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Associations of urinary phthalate metabolites and lipid peroxidation with sperm mitochondrial DNA copy number and deletions

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ABSTRACT

Background: Phthalates, a chemical class of plasticizers, are ubiquitous environmental contaminants that have been associated with oxidative stress. Mitochondria DNA copy number (mtDNAcn) and DNA deletions (mtDNAdel) are emerging biomarkers for cellular oxidative stress and environment exposures.
Objectives: To examine associations of urinary phthalate metabolite and isoprostane concentrations on sperm mtDNAcn and mtDNAdel in male partners undergoing assisted reproductive technologies (ART).
Methods: Ninety-nine sperm samples were collected from male partners undergoing ART at Baystate Medical Center in Springfield, MA as part of the Sperm Environmental Epigenetics and Development Study (SEEDS). Seventeen urinary phthalate metabolite concentrations were analyzed by the Centers for Disease Control using tandem mass spectrometry. Urinary 15-F2t-isoprostane concentrations, a biomarker of lipid peroxidation, were measured using a competitive enzyme-linked immunosorbent assay. A triplex qPCR method was used to determine the relative quantification of mtDNAcn and mtDNAdel.
Results: Sperm mtDNAcn and mtDNAdel were positively correlated (Spearman rho = 0.31; p = .002). Adjusting for age, BMI, current smoking, race, and measurement batch, urinary monocarboxy-isononyl phthalate (MCNP) concentrations were positively associated with mtDNAcn (β = 1.63, 95% CI: 0.14, 3.11). Other urinary phthalate metabolite and isoprostane concentrations were not associated with sperm mtDNAcn or mtDNAdel.
Conclusions: Among this cohort of male ART participants, those with higher MCNP had higher mtDNAcn; other phthalate metabolites and isoprostane were not associated with mtDNAcn and mtDNAdel. Given our relatively small sample size, our results should be interpreted with caution. Future research is needed to replicate the findings in larger studies and among sperm samples obtained from the general population.

1. Introduction

Phthalate diesters are a class of synthetic organic chemicals used in industrial and consumer products that include flexible vinyl plastics, medical equipment, food packaging, and personal care products (Johns et al., 2015; Zota et al., 2014). Phthalate exposure is widespread, such that urinary phthalate metabolites have been detected in the majority of individuals from representative samples within the U.S. general population (CDC, 2010). In humans, urinary phthalate metabolite concentrations have been associated with adverse male reproductive outcomes such as shorter anogenital distance (Suzuki et al., 2012; Swan et al., 2015), abnormal sex steroid hormone synthesis (Meeker et al., 2009), lower serum testosterone (Meeker and Ferguson, 2014), reduced sperm motility, concentration (Cai et al., 2015; Pant et al., 2014), and increased DNA damage (Hauser et al., 2007). Additionally, studies of phthalate exposure animal models reported reduced sex organ weight (Shono and Taguchi, 2014), abnormal Leydig cell aggregation (Mahood et al., 2007), repression of sex determination (Wang et al., 2016), altered sex ratio (Dobrzynska et al., 2011), and delayed sexual maturity (Lee et al., 2004).

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Oxidative stress, characterized by the disruption of redox signaling and control (Jones, 2006), has been proposed a potential mediator in phthalate-associated adverse male reproductive outcome. Oxidative stress measures have been associated with diminished semen quality, including sperm motility, concentration, and morphology (Agarwal et al., 2014). Isoprostanes, formed by the presence of free radicals and commonly used markers of lipid peroxidation in vivo (Kadiiska et al., 2005; Roberts and Morrow, 2002), have been reported to be strongly associated with urinary phthalate metabolite concentrations among pregnant women (Ferguson et al., 2014, 2015; Holland et al., 2016) as well as in our previous study among couples seeking fertility treatment (Wu et al., 2017c). Similarly, a study of couples planning pregnancy reported that select urinary phthalate metabolite concentrations in both males and females were correlated with urinary 8-Oxo-2′-deoxyguanosine concentrations, a biomarker of oxidative stress via DNA oxidation (Guo et al., 2014). It is currently unknown if phthalate exposure directly induces lipid peroxidation, or rather, it involves other intermediate biological targets that induce an imbalance in redox signaling.

