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Urinary Phthalate and Phthalate Alternative Metabolites and Isoprostane among Couples Undergoing Fertility Treatment

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Abstract

Background—Epidemiological data suggest associations between phthalate exposures to a variety of adverse reproductive outcomes including reduced sperm quality and reproductive success. While mechanisms of these associations are not fully elucidated, oxidative stress has been implicated as a potential mediator. We examined associations of urinary metabolites of phthalates and phthalate alternative plasticizers with oxidative stress among couples seeking fertility treatment.

Methods—Seventeen urinary plasticizer metabolites and 15-F2t isoprostane, a biomarker of oxidative stress, were quantified in spot samples from 50 couples seeking fertility treatment who enrolled in the Sperm Environmental Epigenetics and Development Study during 2014–2015.

Results—In multivariable analyses, percent change in isoprostane was positively associated with interquartile range increases for the oxidative metabolites of di-2-ethylhexyl phthalate, [mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP; 20.0%, $p=0.02$), mono-2-ethyl-5-oxohexyl phthalate (MEOHP; 24.1%, $p=0.01$), and mono-2-ethyl-5-carboxypentyl phthalate (MECPP; 24.1%, $p=0.004$)], mono-isobutyl phthalate (MiBP; 17.8%, $p=0.02$), mono-hydroxyisobutyl phthalate (MHiBP; 27.5%, $p=0.003$), and cyclohexane-1,2-dicarboxylic acid mono-hydroxy-isononyl ester (MHINCH; 32.3%, $p=0.002$). Stratification of participants by sex revealed that isoprostane was positively associated with MHiBP (41.4%, $p=0.01$) and monocarboxy-isononyl phthalate (MCNP;

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26.0%, $p=0.02$) among females and MEOHP (35.8%, $p=0.03$), MiBP (29.2%, $p=0.01$), MHBP (34.7%, $p=0.007$) and MHINCH (49.0%, $p=0.002$) among males.

Conclusions—Our results suggest that exposure to phthalates and phthalate replacements are associated with higher levels of oxidative stress in a sex-specific manner. Additional studies are needed to replicate our findings and to examine the potential health implications of the use of phthalates and alternative phthalates in consumer end products.

Keywords

phthalates; endocrine disruptors; EDC; isoprostane; oxidative stress

Introduction

Phthalate diesters are a class of high-volume production synthetic organic chemicals used in industrial and consumer products. The classification of phthalate diesters can be divided by their carbon backbone alkyl chain into high molecular weight (HMW) and low molecular weight (LMW). HMW phthalates are used as plasticizers of polyvinyl chloride (PVC), which is manufactured in many consumer end products including medical equipment, food packaging, and building materials such as flooring and wallboard (Jurewicz and Hanke, 2011). LMW phthalates are typically included in personal care products, solvents, fixatives, or alcohol denaturants (Lyche et al., 2009; Meeker, 2012). Phthalates are not covalently bonded to products and are therefore easily released into the environment (Meeker et al., 2009). Because of this, human exposure is widespread, such that urinary phthalate metabolites have been detected in the majority of individuals from representative samples in the United States general population (CDC, 2015) and worldwide (Becker et al., 2009; Ha et al., 2014; Polanska et al., 2014).

Epidemiologic data suggest that exposure to some phthalates is adversely associated with a variety of reproductive outcomes including lower oocyte yield and lower proportion of cycles resulting in pregnancy as well as live birth (Hauser et al., 2015), poor sperm quality measures in the general population (Bloom et al., 2015b) as well as those seeking fertility treatment (Duty et al., 2003; Hauser et al., 2006; Wang et al., 2015), and longer time to pregnancy (Buck Louis et al., 2014). The direct mechanisms by which phthalates may induce these adverse reproductive outcomes are not clear but there is growing evidence that oxidative stress may be a contributing factor. Oxidative stress is implicated in adverse reproductive conditions including sperm DNA damage (Gavriliouk and Aitken, 2015), endometriosis (Mier-Cabrera et al., 2011; Sharma et al., 2010), and polycystic ovary syndrome (Palacio et al., 2006). Additionally, recent evidence suggests strong positive associations between urinary phthalate metabolite concentrations and biomarkers of oxidative stress among pregnant women (Ferguson et al., 2014; Ferguson et al., 2015; Holland et al., 2016), couples who were planning pregnancy (Guo et al., 2014), and the general US population (Ferguson et al., 2012). Most recently, oxidative stress was shown to partially mediate the association of phthalate exposure on preterm birth in Puerto Rico (Ferguson et al., 2016).

