Phthalate exposure and semen quality in fertile US men

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Abstract

Several experimental and observational studies have demonstrated the antiandrogenicity of several phthalates. However, there is limited evidence of an association between phthalate exposure in adult life and semen quality. The aim of this study was to examine phthalate exposure during adulthood in relation to semen quality in fertile US men. This multi-center cross-sectional study included 420 partners of pregnant women who attended a prenatal clinic in one of five U.S. cities during 1999–2001. Nine phthalate metabolites [mono (2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono (2-ethyl-5-carboxypentyl) phthalate (MECPP)], as well as mono-n-butyl phthalate (MBP) and mono-isobutyl phthalate (MiBP), mono (3 carboxypropyl) phthalate (MCPP), monobenzyl phthalate (MBzP) and monoethyl phthalate (MEP)] were measured in urine collected at the same

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time as the semen sample. We regressed natural log-transformed (ln) sperm concentration, ln(total sperm count), ln(total motile sperm count), percent motile sperm and percent sperm with normal morphology on each of the nine natural log-transformed metabolite concentrations and on the molar-weighted sum of DEHP metabolites in separate models. We fit unadjusted models and models that adjusted for confounders determined *a priori*. In unadjusted models, ln(MiBP) was significantly and positively associated with motility and ln(MBzP) significantly negatively associated with ln(total sperm count). In adjusted linear models, urinary metabolite concentrations of DEHP, DBP, DEP, and DOP were not associated with any semen parameter. We found an inverse association between ln(MBzP) concentrations and sperm motility (β = −1.47, 95% CI: −2.61, −0.33), adjusted for ln(creatinine concentration), geographic location, age, race, smoking status, stress, recent fever, time from sample collection and time to complete analysis. Several sensitivity analyses confirmed the robustness of these associations. This study and the available literature suggest that impacts of adult exposure to phthalates at environmental levels on classical sperm parameters are likely to be small.

Keywords

endocrine disruption; epidemiology; phthalates; reproduction; semen quality

Introduction

Phthalate esters are pervasive environmental chemicals present in food and many commonly used products (plastics, food contact applications, wall coverings, varnishes, lacquers or personal-care products) (ATSDR, 2002) and manufactured in such quantity that exposure is ubiquitous (CDC, 2015). Exposures through ingestion, dermal contact or inhalation are considered the main important routes of exposure (Meeker *et al.*, 2009). Animal studies have shown that exposure to certain phthalates during fetal sexual differentiation reduces fetal testosterone (Welsh *et al.*, 2008). As a result, male pups exhibit a cluster of altered androgen-dependent anatomical features that reflect disordered sex differentiation, including a reduced – that is, a less masculine – anogenital distance (AGD), impaired testicular descent, and reduced testicular size. This cluster of alterations has been referred to as the "phthalate syndrome", which in male rodents, has been shown to have adverse consequences for later sexual development (Foster, 2006). In humans, an association between prenatal exposure to certain phthalates and a similar cluster of reproductive developmental outcomes, most notably a shorter AGD, has been reported in male infants (Swan *et al.*, 2005; Swan, 2008, Swan *et al.*, 2015). Most observational studies show limited or weak evidence of a relationship between impaired semen quality in adulthood and phthalate exposures, including dibutyl phthalates (DBPs), benzyl butyl phthalate (BzBP) and di(2-ethylhexyl) phthalate (DEHP) metabolites (Duty *et al.*, 2003, 2005; Hauser *et al.*, 2006; Han *et al.*, 2014; Herr *et al.*, 2009; Jonsson *et al.*, 2005; Jurewicz *et al.*, 2013; Lenters *et al.*, 2015; Liu *et al.*, 2012; Pant *et al.*, 2014; Specht *et al.*, 2014; Wirth *et al.*, 2008).

In this analysis, we use linear regression to examine the associations between phthalate urinary metabolite concentration in adult men and four measures of semen quality. We use data collected from partners of pregnant women in the Study for Future Families, a large

multi-center study designed to look at geographic variability in semen quality (Redmon *et al.*, 2013). This population differs from those in most previous studies of phthalates exposure and semen quality in which participants were mainly recruited at infertility clinics (Duty *et al.*, 2005; Hauser *et al.*, 2006; Herr *et al.*, 2009; Jonsson *et al.*, 2005; Jurewicz *et al.*, 2013; Pant *et al.*, 2014; Wirth *et al.*, 2008). To the best of our knowledge, these associations have previously been assessed in fertile men in only one European study (Lenters *et al.*, 2015; Specht *et al.*, 2014).

