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A Crossover–Crossback Prospective Study of Dibutyl-Phthalate Exposure from Mesalamine Medications and Serum Reproductive Hormones in Men[☆]

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Abstract

Background—Phthalates, such as dibutyl phthalate (DBP), are endocrine disruptors used in some medication coatings e.g., mesalamine to treat inflammatory bowel disease (IBD).

Objectives—Taking advantage of different mesalamine formulations with/without DBP, we assessed whether DBP from mesalamine ($>1000\times$ background) altered serum hormones.

Methods—Men ($N=73$) with IBD participated in a crossover-crossback prospective study and provided up to 6 serum samples (2:baseline, 2:crossover, 2:crossback). Men on non-DBP mesalamine (background) at baseline crossed-over for 4 months to DBP-mesalamine (high) and then crossed-back for 4 months to non-DBP mesalamine (B_1HB_2 -arm) and vice versa for men on DBP-mesalamine at baseline (H_1BH_2 -arm). We divided H_1BH_2 -arm at the median ($H_1<3$ yrs or $H_1\geq 3$ yrs). We estimated crossover and crossback % changes in serum reproductive hormones using multivariable linear mixed effect models.

Results—When B_1HB_2 -arm (26 men, 134 samples) crossed-over, luteinizing hormone decreased 13.9% (95% confidence interval(CI): $-23.6, -3.0$) and testosterone, inhibin-B, and follicle-stimulating hormone (FSH) marginally decreased; after crossback all increased 8–14%. H_1BH_2 -arm, $H_1\geq 3$ yrs (25 men, 107 samples) had no changes at crossover or crossback whereas in H_1BH_2 -arm, $H_1<3$ yrs (22 men, 100 samples) after crossover, inhibin-B increased 13.2% (CI: 4.2, 22.9), FSH decreased 9.9% (CI: $-17.9, -1.1$) and after crossback, inhibin-B further increased 11.3%, and FSH marginally increased.

Conclusions—High-DBP exposure may disrupt pituitary-gonadal hormones that largely reversed after exposure removal, but only in men with no or short previous high-exposure history. Paradoxically, men with longer duration of high-DBP exposure, exposure removal did not change hormone levels, suggesting that long-term high-DBP exposure may alter the pituitary-gonadal axis and make it insensitive to exposure changes.

Keywords

Phthalate; endocrine disruptor; crossover study; hormones; men

1. Introduction

Ortho-phthalates (hereto referred to as phthalates) are high production volume chemicals¹. Because of their widespread use in consumer and personal care products, phthalate exposure is ubiquitous in the general population².

In experimental studies, several phthalates, including dibutyl phthalate (DBP), have adverse impacts on male reproductive health³. Although banned for several uses, DBP is still used in the enteric coatings of some medications including specific formulations of mesalamine, resulting in high-DBP exposure^{4–10}. Mesalamine, the active ingredient in Asacol[®], Asacol[®]HD, Lialda[®], Pentasa[®], Apriso[®], and Delzicol[®], is a commonly prescribed for inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD). The enteric coating of Asacol[®], and Asacol[®]HD contains DBP as an excipient^{11,12}, whereas it is not used in other mesalamine formulations⁹. Asacol[®] and Asacol[®]HD use leads to high-DBP exposure as measured by urinary monobutyl phthalate (MBP)

concentrations, the primary DBP metabolite^{10,13}, that are approximately 1000 times higher than the median reported for men in the U.S. general population (National Health and Nutrition Examination Survey (NHANES))¹⁴.

Experimental studies have shown that DBP is anti-androgenic and a reproductive toxicant, however, most studies have focused on *in utero* exposure^{3,15,16}. There is limited evidence from epidemiologic studies on the association of background DBP exposure with serum reproductive hormones^{17–21}. However, there are no studies on effects of very high-DBP exposures from medications. Therefore, we took advantage of the difference in DBP-exposure from specific mesalamine formulations and conducted a crossover-crossback prospective cohort study to examine the associations between high-DBP exposure from mesalamine medications and serum reproductive hormones in adult men.

2. Materials and methods

2.1. Participants

As previously described⁵, between 2010–2016, 73 men enrolled in the Mesalamine And Reproductive health Study (MARS) from gastroenterology clinics at three Boston hospitals, Beth Israel Deaconess Medical Center (BIDMC), Brigham and Women's Hospital (BWH) and Massachusetts General Hospital (MGH). Eligible men were 18 to 55 years old, with mild severity IBD, and on mesalamine for at least three months at enrollment. There were no exclusions based on history of infertility. Men with a history of steroid medication use in the last three months, vasectomy, diabetes mellitus, hepatic, or renal diseases were not eligible. MARS was approved by the institutional review boards of Harvard T.H. Chan School of Public Health, BIDMC, BWH and MGH. All men signed informed consents.