U.S. biomonitoring data from 2001–2010 have highlighted temporal changes in the profiles of urinary biomarkers of phthalates as the use of alternative phthalates or phthalate substitutes meant to replace those with potential adverse effects on human health has increased (Zota et al., 2014). For example, the most common HMW phthalate plasticizer, di(2-ethylhexyl) phthalate (DEHP), is being replaced with other phthalates (e.g., di-isononyl phthalate (DiNP) and di-isodecyl phthalate (DiDP)), or non-phthalate plasticizers (e.g., di(isononyl)cyclohexane-1,2-dicarboxylate (DINCH®)). Not surprisingly, urinary metabolite concentrations of these phthalate replacements have been reported to be increasing in recent years (CDC, 2015; Zota et al., 2014). Likewise, changes in LMW phthalate exposure profiles have also been observed, whereby use of di(isobutyl) phthalate (DiBP), an alternative to di(n-butyl) phthalate (DBP), also appeared to be on the rise in the past decade (Zota et al., 2014). Despite these changes, limited data are available in regard to the relationships of exposure to alternative phthalates or phthalate substitutes on oxidative stress. To gain a better understanding on the potential influence of current exposure profiles of phthalates on oxidative stress, we conducted a cross-sectional study among couples seeking reproductive assistance to determine whether preconception exposures to these compounds are associated with urinary isoprostane, a known biomarker of oxidative stress.

Methods

Study population

The Sperm Environmental Epigenetics and Developments Study (SEEDS) is a prospective cohort study aimed at investigating the associations of paternal preconception exposures to endocrine disrupting chemicals, such as phthalates, with sperm epigenetics and subsequent early-life development among couples undergoing fertility treatment at Baystate Medical Center located in Springfield, Massachusetts. Since 2014, the SEEDS cohort has been recruiting couples (men and women 18–55 and 18–40 years of age, respectively) who use their own gametes (sperm and oocytes) for *in vitro* fertilization. For the current study, we included data from the first 50 couples who enrolled in SEEDS. Attending physicians explained the study and obtained written consent from eligible males and females interested in participating. This study was approved by the institutional review boards at Baystate Medical Center and the University of Massachusetts Amherst.

Urinary biomarker measurements

Men and women who agreed to participate provided a spot urine sample in a sterile polypropylene collection cup on the same day of semen sample procurement and oocyte retrieval. Urine samples were vortexed, divided into several aliquots and stored at -80°C . Urine samples were shipped overnight on dry ice to the National Center for Environmental Health of the Centers for Disease Control and Prevention (CDC), where quantification of urinary DINCH and phthalate metabolites was conducted via enzymatic deconjugation of the metabolites, solid-phase extraction, separation and detection using high performance liquid chromatography isotope dilution tandem mass spectrometry as described previously (Silva et al., 2013). Analytical standards, quality control (QC) materials prepared from spiked pooled urine, and reagent blank samples were included in each batch along with study samples. The QC concentrations—averaged to obtain one measurement of high-

concentration QC and one of low-concentration QC for each batch—were evaluated by using standard statistical probability rules (Caudill et al., 2008). The coefficient of variations for the phthalate measurement of QC materials ranged from 6.7 % to 11.7 % (low concentration standard) and 5.0 % to 9.3 % (high concentration standard).

In total, seventeen urinary metabolites were quantified: mono(2-ethylhexyl) phthalate (MEHP); mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP); mono(2-ethyl-5-oxohexyl) phthalate (MEOHP); mono(2-ethyl-5-carboxypentyl) phthalate (MECPP); monocarboxyisooctyl phthalate (MCOP); mono-isononyl phthalate (MNP); monobenzyl phthalate (MBzP); mono(3-carboxypropyl) phthalate (MCPP); monocarboxy-isononyl phthalate (MCNP); mono-n-butyl phthalate (MBP); mono-3-hydroxybutyl phthalate (MHBP); mono-isobutyl phthalate (MiBP); mono-hydroxyisobutyl phthalate (MHiBP); monoethyl phthalate (MEP); monomethyl phthalate (MMP); cyclohexane-1,2-dicarboxylic acid-monocarboxy isooctyl ester (MCOCH); and cyclohexane-1,2-dicarboxylic acid-mono(hydroxy-isononyl) ester (MHINCH). We calculated the molar sum of DEHP metabolites (Σ DEHP) by dividing each metabolite concentration by its molecular weight and then summing: $[\text{MEHP} \times (1/278)] + [\text{MEHHP} \times (1/294)] + [\text{MEOHP} \times (1/292)] + [\text{MECPP} \times (1/308)]$. The limits of detection (LODs) varied for each metabolite, ranging from 0.2 to 0.6 ng/mL. Concentrations below the LOD were assigned a value of LOD divided by the square root of 2. Specific gravity (SG) was measured at room temperature using a digital handheld refractometer (Atago Co., Ltd., Tokyo, Japan), which was calibrated prior to use with deionized water. For analyses utilizing SG-corrected metabolite concentrations, the following formula was used: $P_c = P[(1.02-1)/(SG-1)]$ where P_c is the SG-corrected metabolite concentration (ng/mL), P is the observed metabolite concentration, 1.02 is the SG population median, and SG is the specific gravity of the urine sample.

