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The association between total phthalate concentration and non-communicable diseases and chronic inflammation in South Australian urban dwelling men

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ABSTRACT

Objective: To investigate associations between urinary total phthalate concentration, chronic low-grade inflammation and non-communicable diseases in a cohort of South Australian men.

Methods: 1504 men aged 39–84 years who provided a urinary sample at the follow-up visit of the Men Androgen Inflammation Lifestyle Environment and Stress (MAILES) study, a randomly-selected group of urban-dwelling, community-based men from Adelaide, Australia (n = 2038; study participation rate: 78.1%). Total phthalate concentration was quantified in fasting morning urine samples. Chronic diseases were assessed through self-report questionnaire or directly measured using standardised clinical and laboratory procedures. Inflammatory biomarkers were assayed by ELISA or spectroscopy. Multivariable linear and logistic regression models were applied to determine associations of log-transformed urinary phthalate concentration with inflammation and chronic disease.

Results: Total phthalates were detected in 99.6% of urinary samples; geometric mean (95% CI) was 114.1 (109.5–118.9) μg/g creatinine. Higher total phthalate levels were associated with higher levels of hs-CRP, IL-6 (all $p < 0.05$) and TNF-α but not MPO. Urinary total phthalate concentrations were positively associated with cardiovascular disease, type-2-diabetes and hypertension. Comparing extreme quartiles of total phthalate, prevalence ratios were 1.78 (95% CI 1.17 – 2.71, p-trend = 0.001) for cardiovascular disease and 1.84 (95%CI 1.34 – 2.51, p-trend = 0.001) for type-2-diabetes and 1.14 (95%CI 1.01 – 1.29, p-trend = 0.013) for hypertension. Total phthalates and asthma and depression were not significantly associated.

Conclusion: A positive association between total phthalates and cardiovascular disease, type-2-diabetes, hypertension and increased levels of chronic low-grade inflammatory biomarkers was observed in urban-dwelling Australian men.

1. Introduction

A non-communicable disease (NCD) is a medical condition or disease that is not caused by infectious agents (non-infectious or non-transmissible) but lasts for a long period and progresses slowly (World Health Organization, 2010). NCDs have become the world's leading cause of death and a major cause of personal morbidity and healthcare system costs (Alwan et al., 2011). The 2004–2005 Australian National Health Survey (NHS) reported that over seven million people had at least one NCD (AIHW, 2006) and 99% of those aged 15 years and over had at least one NCD risk factor, with approximately 14% reporting five or more risk factors (AIHW.).

NCDs are referred as “Lifestyle” diseases because majority of these diseases are due to unhealthy lifestyle. Many clinical factors have been linked to an increased risk or severity of NCDs; these include smoking, physical inactivity, overweight or obesity and an unhealthy dietary pattern (Alwan et al., 2011). In recent times, environmental risk factors have emerged as risk factors for NCDs worldwide (Prüss-Üstün and Corvalán, 2006) including in Australia (AIHW, 2012). Phthalates, a group of chemicals used widely in common consumer products such as food packaging, toys, medical devices, medications and personal care

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products (National Industrial Chemicals Notification and Assessment Scheme (NICNAS), 2008) are a potential environmental risk factor for NCDs (AIHW, 2011). Following exposure, phthalates are hydrolysed to their corresponding monoesters through a step-wise metabolic transformation (Meeker et al., 2012). The shorter chain mono-esters are excreted into urine mainly unchanged while the longer chain mono-easters are more likely to be oxidized and/or conjugated with glucuronic acid prior to their excretion in urine (Frederiksen et al., 2007). These phthalate metabolites are detectable in urine (Kato et al., 2004).

The toxic effects of phthalate exposure have been demonstrated in animal studies (Posnack, 2014; Mangala Priya et al., 2013; Zuo et al., 2014). A small number of epidemiological studies have investigated the association of NCDs such as cardiovascular disease (Trasande et al., 2014; Shiue, 2013; Olsen et al., 2012; Lind and Lind, 2011), type-2-diabetes (Sun et al., 2014; Huang et al., 2014; Kuo et al., 2013), hypertension (Shiue, 2014a; Trasande et al., 2013), asthma (Jaakkola et al., 2006; Hoppin et al., 2013) and depression (Kobrosly et al., 2014) with specific phthalate metabolites (Goodman et al., 2014). All these cross-sectional studies found statistically significant or marginally significant associations between some of the phthalate metabolites and NCDs (Zuo et al., 2014; Trasande et al., 2014; Shiue, 2013; Olsen et al., 2012; Lind and Lind, 2011; Sun et al., 2014; Huang et al., 2014; Kuo et al., 2013; Shiue, 2014a; Trasande et al., 2013; Jaakkola et al., 2006; Hoppin et al., 2013; Kobrosly et al., 2014; Goodman et al., 2014). However, existing studies have predominantly focused on women, children and elderly populations and the health effects of phthalates have not been explored in a representative cohort of community dwelling middle-aged and elderly men.

NCDs are often associated with chronic low-grade inflammation (Pawelec et al., 2014; Burmeister et al., 2014), and the pro-inflammatory effects of phthalates have recently been a focus of attention (Ferguson et al., 2011). An *in vitro* study has demonstrated that phthalates promote interleukin-6 (IL-6) and interleukin-8 (IL-8) production (Jepsen et al., 2004). Continuous exposure to phthalates may be pro-inflammatory, with the potential to interact with other risk factors worsening NCD outcomes (Burmeister et al., 2014; Ferguson et al., 2012). However, epidemiological studies in the area are limited, and none have been conducted in Australia.

