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Phthalate exposure and reproductive parameters in young men from the general Swedish population

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ABSTRACT

Background: In animals, exposure to certain phthalates negatively affects the male reproductive function. Human results are conflicting and mostly based on subfertile males, in whom the association between exposure and reproductive function may differ from the general population.
Objectives: To study if levels of phthalate metabolites were associated with semen quality and reproductive hormones in general Swedish men.
Methods: We recruited 314 young men delivering semen, urine and blood samples at the same visit. We analyzed reproductive hormones and several semen parameters including progressive motility and high DNA stainability (HDS)—a marker for sperm immaturity. In urine, we analyzed metabolites of phthalates, including diethylhexyl phthalate (DEHP). We studied associations between urinary levels of the metabolites and seminal as well as serum reproductive parameters, accounting for potential confounders.
Results: DEHP metabolite levels, particularly urinary mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), were negatively associated with progressive sperm motility, which was 11 (95% CI: 5.0–17) percentage points lower in the highest quartile of MECPP than in the lowest. Further, men in the highest quartile of the DEHP metabolite monoethylhexyl phthalate had 27% (95% CI: 5.5%–53%) higher HDS than men in the lowest quartile.
Conclusions: DEHP metabolite levels seemed negatively associated with sperm motility and maturation.

1. Introduction

Several phthalates that are common constituents of consumer products (Wittassek et al., 2011) are reported to disturb the reproductive function of male animals (Martino-Andrade and Chahoud, 2010). Those effects, of exposure doses above those encountered in humans, include a reduced sperm production and motility (Glombik et al., 2014) and reduced levels of testosterone (Agarwal et al., 1986; Miura et al., 2007; Oishi, 1985). However, the few studies performed in primates indicate a lower sensitivity, in comparison with rodents (Martino-Andrade and Chahoud, 2010). Testicular toxicity of phthalates is mainly apparent for those with a side-chain of medium length, such as dibutyl phthalate (DBP), or with a longer side-chain that is branched, such as diethylhexyl phthalate (DEHP).

The long-chain phthalates are often used as plasticizers such as in PVC, whereas the shorter-chain phthalates have other applications such as in personal-care products, paints, adhesives and enteric-coated tablets (Wittassek et al., 2011). Humans are exposed to long-chain phthalates mainly through the diet, whereas the shorter-chain phthalates seem to have additional sources of a higher importance (Wittassek et al., 2011).

Human phthalate exposure is most often assessed through levels of metabolites in urine, and has in some studies been associated with an impaired semen quality (Kay et al., 2014), but this has mostly been studied in subfertile men. The evidence for associations with the levels of reproductive hormones in humans is weak, or limited for testosterone levels (Kay et al., 2014).

In subfertile men, due to a more vulnerable reproductive system, the association between exposure and semen quality may differ from that in the general population. Studies in men who are not subfertile are scarce and rather conflicting.

Thus, in men with an occupational exposure to phthalates, DEHP has been linked with a decreased sperm motility and with an increased sperm DNA fragmentation (Huang et al., 2011, 2014) and, further, with reduced levels of free testosterone in one study (Pan et al., 2006) but with increased levels of estradiol in another (Fong et al., 2015).

In men without an occupational exposure to phthalates, one study reported a decrease in sperm motility with increasing diethyl phthalate (DEP) metabolite levels (Jönsson et al., 2005). Another study found no associations (Joensen et al., 2012), whereas a third study reported that

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metabolite levels of DBP were negatively associated with sperm concentration (Han et al., 2014). In a more recent study, metabolite levels of DEHP and di-isonyl phthalate (DiNP) were negatively associated with semen volume, and DEHP metabolite levels were negatively associated also with the total sperm count (Specht et al., 2014).

The available human data based on non-infertile men differ in design, not all being based on measurements of secondary urinary metabolites (Jönsson et al., 2005) considered to be the most reliable exposure markers of longer-chain phthalates (Frederiksen et al., 2010; Wittassek et al., 2011), and which were measured only in the later studies. Furthermore, despite half-lives in hours, metabolite levels were not always sampled the same day as the semen, and not always in urine (Specht et al., 2014). In addition, not all studies included adjustment for time of abstinence and the urinary dilution (Joensen et al., 2012). All these factors may influence potential associations between metabolite levels and semen parameters. Therefore, we wanted to study associations between levels of several (including secondary) phthalate metabolites and markers of male reproductive function, through samples of urine, serum and semen, collected at the same visit in a homogenous group of 314 young Swedish men from the general population. We also wanted to adjust for both abstinence time and the urinary dilution.

In addition, in order to validate the use of serum as a matrix for measurements of phthalate metabolites, we wanted to study correlations between metabolite levels in urine and serum, the latter being more frequently available from biobanks.

2. Methods

For a previous study of reproductive function in Swedish adolescent men (Axelsson et al., 2011), between 2008 and 2010 we included 314 men (241 men [14% of 1681 invited] from the health board prior to military service and 73 men through announcement in schools).