Urinary isoprostane measurements

Urinary isoprostane (15-F_{2t}-Isoprostane/8-epi-PGF₂ alpha) was measured using a competitive enzyme-linked immunoassay (ELISA) kit according to the manufacturer's protocol (Cat #: EA85, Oxford Biomedical Research) and read on a SpectraMax M2 microplate reader (Molecular Devices). A significant amount of urinary isoprostane is excreted as glucuronide conjugates (Yan et al., 2010); therefore, urine samples were pretreated with beta-glucuronidase to allow for the measurement of total urinary isoprostane. All samples were run in duplicate with repeated analyses for duplicate results with a coefficient of variation (CV) >15%. Final urinary isoprostane concentrations were SG-corrected as described above. Control urines were utilized to monitor plate-to-plate variations; intra-day and inter-day CVs were 5.1% and 6.5%, respectively.

Statistical methods

Geometric means and selected percentiles were calculated for both males and females to describe the distributions of both SG-adjusted urinary metabolites and isoprostane for comparison to other published reports. Sex differences for continuous and categorical variables were assessed using the Wilcoxon paired signed-rank and chi-square tests, respectively. Both isoprostane and urinary metabolite concentrations were log transformed for statistical analysis. Spearman's rank correlations were calculated to assess relationships

of urinary concentrations of phthalate metabolites and isoprostane between couples. Generalized linear regression models (GLM) were used to describe the relationship between isoprostane and phthalate/DINCH metabolite concentrations. No influential points were observed, as determined by Cook's distance. Owing to the low detection frequencies of DINCH metabolites, GLM models were fitted as a bivariate variable as above or below LOD. In adjusted models, covariates were included on the basis of biological plausibility or if previously associated with isoprostane and phthalate metabolites (Ferguson et al., 2014; Ferguson et al., 2015). Bivariate analyses showed no association for smoking and race/ethnicity with phthalate metabolite concentrations and isoprostane; therefore, these variables were not added to models as covariates. The included covariates were age, body mass index (BMI, $<30 \text{ kg/m}^2$, 30 kg/m^2), and SG. A biomarker metabolite by sex interaction term was added to test for sex differences in the effect of each biomarker on isoprostane. To explore nonlinear relationships, we fitted generalized additive models; however, no nonlinear relationships existed (data not shown). To further explore sex modification, we stratified the above analysis based on participant sex. Analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). P-values < 0.05 were used to define statistical significance.

Results

The current study population consists of 50 male and 49 female partners seeking infertility treatment. Of the 50 couples, one female urine sample was missing. Demographics of our study population are presented in Table 1. On average, male partners were 1.7 years older than their female partners (35.8 ± 5.3 years vs. 34.2 ± 4.2 years; $p = 0.02$) and were more likely to be over the age of 40 while female partners were more likely to be between 30–40 years of age ($p = 0.007$). The mean BMI was $28.9 \pm 5.3 \text{ kg/m}^2$ for males with 36% classified as overweight (BMI = $25\text{--}29.9 \text{ kg/m}^2$) and 38% classified as obese (BMI $>30 \text{ kg/m}^2$). For females, the mean BMI was $28.9 \pm 6.8 \text{ kg/m}^2$ with 30% and 36% classified as overweight and obese, respectively. Based on 2010 World Health Organization reference values for semen quality (Cooper et al., 2010), 28% of male partners were diagnosed infertile, whereas 56% of female partners were diagnosed with known causes of fertility, including polycystic ovarian syndrome, premature ovarian failure, anovulation, diminished ovarian reserves, endometriosis, and uterine factor infertility.

The distributions of specific gravity-adjusted urinary metabolites and isoprostane concentrations for male and female partners are presented in Table 2. The majority of urinary phthalate metabolites (MEHHP, MEOHP, MECPP, MBzP, MiBP, MHiBP, MEP, MCPP, MCNP, and MCOP) had detection frequencies greater than 90% among couples, in addition to MnBP and MMP for males. MHINCH and MCOCH, metabolites of DINCH, were detectable in 43% and 16% of urine samples, respectively. Female partners had higher urinary concentrations than male partners for the four metabolites of DEHP (MEHP, MEHHP, MEOHP, and MECPP; $p < .0001$); MCNP, MHBP and MHiBP ($p < 0.01$); and MEP and MCOP ($p < 0.05$). Urinary isoprostane was detected in 100% of participants and the geometric mean was higher in female ($3.6 \pm 1.9 \text{ ng/mL}$) than male ($2.9 \pm 1.6 \text{ ng/mL}$) partners, although this difference did not achieve statistical significance ($p = 0.09$).