Material and Methods

Study Population

The Study for Future Families (SFF) is a multi-center study of partners of pregnant women recruited in Los Angeles, CA (Harbor-UCLA Medical Center and Cedars-Sinai Medical Center), Minneapolis, MN (University of Minnesota Medical Center), Columbia, MO (University Physicians), Iowa City, IA (University of Iowa), and New York City, NY in 1999–2001. Only couples whose pregnancies were conceived without medical intervention were eligible. Men were asked to give two semen samples and one blood and one urine sample. In this analysis we report on semen parameters from the first semen sample in 420 men and phthalates in urine samples provided on the same day.

Ethical approval

Human subject committees at all participating institutions approved SFF and all subjects signed informed consents. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research. Further details of the study design have been described previously (Swan *et al.*, 2003, Stokes-Riner *et al.*, 2007, Redmon *et al.*, 2013).

Urine collection and phthalate metabolite analysis

Men were requested to give a urine sample at the first study visit. Urine samples were collected in phthalate-free polypropylene cups and all collection and storage materials were shown to be phthalate-free. Samples were stored at −20°C or −80°C before shipment to the Division of Laboratory Sciences, National Center for Environmental Health, CDC. Metabolites were measured at the Division of Laboratory Sciences, National Center for Environmental Health, CDC (Atlanta, GA), where laboratory staff had no access to participant data. The laboratory analysis involved the enzymatic deconjugation of the phthalate metabolites from their glucuronidated form, automated on-line solid-phase extraction coupled with separation with high-performance liquid chromatography, and detection by isotope-dilution tandem mass spectrometry. This high-throughput method allows for the concurrent quantification in human urine of the phthalate metabolites reported here. Further details of the analytical approach have been described previously (Silva *et al.*, 2007). For this analysis we considered nine phthalate metabolites measured in SFF: four metabolites of DEHP [mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)], as well as mono-n-butyl phthalate (MBP), and mono-isobutyl phthalate (MiBP), metabolites of dibutyl phthalate (DBP);

mono(3 carboxypropyl) phthalate (MCPP), a metabolite of di(n-octyl) phthalate (DOP), monobenzyl phthalate (MBzP), a metabolite of butylbenzyl phthalate (BBzP) and monoethyl phthalate (MEP), a metabolite of diethyl phthalate (DEP). We also created a molar-weighted sum of DEHP metabolites, where the weight for each of the four DEHP metabolites is the inverse of its molecular weight. Limits of detection (LOD) ranged from 0.2 to 1.2 ng/ml.

Semen collection and analysis

Men were requested to observe a two to five day abstinence period before providing a semen sample, and at the time of the visit the importance of accurately reporting the actual abstinence period was stressed. At the study visit, men collected semen samples by masturbation in the clinic, and on average the samples were analyzed within 27 minutes of collection (Stokes-Riner *et al.*, 2007). We examined five semen quality parameters: sperm concentration, percent motile sperm, percent sperm with normal morphology, total sperm count (TSC) (specimen weight × sperm concentration), and total motile sperm count (TMSC) (specimen weight × sperm concentration × motility). Methods used for semen evaluation and quality control of laboratory methods have been described previously (Brazil *et al.*, 2004). Briefly, ejaculate volumes were estimated by specimen weight, assuming a semen density of 1.0 g/mL and concentration assessments were evaluated manually using MicroCell counting chambers. The percent motile sperm was determined in a MicroCell (Conception Technologies, San Diego, California) chamber and refers to total motility: the percent of sperm with any flagellar movement, whether twitching or progressive (Overstreet and Brazil, 1997). For MicroCell counting, semen was diluted with equal parts of fixative to immobilize the sperm. The final concentration was the mean of the concentration values from the separate dilutions of 2 drops, when these differed by less than 10%. If the duplicate concentrations varied by more than 10%, a third dilution was prepared and counted and the median of the 3 values used as the estimate of concentration. On average, 200–300 sperm were counted for each dilution. Seminal smears were prepared at the clinical centers and shipped to the Andrology Coordinating Center at the University of California, Davis, for Papanicalou staining, analysis, and storage, where a single technician assessed sperm morphology using the strict morphology method, recommended by the World Health Organization (Guzick *et al.*, 2001; WHO, 1999).

