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Associations between serum phthalates and biomarkers of reproductive function in 589 adult men

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ABSTRACT

Phthalates which are widely used, are ubiquitous in the environment and in some human tissues. It is generally accepted that phthalates exert their toxic action by inhibiting Leydig cell synthesis of testosterone, but in vitro studies have also shown anti-androgenic effects at the receptor level. Some cross-sectional studies have shown inverse associations between urinary levels of phthalates and reproductive hormones, but results are conflicting and the evidence base is limited. The aim of this study was to investigate if levels of di-2-ethylhexyl phthalate (DEHP) and diisononyl phthalate (DiNP) metabolites in serum are associated with serum concentrations of male reproductive hormones and semen quality. A secondary aim was to investigate metabolic pathways of DEHP and DiNP on semen quality and reproductive hormones.

A cross-sectional sample of 589 spouses of pregnant women from Greenland, Poland and Ukraine were enrolled between 2002 and 2004. The men gave semen and blood samples and were interviewed. Six phthalate metabolites of DEHP and DiNP were measured by liquid chromatography tandem mass spectrometry in serum. The metabolites were summed according to their molar weight.

We observed significant inverse associations between serum levels of the metabolites, the proxies and serum testosterone. Negative associations were also discovered between some metabolites and sex hormone-binding globulin, semen volume and total sperm count.

Findings are compatible with a weak anti-androgenic action of DEHP metabolites, but less so for DiNP metabolites. Metabolic pathways differed significantly between the three study sites, but without major effect on semen quality or reproductive hormones.

1. Introduction

The hypothalamic–pituitary–gonadal axis and associated hormones are imperative in the regulation of spermatogenesis. Spermatogenesis may consequently be affected by exogenous compounds if they interfere with the endogenous hormone regulation (Gazouli et al., 2002). The ubiquitous phthalates show anti-androgenic action in animal models and in vitro. They therefore represent a class of chemicals that is suspected to harm male fertility in humans (Borch et al., 2006; Christen et al., 2010; Dalsenter et al., 2006). For example, di-2-ethylhexyl phthalate (DEHP) exposure in the pre- and post-pubertal male rat decreases serum testosterone levels and adversely affects Leydig cell proliferation in the adult rat testis (Li et al., 2012; Noriega et al., 2009), but DEHP has also been found to antagonize the androgen receptor in vitro (Christen et al., 2010; Shen et al., 2009).

Abbreviations: DEHP, di-2-ethylhexyl phthalate; DiNP, diisononyl phthalate; MEHP, mono-2-ethylhexyl phthalate; MiNP, monoisononyl phthalate; 5OH-MEHP, 2-ethyl-5-hydroxy-hexyl phthalate; 5oxo-MEHP, 2-ethyl-5-oxyhexyl phthalate; 5cx-MEPP, 5-carboxy-mono-2-ethylpenty phthalate; 7OH-MMeOP, mono-4-methyl-7-hydroxy-octyl phthalate; 7oxo-MMeOP, mono-4-methyl-7-oxo-octyl phthalate; 7cx-MMeOP, mono-4-methyl-7-carboxyheptyl phthalate; SHBG, sex hormone-binding globulin; FAI, Free Androgen Index; LH, luteinizing hormone; FSH, follicle stimulation hormone; NAG, Neutral α-glucosidase; PSA, Prostate-Specific Antigen; LOD, limit of detection; BMI, body mass index; CV, coefficients of variation; WHO, World Health Organization; PPAR, peroxisome proliferator activated receptors; GLM, general linear models; DMP, dimethyl phthalate; DEP, diethylphthalate; DBP, dibutyl phthalate.

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Phthalates are di-esters of 1,2-benzenedicarboxylic acid (phthalic acid). They have low water solubility, which decreases with increasing length of the side chain and with higher molecular weight (Lyche et al., 2009). The use of phthalates in industrial production mainly depends on their molecular weight. High molecular weight phthalates (ester side-chain lengths with five or more carbons), such as DEHP and diisononyl phthalate (DiNP), are used as plasticizers in numerous polyvinyl chloride products, such as raincoats and footwear, wall coverings and flooring, food packaging, gloves, toys and medical devices (Kavlock et al., 2002a, 2002b; Rock et al., 1986). Low weight phthalates, such as dimethyl phthalate (DMP), diethyl phthalate (DEP) and dibutyl phthalate (DBP), are used as solvents in personal care products, lacquers, insecticides and coatings (Duty et al., 2005a). Humans are mainly exposed to phthalates through ingestion and dermal contact while inhalation is of minor importance outside the occupational setting (Bradley et al., 2013; Wormuth et al., 2006). The compounds or their metabolites have been detected in urine in more than 95% of the investigated men and women in Western parts of the world (Wittassek et al., 2007).