Table 3 shows Spearman correlations of urinary plasticizer metabolites and isoprostane concentrations between male and female partners. We observed a significant positive correlation between partners for the phthalate metabolites MBzP, MiBP, MHiBP, MEP, MCNP, MCOP, MMP and MNP; statistically significant negative correlations were observed between partners for two DEHP metabolites, MEHHP and MEOHP. Urinary isoprostane between partners was positively correlated but did not reach statistical significance ($r = 0.26$; $p = 0.07$).

In order to compare our results to previous studies (Ferguson et al., 2014; Ferguson et al., 2015), linear regression models presented in Table 4 provide estimates of the percent change in urinary isoprostane associated with an IQR increase for each untransformed metabolite concentration adjusting for SG, age, sex and BMI. Isoprostane was positively associated with IQR increases of all oxidative metabolites of DEHP: MEHHP (20.0%, 95% CI: 2.7 – 40.3%; $p = 0.02$); MEOHP (24.1%, 5.3–46.2%; $p = 0.01$); and MECPP (24.1%, 6.9–41.4%; $p = 0.004$) as well as the molar sum of DEHP metabolites (26.1%, 5.8 – 49.5%; $p = 0.01$). While isoprostane was not associated with DBP metabolites, MnBP and MHP, isoprostane was positively associated with the metabolites of its alternative DiBP (MiBP: 17.8%, 1.0 – 37.8%; $p = 0.02$ and MHiBP: 27.5%, 8.4–50.0%; $p = 0.003$). Furthermore, MHINCH, one of the metabolites of the phthalate alternative DINCH, was positively associated with isoprostane (32.3%; 12.5– 52.0%; $p = 0.002$), such that individuals with detectable concentrations ($>LOD$) of MHINCH had 38% higher concentrations of isoprostane than those below the detection limit ($<LOD$). In the multiple regression models, we consistently observed that sex was highly significant ($p < 0.0002$) across all phthalate and DINCH metabolite models. A metabolite by sex interaction term was found to be significant for MEOHP (p for interaction = 0.04) and borderline significant for MiBP and MHiBP (p for interaction 0.1). In addition to phthalate exposure, infertility conditions have been associated with increased oxidative stress (Gavriliouk and Aitken, 2015; Mier-Cabrera et al., 2011; Palacio et al., 2006). Thus, we also explored the possibility for interactions by infertility diagnoses in our models; however, no significant interaction terms were observed ($p > 0.1$).

To further examine the effect of sex on the relationship between isoprostane and exposure to phthalates and DINCH, data were stratified for sex-specific analyses (Table 5). For females, isoprostane was positively associated with an IQR increase in MHiBP (41.4%; 95% CI: 7.2, 84.0%; $p = 0.01$) and MCNP (26.0%; 95% CI: 3.9, 50.8%; $p = 0.02$). Several other positive associations were observed between isoprostane and several phthalate metabolites (MECPP, Σ DEHP, MEP, MCPP and MNP); however, these did not achieve statistical significance ($p = 0.15$). For males, percent changes in isoprostane concentrations were associated with IQR increases for MEOHP (35.8%; 95% CI: 2.9, 80.8%; $p = 0.03$), MiBP (29.2%; 95% CI: 5.5, 56.7%; $p = 0.01$), and MHiBP (34.7%; 95% CI: 9.4, 66.0%; $p = 0.007$). Furthermore, stratification of MHINCH by above/below LOD revealed a significant positive association with percent change in isoprostane (49.0%; 95% CI: 20.0, 79.0; $p = 0.002$). Similar to females, DEHP metabolites (MEHHP, MECPP and Σ DEHP) in males displayed a positive association with isoprostane but failed to reach statistical significance ($p < 0.15$).

Discussion

In this cross-sectional study of 50 couples seeking infertility treatment, we found that the oxidative metabolites of DEHP (MEHHP, MEOHP and MECPP), DiBP metabolites (MiBP and MHiBP), and the DINCH metabolite MHINCH were positively associated with isoprostane. We also observed sex-specific effects, such that urinary isoprostane was positively associated with MHiBP and MCNP among females and with MEOHP, MiBP, MHiBP and MHINCH among males. Although many associations did not achieve statistical significance in stratified analyses, urinary concentrations of DINCH and phthalate biomarkers were overall positively associated with oxidative stress. In addition, we found statistically significant positive correlations for nine phthalate metabolites among couples, suggesting shared sources of exposure among couples; however, we also observed that two oxidative metabolites of DEHP, MEHHP and MEOHP, were inversely correlated among couples.