2.2. Study design

Men participated in up to six visits (v) (baseline: v1&v2, crossover: v3&v4 and crossback: v5&v6). Men who started on non-DBP mesalamine (background-DBP exposure) crossed-over to high-DBP mesalamine (high-DBP exposure) then crossed-back to non-DBP mesalamine (background) (B₁HB₂-arm; Background₁-High-Background₂) and vice versa in men who started on DBP mesalamine (H₁BH₂-arm; High₁-Background-High₂) (Figure 1). Crossover and crossback periods (between v2&v3 and v4&v5) were designed to be four months and durations between v1&v2, v3&v4 and v5&v6 were designed to be two weeks. We a priori chose the four month periods to be longer than the spermatogenesis cycle (around 70 days)²², as one of our aims was to study semen parameters⁵. At baseline, men reported demographic, lifestyle and health information on questionnaires and height and weight were measured. At each visit, men reported fever and illness in the previous three months and gave blood, semen and urine samples. 13 out of the 47 men in the H₁BH₂-arm participated only in a short protocol consisting of up to four visits that required only crossover without crossback as previously described⁵.

2.3. Exposure assessment

High-DBP exposure was defined by medication type i.e., DBP-containing mesalamine versus non-DBP mesalamine. We relied on self-reported use of mesalamine medications as prescribed over the study period²³.

2.4. Serum hormone analysis

Nurses collected non-fasting blood samples at each visit between 7 am and 2 pm and noted the exact time. All samples were shipped in a single batch for analysis to the laboratories at Ansh (inhibin-B and sex hormone-binding globulin (SHBG)), National Institute of Environmental Health Sciences (NIEHS) (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)), and Mayo Clinic (total testosterone (TT) and albumin).

SHBG was measured by enzyme linked immunosorbent assay (ELISA) (quantitative two-step sandwich type immunoassay). Testosterone was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Agilent Technologies, Santa Clara, CA 95051). Albumin, used to calculate the free testosterone (FT) concentration, was measured by an enzymatic colorimetric assay on the Roche Cobas c311 chemistry analyzer (Roche Diagnostics, Indianapolis, IN 46250). Inhibin-B was measured by ELISA (quantitative three-step sandwich type immunoassay). Ansh laboratory has developed highly specific antibodies for inhibin-B with no detectable cross-reactivity to inhibin-A or activin-A and AB, and negligible cross-reactivity to activin-B (0.04% at 50 ng/ml). LH and FSH concentrations were measured by chemiluminescent microparticle immunoassay (CMIA) technology (Architect i100SR Immunoassay Analyzer, Abbott Diagnostics, Abbott Park, IL).

The intra- and inter-assay coefficients of variation (CV) were for SHBG: <9.0% and <8.0%, testosterone: 9.0% and 8.9%, albumin: 1.4% and 2.1%, Inhibin-B: <4.4% and <7.4%, LH: 2.8% and 5.7%, and FSH: 1.8% and 12.7%, respectively. All laboratories were blinded to participant information. We calculated FT from the measured concentrations of TT, SHBG, and albumin using the method of Vermeulen et al.²⁴. We also calculated TT/LH, FT/LH, and Inhibin-B/FSH ratios.

2.5. Statistical Analysis

In primary analyses, to define exposure we used a six-level indicator variable cross-classifying each observation based on the medication type (high versus non-DBP) at each period (baseline, crossover and crossback) for the two study arms (H_1BH_2 and B_1HB_2). We modeled the hormone concentrations as natural log-transformed continuous outcomes.

We performed descriptive statistics and tested for any differences between men in the different arms using Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables.

We selected the covariates based on directed acyclic graphs and statistical considerations (>10% change in the effect estimate). The final model included age (continuous), body mass index (BMI) (continuous), sample drawing time (continuous, time varying), season of the sample collection (warm and cold, time varying), race (Caucasian or not), current smoking

(binary), and duration on high-DBP mesalamine as self-reported at baseline (continuous). In preliminary models, we considered adjustment for IBD severity, IBD diagnosis (UC/CD), duration since diagnosis, history of reproductive diseases or surgeries, education, alcohol consumption, fever and illness in the previous three months but they were not confounders and thus not retained in the final models.

We estimated the DBP crossover (crossover versus baseline), crossback (crossback versus crossover), and carryover (crossback versus baseline) percent changes in the hormone parameters within each arm, as well as the cross-sectional differences at baseline between arms. We used linear mixed effect models (LMEM) with a random intercept to account for within-man correlation among longitudinal measures of a given hormone parameter arising from man-to-man heterogeneity across the study participants.

Because men in H₁BH₂-arm were on their high-DBP mesalamine at baseline for a wide range of durations (4 months to 24 years), in post-hoc analyses we explored whether the duration on high-DBP mesalamine at baseline modified associations by adding an interaction term. Additionally, we subdivided men in the H₁BH₂-arm based on the median duration of three years on high-DBP mesalamine at baseline i.e., (H₁BH₂-arm, H₁<3 yrs) and (H₁BH₂-arm, H₁ ≥ 3 yrs) and conducted analyses based on the resulting three arms using the same method as the two-arm analyses above.

As a sensitivity analysis, we assessed LMEM model sensitivity to the covariance structure implied by the random intercept model, using the robust empirical standard errors²⁵ in both two-arm and three-arm analyses. We considered two-sided alpha <0.05 as statistically significant. We conducted all statistical analyses using SAS version 9.4 (SAS Institute Inc., Cary, NC) and used the R package ggplot for generation of graphical output.

3. Results

Of the 215 eligible men who were contacted, 73 (mean age 34.6 years) agreed to participate and provided 341 blood samples (average: five samples/man) (Supplementary Fig. 1). B₁HB₂-arm (26 men, 134 samples) were on non-DBP mesalamine medication at baseline for a median of 1 year. H₁BH₂-arm (47 men, 207 serum samples) were on their high-DBP mesalamine medication at baseline (H₁) for a median duration of 3 years. Men in the two arms had comparable characteristics (Table 1 and Figure 1).