The biological half-life of phthalates is short and therefore they do not accumulate in the body. A study of a healthy man intentionally ingesting DEHP observed 65–70% urinary excretion 44 h after oral intake (Koch et al., 2004). For DiNP, the urinary excretion was 43.6% after 49 h (Wittassek and Angerer, 2008). The di-ester phthalates are rapidly hydrolyzed into mono-esters during absorption. The mono-esters of the less polar phthalates are further metabolized at different positions in the ester chain by oxidation and hydroxylation into secondary metabolites, which are excreted in the urine as such or after conjugation to glucuronic acid (Hans-Detlev Gilsing et al., 2002; Lyche et al., 2009). The primary metabolite of DEHP, the mono-ester mono-2-ethylhexyl phthalate (MEHP), can be metabolized to, among others, 2-ethyl-5-hydroxy-hexyl phthalate (5OH-MEHP) and 2-ethyl-5-oxyhexyl phthalate (5oxo-MEHP) or 5-carboxy-mono-2-ethylpenty phthalate (5cx-MEPP) (Fig. 1). In humans, urinary levels of the DEHP secondary metabolites 5OH-MEHP and 5oxo-MEHP are several folds higher than the levels of MEHP (Koch et al., 2003). Analysis of these metabolites may therefore increase the analytical sensitivity compared to analysis of MEHP (Koch et al., 2003). Furthermore, the chance of contamination during preparation and analysis of urine samples is greater for MEHP than for the secondary metabolites (Frederiksen et al., 2010; Koch et al., 2003). Finally, most reproductive effects are exerted by the primary- or secondary mono-ester metabolites and not by the di-ester itself (Martino-Andrade and Chahoud, 2010). The metabolism of DiNP is similar to that of DEHP (Fig. 1). Metabolic pathways may impact the toxicity of the phthalate, but only a few studies regarding metabolism and reproductive health exist (Joensen et al., 2012; Meeker et al., 2009).

In most in vitro and rodent studies of phthalate toxicity, the dose-levels are orders of magnitude above environmental levels. Interestingly, low-level dose animal studies have found the same anti-androgenic effects on male rat development (Christiansen et al., 2010). It has been questioned whether it is possible to extrapolate animal data to humans. A recent study of xenografted human or rat fetal testis tissue into castrate male mice treated with DBP or monobutyl phthaltate (a metabolite of DBP) found that contrary to the rat, steroidogenesis of the human fetal testis was not suppressed by DBP (Mitchell et al., 2012). In contrast, an in vitro study based on cultured adult human testis tissue showed that testosterone production was inhibited by DEHP and MEHP (Dsdoits-Lethimonier et al., 2012). Validation studies as well as studies of other phthalates are needed, but these findings indicate that research into the effect of DEHP on testicular function is needed.

The number of epidemiologic studies of male reproductive function and environmental and occupational phthalate exposure has increased during the last few years. Several studies indicate that urinary DEHP metabolites may reduce total and free testosterone levels (Joensen et al., 2012; Meeker et al., 2009; Mendiola et al., 2012; Pan et al., 2006), also decreased estradiol and follicle stimulation hormone (FSH) have been reported (Joensen et al., 2012; Meeker et al., 2009). Studies on semen quality have, however, yielded conflicting results. One study investigating DEHP in semen found significantly negative associations with sperm concentration, normal morphology and motility (Pant et al., 2008), but these results have not been replicated in studies investigating DEHP metabolites in other media (Han et al., 2013; Hauser et al., 2006; Herr et al., 2009; Liu et al., 2012; Toshima et al., 2012). Altogether, these results point towards adverse, although inconsistently so, effects of DEHP and DiNP on reproductive function.

Hypothesizing that DEHP and DiNP metabolites exert anti-androgenic effects in humans, the aim of this study was to assess the associations of semen quality and male reproductive hormones relative to serum concentrations of the phthalates. Second, the aim was to investigate whether

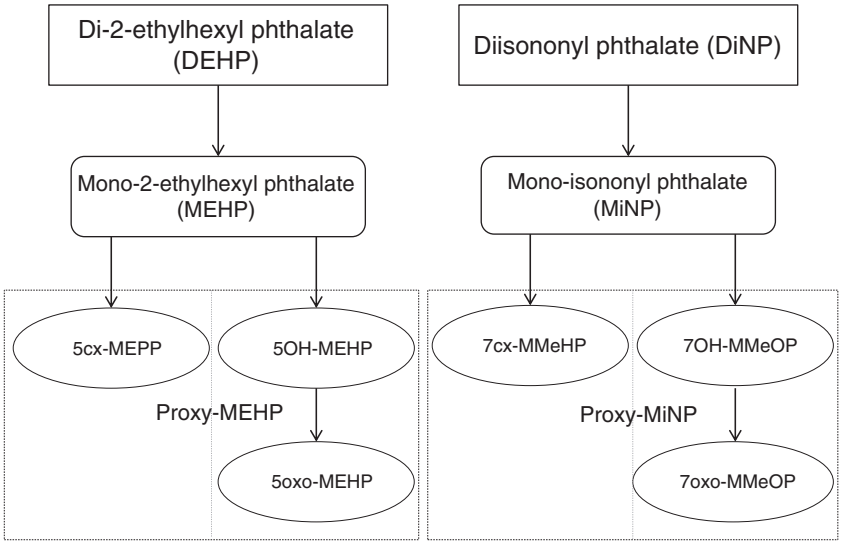

Fig. 1. Metabolic pathways of DEHP (Koch et al., 2003) and DiNP (Koch et al., 2007) and the metabolites addressed in the present study. 5cx-MEHP, 5OH-MEHP and 5oxo-MEHP were summed according to their molar weight and given the name Proxy-MEHP. 7cx-MMeHP, 7OH-MMeOP and 7oxo-MMeOP were summed according to their molar weight and given the name Proxy-MiNP.