The preconception period is recognized as one of the earliest susceptible windows of human development to environmental exposures (Chapin et al., 2004). In women, the duration of oocyte maturation, marked from initiation of growth to ovulation, is estimated at 85 days in humans (Telfer and McLaughlin, 2007) and has been shown to be susceptible to endocrine disrupting chemical exposure (Hunt et al., 2003; Hunt et al., 2009). Spermatogenesis is considered to be the final process of germ cell development that entails the progression from diploid spermatogonia to haploid spermatozoa. While undergoing dynamic epigenetic reprogramming during this process, the production of viable sperm for fertilization and is estimated to take around 74 days (Wu et al., 2015). Although sperm has been traditionally considered vehicle only for the delivery of the paternal genome upon fertilization, compelling animal data demonstrate that paternal nutritional manipulation during adulthood can alter sperm epigenetic marks (Lambrot et al., 2013; Wei et al., 2014) and noncoding RNA (Sharma et al., 2016) that may affect offspring health and development. Despite the lack of mechanistic data, a growing body of human observational studies show that preconception exposures may be associated with adverse reproductive health (Bae et al., 2015; Bloom et al., 2015a; Murphy et al., 2010; Robledo et al., 2015; Smarr et al., 2016). Most notably are results indicating that male partner urinary concentrations of MMP, MnBP, and MBzP were associated with a 20% reduction in fecundity as measured by time-to-pregnancy in U.S. couples (Buck Louis et al., 2014).

There is accumulating experimental and epidemiologic evidence that phthalate exposures contribute to oxidative stress conditions by increasing reactive oxygen species (ROS). Exposure of endometrial cells to DEHP at high concentrations (1000 pmol) increased ROS production and decreased the expression of antioxidant enzymes, glutathione peroxidase, heme oxygenase and catalase (Cho et al., 2015) *in vitro*. Among vinyl chloride workers, urinary MEHP, MEHHP and MEOHP were positively associated with the production of superoxide anion in sperm (Huang et al., 2014). Utilizing data from the National Health and Nutrition Examination Survey (NHANES), a nationally representative survey of the U.S. general population, the potent antioxidant bilirubin was shown to be inversely related to urinary metabolites of DEHP and DBP as well as MCPP and MBzP (Ferguson et al., 2012). Similarly, urinary 8-OHdG, an oxidative stress biomarker of DNA damage, was positively

correlated with MEHP, MBzP, and MEP in females and with MiBP and MEHP in male partners of couples planning pregnancy (Guo et al., 2014). Previous studies among pregnant women have shown a strong positive association between urinary isoprostane and nearly all phthalate metabolites, such that isoprostane levels were 14% – 56% higher per IQR increases in metabolite concentrations (Ferguson et al., 2014; Ferguson et al., 2015). Although only a few metabolites showed statistically significant associations (p -value < 0.05) in our study, the elevated levels of isoprostane associated with IQR increases in metabolite concentrations were similar to those reported previously (Ferguson et al., 2014; Ferguson et al., 2015). Thus, while our effect estimates were comparable to other studies with larger sample sizes, we did not achieve statistical significance for several biomarkers likely owing to limited statistical power from our modest sample size. Another plausible explanation that can account for disparities in results among studies may be attributable to the use of different commercially available ELISA kits which require different sample preparations. However, measurement error as introduced by different kits may contribute to imprecision, introduce non-differential misclassification, and bias the results toward the null.

In our study, concentrations of all four DEHP metabolites were significantly higher in female than male participants. Unadjusted concentrations of all DEHP metabolites of our female participants were also higher than those reported for NHANES 2011–2012 (CDC, 2015). However, in comparison with SG-adjusted values of women seeking infertility care who took part in the Environment and Reproductive Health study (Messerlian et al., 2016), MEHP concentrations in our female participants were twofold higher (8.0 ng/mL vs. 3.8 ng/mL), while concentrations of MEOHP (13.8 ng/mL vs. 12.1 ng/mL), MEHHP (19.7 ng/mL vs 18.8 ng/mL), and MECPP (27.8 ng/mL vs. 31.6 ng/mL) were similar. Medical interventions have previously been shown to contribute to urinary DEHP metabolite concentrations (Koch et al., 2005; Vandentorren et al., 2011; Yan et al., 2009) due to the presence of DEHP as a plasticizer in medical tubing and intravenous (IV) bags. We speculate that the higher concentrations of DEHP metabolites among females in our study are likely attributable to the timing of urine collection, which occurred either before or shortly after administration of IV tubing for oocyte retrieval procedures.

Because of the potential negative impacts on human health, certain phthalates have been substituted for compounds deemed as safer alternatives. For example, DEHP, the primary PVC plasticizer, is being replaced by other HMW phthalates, such as DiNP and DiDP, as well as the non-phthalate plasticizer DINCH®, which was introduced in 2002 as an alternative to sensitive phthalate-based PVC applications such as toys, medical devices and food packaging (Calafat et al., 2015). Not surprisingly, these shifts in phthalate production and consumer product usage are reflective of concentration profiles of phthalate metabolites observed via biomonitoring data in the United States and Germany. In Americans, urinary concentrations of DEHP metabolites have declined by 50% in 2009–2010 compared to 2005–2006, while metabolites of DiNP and DiDP have increased 2.6 and 1.1 times, respectively (Zota et al., 2014). Moreover, while not detected before 2002 (Calafat et al., 2015), MHINCH, the metabolite of DINCH, was detected in the urine of 20% of U.S. residents in 2011–2012 (CDC, 2015) and in 43% of our participants. For LMW phthalates, urinary MiBP concentrations have increased threefold over a ten year span (Zota et al., 2014) as its parent compound, DiBP, has been used as an alternative to DBP. Of particular