Statistical methods

Phthalate metabolites concentrations were natural log-transformed to normalize their distribution. Sperm concentration, TSC, and TMSC were also natural log-transformed in order to better satisfy model assumptions. Statistical programming was performed in SAS (SAS, Cary, N.C) and R 3.0.2 (R Foundation for Statistical Computing, www.r-project.org).

Each of the five semen parameters [ln(concentration), percent motile sperm, percent sperm with normal morphology ln(TSC), and ln(TMSC)] was regressed on each of the nine natural log-transformed metabolites concentrations [ln(MEHP), ln(MEHHP), ln(MEOHP), ln(MECPP), ln(MBP), ln(MiBP), ln(MCPP), ln(MBzP), ln(MEP)] and on the molar-weighted sum of DEHP metabolites in separate models; metabolite concentrations below the LOD were assigned the value LOD/sqrt(2) for data analysis (Hornung and Reed, 1990). With the exception of ln(MBP), all creatine-adjusted ln(phthalate metabolites) differed

significantly across locations (data not shown). In addition, since sperm concentration and motility were determined locally and some differences remained despite tight quality control procedures (Brazil *et al.*, 2004), we adjusted for geographic location (MO, CA, MN, NY, IA, coded as dummy variables) in all primary models. We also adjusted for age (years), and used binary variables for race (black/not black), current smoking status (yes/no), stress [number of stressful life events in 3 months prior to sample collection (< 2 vs.   2 events) (Gollenberg et al. 2010)] and recent fever (yes/no). The natural logarithm of creatinine concentration was used to control for urine sample dilution. Ln(creatinine) was used because the relationships between metabolites and creatinine were only linear when both were natural log-transformed, and this transformation is consistent with other papers (Mendiola *et al.*, 2010). In addition, models for ln(sperm concentration), ln(TSC) and ln(TMSC) were adjusted for abstinence time (as a continuous variable); models for motility were adjusted for time from sample collection to analysis, and time to complete analysis because motility decreases with time from sample collection; and models for morphology were adjusted for time to complete analysis. Covariates were selected *a priori* to be consistent with previous decisions about covariate adjustment for these outcomes in the SFF cohort (Swan *et al.*, 2003, Mendiola *et al.*, 2010; Redmon *et al.*, 2013); all covariates selected were included in the final models. Standard residual-based plots were examined to assess the regression modeling assumptions of constant residual variance, linearity between covariates and outcome and normality of the residuals. We also flagged extreme outliers and influential observations (as assessed by Cook's distance), and if warranted, refit the model without these observations (Weisberg, 2005).

In order to assess the influence of several factors that might have affected results, we carried out four sensitivity analyses for the three primary outcomes ln(concentration), motility, and morphology. We fit models without adjusting for geographic location. Because only eight subjects had a fever, we also fit models that did not adjust for fever. A third sensitivity analysis excluded men whose creatinine concentration was below 30 or above 300 (WHO, 1996). Finally, to assess sensitivity to using a fixed value of metabolites below the LOD, we fit models that excluded subjects with values below the LOD for the metabolite in the model.

Results

Of the 950 men who participated in SFF, 805 gave at least one semen sample. Because urine was not collected until the second year of the study only 533 men provided urine samples. Phthalate metabolite measurements and semen quality were available for 441 of these 533 men. Of these, eight subjects who reported abstinence time greater than 240 hours were excluded. We excluded an additional 13 subjects who were missing data on one or more model covariates. Demographic characteristics and summaries of semen quality parameters of the 420 SFF participants included in this analysis are presented in Table 1. Summary statistics of urinary concentrations of phthalate metabolites are presented in Table 2. Correlations between the natural logarithm of the urinary concentrations of the four DEHP metabolites ranged from 0.84 to 0.99 and correlations between the logarithm of pairs of metabolites from different phthalates ranged from 0.23 to 0.77 for ln(MBP) and ln(MiBP). After adjusting for ln(creatinine), correlations between metabolites were somewhat lower,

ranging from 0.84 to 0.95 for pairs of DEHP metabolites, and from −0.06 (for ln(MEP) and most other metabolites) to 0.53 for ln(MBP) and ln(MiBP).