Among the 60 men who participated in the full-protocol, 51 men (85%) crossed-over and 47 (78%) crossed-back; 89% and 77%, respectively, in B₁HB₂-arm, 82% and 79%, respectively, in H₁BH₂-arm. During the follow-up, nine men were lost follow-up, three in B₁HB₂-arm and six in H₁BH₂-arm (p-value= 0.64). The median crossover duration (between v2 & v3), and crossback duration (between v4 & v5) was 121 days. The median duration between visits on the same medication (v1 & v2), (v3 & 4) and (v5 & v6) was 16 days. All serum samples had detectable concentrations for all hormones (Table 2).

In the adjusted two-arm analyses (Table 3 & Supplementary Fig. 3), on average, when men in B₁HB₂-arm (Table 3A) were newly-exposed to high-DBP mesalamine, LH decreased by 13.9% (95% confidence interval (CI): -23.6, -3.0) and TT, FT, SHBG, inhibin-B, and FSH

decreased though not significantly. After crossback to their original non-DBP mesalamine, apart from SHBG which continued to decrease non-significantly, LH increased by 13.8% (CI: 0.5, 28.9), TT by 10.4% (CI: 0.4, 21.4), FT by 10.4% (CI: 0.2, 21.6), and inhibin-B and FSH marginally significantly increased. There was no carryover effect in B₁HB₂-arm. When men in H₁BH₂-arm (Table 3B) crossed-over to non-DBP mesalamine, on average, inhibin-B increased by 8.5% (CI: 2.4, 15.1) and continued to increase by an additional 7.7% (CI: 1.3, 14.5) after crossback to their original high-DBP mesalamine. LH and FSH decreased by 10.1% and 8.4%, respectively after crossover and increased by 9 to 10% after crossback (significantly for FSH). TT/LH, FT/LH and inhibin-B/FSH ratios increased significantly by 11 to 18% after crossover and decreased non-significantly after crossback despite non-significant changes in TT and FT.

In the cross-sectional analysis of values at baseline, on average, men in H₁BH₂-arm had lower TT, FT, LH, and FSH than men in the B₁HB₂-arm. Because of the wide range in duration on high-DBP mesalamine and to further explore duration on high-DBP mesalamine at baseline we divided the men in the H₁BH₂-arm at the median. At baseline, men on high-DBP mesalamine for less than 3 years (H₁<3 yrs) had higher TT, FT, and LH by 15–20% and lower by 18% for FSH (although none were statistically significant) than men in H₁BH₂-arm on high-DBP mesalamine for 3 years or more (H₁ ≥ 3 yrs). There was effect modification of the DBP exposure effect by the duration of high-DBP mesalamine at baseline for TT (p-value= 0.06), LH (p-value= 0.002) and FSH (p-value= 0.08) (data not shown).

Based on these results, we conducted a post-hoc three-arm analysis: H₁BH₂-arm, H₁<3 yrs (25 men, 107 serum samples) had an average H₁ duration of 1.2 years, H₁BH₂-arm, H₁ ≥ 3 yrs (22 men, 100 serum samples) had an average H₁ duration of 11.1 years (Supplementary Table 1 & Supplementary Fig. 2) and B₁HB₂-arm (Figure 2 & Supplementary table 2). We found heterogeneity of the exposure responses among the three arms. Although on average, men in H₁BH₂-arm, H₁ ≥ 3 yrs had no significant changes in hormones in crossover or crossback, men in H₁BH₂-arm, H₁<3 yrs had significant changes similar to men in the B₁HB₂-arm. When men in H₁BH₂-arm, H₁<3 years crossed-over, on average, inhibin-B increased by 13.2% (CI: 4.2, 22.9), and FSH decreased by 9.9% (CI: -17.9, -1.1), while TT/LH, FT/LH and inhibin-B/FSH ratios significantly increased by 18 to 26%. After crossback, inhibin-B had an additional increase of 11.3% (CI: 2.2, 21.3) and FSH marginally significantly increased by 9.7% (CI: -0.3, 20.8). Although not significant, there was a pattern after crossover that TT, FT, and SHBG increased and LH decreased. After crossback, SHBG decreased, TT, and FT had smaller additional increases, and LH increased. There was a significant carryover effect for FT, inhibin-B and inhibin-B/FSH ratio. Results were consistent across the adjusted and unadjusted analyses in both the primary and sensitivity analyses (Supplementary Tables 3 to 6, Figure 2 and Supplementary Figure 3).

4. Discussion

In our crossover-crossback study, on average men on non-DBP mesalamine at baseline (B₁HB₂-arm) and newly-exposed to high-DBP had decreased serum levels of testicular hormones (testosterone and inhibin-B) and gonadotropins (LH and FSH). After crossback to

their non-DBP mesalamine, both testicular hormone and gonadotropin concentrations increased exceeding baseline concentrations. In our post-hoc three-arm analysis, in the H₁BH₂-arm the duration on high-DBP mesalamine at baseline modified the hormone response. The hormone changes were only found in men who were exposed to high-DBP mesalamine for < 3yrs (H₁BH₂-arm, H₁<3 yrs) while there was no effect in those who had been receiving high-DBP mesalamine for 3 years (H₁BH₂-arm, 3 yrs). When men in the H₁BH₂-arm, <3 yrs crossed-over to non-DBP mesalamine, testicular hormones increased and gonadotropins decreased, suggesting a functioning pituitary negative feedback response. When they crossed-back to their original high-DBP mesalamine, their testicular hormones continued to increase but at slower rates while gonadotropins increased. TT/LH, FT/LH and inhibin-B/FSH ratios increased after crossover to non-DBP mesalamine, suggesting improved Leydig and Sertoli cell functions.