relevance to these recent shifting trends of phthalate exposures profiles are our findings suggesting that metabolite concentrations of several phthalate replacements are associated with higher levels of oxidative stress biomarkers. For example, IQR increases in MHBP concentrations were associated with 41.4% and 34.7% higher levels of isoprostane among female and male partners, respectively. Interestingly, we observed that all of the non-DEHP HWM phthalate metabolites were associated with isoprostane in a sex-specific manner. MCP, MCNP, MCOP and MNP displayed significant or borderline significant associations with isoprostane in females; however, no such apparent relationships were observed in male partners. However, while MHINCH was associated with a higher oxidative stress biomarkers in the total study population, stratification analyses revealed that this association was driven by male partners, whereby an IQR increase in MHINCH was associated with a 49% higher isoprostane level. A biological explanation for these observed sex-specific associations is unknown; however, such associations between other phthalate metabolites and urinary 8-OHdG, a marker of oxidative DNA damage, have been previously shown (Guo et al., 2014).

In addition to a modest sample size, our study has several other limitations. We recognize that a single spot urine may pose a concern for accurate exposure assessment of compounds with a short half-life such as phthalates and DINCH. However, studies have suggested that a single spot urine may sufficiently represent phthalate exposure over several months (Hauser et al., 2004; Teitelbaum et al., 2008). Also, LMW phthalates including DBP, DEP, DiBP, and BBzP may have greater temporal stability than the HMW phthalates DEHP, DiDP, and DiNP (Johns et al., 2015). Given the cross-sectional nature of the study, we also cannot rule out the possibility of inverse causality by which some unmeasured factor (e.g., chronic disease and P450 genetic polymorphism) could be related to both oxidative stress and the metabolism of phthalates or non-phthalates. In addition, it is essential to consider the half-life of the outcome biomarker. Pharmacokinetic studies have estimated the half-life of isoprostane to be 16 minutes (Kaviarasan et al., 2009); however, reliability studies have shown that isoprostane concentrations from single urine samples did not differ from 24 hour urine samples (Basu and Helmersson, 2005). Although several factors are likely to influence isoprostane, such as antioxidant supplementation (Guertin et al., 2016; Sutherland et al., 2007), urinary concentrations of isoprostane may also be influenced by recent (~ 24 hours) exposures to phthalates. Finally, given the higher than anticipated concentrations of DEHP metabolites among female participants in our study, most likely because of exposure to DEHP associated with oocyte retrieval, the generalization of these results to other populations should be interpreted cautiously, particularly in light of any potential differences between the IVF population and the general population, such as socioeconomic factors or health-related behaviors that may impact exposure levels of phthalates.

Conclusions

Our initial findings among 50 couples seeking fertility treatment supports the existing literature suggesting associations between phthalate exposure and oxidative stress. Moreover, our study provides the first data, to our knowledge, to investigate the relationship of DINCH, a phthalate replacement, with oxidative stress. While this study was limited by a small sample size, the effect estimates between urinary phthalate metabolite concentrations and isoprostane are in line with those reported previously among pregnant women.

Additional studies are needed to examine the potential health implications of the increased use of phthalates and their replacements in consumer products.

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Highlights

- Phthalates were positively associated with isoprostane in an IVF population.
- The observed associations were sex-specific.
- Phthalate replacements were associated with isoprostane in males.
- While limited by sample size, findings are concordant with previous reports.

Table 1

Demographic and clinical characteristics of SEEDS couples (N=50).

Individual Characteristics	Males	Females
Age	35.8 ± 5.3	34.1 ± 4.2 *
<30	10 (20%)	9 (18%)
30–40	27 (54%)	38 (76%)
40+	13 (26%)	3 (6%) [#]
Race		
White	39 (78%)	47 (94%)
Non-White	4 (8%)	2 (4%)
Unknown/Refused	7 (14%)	1 (2%)
BMI†	28.8 ± 5.0	28.9 ± 6.8
<25	11 (22.4%)	17 (34%)
25–30	19 (38.8%)	15 (30%)
30+	19 (38.8%)	18 (36%)
Infertility	14 (28%)	28 (56%)

Values are mean ± SD or N (%)

† Male Body Mass Index (BMI) (N = 49)

P-values were determined for continuous and categorical data via Wilcoxon paired signed-rank and chi-square tests, respectively.

* p-value < 0.05;

[#] p-value < 0.01.