Measures of the sperm mitochondrial genome may provide useful markers of oxidative stress, male fertility and environmental exposures. The mitochondrial genome is highly susceptible to oxidative damage due to its proximity to reactive oxygen species produced during oxidative phosphorylation and lack of protective defenses such as histones and DNA repair mechanisms (Phillips et al., 2014a; Sutovsky et al., 2003). Mitochondria contain their own double-stranded 16.6 kb, circular, maternally-inherited genome that encodes 37 genes, which are involved in a host of biological functions including, most notably, ATP production and other homeostatic and signaling processes (Chen et al., 2012; Phillips et al., 2014b). The number of mitochondria vary widely across cell types and each mitochondrion can harbor between 1 and 10 copies of its genome (Phillips et al., 2014b; Robin and Wong, 1988). Sperm mitochondrial copy number (mtDNAcn), the ratio of the number of mitochondrial genome copies to nuclear DNA (nDNA) copies, and percent of large deletions in the mitochondrial genome (mtDNAdel) have both been associated with oxidative stress measures (Abasalt et al., 2013; Bonanno et al., 2016), and have also been associated with male infertility (Rajender et al., 2010). In addition, leukocyte mtDNAcn and mtDNAdel have also been associated with markers of oxidative stress (Lee et al., 2000a; Liu et al., 2003a; Wang et al., 2011), further supporting the sensitivity of mtDNAcn and mtDNAdel to oxidative stress and their potential use as biomarkers of this imbalance. One hypothesis suggests that increases in mtDNAcn may be a compensatory mechanism by which oxidative damage or mtDNAdel induce the propagation of the mitochondrial genome (Lee et al., 2000b). Furthermore, one recent study reported that urinary polycyclic aromatic hydrocarbons (PAH) metabolite concentrations were inversely associated with sperm mtDNAcn among young Chinese men (Ling et al., 2017), indicating the potential responsiveness of sperm mtDNAcn to environmental exposures as has been shown with leukocyte mtDNAcn and air pollution (Hou et al., 2010, 2013; Zhong et al., 2016).

To further characterize the relation of phthalates with measures of male reproductive health and the role of oxidative stress, we examined the associations of urinary phthalate metabolite and isoprostane concentrations with mtDNAcn and mtDNAdel in sperm among couples seeking reproductive assistance.

2. Methods and materials

2.1. Study population

Couples were recruited as part of the Sperm Environmental Epigenetics and Development Study (SEEDS) from the Baystate Medical Center In Vitro Fertility (IVF) Program in Springfield, Massachusetts between 2014 and 2017. Inclusion criteria for male participants were 18–55 years of age without vasectomy, and for female participants were ≤ 40 years old. Furthermore, both males and females were required to use their own gametes to be eligible to participate in the study. Written consents from eligible males and females interested in participating were obtained by attending physicians. This study was approved by the institutional review boards at Baystate Medical Center and at the University of Massachusetts Amherst.

2.2. Covariate assessments

Relevant demographic (race, age, height, weight) and medical history data (diagnosis of infertility) were collected by clinic personnel during the course of an IVF cycle for both partners. In addition, each partner was asked to complete an intake questionnaire regarding lifestyle factors (current and past cigarette and alcohol use) prior to sample collection. Current smoking status was determined using a Cotinine ELISA kit (Calbiotech Cat#: CO096D) on a binary basis of smoker or non-smoker.

2.3. Urinary biomarker measurements

2.3.1. Phthalates

Men who agreed to participate provided a spot urine sample in a sterile polypropylene collection cup on the same day of semen sample procurement. 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 1,2-Cyclohexane dicarboxylic acid diisononyl ester (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 (Kato et al., 2005; Silva et al., 2013). The following urinary phthalate and DINCH metabolites and were analyzed: 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); Monocarbxyisooctyl 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); Mono-ethyl phthalate (MEP); Mono-methyl phthalate (MMP); cyclohexane-1,2-dicarboxylic acid-monocarboxy isooctyl ester (MCOCH); and cyclohexane-1,2-dicarboxylic acid-mono(hydroxyisononyl) ester (MHiNCH).

Limits of detection (LOD) varied between batches and ranged from 0.1 to 0.9 ng/mL across all metabolites. Values falling under the LOD for each metabolite on each plate were replaced by the LOD/sqrt(2). 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 with 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 5.0–9.3% (high concentration standard).

Specific gravity was measured in urine by the CDC or by Baystate Reproductive Clinic using a handheld refractometer for participants (Atago Co., Ltd., Toko, Japan). The formula for dilution normalization of phthalate measurement by specific gravity is $Pc = P[(SGm-1)/(SG-1)]$ where Pc is the SG-corrected metabolite concentration (ng/mL), P is the measured phthalate metabolite concentration, SGm is the median SG value of all samples, and SG is the specific gravity value for that individual urine sample. Values were log transformed for analyses.