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A crossover–crossback prospective study of dibutyl-phthalate exposure from mesalamine medications and semen quality in men with inflammatory bowel disease

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ABSTRACT

Background: Phthalates are widely used chemicals with ubiquitous exposure. Dibutyl-phthalate (DBP), a male reproductive toxicant in animals, is understudied in humans. Some mesalamine medications used to treat inflammatory bowel disease (IBD) have DBP in their coating, whereas other mesalamine formulations do not.
Objectives: Taking advantage of differences in mesalamine formulations, we investigated whether high-DBP exposure from mesalamine medications was associated with decreased semen parameters.
Methods: 73 men with IBD taking mesalamine participated in a crossover-crossback prospective study. Men taking non-DBP containing mesalamine at baseline i.e., background exposure, crossed-over for four months to high-DBP mesalamine and then crossed-back for four months to their non-DBP mesalamine (B_1HB_2-arm;Background$_1$-High-Background$_2$) and vice versa for men taking high-DBP mesalamine at baseline (H_1BH_2-arm;High$_1$-Background-High$_2$). Men provided up to six semen samples (2: baseline, 2: crossover and 2: crossback).
Results: We estimated crossover, crossback and carryover effects using linear mixed models adjusted for abstinence time, age, season and duration on high-DBP mesalamine at baseline. Semen parameters in B_1HB_2-arm (26 men, 133 samples) decreased after high-DBP mesalamine exposure (crossover versus baseline), especially motility parameters, and continued to decrease further even after crossback to non-DBP mesalamine (crossback versus crossover). The cumulative carryover effect of high-DBP (crossback versus baseline) was a decrease of % total sperm motility by 7.61(CI:−13.1, −2.15), % progressive sperm motility by 4.23(CI:−8.05, −0.4) and motile sperm count by 26.0% (CI:−46.2%, 1.7%). However, H_1BH_2-arm (47 men, 199 samples) had no significant change during crossover or crossback.
Conclusions: Men newly exposed to high-DBP mesalamine for four months had a cumulative reduction in several semen parameters, primarily sperm motility, that was more pronounced and statistically significant even after exposure ended for four months.

Abbreviations: DBP, dibutyl phthalate; FDA, Food and Drug Administration; 5-ASA, 5-aminosalicylic acid; IBD, inflammatory bowel disease; UC, ulcerative colitis; CD, Crohn's disease; MBP, monobutyl phthalate; NHANES, National Health and Nutrition Examination Survey; MARS, Mesalamine And Reproductive health Study; BIMC, Beth Israel Deaconess Medical Center; BWH, Brigham and Women's Hospital; MGH, Massachusetts General Hospital; IRB, institutional review board; B_1HB_2, Background$_1$-High-Background$_2$ DBP exposure; H_1BH_2, High$_1$-Background-High$_2$ DBP exposure; QC, quality control; BMI, body mass index; kg, kilogram; m, meter; SD, standard deviation; LMEM, mixed effects models; FEM, fixed effect models; N, number of men; WHO, World Health Organization; EPA, Environmental Protection Agency.

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1. Introduction

Over the last several decades, accumulating evidence suggests a downward trend (Carlsen et al., 1992; Centola et al., 2016; Skakkebaek et al., 2016) and geographic variability in semen quality (Swan et al., 2003), a surrogate for male fertility. These trends raise concern that lifestyle or environmental exposures may affect semen quality and male fertility (Sharpe and Skakkebaek, 1993). One class of environmental chemicals for which there is concern about potential adverse male reproductive health effects are phthalates (Congress PL-t, 2008). In experimental animal studies, several phthalates including dibutyl-phthalate (DBP) were anti-androgens and male reproductive toxicants, adversely affecting testicular function (Cater et al., 1977; Park et al., 2002; Shono and Taguchi, 2014; Li et al., 2014; van den Driesche et al., 2014). The most studied window of exposure is in-utero exposure which led to male reproductive tract malformations in rats (Foster, 2006; Kim et al., 2010; Motohashi et al., 2015). Less well-studied are puberty and adulthood exposure. Studies in rats have shown effects of post-natal exposure to DBP on the male reproductive tract (Bao et al., 2011; Moody et al., 2013; Lee et al., 2008; Tsutsumi et al., 2004) and to butyl benzyl phthalate (BBzP) (Nagao et al., 2000). There are several epidemiologic studies in adult men that explored cross-sectional associations between background low-DBP environmental exposure, and other phthalates, with semen quality. Most of these studies were conducted in men recruited from infertility clinics (Kranvogl et al., 2014; Pant et al., 2014; Hauser et al., 2006; Liu et al., 2012; Tranfo et al., 2012; Wirth et al., 2008; Toshima et al., 2012; Wang et al., 2015), and although some studies found associations of DBP (Hauser et al., 2006; Tranfo et al., 2012) and other phthalates (Kranvogl et al., 2014; Pant et al., 2014; Tranfo et al., 2012; Wirth et al., 2008; Axelsson et al., 2015) with lower semen quality, others did not (Liu et al., 2012; Bloom et al., 2015; Thurston et al., 2015).

