 (2.89, 5.92)	11.6 (7.65, 15.9)	3.55 (1.87, 6.05)	7.01 (3.91, 11.2)	5.15 (3.39, 7.84)
60 to 80	1.08 (0.58, 2.09)	7.76 (5.08, 11.8)	5.10 (3.42, 7.92)	12.8 (8.45, 19.7)	4.13 (2.44, 7.32)	9.92 (4.89, 15.8)	6.02 (3.25, 10.6)
Female							
6 to <12	1.84 (1.06, 3.18)	14.8 (8.77, 26.4)	10.0 (6.00, 16.4)	26.1 (16.7, 41.0)	12.4 (6.53, 25.8)	17.6 (10.7, 29.9)	13.1 (9.05, 20.6)
12 to <20	1.47 (0.92, 2.99)	7.55 (5.12, 14.9)	5.60 (3.81, 9.71)	14.4 (9.88, 23.1)	7.00 (4.00, 13.0)	12.4 (6.29, 20.7)	8.09 (5.27, 13.1)
20 to <40	1.94 (1.07, 3.57)	8.17 (5.44, 14.6)	5.76 (3.62, 10.0)	14.3 (9.06, 21.7)	5.36 (2.78, 9.73)	8.99 (4.65, 15.6)	7.14 (4.56, 11.6)
40 to <60	1.49 (0.90, 2.90)	11.8 (5.82, 15.4)	6.73 (3.73, 10.0)	16.1 (10.4, 23.9)	5.34 (3.09, 9.91)	9.89 (6.06, 18.6)	7.63 (4.81, 13.4)
60 to 80	1.04 (0.70, 1.94)	9.05 (5.27, 13.7)	6.20 (3.41, 8.38)	15.1 (9.98, 23.9)	4.00 (2.82, 7.89)	8.70 (5.24, 15.9)	5.58 (4.02, 10.2)
	MEP	MCCP	MCNP	MCOP	MiNP	MnMP	T
Male							
6 to <12	25.4 (16.1, 48.6)	4.32 (2.68, 8.51)	3.48 (2.10, 5.63)	24.9 (13.1, 45.5)	1.24 (0.69, 2.53)	2.86 (1.21, 7.88)	3.43 (2.06, 5.10)
12 to <20	25.9 (14.0, 46.9)	1.77 (1.14, 4.11)	2.37 (1.59, 4.09)	14.0 (7.76, 43.1)	0.79 (0.43, 2.00)	1.03 (0.54, 2.05)	380 (206, 485)
20 to <40	27.0 (12.2, 74.7)	2.66 (1.46, 6.37)	2.41 (1.60, 4.95)	23.1 (9.28, 57.0)	1.33 (0.48, 3.15)	1.19 (0.55, 2.67)	412 (325, 497)
40 to <60	21.3 (11.9, 67.7)	2.67 (1.30, 5.24)	2.22 (1.23, 4.25)	20.4 (7.23, 58.3)	0.96 (0.40, 2.85)	1.02 (0.51, 2.02)	354 (265, 478)
60 to 80	55.1 (25.1, 146)	2.47 (1.38, 5.41)	2.02 (1.41, 3.78)	14.5 (7.18, 39.3)	0.72 (0.35, 2.11)	1.05 (0.36, 2.82)	371 (271, 506)
Female							
6 to <12	30.1 (16.7, 60.9)	3.73 (2.76, 6.13)	2.95 (2.13, 5.00)	19.1 (13.6, 32.8)	1.13 (0.66, 2.29)	3.40 (1.09, 6.43)	5.91 (2.82, 13.6)
12 to <20	35.1 (18.4, 100)	2.73 (1.77, 6.64)	2.39 (1.45, 5.65)	19.9 (10.8, 52.8)	1.59 (0.64, 3.99)	1.49 (0.83, 4.05)	21.8 (15.7, 30.0)
20 to <40	32.1 (17.2, 89.8)	2.75 (1.59, 6.13)	2.42 (1.52, 4.35)	21.9 (10.0, 60.0)	1.38 (0.65, 3.92)	1.31 (0.65, 2.86)	25.7 (17.4, 38.2)
40 to <60	54.0 (22.9, 124)	2.97 (1.65, 5.94)	3.01 (1.65, 5.58)	23.0 (8.89, 52.2)	1.29 (0.60, 2.74)	1.41 (0.56, 2.71)	18.6 (13.2, 26.3)
60 to 80	49.8 (24.3, 124)	3.05 (1.67, 6.27)	2.73 (1.77, 4.61)	17.4 (6.76, 64.7)	0.83 (0.41, 2.31)	1.08 (0.56, 2.03)	13.9 (9.74, 23.0)