Coefficients and corresponding 95% confidence intervals for the association between each semen parameter and each ln(metabolite) concentration, from separate models, are shown without adjustment for other variables in Table 3, and in models that adjusted for confounding variables in Table 4. In the unadjusted models, ln(MiBP) was positively associated with motility and ln(MBzP) was negatively associated with total sperm count. In adjusted models, we found no statistically significant associations between any of the semen parameters and any of the metabolites concentrations, with the exception of a inverse relationship between ln(MBzP) and sperm motility. Our checks for the regression assumptions showed that model assumptions were reasonably well met. There were no extreme outliers (all standardized residuals were less than 4 in absolute value) and no influential observations (Cook's distances were all less than 0.11). Figure 1 shows the relationships between each ln(metabolite) and ln(concentration), morphology, and motility from the adjusted models, when all variables are put on a common scale by subtracting the variable-specific mean and dividing by the variable-specific standard deviation. Figure 2 shows the relationship between motility and ln(MBzP) with the superimposed regression line and 95% confidence interval from the covariate-adjusted regression.

In models using ln(molar-weighted DEHP metabolites), stress was associated with a lower ln(concentration) (slope: −0.20, 95% CI: −0.37, −0.02), fever was associated with a lower ln(concentration) (slope: −0.72, 95% CI: −1.26, −0.18), abstinence time was associated with a higher ln(concentration) (slope: 0.005, 95% CI: 0.002, 0.007), and time to complete the analysis was associated with a lower motility (slope: −0.38, 95% CI: −0.51, −0.26). Center was also a very significant predictor of sperm motility (p<0.0001 from the 4 degree of freedom test), with Missouri associated with much lower motility than the other centers. The covariate associations were similar when other metabolites were used in place of the molar-weighted sum of DEHP metabolites.

Results from the four sensitivity analyses showed that there were no significant associations between semen parameters and phthalate metabolites in any of the covariate-adjusted models considered other than the inverse relationship between ln(MBzP) and sperm motility. Ln(MBzP) was a significant predictor of motility in all sensitivity analyses.

Discussion

The present study did not find any association between urinary concentrations of phthalate metabolites and any classical semen quality parameters in multiple regression models, adjusted for appropriate covariates except for an inverse association between ln(MBzP) and sperm motility. While the robustness of this latter association was confirmed by several sensitivity analyses, based on the number of tested associations we cannot rule out the possibility that the observed significant association could be a chance finding. This is the first study to examine this association in a U.S. population of fertile men. Our results extend previous findings in selected populations, mainly of infertile men.

Several animal toxicology studies report that a number of phthalates are testicular toxicants and impair spermatogenesis (Aly *et al.*, 2015; Uren-Webster *et al.*, 2010). Based on results in several animal species DEHP and DBP metabolites appear to have the capacity to disrupt normal reproduction (semen quality, etc.) (Fabjan et al., 2006). However, previous results and ours show only weak or no associations between human adult exposure to phthalates and impaired semen quality. Several investigations found inconsistent or weak associations between sperm concentration, motility or morphology and urinary phthalate metabolites (mainly DEHP, DBPs or DEP) in young unselected men (Jonsson *et al.*, 2005; Joensen et al., 2012), men from the general population (Han *et al.*, 2014), fertile men (Lenters *et al.*, 2015; Specht *et al.*, 2014) or male partners of subfertile couples (Wirth *et al.*, 2008; Liu *et al.*, 2012). These results are consistent with Herr et al. (2009) who looked at this question in men referred for fertility work-up and found no association between the sum of four urinary DEHP metabolites (MEHP, MEHHP, MEOHP and MECPP) and any sperm parameter. In contrast, significant inverse relationships between exposure to DBPs and both sperm concentration and motility were seen in a study of men who are part of a couple seeking an infertility work-up (Hauser *et al.*, 2006). Using data from an earlier sample of these men, Duty *et al.* (2003) described a dose-response relation between exposure to DBPs and sperm motility and concentration. In similar potentially infertile male populations, Jurewicz and collaborators (2013) and Pant and coworkers (2014) showed an inverse association between urinary DEHP metabolites and sperm motility. Finally, in a recent meta-analysis (14 studies) looking at the associations between phthalates or their metabolite levels and human semen quality, Cai and colleagues (2015) found some evidence of an inverse relationship between specific phthalate exposures and impaired semen quality (e.g. MBP, MBzP or MEHP), but also noted that there were several cases where associations between phthalates and semen quality were not found (e.g. MMP).

These differences across studies may reflect population differences, or chance. The SFF cohort only included partners of pregnant women, who have better than average semen quality, while other cohorts included men from couples presenting to infertility clinics, who tend to have poorer semen quality. These data, coupled with other published literature, suggest that phthalate exposure during adulthood is not a major effector of classical parameters of semen quality. However, phthalates might alter other measures related to sperm quality (e.g. measures of DNA damage, alteration of gene expression) (Spade *et al.*, 2014).