The decreases in testosterone and inhibin-B with high-DBP mesalamine new exposure in the B₁HB₂ arm are consistent with the decreases in semen quality parameters, primarily sperm motility seen in this same cohort⁵. However, after exposure removal for four months, in contrast to the observed reversal of hormonal changes, the semen quality decline was not reversed and instead further declined⁵. This may be explained by the long process of spermatogenesis²² which potentially may result in a lagged response to removal of high-DBP exposure. However, studies that follow sperm production over a longer follow up period will be required to confirm this hypothesis. Interestingly among men exposed to high-DBP mesalamine for a long duration before study entry (H₁BH₂-arm, H₁ 3 yrs), the four month period during the crossover to non-DBP mesalamine was inadequate to ‘reverse’ the inhibitory effect of high-DBP exposure on LH, FSH, testosterone and inhibin-B. Similarly, semen quality did not change in H₁BH₂-arm after removal of high-DBP exposure⁵. In a cross-sectional analysis, men exposed to high-DBP for a longer duration (H₁BH₂-arm, H₁ 3 yrs) had lower TT, FT, and LH concentrations than men exposed for a shorter duration (H₁BH₂-arm, H₁<3 yrs) or men who were not exposed at baseline (B₁HB₂-arm).

In experimental studies, peri-pubertal DBP-exposure altered differential expression of proteins involved in spermatogenesis and changed number and function of Sertoli and Leydig cells^{26,27}. Other studies in older animals showed that DBP preferentially targeted Sertoli cells²⁸. Studies during fetal development showed that DBP was anti-androgenic resulting in decreased testosterone production by Leydig cells due to interference with steroidogenesis, down-regulating gene and/or protein expression essential for the steroidogenic pathway, and cholesterol transport and metabolism^{29–31}. Suppression of both gonadotropin and testosterone production as well as decreased semen quality were observed in di(2-ethylhexyl)phthalate (DEHP) newly-exposed pre-pubertal and mature animals^{32,33}, similar to our results on men newly exposed to high-DBP mesalamine. In adult DEHP-exposed animals, pituitary LH and FSH protein expression decreased significantly^{34,35}. Decreased testosterone may indicate suppression of Leydig cell function and decreased inhibin-B may indicate suppression of Sertoli cell function, as well as germ cell production in adult testis³⁶.

In humans, both inhibin-B and FSH decreased with higher-DEHP exposure³⁷ suggesting that the endocrine communication between the pituitary and the testis might be

disrupted^{27,37} hence disruption of the hypothalamo-pituitary-testis (HPT) axis. Similarly, based on our results of the suppression of both testicular hormones and gonadotropins, we speculate that high-DBP exposure from mesalamine was associated with HPT axis disruption targeting the gonads and/or the pituitary in the newly exposed men. In the H₁BH₂-arm, H₁<3 yrs, gonadotropin negative feedback was partially regained after a relatively short removal of high-DBP exposure and partially compensated the testicular suppression after re-exposure. However, after longer durations of high-DBP exposure, as we observed in men in H₁BH₂-arm, H₁ ≥ 3 yrs, the reproductive hormones were suppressed and the hormonal responses may be desensitized to DBP exposure removal. Therefore, our results from the three arms suggest that there may be different effects of the hormone responses modified by the duration of high-DBP mesalamine history. One caveat is that there are limited comparable animal data to explain modes of action of high-DBP exposure in adult life, especially with such unique crossover-crossback design, and no data on the potentially modified hormone responses by the duration of exposure. In addition, many of the experimental animal studies only measured testosterone and not gonadotropins.

In epidemiological studies on the association between environmental DBP-exposures and reproductive hormones in adult men, the results were inconsistent with the main focus on testosterone. Urinary MBP was negatively correlated with testosterone among occupationally exposed Chinese men³⁸ and in infertility clinics in Slovenia³⁹, China⁴⁰, and India (only in the unadjusted analysis)⁴¹ but not in a study from Poland⁴². Both pooled and non-pooled analyses of infertile and fertile men in the US failed to find statistically significant associations^{17–19}. Only a few studies included men from the general population and found that urinary MBP was inversely associated with testosterone only in men aged 40–60 years from NHANES²⁰, but no evidence from studies in Denmark (only a significant increase in LH)²¹, Sweden⁴³, or China⁴⁴. All the studies described above were cross-sectional and used a single serum sample to measure the hormone concentrations at the same time when urinary MBP was measured. Janjua et al⁴⁵ conducted the only human study with some similarity to our design examining the association between DBP-exposure (dermally) and short term effects on reproductive hormones. They did not find associations of topical DBP-exposure with reproductive hormones. These results must be interpreted cautiously due to much lower anticipated exposure levels with topical exposure compared to our study, likely too short washout period (one week), and a small sample size (n=26).