DEHP metabolites and MBzP (Supplemental Table 4). In males ages 12–20, effect estimates were negative for all phthalate metabolites except MEP, but were weaker in magnitude and were generally not statistically significant except for MCOP (Table 3). Within the 20–40 and 60- to 80-year age groups, associations were generally inverse but were not statistically significant. However, in the 40- to 60-year age group, associations were somewhat stronger. An IQR increase in urinary Σ DEHP metabolite and MBP concentrations was associated with 7.84% (95% CI, –15.8, 0.85) and 12.9% (95% CI, –20.3, –4.87) decreases in serum T, respectively.

Discussion

We found numerous significant inverse associations between multiple phthalates and serum total T in various age

and sex strata among NHANES 2011–2012 participants. This study serves to confirm some previously reported associations, while improving upon limitations of previous studies such as having access to a large sample size and the inclusion of a more general population of women, children, and older adults in our analysis.

The impact of phthalates on endocrine function in females has been less studied compared to males. In this study, we observed a significant inverse relationship between several phthalate metabolites (MBzP, MCCP, MCNP, MCOP), but not DEHP metabolites, among girls 6–12 or 12–20 years old. In a large study of 725 healthy Danish girls aged 5–19 years, Frederiksen et al (34) reported no associations between MBP, MBzP, or metabolites of DEHP and di-iso-nonyl phthalate in relation to circulating T levels. However, a 5-year longitudinal study

Table 3. Urinary Phthalate Metabolite Associations With T in Females, NHANES 2011–2012 (Adjusted)

	Ages 6–12 (n = 141)		Ages 12–20 (n = 155)		Ages 20–40 (n = 262)		Ages 40–60 (n = 230)		Ages 60–80 (n = 204)	
	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P
Σ DEHP	–4.27 (–22.7, 18.5)	.689	–6.14 (–18.9, 8.68)	.399	–1.27 (–11.5, 10.1)	.818	–20.1 (–30.9, –7.72)	.003	–2.24 (–21.0, 20.9)	.835
MBzP	18.7 (–5.13, 48.5)	.136	–19.4 (–31.9, –4.61)	.013	3.34 (–10.0, 18.7)	.642	–24.0 (–35.0, –11.2)	.001	10.3 (–10.9, 36.6)	.368
MBP	–7.27 (–23.5, 12.4)	.444	–13.3 (–28.0, 4.36)	.134	6.18 (–5.72, 19.6)	.323	–22.3 (–31.4, –12.0)	<.001	–0.39 (–15.9, 18.0)	.964
MiBP	4.65 (–15.2, 29.2)	.673	–13.5 (–26.2, 1.40)	.076	2.18 (–10.8, 17.1)	.756	–22.8 (–32.4, –11.8)	<.001	–1.40 (–18.2, 18.8)	.883
MEP	11.6 (–10.4, 39.1)	.329	–5.45 (–18.0, 8.97)	.440	0.31 (–9.95, 11.8)	.955	–10.8 (–19.5, –1.20)	.029	5.26 (–9.18, 22.0)	.497
MCCP	–4.92 (–19.6, 12.4)	.556	–11.9 (–21.6, –1.03)	.035	–4.12 (–12.1, 4.58)	.344	–13.5 (–21.9, –4.31)	.005	–14.0 (–24.1, –2.58)	.019
MCNP	–34.9 (–47.1, –19.8)	<.001	–1.67 (–14.1, 12.5)	.807	–11.2 (–20.4, –0.91)	.035	–8.28 (–17.7, 2.18)	.118	–1.61 (–14.4, 13.1)	.820
MCOP	–20.8 (–36.3, –1.55)	.038	–7.35 (–19.4, 6.45)	.283	–8.87 (–17.7, 0.91)	.075	–9.09 (–20.1, 3.43)	.149	–11.9 (–23.8, 1.83)	.088
MiNP	–13.1 (–27.9, 4.83)	.145	–10.0 (–19.6, 0.73)	.069	–4.74 (–12.9, 4.22)	.291	–11.0 (–21.0, 0.18)	.055	–4.53 (–20.0, 13.9)	.607
MnMP	–1.02 (–19.4, 21.5)	.922	–2.85 (–18.9, 16.4)	.754	9.63 (–3.77, 24.9)	.168	–13.6 (–25.5, 0.16)	.054	6.31 (–14.7, 32.4)	.586

For ages <20 years, models were adjusted for urinary creatinine, age, PIR, BMI (categories based on CDC sex-specific 2000 BMI-for-age growth charts for US), race/ethnicity, and session of sample collection (morning, afternoon, or evening). For ages >20, models were adjusted for urinary creatinine, age, PIR, BMI, race/ethnicity, and session of sample collection (morning, afternoon, or evening).