We compared unadjusted urinary concentrations of the BBzP metabolite in our men to those from male participants in the 2001–2002 National Health and Nutrition Examination Survey (NHANES) (CDC, 2015). Geometric mean MBzP concentration was almost equal in both populations; 11.2 ng/mL compared with 11.1 ng/mL for male NHANES and our study population, respectively. Several previous studies have also suggested that exposure to phthalates may affect reproductive hormones. These results, observed across different designs and study populations (young, infertile or fertile men) indicate harmful effects of DEHP and diisononyl phthalate metabolites on the reproductive axis, in particular with regard to free and total testosterone and estradiol levels (Joensen et al., 2012; Meeker et al., 2009; Mendiola et al. 2011, 2012; Specht et al., 2014).

Our data were limited by the use of a single spot urine sample to assess phthalate exposure and a single semen sample, which does not capture the day-to-day variability within the same subject. Furthermore, the time when spermatogenesis occurred likely preceded the time urine was collected and exposure assessed. However, while urinary concentrations of phthalate metabolites are variable, because exposure is ubiquitous metabolite concentrations in multiple samples from the same subject tend to fall in the same quartile of their distribution (Hauser et al., 2004).

With regard to use of a single semen sample per man, there is evidence that as long as the model adjusts for important covariates, semen parameters from the first and the second sample are not significantly different from each other (Stokes-Riner *et al.* 2007). Future analyses could extend the modeling to accommodate repeated semen samples and phthalate metabolite concentrations from each participant.