Several studies have characterized DBP and other phthalates in coatings of many licensed medications including over-the-counter medications in the US^{9,10,13}, Canada⁴, United Kingdom⁶, Germany⁸ and China⁷. The urinary MBP in patients taking Asacol® was approximately 1000 times higher than the median levels reported for men in the US general population (NHANES)¹⁴. Therefore, we used a priori medication type as a proxy for high-DBP exposure⁵ rather than measuring urinary MBP concentrations which represent only exposure over the past several hours due to the short DBP half-life⁴⁵.

Our study had several potential limitations including the lack of randomization to mesalamine formulations at baseline. However, the cross-sectional results were consistent with the longitudinal associations. In addition, physicians do not prescribe mesalamine based on DBP in the enteric coating or in relation to hormonal concentrations or semen

quality, arguing against potential confounding by medication at baseline. Although our sample size was limited due to the innovative study and length of participation, the power of the study is derived from the unique design and the use of subjects as their own control. This avoids the purely cross-sectional analysis that may be confounded by inter-individual variability and lacks temporality. Post-hoc power analysis showed that the study had a sufficient number of participants to provide 80% power for detecting at least a 7% change in TT or FT, 8% in SHBG, 6% in inhibin-B and FSH and 9% in LH which were reasonable for a study of this design. Although there may be a concern of generalizing results from men with IBD, all men were classified as having mild IBD.

Our study had several important strengths including a unique innovative prospective design rarely conducted in environmental epidemiology studies. We were able to compare, within the same men, their hormone parameters during periods of high-DBP to background-DBP exposure and vice versa accounting for confounding by measured and unmeasured non-time-varying characteristics⁵. This is a major strength compared to cross-sectional studies. We had repeated measures up to six time points per man which increased the reliability of the measures. The different orders of exposure and non-exposure in the different arms gave us an additional opportunity to observe the reversibility of the changes under the study period. Our study was not restricted to infertile men compared to most of the previous literature. Confounding by indication was unlikely because mesalamine medications (with or without DBP coating) prescribed for treating IBD have the same active ingredient (mesalamine). We used the gold standard methods for hormone analyses especially for testosterone, which was accurately and precisely measured using LC-MS/MS methodology⁴⁶. Finally, we had the opportunity to examine high-DBP exposure (1000 times background) compared to environmental background exposure.

Our results are of a concern because testosterone deficiency in adult men is a risk factor not only for decreased semen quality and infertility but also for decreased libido, energy, bone mass, and muscle strength, and is associated with erectile dysfunction, osteoporosis, metabolic syndrome, and, cardiovascular disease, and hence increased mortality⁴⁷. Although manufacturers are required to disclose a listing of inactive ingredients in drug product labeling, they are not required to do so if the composition is a confidential trade secret i.e., used in patented delivery mechanisms (U.S. Pharmacopeial Convention 2009). The FDA may have information on the type and amount of phthalates used in specific approved drug products, but they do not make the information publically available because of its proprietary nature⁹. Recently, there was a consensus that women with IBD who are pregnant or might become pregnant may need to avoid high-DBP mesalamine⁴ leaving men of reproductive age with no recommendations and no guidance about any other medications that contain phthalate coating. Our results suggest the need to reconsider the use of DBP-containing mesalamine and other DBP-containing medications for men as well as improved education and access to information.

5. Conclusions

Although, some medications are a source of high-DBP exposure this source is relatively overlooked. Most studies of endocrine disrupting chemicals in men and women have

focused on background environmental exposures. To our knowledge, MARS is the only and the first study that examined the association between such high-DBP exposure and health outcomes. Our results present evidence that high-DBP exposure disrupted serum levels of pituitary and gonadal hormones that largely reversed after removal of exposure, but only in men with no history of or a short duration (<3 yrs) of previous high-exposure. Paradoxically, among men with longer durations of high-DBP exposure, removal of exposure did not reverse these effects within a 4 month follow up period, suggesting that long-term high-DBP exposure may alter the pituitary-gonadal axis and make it insensitive to changes in exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

DBP	dibutyl phthalate
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
BIDMC	Beth Israel Deaconess Medical Center
BWH	Brigham and Women's Hospital
MGH	Massachusetts General Hospital
B₁HB₂	Background ₁ -High-Background ₂ DBP exposure
H₁BH₂	High ₁ -Background-High ₂ DBP exposure

H₁BH₂-arm H₁<3 yrs, H₁BH₂-arm with duration on DBP-containing mesalamine medication at baseline <3 years

H₁BH₂-arm H₁ 3 yrs, *H₁BH₂-arm with duration* on DBP-containing mesalamine medication at baseline 3 years

BMI body mass index

Kg Kilogram

m meter

SD standard deviation

LMEM mixed effects models

N number of men

95% CI 95% Confidence Interval

TT total testosterone

FT free testosterone

LH luteinizing hormone

FSH follicle-stimulating hormone

SHBG sex hormone-binding globulin

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Highlights

- Mesalamine medications with coatings that contain dibutyl phthalate (DBP) lead to very high DBP exposure.
- New exposure to high DBP from mesalamine medications disrupted pituitary-gonadal hormones in adult men.
- Among men with no or short previous high DBP exposure, the hormone disruption was reversed after exposure removal.
- Long term high DBP exposure may alter the pituitary-gonadal axis and make it insensitive to removal of exposure.