Participation criteria were: a) being 17 to 20 years old; b) living within 60 km from the city of Malmö in Southern Sweden; c) participant and mother born and raised in Sweden.

All participants received 500 SEK (55 Euro), signed an informed consent, and answered questionnaires about length and weight, current smoking, parental smoking during pregnancy and previous disease and medications. At the same visit, they delivered samples of urine, blood and semen before noon, and reported their abstinence time. Urinary samples were portioned in 10 mL tubes with 1–2 mL in each.

The study was approved by the regional ethical review board of Lund University.

2.1. Semen analysis

Semen samples, collected upon masturbation at the hospital, were examined for volume, sperm concentration, total sperm count, proportion of morphologically normal sperm and progressive motility, all according to the WHO guidelines (World Health Organization, 1999). We additionally analyzed the DNA fragmentation index (DFI) and high DNA stainability (HDS) using the Sperm Chromatin Structure Assay (Evenson et al., 2002).

2.2. Analysis of reproductive hormones

Blood samples were drawn before noon. Serum was analyzed at the clinical chemistry laboratory of Skåne University Hospital, Sweden by means of ElectroChemiLuminiscenceImmunoassay (Roche Cobas) for concentrations of follicle-stimulating hormone, luteinizing hormone, sex hormone-binding globulin and testosterone and by use of an immunofluorometric method (Delfia, Perkin-Elmer) for estradiol. Coefficients of variation (CV) and level of detection (LOD) were as follows: follicle-stimulating hormone had CV 5.5% at 5.0 IU/L and 6.3% at 75 IU/L and LOD at 0.10 IU/L; luteinizing hormone had CV 3.2% at 7.0 IU/L and 3.1% at 82 IU/L and LOD 0.10 IU/L; sex hormone-binding globulin had CV 1.2% at 16 nmol/L and 1.4% at 34 nmol/L and LOD 0.35 nmol/L; testosterone had CV 3.8% at 3.2 nmol/L and 1.6% at 25 nmol/L and LOD 0.087 nmol/L; estradiol had CV 20% at 30 pmol/L and 10% at 280 pmol/L, LOD 8 pmol/L.

Concentration of free testosterone was calculated according to Vermeulen et al. (1999).

2.3. Analyses of phthalate metabolites

LODs and CVs are shown in the Supplemental Material, Table S1. Levels below LOD were given the value of LOD divided by 2.

2.3.1. Urinary levels

Urinary samples were processed through an automated solid-phase extraction technique and analyzed by liquid chromatography–tandem mass spectrometry (LC/MS/MS) according to a previously published method (Toft et al., 2012). In brief, internal standards were added, and samples thereafter treated with glucuronidase, evaporated and dissolved in water containing acetic acid before analysis using the LC/MS/MS. In each series, an internal control urinary sample was analyzed. We analyzed ten metabolites of five different phthalates.

For DEHP, we analyzed the primary metabolite mono-(2-ethylhexyl) phthalate (MEHP) and the secondary metabolites: mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(2-ethyl-5-hydroxylhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP). For DiNP, we analyzed the secondary metabolites: mono-(carboxy-iso-octyl) phthalate (MCiOP), mono-(hydroxy-iso-nonyl) phthalate (MHiNP) and mono-(oxo-iso-nonyl) phthalate (MOiNP). For DBP, we analyzed monobutyl phthalate (MBP). For butylbenzyl phthalate, we assessed monobenzyl phthalate (MBzP), and for DEP, we measured monoethyl phthalate (MEP).

We adjusted for the urinary dilution by dividing the molar concentrations of the metabolites with that of creatinine.

2.3.2. Serum levels

In serum, we analyzed the secondary DEHP- and DiNP metabolites listed above according to a previously described LC/MS/MS-based method (Specht et al., 2014). In brief, isotopically labeled internal standards were added to aliquots of 100 μL serum for all the evaluated compounds. Samples were digested with glucuronidase and proteins precipitated with acetonitrile. Analyses were performed in negative ion mode. The quality of the analysis was checked by inclusion of chemical blank samples and an in-house quality control in the analyzed sample batches. Each sample was analyzed three times in three different analytical batches.

2.4. Statistics

We used SPSS version 18 to 22 for statistic calculations and considered $p < 0.05$ as statistically significant.

By use of Spearman's rank correlation test, we studied to which degree the different urinary and serum metabolite levels were correlated with each other. Furthermore, for those metabolites for which both serum and urinary levels were available (see above) the correlations between these two measurements were tested.

In order to compare our results with those of a previous study (Joensen et al., 2012), we calculated %MEHP. This was defined as the urinary concentration of the primary metabolite MEHP as a proportion of the sum of the urinary levels of the DEHP metabolites (MEHP, MECPP, MEHHP and MEOHP).

To achieve a more normal distribution of residuals, and a better statistical prediction (Tabachnick and Fidell, 2013), we transformed sperm concentration and total sperm count by the cubic root (Sanchez-Pozo et al., 2013) and semen volume, HDS, DFI, testosterone, free testosterone, luteinizing hormone, follicle-stimulating hormone, estradiol and