In conclusion, we did not find associations between phthalate exposure in adulthood and classical semen quality parameters in fertile US men, with the exception of an association between MBzP and decreased motility. This study and the available literature suggest that impacts of adult exposure to phthalates at environmental levels on classical sperm parameters are likely to be small.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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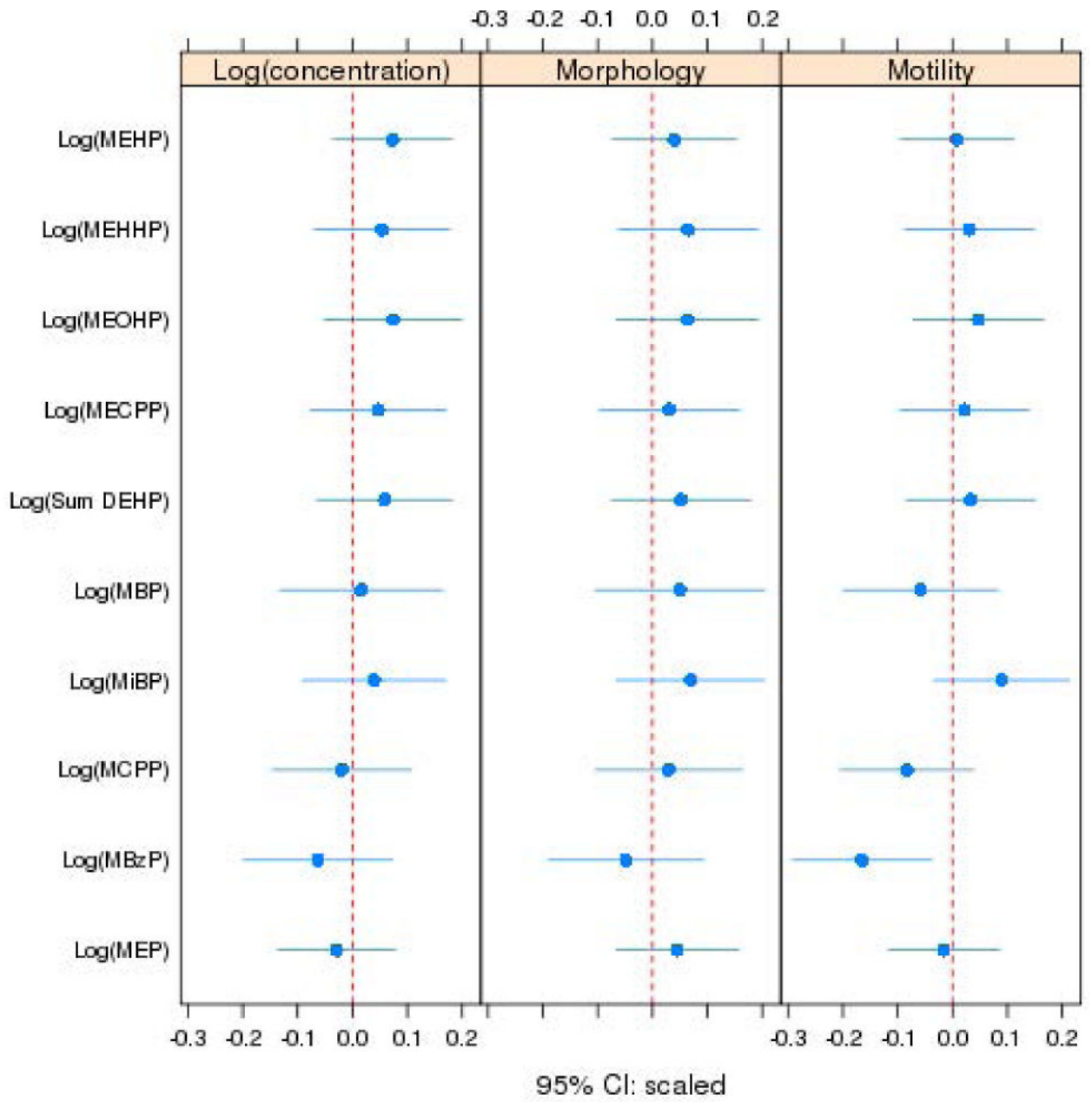
Figure 1.
Point estimates and 95% confidence intervals for the relationship between the ln-metabolites and each semen quality outcome from adjusted regression models, with all variables centered and scaled.
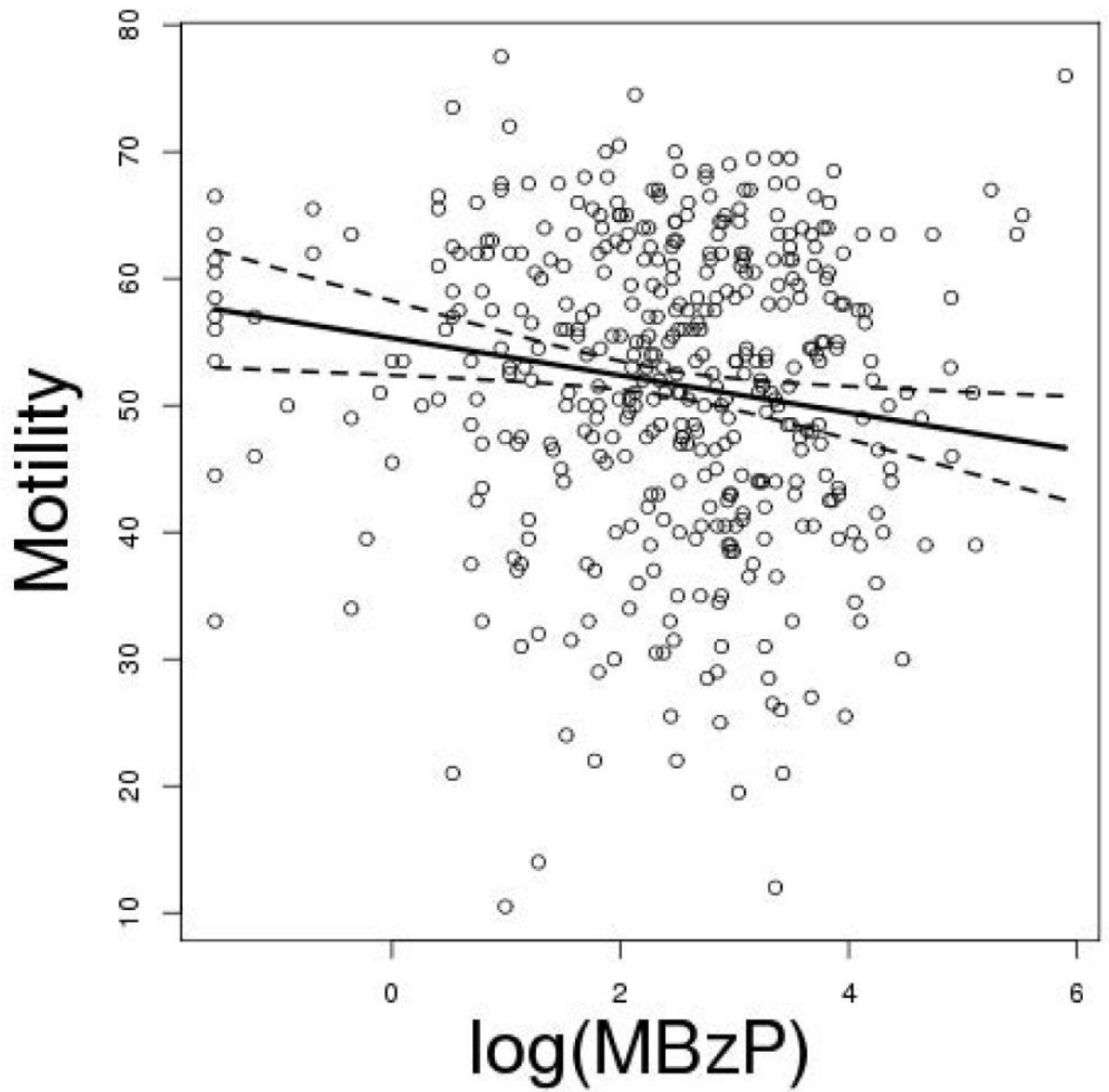
Figure 2.
Scatterplot of motility versus ln(MBzP), with superimposed regression line and 95%
confidence interval from the covariate-adjusted model.