Figure 1. Mesalamine And Reproductive health Study (MARS) Design

B₁HB₂-arm: **B₁** represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B₂** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H₁BH₂-arm:** **H₁** represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H₂** represents high-DBP exposure after crossback; included 47 men taking mesalamine containing DBP at baseline (25 men for less than 3 years and 22 men with 3 years or more).

Abbreviations: MARS, Mesalamine And Reproductive health Study; **DBP**, dibutyl phthalate.

Panel 2A. Unadjusted geometric means (95%CI)

Panel 2B. Adjusted geometric means (95%CI)

Panel 2A. Unadjusted geometric means (95%CI)

Panel 2B. Adjusted geometric means (95%CI)

Panel 2A. Unadjusted geometric means (95%CI)

Panel 2B. Adjusted geometric means (95%CI)

Figure 2. Geometric means (95%CI) of reproductive hormone parameters at baseline, crossover and crossback for the 3 arms (B₁H₁B₂-arm, H₁BH₂-arm with duration on DBP-containing mesalamine medication at baseline <3 years and H₁BH₂-arm with duration ≥ 3 years) –MARS Figure 2 shows the adjusted geometric means and 95% CI [adjusted for race (Caucasian or not), age (continuous), BMI (continuous), hour of sample draw (continuous), season (warm or cold), current smoking, age (continuous), and duration on DBP-containing mesalamine medication at baseline (in years)]. **B₁H₁B₂-arm**, **B₁** represents background-exposure at baseline, **H** represents high-DBP exposure after crossover and **B₂** represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline. **H₁BH₂-arm**, **H₁** represents high-DBP exposure at baseline, **B** represents background-exposure after crossover and **H₂** represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline. H₁BH₂-arm is subdivided into two arms based on the median duration on DBP-containing mesalamine medication at baseline (**period**) i.e., <3 years and ≥ 3 years

The grey lines represent B₁H₁B₂ arm, the black line represents H₁BH₂-arm, H₁<3 yrs, and the red line represents H₁BH₂-arm, H₁ ≥ 3 yrs.

Abbreviations: MARS, Mesalamine And Reproductive health Study; **95% CI**, 95% Confidence Interval; **DBP**, dibutyl phthalate; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone.

Table 1

Demographics of 73 men contributing 341 blood samples in the MARS Study by arm

	B₁HB₂-arm (26 men, 134 visits)	H₁BH₂-arm (47 men, 207 visits)	Total (73 men, 341 visits)	
	N(%) / Mean (SD), [Range]	N(%) / Mean (SD), [Range]	N(%) / Mean (SD), [Range]	P-value^b
Baseline characteristics (at Visit 1), # men (N)^a				
Duration on High-DBP mesalamine at baseline (H₁, years)	0	5.86 (6.16), [0.33, 24.1]	3.77 (5.68), [0, 24.1]	–
Age (Years)	33.9 (8.97), [20.2, 54.6]	34.9 (9.66), [19.6, 55.7]	34.6 (9.37), [19.6, 55.7]	0.82
Race				0.27
Caucasian	25 (96%)	38 (81%)	63 (86%)	–
Black/African American	1 (4%)	2 (4%)	3 (4%)	–
Asian	0	4 (9%)	4 (6%)	–
Other	0	3 (6%)	3 (4%)	–
BMI (Kg/m²)	25.3 (3.56), [19.4, 32.7]	26.4 (6.39), [19.5, 54.0]	26.0 (5.54), [19.4, 54.0]	0.88
BMI-categories				0.99
Normal weight (18.5 BMI<25)	14 (54%)	24 (51%)	38 (52%)	–
Overweight (25 BMI<30)	9 (35%)	16 (34%)	25 (34%)	–
Obese (BMI 30)	3 (11%)	7 (15%)	10 (14%)	–
Education				0.82
Below college	3 (14%)	6 (14%)	9 (14%)	–
College graduate	10 (48%)	16 (38%)	26 (41%)	–
Graduate degree	8 (38%)	20 (48%)	28 (45%)	–
Smoking status				0.01
Never smoker	22 (85%)	36 (77%)	58 (80%)	–
Former smoker	1 (4%)	11 (23%)	12 (16%)	–
Current smoker	3 (11%)	0	3 (4%)	–
Warm season at baseline^c	5 (19%)	20 (43%)	25 (34%)	0.07
IBD diagnosis				0.61
Ulcerative colitis	16 (62%)	32 (68%)	48 (66%)	–
Crohn's disease	10 (38%)	15 (32%)	25 (34%)	–
IBD score^d	1.65 (1.38), [0, 4]	1.19 (1.35), [0, 5]	1.36 (1.37), [0, 5]	0.13
Duration since IBD diagnosis (years)	9.00 (7.84), [0.28, 29.5]	10.8 (9.24), [0.7, 39.0]	10.1 (8.76), [0.28, 39.0]	0.41
Time-varying characteristics, # visits^a				
Warm season of blood draw^c	60 (45%)	94 (45%)	154 (45%)	0.91
Hour of the blood draw	9.68 (1.62), [7.25, 13.9]	9.72 (1.61), [7.17, 13.5]	9.71 (1.61), [7.17, 13.9]	0.81
Hour of the blood draw-categories				0.74
7 am to 10 am	74 (55%)	110 (53%)	184 (54%)	–
After 10 am to 2 pm	60 (45%)	97 (47%)	157 (46%)	–

B₁HB₂-arm, B₁ represents background-exposure at baseline, H represents high-DBP exposure after crossover and B₂ represents background-exposure after crossback; included 26 men taking mesalamine that did not contain DBP at baseline; 16 men (62%) on Lialda[®], 8 men (31%) on Pentasa[®], one man (4%) on Apriso[®] and one man (4%) was not on mesalamine medication at the time of recruitment.