Table 4. Urinary Phthalate Metabolite Associations With T in Males, NHANES 2011–2012 (Adjusted)

	Ages 6–12 (n = 134)		Ages 12–20 (n = 160)		Ages 20–40 (n = 267)		Ages 40–60 (n = 221)		Ages 60–80 (n = 219)	
	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P	% Change (95% CI)	P
ΣDEHP	−29.3 (−46.8, −6.10)	.018	−8.42 (−24.5, 11.0)	.372	0.42 (−5.84, 7.09)	.899	−7.84 (−15.8, 0.85)	.077	−4.86 (−17.0, 9.01)	.474
MBzP	−18.5 (−40.7, 12.0)	.210	−21.1 (−38.6, 1.30)	.065	−4.92 (−12.9, 3.82)	.262	−2.02 (−10.9, 7.77)	.675	1.54 (−12.7, 18.2)	.843
MBP	−12.9 (−36.9, 20.2)	.401	−19.3 (−37.0, 3.34)	.091	−3.85 (−11.0, 3.89)	.321	−12.9 (−20.3, −4.87)	.002	9.99 (−5.75, 28.4)	.228
MiBP	−12.4 (−31.2, 11.7)	.289	−19.9 (−39.4, 5.83)	.121	0.39 (−6.26, 7.52)	.911	−6.17 (−13.6, 1.96)	.134	0.57 (−12.6, 15.7)	.937
MEP	27.5 (−14.8, 90.9)	.240	9.46 (−17.1, 44.5)	.525	−3.30 (−9.81, 3.67)	.345	3.07 (−4.84, 11.6)	.459	13.4 (−1.22, 30.1)	.076
MCPP	2.92 (−23.0, 37.6)	.846	−8.13 (−22.2, 8.50)	.319	−2.81 (−8.14, 2.82)	.322	1.77 (−5.14, 9.19)	.625	−4.30 (−15.5, 8.35)	.488
MCNP	−9.02 (−31.3, 20.4)	.510	−18.8 (−34.6, 0.93)	.063	0.97 (−5.61, 8.01)	.779	−1.99 (−9.62, 6.28)	.627	2.04 (−12.1, 18.4)	.791
MCOP	−7.83 (−32.3, 25.5)	.605	−22.5 (−36.7, −5.18)	.014	−0.33 (−7.09, 6.92)	.926	7.40 (−0.83, 16.3)	.081	−3.22 (−16.8, 12.5)	.671
MiNP	−11.9 (−34.9, 19.1)	.412	−10.3 (−26.2, 9.03)	.277	−0.38 (−6.98, 6.69)	.914	4.82 (−4.07, 14.5)	.299	−3.94 (−18.2, 12.81)	.625
MnMP	15.7 (−14.0, 55.6)	.337	−1.35 (−21.9, 24.6)	.909	−3.00 (−11.8, 6.69)	.531	3.63 (−6.66, 15.1)	.505	−11.1 (−25.5, 6.11)	.194

For ages <20 years, models were adjusted for urinary creatinine, age, PIR, BMI (categories based on CDC sex-specific 2000 BMI-for-age growth charts for United States), race/ethnicity, and session of sample collection (morning, afternoon, or evening). For ages >20, models were adjusted for urinary creatinine, age, PIR, BMI, race/ethnicity, and session of sample collection (morning, afternoon, or evening).

among a subset of these children who were between the ages of 6 and 13 years at enrollment reported significantly lower T and dehydroepiandrosterone sulfate in relation to the sum of urinary MBP and MiBP at 13 years of age (35), which was consistent with our crude results (Supplemental Table 3). Among adult women 40 to 60 years of age we found significant inverse associations with most phthalate metabolites. Conversely, few associations were observed among women ages 20–40 or 60–80. The only other study of phthalates and T among women that we are aware of, which was conducted among 180 pregnant women in the United States between 1999 and 2002, found significant inverse relationships between DEHP metabolites and T among all women, and between MBP and T among women carrying female fetuses (25). The novel findings presented here for reduced T among women may be of high public health significance because androgen deficiency among women may impair sexual function, libido, energy, cognitive functions, bone density, cardiovascular function, and overall well-being (36).