Distribution of specific gravity-adjusted urinary metabolite (ng/mL) and isoprostane (ng/mL) concentrations among females (N=49)[†] and males (N=50) enrolled in SEEDS between 2014 and 2015.

Table 2

Metabolite	Sex	LOD ² (%>LOD)	GM (95% CI)	Percentiles				
				25 th	50 th	75 th	95 th	
MEHP	Males	0.5 (78.0)	1.1 (0.9, 1.3)	0.7	1.1	1.7	3.7	
	Females	(87.8)	8.0 (5.0, 12.7) [#]	2.4	10.2	22.6	64.8	
MEHHP	Males	0.2 (100)	6.0 (5.2, 7.0)	4.2	6.2	8.7	13.6	
	Females	(100)	19.7 (13.8, 28.1) [#]	10.0	19.7	40.9	86.4	
MEOHP	Males	0.2 (100)	4.0 (3.5, 4.5)	3.0	4.0	5.2	8.3	
	Females	(100)	13.8 (10.0, 18.8) [#]	6.7	14.5	25.6	48.7	
MECPP	Males	0.2 (100)	8.8 (7.6, 10.2)	6.5	9.2	12.7	18.4	
	Females	(100)	27.8 (20.7, 37.4) [#]	15.0	27.9	49.3	118.2	
ΣDEHP-3	Males		0.07 (0.06, 0.08)	0.05	0.07	0.10	0.15	
	Females		0.25 (0.18, 0.35) [#]	0.11	0.30	0.50	0.90	
MCOP	Males	0.2 (100)	24.5 (18.1, 33.3)	10.4	21.5	47.4	179.3	
	Females	(100)	34.9 (23.6, 51.6) [*]	12.7	37.5	94.6	214.0	
MNP	Males	0.5 (62.0)	0.9 (0.7, 1.2)	<LOD	0.7	1.6	6.4	
	Females	(57.1)	1.0 (0.8, 1.3)	<LOD	1.0	2.1	5.6	
MBzP	Males	0.3 (98.0)	3.7 (2.8, 4.9)	2.0	3.7	7.8	20.8	
	Females	(98.0)	4.4 (3.1, 6.4)	1.8	4.6	9.2	49.3	
MCP	Males	0.2 (98.0)	2.1 (1.6, 2.8)	1.0	1.8	3.8	13.5	
	Females	(91.8)	2.4 (1.8, 3.2)	1.2	2.1	4.2	10.8	
MCNP	Males	0.2 (100)	2.9 (2.4, 3.5)	1.8	2.6	4.4	10.1	
	Females	(100)	3.8 (3.0, 4.8) [#]	2.1	3.8	5.8	14.4	

Metabolite	Sex	LOD ² (%>LOD)	GM (95% CI)	Percentiles		
				25 th	50 th	95 th
MnBP	Males	0.4 (96.0)	6.9 (5.5, 8.7)	5.6	7.4	11.2
	Females	(89.8)	6.7 (5.0, 9.0)	4.3	7.9	13.9
MHBP	Males	0.4 (74.0)	0.6 (0.5, 0.7)	<LOD	0.6	0.9
	Females	(65.3)	1.0 (0.8, 1.2) [#]	<LOD	0.6	0.9
MiBP	Males	0.2 (100)	6.2 (5.0, 7.6)	4.1	6.6	10.2
	Females	(100)	5.8 (4.5, 7.4)	3.1	6.4	10.3
MHiBP	Males	0.4 (98.0)	1.9 (1.6, 2.3)	1.4	1.9	2.7
	Females	(93.9)	2.8 (2.3, 3.4) [#]	1.8	2.8	4.0
MEP	Males	0.6 (100)	25.1 (17.0, 37.1)	9.2	19.5	49.9
	Females	(100)	43.1 (30.1, 61.9) [*]	17.2	38.7	85.1
MMP	Males	0.5 (94.0)	2.1 (1.7, 2.7)	1.3	1.9	3.9
	Females	(87.8)	2.2 (1.8, 2.9)	1.3	2.3	3.3
MCOCH ⁴	Males	0.5 (18.0)		<LOD	<LOD	<LOD
	Females	(14.3)		<LOD	<LOD	<LOD
MHINCH ⁴	Males	0.4 (44.0)		<LOD	<LOD	0.9
	Females	(42.9)		<LOD	<LOD	0.5
Isoprostane ⁵	Males	10.0 (100)	2.9 (2.5, 3.3)	2.4	3.1	3.9
	Females	(100)	3.6 (3.0, 4.3)	2.4	3.9	5.2

LOD: limit of detection (in ng/mL); GM: geometric mean; CI = confidence interval.

¹Urine sample not available for one female participant

²Percentage of metabolites and isoprostane concentrations above limit of detection.

³ΣDEHP: Molar sum of DEHP metabolites (MEHP, MEHHP, MEOHP, MECPP) expressed in μmol/L.