In the current study we evaluated the relationship between urinary total phthalate concentration, NCDs and markers of inflammation in South Australian community-dwelling men.

2. Material and methods

2.1. Study design and participants

Fig. 1 shows that the participants included 1504 men aged 39–84 who provided a urinary sample at the follow-up visit (2007 – 2010) of the Men Androgen Inflammation Lifestyle Environment and Stress (MAILES) study (Grant et al., 2013), a randomly-selected group of urban-dwelling, community-based men from Adelaide, Australia (n = 2038; study participation rate: 78.1%). The MAILES cohort has been previously shown to be broadly representative of the target population. The mean BMI of the analytic subset was similar with the entire MAILES cohort (28.4 vs 28.5). The mean age of the subset was slightly younger than the entire cohort at baseline (54.8 vs 55.7 years), but otherwise representative of the MAILES cohort. Participants were asked to attend a clinic for a physical examination, to complete detailed questionnaires to document socio-demographic status and health related behaviours (including food intakes), as well as health status and medication use and to donate fasting blood and urine samples. The study was approved by the research ethics committees of the Royal Adelaide Hospital and the North Western Adelaide Health Service, and written informed consent was obtained from all participants.

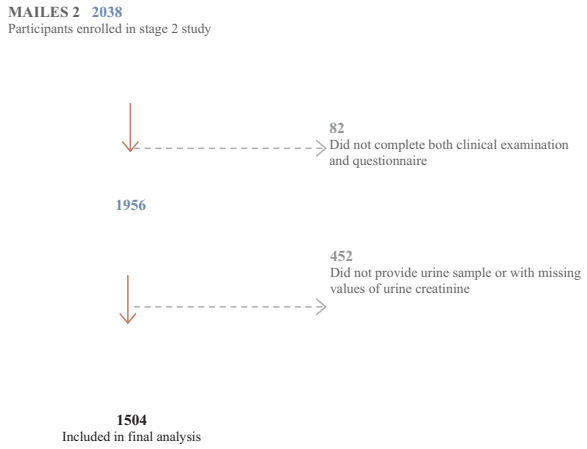

Fig. 1. Consort diagram of the study.

2.2. Measurements of phthalates

The fasting morning void urine samples were collected immediately post-uroflowmetry testing between 7 and 11 a.m. in the clinic and stored in 5 mL polypropylene tubes at −80 °C. Total phthalates were assayed as phthalic acid extracted from the participant urine sample using liquid chromatography tandem mass spectrometry (LC-MS/MS) as previously described (Bai et al., 2015). The method extracts phthalic acid (PA) from participant urine samples (total PA, after hydrolysis of the glucuronides with 3 mol/L HCL), calibration standards (containing $^{13}C_4$-PA, added as an external calibration standard and $^{2}H_4$-PA, added as internal standard to all samples) and quality controls (containing $^{13}C_4$-PA and $^{2}H_4$-PA) using solid phase extraction (Strata-X polymeric reversed-phase, Phenomenex, Lane Cove, NSW, Australia). The analytes were separated by HPLC on a phenyl hexyl column (Luna, 50 × 2.0 mm, 3 µ, Phenomenex, Lane Cove, NSW, Australia) preceded by a 2 mm cartridge of the same material, using a gradient of mobile phase A (5% methanol and 0.1% formic acid in Mill-Q water) and B (95% methanol and 0.1% formic acid in water) pumped at 0.2 mL/min. The gradient was programmed as follows: 0 – 0.5 min (5% mobile phase B), 0.50 – 6.51 min (70% mobile phase B), 6.52–6.99 min (100% mobile phase B), 7 min (revert to 5% mobile phase B). The eluate was monitored with an API3000 MS/MS (AB Sciex, Mulgrave, Vic, Australia) operating in negative MRM mode. The singly-charged Q1/Q3 transition for PA is 165.0/121.0/77.0 amu (amu), for $^{13}C_4$-PA is 169.1/124.0/79.0 and 169.0/124.0/79.0 amu for $^{2}H_4$-PA. The concentration of PA was calculated from a calibration curve of the ratio of the peak area of $^{13}C_4$-PA/$^{2}H_4$-PA versus concentrations of $^{13}C_4$-PA. The data were acquired and processed with Analyst 1.4 linked directly to the mass spectrometer. Accuracy and precision for inter-day quality controls (low, medium and high) were within 6.6% of nominal values and within 10.0%, respectively; the respective values for the lower limit of quantification (LLOQ, 9.3 ng/mL) were 7.9% and 8.6%. The assay range was from 9.3 to 1320 ng/mL. To prevent contamination from plastics during processing, contact with plastic lids or containers was minimized. Those samples below the LLQQ of total phthalate (n = 6) were assigned a value for total phthalates of half the limit of quantification (Saravanabhavan et al., 2013). The inter assay CV was 4.6%.

Urine albumin was quantified by immuno-turbidimetric assay (OSR6167, Beckman Coulter) and the IDMS traceable Kinetic Jaffe (compensated) method was used to measure creatinine in serum or urine (OSR6578, Beckman Coulter). The inter assay CV was 4.4%.

In all the analyses, we used urine creatinine adjusted total phthalates (µg per g creatinine) as an exposure variable.