Among males, our finding for an inverse association between DEHP metabolites and T among young boys 6–12 years of age is consistent with our recent study in Mexico among 113 boys aged 8–14, where we found suggestive inverse relationships between DEHP metabolites (as well as MBzP and MiBP) and both total and free T (24). The longitudinal study of Danish children described earlier reported that MBzP and metabolites of DEHP and di-iso-nonyl phthalate were associated with lower T levels in the boys at age 13 (35). The lack of association between phthalates and T among adolescent boys (ages 12–20) in the present study may be due to the widely varying levels of T in this age group and our inability to account for pubertal development in our analysis.

For men in the present study, the only evidence for significant or suggestive inverse associations between phthalates (DEHP metabolites and MBP) and T were observed among men ages 40–60. These results among men

in this age group may have important public health implications because declined T levels in aging men can have adverse effects on muscle strength, energy, bone mass, leanness, intellectual capacity, and libido, and may be associated with increased risk of osteoporosis, obesity, type 2 diabetes, metabolic syndrome, cardiovascular disease, and erectile dysfunction (2, 37). Most studies to date on phthalates and T have been conducted among men of reproductive age. Significant declines in total T, free T, and/or free androgen index in relation to DEHP metabolites, but not other phthalates (eg, MBzP, MBP, or MEP), have been reported among men attending infertility clinics in the United States (17) and Poland (18), men with proven fertility in three European countries (23) and the United States (20), and a pooled analysis combining data from the two US studies (21). Both MEHP and MBP were negatively correlated with free T levels among men occupationally exposed to phthalates in China (19, 38). Significantly reduced T was also associated with both DEHP and dibutyl phthalate measured in semen in a study of men attending an infertility clinic in India (22). On the other hand, several relatively small studies did not report significant relationships between biomarkers of phthalate exposure and T in men, although most did report negative effect estimates (39–41).

To our knowledge concentrations of urinary phthalate metabolites measured in this 2-year cycle of NHANES (2011–2012) have not been reported elsewhere to date. When comparing data from this analysis to phthalate metabolite data from previous cycles in general, concentrations from this more recent cycle were lower than early cycles for DEHP metabolites, MBP, and MBzP, but higher for MiBP and MCOP (27). This is consistent with a recent report of phthalate metabolite trends in NHANES data from 2001 through 2010 and may reflect recent changes in phthalate usage and product formulations (42).

Strengths of this analysis include sensitive, isotope dilution liquid chromatography tandem mass spectrometry-

based measurement of urinary phthalate metabolites as well as total T. This resulted in much greater detection rates of T in women and children compared to immunoassay-based methods, as well as much greater overall data accuracy and precision (43, 44). All previous studies of phthalates in relation to T have utilized immunoassay-based methods and thus may have underestimated effect estimates or failed to detect true associations due to random measurement errors. The present analysis also involved a much broader population compared to previous studies and a large sample size. However, sample size became more limited when stratifying by age and sex.

Some weaknesses of this study include the cross-sectional nature of the data, which restricts interpretation of causality, and unmeasured or residual confounding (eg, diet, medications) and reverse-causation cannot be ruled out as potential alternative explanations of our findings. In addition, the study design does not allow for investigation of the effects of early life exposures on later development and health, which is of concern for endocrine-disrupting chemicals such as phthalates. However, recent research has demonstrated an example where phthalates may reduce T production in human adult testis (45) but not during the fetal stage (46). Also, phthalates are metabolized rapidly, and urinary concentrations can fluctuate within-individual over time, which likely leads to substantial measurement error (13), although we would expect this error to be random with respect to T levels, which would bias our results toward the null. Although the method used to measure total T was a strength of the study, we did not have data on free (bioavailable) T, SHBG, and other hormones or markers that may provide clues into mechanisms and/or sites of action for phthalate antiandrogenic effects. However, the patterns of the observed associations with total T presented here by age group and by sex may have some utility in this regard. Finally, due to the exploratory nature of our analysis, particularly among women and children, many statistical comparisons were made. Thus, we cannot rule out the possibility that some of the statistically significant results reported here were due to chance.

In conclusion, multiple phthalates were associated with significantly reduced levels of circulating T in both males and females in differing age groups. Because T plays an important role in all life stages for both sexes, future efforts should focus on better defining and possibly intervening to reduce the impacts of these relationships.

Acknowledgments

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