Table 1

Characteristics of study population, partners of pregnant women in five US cities (N = 420)

Characteristics	Mean	SD	Min	Median	Max
Age (years)	32	6	18	32	53
Abstinence time (hours)	77	30	9	72	227
Time to start semen analysis (minutes)	27	10	0	28	90
Time to complete semen analysis (minutes)	58	9	24	58	97
Sperm concentration (10^6/ml)	66	45	3	59	316
Sperm motility (%)	52	12	10	54	78
Morphology (%)	11	5	0.5	10	26
Total sperm count (10^6)	297	211	6	246	1218
Total motile sperm count (10^6)	134	103	0.5	114	627
Creatinine concentration (mg/dl)	143	81	7	138	509
	N	%			
WHO reference values[a]					
Sperm concentration (10^6/ml) <20×10^6/ml	46	11			
Sperm motility (%) < 50%	160	38			
Morphology (%) < 4%	33	8			
Total sperm count (10^6) <40×10^6/ml	27	6			
Center: CA	114	27			
Center: MN	127	30			
Center: MO	88	21			
Center: NY	18	4			
Center: IA	73	17			
Race: Black	35	8			
Current smoker	91	22			
Stress	100	24			
Recent fever	8	2			

SD: Standard deviation.

[a] Number (%) of subjects below the WHO (1999) reference values for each semen parameter.

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Table 2

Summary statistics for the urinary phthalate metabolites (in ng/mL)

Metabolite	Geometric mean	Min	Max	Limit of Detection (LOD)	%> LOD
MEHP	3.61	0.85	403	1.20	77
MEHHP	22.87	0.49	2600	0.70	99
MEOHP	12.59	0.49	1320	0.70	97
MECPP	32.78	0.90	3250	0.60	100
MBP	15.28	0.42	388	0.60	98
MiBP	2.80	0.21	83	0.30	92
MCPP	2.09	0.14	86	0.20	96
MBzP	11.13	0.21	364	0.30	98
MEP	200.96	0.80	14700	0.80	100

Table 3

Estimated coefficients (95% confidence intervals) from separate unadjusted linear regression models of each semen parameter on the natural logarithm of each phthalate metabolite concentration (N=420). Significant associations (p<0.05) are bolded.