⁴GM was not calculated due to low detection frequency.

10'0>d

'50'0>d
*
LOD is is DOT
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Table 3

Spearman correlations coefficients (r) of urinary metabolite and isoprostane concentrations between partners in SEEDS couples (N = 49).

	r	p-values
MEHP	−0.18	0.23
MEHHP	−0.41	<0.005
MEOHP	−0.34	0.02
MECPP	−0.20	0.18
MCOP	0.50	<0.001
MNP	0.47	<0.001
MBzP	0.50	<0.001
MCCP	0.31	0.03
MCNP	0.40	<0.005
MnBP	0.01	0.93
MHBP	−0.11	0.43
MiBP	0.36	0.01
MHiBP	0.30	0.03
MEP	0.32	0.03
MMP	0.47	<0.001
Isoprostane	0.26	0.07

Table 4

Adjusted linear regression models^a of percent difference (95% CIs) in isoprostane concentrations in association with interquartile range (IQR) increase in metabolite concentrations.

Metabolite	Total (N=99)	
	Beta (95% CI)	p-value
MEHP	11.4 (−7.1, 34.2)	0.24
MEHHP	20.0 (2.7, 40.3)	0.02
MEOHP	24.1 (5.3, 46.2)	0.01 [*]
MECPP	22.8 (6.9, 41.4)	0.004
ΣDEHP	26.1 (5.8, 49.5)	0.01
MCOP	10.3 (−5.7, 29.1)	0.19
MNP	9.6 (−5.9, 27.5)	0.23
MBzP	8.9 (−7.1, 28.3)	0.31
M CPP	11.3 (−2.6, 25.5)	0.11
MCNP	10.4 (−4.3, 26.0)	0.18
MnBP	−1.9 (−15.1, 13.4)	0.79
MHBP	4.5 (−18.4, 33.8)	0.71
MiBP	17.8 (1.0, 37.8)	0.02 [#]
MHiBP	27.5 (8.4, 50.0)	0.003 [#]
MEP	13.7 (−2.4, 32.2)	0.10
MMP	9.6 (−5.1, 26.6)	0.23
MHINCH ^b	32.3 (12.5, 52.0)	0.002 [#]
MCOCH ^b	13.9 (−12.0, 40.0)	0.30

^aModels adjusted for specific gravity, age, sex, and body mass index.

^bModels with below/above the limits of detection.

^{*} p < 0.05 and

[#] p < 0.1 for metabolite by sex interaction

Table 5

Adjusted linear regression models of percent difference (95% CIs) in isoprostane concentrations in association with interquartile range (IQR) increase in metabolite concentrations.

Metabolite	Females (N=49)		Males (N=50)	
	Beta (95% CI)	p-value	Beta (95% CI)	p-value
MEHP	11.8 (−6.5, 30.8)	0.22	3.9 (−14.0, 25.4)	0.68
MEHHP	13.5 (−3.1, 32.9)	0.13	22.1 (−5.8, 58.2)	0.13
MEOHP	13.9 (−2.9, 35.4)	0.12	35.8 (2.9, 80.8)	0.03
MECPP	18.9 (−0.4, 43.5)	0.06	24.8 (−4.9, 63.8)	0.12
ΣDEHP	10.7 (−1.0, 23.7)	0.07	27.5 (−4.9, 72.7)	0.11
MCOP	18.9 (−4.8, 52.3)	0.11	6.0 (−12.3, 28.0)	0.50
MNP	16.5 (−1.4, 39.5)	0.08	3.1 (−16.7, 29.5)	0.74
MBzP	11.6 (−7.1, 34.1)	0.25	10.5 (−12.5, 41.8)	0.39
MCCP	16.2 (−3.0, 39.2)	0.10	7.8 (−10.7, 28.5)	0.44
MCNP	26.0 (3.9, 50.8)	0.02	−1.0 (−20.9, 22.7)	0.89
MnBP	7.3 (−19.0, 44.7)	0.61	−0.9 (−16.2, 16.1)	0.89
MHBP	14.4 (−20.9, 68.3)	0.46	6.0 (−24.4, 48.7)	0.75
MiBP	20.3 (−6.0, 51.6)	0.13	29.2 (5.5, 56.7)	0.01
MHiBP	41.4 (7.2, 84.0)	0.01	34.7 (9.4, 66.0)	0.007
MEP	16.1 (−3.7, 40.0)	0.10	3.6 (−16.2, 25.9)	0.78
MMP	13.4 (−7.2, 36.8)	0.21	8.1 (−13.2, 32.9)	0.50
MHINCH ^b	15.0 (−12.0, 41.0)	0.28	49.0 (20.0, 79.0)	0.002
MCOCH ^b	−2.0 (−39.0, 35.0)	0.90	23.0 (−15.0, 61.0)	0.23

^a Models adjusted for specific gravity, age, and body mass index.

^b Models with below/above LOD