In addition to widespread general population DBP exposure from personal care and consumer products (Wittassek et al., 2011; Silva et al., 2004), some medications such as specific mesalamine formulations have enteric coatings that contain DBP (Wittassek et al., 2011; Nguyen et al., 2016; Hernandez-Diaz et al., 2009; Seckin et al., 2009; Kelley et al., 2012) despite the recent US Food and Drug Administration (FDA) recommendation against the use of phthalates in drug delivery vehicles (FDA, 2012a). Mesalamine or 5-aminosalicylic acid (5-ASA) is a commonly prescribed maintenance therapy for inflammatory bowel diseases (IBD), specifically ulcerative colitis (UC) and Crohn's disease (CD) (Song et al., 2012). Our research and others have shown that mesalamine medications with coatings that contain DBP contribute to high-DBP exposure as measured by concentrations of urinary monobutyl phthalate (MBP), the primary DBP metabolite (Hauser et al., 2004; Hait et al., 2014). Specifically, in individuals taking mesalamine medications that contain DBP, their urinary levels of MBP were approximately 1000 times higher than the median levels reported for men in the US general population (National Health and Nutrition Examination Survey (NHANES)) (CDC, 2015). Therefore, patients with IBD taking DBP-containing mesalamine will have chronic high exposure to DBP because the medication is taken daily to treat IBD.

Mesalamine is the active ingredient in Asacol® and Asacol®HD and DBP is an excipient in their enteric coating (FDA, 2012b). Other mesalamine formulations such as Pentasa®, Lialda®, Apriso®, and Delzicol® do not contain DBP (Kelley et al., 2012; Gallinger and Nguyen, 2013). Asacol®, widely used to treat IBD in adults and children, was a first line of therapy for patients with UC and often used in pregnant women with IBD (Gallinger and Nguyen, 2013; Khan et al., 2013). The aim of the study was to investigate the effect of high-DBP exposure on semen quality, taking advantage of the difference in mesalamine formulations to conduct a crossover-crossback prospective study in adult men with IBD.

2. Materials and methods

2.1. Study population

We conducted a crossover-crossback prospective study in adult men with IBD (Mesalamine And Reproductive health Study (MARS)). Eligibility for participants in the MARS was 18 to 55 years of age and taking oral mesalamine for at least the past three months. All men must have had a mild IBD score on the simple clinical colitis activity index (Walmsley et al., 1998) (five or less for UC) or Harvey-Bradshaw index (Harvey and Bradshaw, 1980) (four or less for CD). Men were recruited from gastroenterology clinics at three Boston hospitals; Beth Israel Deaconess Medical Center (BIMC), Brigham and Women's Hospital (BWH) and Massachusetts General Hospital (MGH) from October 2010 through October 2015. MARS was approved by the institutional review boards (IRBs) of Harvard T.H. Chan School of Public Health, BIMC, BWH and MGH. All men signed informed consents.

2.2. Study design

Eligible men were invited to participate in up to six visits; to account for within-person variability in semen parameters. Each man was asked to participate in two visits at baseline, after crossover and after crossback. At each of the two baseline visits, participants provided semen, urine and blood samples collected two weeks apart (visits 1 and 2). Men were then asked to crossover to another formulation of mesalamine; men who were taking non-DBP mesalamine at baseline crossed-over to DBP-containing mesalamine medication (i.e., Asacol®) and vice versa for men prescribed DBP-containing mesalamine at baseline crossed-over to non-DBP mesalamine. After crossover for four months, two sets of semen, urine and blood samples were collected two weeks apart (visits 3 and 4). Participants were then asked to crossback for four months to their original mesalamine medications after which two sets of semen, urine and blood samples were collected two weeks apart (visits 5 and 6) (Fig. 1).

In brief, men who were prescribed non-DBP mesalamine with background exposure from other sources crossed-over to high-DBP mesalamine then crossed-back to non-DBP mesalamine (B_1HB_2-arm: $Background_1$-$High$-$Background_2$). Men who were prescribed high-DBP mesalamine crossed-over to non-DBP mesalamine then crossed-back to high-DBP mesalamine (H_1BH_2-arm: $High_1$-$Background$-$High_2$). The 'wash-in' and 'wash-out' periods between crossover and crossback were four months to extend beyond the 70 days average period of spermatogenesis (Heller and Clermont, 1963). Questionnaires about lifestyle factors, medical history and ejaculation abstinence time were administered at every visit.

Among the 47 men in the H_1BH_2-arm, 13 men participated only in a short protocol defined as up to four visits. These 13 men did not want to change medication but because the manufacturer was reformulating Asacol® to remove DBP, we anticipated that they would be 'switched' to a non-DBP mesalamine when this came to market. Warner Chilcott discontinued Asacol® in 2013 and introduced Delzicol® (non-DBP mesalamine) to the market. However, Asacol®HD (containing DBP) remained on the market. For 10 of the 13 men, their physician changed their medications to Asacol®HD, thus they never crossed-over to non-DBP mesalamine and only contributed to the baseline visits while three men changed medication to Delzicol® i.e. crossed-over. However, by design none of the men in the short protocol crossed-back.

Men were asked to abstain from ejaculation for 2–5 days before providing semen samples, collected by masturbation at the MGH andrology laboratory into a sterile container and analyzed using standardized clinical protocols and quality control (QC) as described previously (Hauser et al., 2006; Perry et al., 2011). Briefly, semen was allowed to liquefy at 37 °C for 20 min. The physical properties of the semen were reported, including the sample volume, pH, color and viscosity. Ejaculate volume was measured using a graduated serological pipet. Sperm concentration

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