H₁BH₂-arm, H₁ represents high-DBP exposure at baseline, B represents background-exposure after crossover and H₂ represents high-DBP exposure after crossback; included 47 men taking mesalamine-containing DBP at baseline; 24 men (51%) on Asacol[®] and 23 men (49%) on Asacol[®]HD.

^aN (%) for categorical/binary variables and mean (SD), [Range] for continuous variables

^bP-values are based on Fisher exact test for categorical variables and Kruskal Wallis test for continuous variables.

^cSeason of sample collection, Warm: April Through September

^dIBD score: included bowel frequency and urgency, presence of blood in the stool and general wellbeing. Mild IBD score: 5 or less on the simple clinical colitis activity index for UC and 4 or less on the Harvey-Bradshaw index for CD.

Abbreviations: MARS, Mesalamine And Reproductive health Study; BMI, body mass index; Kg, Kilogram; m, meter; SD, standard deviation; IBD, Inflammatory Bowel Disease; UC, ulcerative colitis; CD, Crohn's disease; N, number of men; DBP, dibutyl phthalate; B₁HB₂, Background₁-High-Background₂ DBP exposure; H₁BH₂, High₁-Background-High₂ DBP exposure.

Table 2

Hormone concentrations for 73 men (341 blood samples) in the MARS study

Hormone Parameters	Mean	SD	Geometric Mean	Minimum	5 th Perc	25 th Perc	50 th Perc	75 th Perc	95 th Perc	Maximum	QR
Total Testosterone (nmol/L)	17.2	6.18	16.1	4.41	8.36	13.3	16.3	21.1	28.4	40.5	7.88
Free Testosterone (pmol/L)	345	105	329	44.3	198	275	333	400	541	730	125
SHBG (nmol/L)	34.3	16.6	30.2	3.89	11.2	22.8	31.5	44.5	64.7	98.7	21.7
Inhibin-B (pg/mL)	191	77.6	176	52.0	81.8	134	171	241	334	432	107
LH (IU/L)	3.23	1.37	2.96	0.90	1.42	2.22	3.04	3.94	5.64	10.8	1.72
FSH (IU/L)	3.20	1.74	2.77	0.62	1.13	1.85	2.97	4.04	6.73	9.99	2.19
Total Testosterone/LH Ratio	6.10	2.94	5.45	1.78	2.46	3.93	5.40	7.92	11.7	17.7	3.99
Free Testosterone/LH Ratio	123	56.5	111	23.8	51.6	81.6	111	155	223	360	73.0
Inhibin-B/FSH Ratio	90.1	78.4	63.4	7.59	15.6	37.5	61.9	120	265	391	82.7

Abbreviations: MARS, Mesalamine And Reproductive health Study; **SD**, standard deviation; **Perc**, percentile; **QR**, quartile range; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone.

Adjusted means and percent changes (95% CI) of crossover, crossback and carryover on hormone concentrations among men starting on mesalamine medication not containing dibutyl phthalate (B₁HB₂-arm)^a

Adjusted B₁HB₂-arm [Background (B₁)- High (H)- Background (B₂) dibutyl phthalate exposure]: 26 men, 134 blood samples

Table 3A

Hormone serum concentrations	Fitted Geometric Means (95% CI)				Comparisons		
	Baseline (B ₁)	Crossover (H)	Crossback (B ₂)	Crossover % Change (95% CI) H-B ₁	P value	Crossback % Change (95% CI) B ₂ -H	Carryover % Change (95% CI) B ₂ -B ₁
Total Testosterone (nmol/L)	16.3 (14.2, 18.8)	15.6 (13.6, 18.0)	17.2 (14.9, 19.9)	-4.28 (-12.7, 4.88)	0.35	10.4 (0.37, 21.4)	5.64 (-4.29, 16.6)
Free Testosterone (pmol/L)	330 (292, 373)	322 (284, 365)	355 (312, 404)	-2.36 (-11.1, 7.19)	0.61	10.4 (0.16, 21.6)	7.77 (-2.53, 19.2)
SHBG (nmol/L)	29.6 (24.5, 35.7)	29.4 (24.3, 35.6)	28.8 (23.7, 34.9)	-0.52 (-10.3, 10.3)	0.92	-2.14 (-12.1, 8.91)	-2.65 (-12.9, 8.85)
Inhibin-B (pg/mL)	162 (137, 192)	161 (136, 190)	173 (146, 205)	-0.89 (-7.57, 6.27)	0.80	7.28 (-0.21, 15.3)	6.32 (-1.44, 14.7)
LH (IU/L)	3.29 (2.81, 3.85)	2.83 (2.42, 3.32)	3.22 (2.73, 3.80)	-13.9 (-23.6, -3.02)	0.01	13.8 (0.53, 28.9)	-2.04 (-13.8, 11.4)
FSH (IU/L)	3.23 (2.57, 4.07)	3.05 (2.42, 3.84)	3.30 (2.62, 4.17)	-5.79 (-12.8, 1.82)	0.13	8.35 (-0.04, 17.4)	2.08 (-6.19, 11.1)
Total Testosterone/L H Ratio	4.95 (4.13, 5.92)	5.50 (4.59, 6.6)	5.33 (4.42, 6.42)	11.1 (-1.11, 24.9)	0.08	-3.17 (-14.2, 9.33)	7.62 (-5.13, 22.1)
Free Testosterone/L H Ratio	100 (84.7, 119)	114 (95.9, 135)	110 (92.4, 132)	13.4 (-0.71, 29.6)	0.06	-3.04 (-15.6, 11.4)	9.98 (-4.68, 26.9)
Inhibin-B/FSH Ratio	50.0 (35.0, 71.5)	52.7 (36.8, 75.3)	52.3 (36.5, 75.0)	5.32 (-4.45, 16.1)	0.30	-0.68 (-10.2, 9.88)	4.61 (-5.93, 16.3)