Metabolite	Log(Conc)	95% CI	Motility	95% CI	Morphology	95% CI	Log(TSC)	95% CI	Log(TMSC)	95% CI
MEHP	0.02	(−0.04, 0.08)	0.48	(−0.42, 1.38)	0.18	(−0.21, 0.57)	0.00	(−0.06, 0.06)	0.01	(−0.07, 0.09)
MEHHP	0.01	(−0.05, 0.07)	0.67	(−0.18, 1.52)	0.18	(−0.19, 0.55)	−0.01	(−0.07, 0.05)	0.01	(−0.07, 0.09)
MEOHP	0.02	(−0.04, 0.08)	0.77	(−0.08, 1.62)	0.18	(−0.19, 0.55)	0.00	(−0.06, 0.06)	0.02	(−0.06, 0.10)
MECPP	0.00	(−0.06, 0.06)	0.68	(−0.24, 1.60)	0.12	(−0.29, 0.53)	−0.01	(−0.09, 0.07)	0.00	(−0.08, 0.08)
Molar Sum DEHP	0.01	(−0.05, 0.07)	0.72	(−0.16, 1.60)	0.17	(−0.22, 0.56)	−0.01	(−0.07, 0.05)	0.01	(−0.07, 0.09)
MBP	−0.01	(−0.07, 0.05)	0.35	(−0.61, 1.31)	0.13	(−0.30, 0.56)	−0.04	(−0.12, 0.04)	−0.03	(−0.11, 0.05)
MiBP	0.01	(−0.05, 0.07)	**1.01**	**(0.13, 1.89)**	0.17	(−0.22, 0.56)	−0.02	(−0.08, 0.04)	0.00	(−0.08, 0.08)
MCPP	−0.03	(−0.11, 0.05)	−0.19	(−1.25, 0.87)	0.08	(−0.39, 0.55)	−0.04	(−0.12, 0.04)	−0.04	(−0.14, 0.06)
MBzP	−0.04	(−0.10, 0.02)	−0.50	(−1.36, 0.36)	−0.07	(−0.44, 0.30)	**−0.07**	**(−0.13, −0.01)**	−0.07	(−0.15, 0.01)
MEP	−0.03	(−0.09, 0.03)	0.31	(−0.46, 1.08)	0.17	(−0.16, 0.50)	−0.05	(−0.11, 0.01)	−0.03	(−0.09, 0.03)

Table 4

Estimated coefficients (95% confidence intervals) from separate covariate-adjusted linear regression models of each semen parameter on the natural logarithm of each phthalate metabolite concentration (N=420)[1].

Metabolite	Log(Conc)	95% CI	Motility	95% CI	Morphology	95% CI	Log(TSC)	95% CI	Log(TMSC)	95% CI
MEHP	0.05	(−0.02, 0.11)	0.08	(−0.89, 1.05)	0.16	(−0.31, 0.63)	0.04	(−0.04, 0.11)	0.04	(−0.05, 0.13)
MEHHP	0.03	(−0.04, 0.10)	0.27	(−0.76, 1.31)	0.25	(−0.25, 0.74)	0.03	(−0.04, 0.11)	0.04	(−0.06, 0.13)
MEOHP	0.04	(−0.03, 0.12)	0.41	(−0.64, 1.46)	0.24	(−0.26, 0.74)	0.05	(−0.03, 0.13)	0.06	(−0.04, 0.15)
MECPP	0.03	(−0.05, 0.11)	0.21	(−0.92, 1.35)	0.13	(−0.41, 0.67)	0.03	(−0.05, 0.12)	0.03	(−0.07, 0.14)
Molar Sum DEHP	0.04	(−0.04, 0.11)	0.30	(−0.80, 1.41)	0.21	(−0.32, 0.74)	0.04	(−0.04, 0.12)	0.04	(−0.06, 0.14)
MBP	0.01	(−0.09, 0.11)	−0.58	(−2.00, 0.84)	0.22	(−0.46, 0.91)	0.00	(−0.11, 0.11)	−0.02	(−0.15, 0.11)
MiBP	0.02	(−0.06, 0.11)	0.82	(−0.31, 1.96)	0.28	(−0.27, 0.83)	0.01	(−0.07, 0.10)	0.02	(−0.08, 0.13)
MCPP	−0.01	(−0.11, 0.08)	−0.92	(−2.28, 0.43)	0.15	(−0.51, 0.80)	0.01	(−0.09, 0.11)	−0.03	(−0.15, 0.10)
MBzP	−0.04	(−0.12, 0.04)	**−1.47**	**(−2.61, −0.33)**	−0.19	(−0.75, 0.36)	−0.06	(−0.14, 0.03)	−0.09	(−0.19, 0.02)
MEP	−0.02	(−0.07, 0.04)	−0.13	(−0.95, 0.69)	0.16	(−0.24, 0.55)	0.01	(−0.05, 0.07)	0.01	(−0.07, 0.09)

[1] All models adjusted for the natural logarithm of creatinine concentration, geographic location, age, race, smoking status, stress and recent fever. Models for sperm concentration, total sperm count (TSC) and total motile sperm count (TMSC) also adjusted for abstinence time; models for motility also adjusted for time from sample collection to analysis and time to complete analysis; and models for morphology also adjusted for time to complete analysis. Phthalate metabolites and sperm counts were natural log-transformed. Significant associations (p<0.05) are bolded.