Table 3B

Adjusted means and percent changes (95% CI) of crossover, crossback and carryover on hormone concentrations among men starting on mesalamine medication containing dibutyl phthalate (H₁BH₂-arm)^a

H₁BH₂-arm[High (H₁)-Background (B) - High (H₂)] dibutyl phthalate exposure]: 47 men, 207 blood samples

Hormone serum concentrations	Fitted Geometric Means (95% CI)					Comparisons		
	Baseline (H ₁)	Crossover (B)	Crossback (H ₂)	Crossover % Change (95% CI) B- H ₁	P value	Crossback % Change (95% CI) H ₂ -B	P value	Carryover % Change (95% CI) H ₂ -H ₁
Total Testosterone (nmol/L)	16.0 (14.5, 17.7)	15.9 (14.3, 17.7)	17.0 (15.3, 19.0)	-0.58 (-7.86, 7.27)	0.88	7.13 (-1.18, 16.1)	0.09	6.50 (-1.59, 15.3)
Free Testosterone (pmol/L)	316 (290, 345)	324 (294, 358)	343 (311, 380)	2.52 (-5.1, 10.8)	0.53	5.90 (-2.49, 15.0)	0.17	8.57 (0.18, 17.7)
SHBG (nmol/L)	31.7 (27.7, 36.3)	33.2 (28.8, 38.3)	31.7 (27.4, 36.6)	4.65 (-3.97, 14.1)	0.30	-4.50 (-12.8, 4.58)	0.32	-0.05 (-8.60, 9.30)
Inhibin-B (pg/mL)	171 (152, 193)	185 (164, 210)	200 (176, 226)	8.52 (2.37, 15.1)	0.006	7.71 (1.31, 14.5)	0.02	16.9 (10.0, 24.2)
LH (IU/L)	2.96 (2.65, 3.31)	2.66 (2.35, 3.01)	2.90 (2.55, 3.29)	-10.1 (-18.6, -0.78)	0.04	8.93 (-1.98, 21.1)	0.11	-2.09 (-11.7, 8.51)
FSH (IU/L)	2.64 (2.24, 3.11)	2.42 (2.05, 2.87)	2.65 (2.24, 3.14)	-8.39 (-14.2, -2.23)	0.009	9.63 (2.40, 17.4)	0.008	0.43 (-6.14, 7.46)
Total Testosterone/LH Ratio	5.41 (4.76, 6.15)	6.01 (5.23, 6.90)	5.90 (5.12, 6.80)	11.0 (0.73, 22.3)	0.04	-1.80 (-11.4, 8.86)	0.73	9.01 (-1.47, 20.6)
Free Testosterone/LH Ratio	107 (94.9, 120)	122 (107, 139)	118 (103, 136)	14.0 (2.07, 27.2)	0.02	-2.74 (-13.6, 9.42)	0.64	10.8 (-1.16, 24.3)
Inhibin-B/FSH Ratio	64.7 (50.1, 83.4)	76.6 (59.1, 99.2)	75.2 (58.0, 97.5)	18.4 (9.12, 28.5)	<0.0001	-1.80 (-9.84, 6.96)	0.68	16.3 (6.82, 26.6)

^a Adjusted for race (Caucasian or not), age (continuous), BMI (continuous), hour of sample draw (continuous), season (warm or cold), current smoking, age (continuous), and duration on DBP-containing mesalamine medication at baseline (in years) in the mixed effect model.

^b Percentage changes are presented by exponentiating the beta coefficient. Negative sign means % decrease for the corresponding outcome compared to the measure in the previous period.

^c B₁BH₂-arm: B₁ represents background low-DBP exposure at baseline, H represents high-DBP exposure after crossover and B₂ represents background low-exposure after crossback.

^d H₁BH₂-arm: H₁ represents high-DBP at baseline, B represents background-DBP exposure after crossover and H₂ represents high-DBP after crossback.

Abbreviations: MARS, Mesalamine And Reproductive health Study; N, number of men; B₁HB₂, Background1-High-Background2 DBP exposure; H₁BH₂, High1-Background-High2 DBP exposure; 95% CI, 95% Confidence Interval; **DBP**, dibutyl phthalate; **SHBG**, sex hormone-binding globulin; **LH**, luteinizing hormone; **FSH**, follicle-stimulating hormone.