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Urinary phthalate metabolites are associated with insulin resistance in obese subjects ☆

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ABSTRACT

Phthalates are potentially involved in the development of type 2 diabetes mellitus. In a cohort of 123 obese subjects, 10 phthalate metabolites were analyzed. An oral glucose tolerance test was performed and various estimates of insulin resistance and beta-cell function were calculated. After adjustment for age, physical activity level, smoking behavior, medication use and body mass index, several phthalate metabolites were linked to markers of glucose tolerance and insulin resistance.

1. Introduction

The development of type 2 diabetes mellitus is a multifactorial problem for which many risk factors have been established (Bi et al., 2012). Obesity, physical inactivity and cigarette smoking are amongst the known risk factors. Recently, certain chemicals are under scrutiny for their potential causative role in type 2 diabetes development (Hectors et al., 2011). One of these are phthalates, commonly used in plastics as they lend flexibility and durability to the material (ATSDR, 1995, 2001, 2002). Innumerable consumer devices therefore contain phthalates. Phthalates do not bind to the plastic itself, thus easily leaching and transferring to food and air. As a consequence, one of the major routes for human exposure is ingestion, next to dermal absorption of phthalates in personal-care products. Given their hydrophylicity, phthalates are not stored within the human body but quickly metabolized through Phase I and Phase II reactions, followed by urinary excretion within 24–48 h after exposure. Phthalates are ubiquitously present and the majority of the Western population has measurable levels of several urinary phthalate metabolites (Koch and Calafat, 2009; Wittassek et al., 2011).

Phthalates are known agonists of the nuclear peroxisome proliferator-activated receptors (PPARs), both alpha and gamma, key factors in the regulation of glucose homeostasis and adipogenesis (Casals-Casas et al., 2008). Exposure to phthalates has been postulated to increase the risk of obesity and type 2 diabetes development in humans (Casals-Casas et al., 2008; Huang et al., 2014). In fact, mainly for the monoester metabolites, formed through phase I reactions, adverse health effects have been documented. A few cross-sectional studies have identified relationships between phthalate metabolite levels and abdominal obesity, insulin resistance and type 2 diabetes (James-Todd et al., 2012; Kim et al., 2013; Lind et al., 2012a, 2012b; Stahlhut et al., 2007; Svensson et al., 2011). In these studies, the diagnosis of type 2 diabetes relied on self-reporting or a fasting plasma glucose $>$ 126 mg/dl. A recent review by the US National Toxicology Program highlighted the need for more detailed studies on the link between type 2 diabetes and so-called endocrine disrupting chemicals(Thayer et al., 2012).

Our study aimed at evaluating the relationship between urinary levels of several phthalate metabolites and glucose metabolism, assessed by a standard 75 g oral glucose tolerance test (OGTT), both in an overweight and obese population.

☆This study was registered at clinicaltrials.gov (number NCT01778868)

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2. Subjects and methods

Study population A group of 123 adult obese subjects without a known history of type 2 diabetes was selected when visiting the weight management clinic of the Antwerp University Hospital between November 2009 and February 2012. This study was approved by the local Ethical Committee (Belgian Registry number B30020097009) and registered at clinicaltrials.gov (number NCT01778868). All participants provided their written informed consent.

Body composition Anthropometric measures were taken in the morning with patients in a fasting state and undressed. Height was measured to the nearest 0.5 cm; body weight was measured to the nearest 0.2 kg.

Physical activity level (PAL) was estimated using a validated, self-reported questionnaire, combining estimates of energy expenditure during professional working hours and leisure time (Baecke et al., 1982).

Current medication use, defined as taking tablets on a daily basis, was registered, including both prescribed as over-the-counter drugs. A medical doctor interviewed patients, asking what tablets patients had taken during the week prior to the urine sampling. This was taken into account as phthalates are known components of the enteric coatings of some drugs.

Current smoking behavior was registered during the interview with the medical doctor.

Urine and blood sampling The day prior to the in-hospital investigations, 24-h urine was collected at home in 1 phthalate free container. The 24 h urine collection started with the second void on the day prior to the in-hospital investigation and ended with the first void on the day of the in-hospital investigation. All voids were collected in 1 container. All other investigations described in this methods section (anthropometry, blood sampling, questionnaires) were performed in hospital, on the same day. Venous blood samples were obtained in the fasting state between 08.00 a.m. and 10.00 a.m. into sterile BD Vacutainer tubes (BD Biosciences, Erembodegem, Belgium). Creatinine was measured at the hospital laboratory, and the MDRD-formula was used to estimate creatinine clearance. An OGTT was performed in all subjects (American Diabetes Association, 2014). HbA1c, glucose, insulin and c-peptide were measured at the hospital laboratory. Samples were taken at baseline and at 15 (glucose only), 30, 60, 90, 120, 150 (glucose only) and 180 min. Diabetes, impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and normal glucose tolerance (NGT) were classified according to the American Diabetes Association (2014). We calculated area under the curve (AUC) for glucose and insulin levels using the trapezoid method. Fasting based estimates of insulin resistance (HOMA-IR) and beta-cell function (HOMA-B) (Matthews et al., 1985) and OGTT based estimates of insulin resistance (Belfiore Index), insulin sensitivity (Matsuda Index) and beta-cell function (Insulinogenic Index and the ratio AUC insulin/AUC glucose) were calculated as well (Belfiore et al., 1998).

Toxicological analyses The analysis of major urinary mono-ester PMs (mono-methyl-phthalate (MMP), mono-carboxypropyl-phthalate (MCPP), mono-ethyl-phthalate (MEP), mono-(2-ethyl-5-carboxypentyl)-phthalate (5Cx-MEPP), mono-(2-ethyl-5-hydroxyhexyl)-phthalate (5HO-MEHP), mono-*iso*butyl-phthalate (M*i*BP), mono-(2-ethyl-5-oxohexyl)-phthalate (5Oxo-MEHP), mono-*n*-butyl-phthalate (M*n*BP), mono-benzyl-phthalate (MBzP), mono-(2-ethylhexyl)-phthalate (MEHP)) implied enzymatic deconjugation and solid phase extraction as the main steps for sample preparation, while instrumental analysis was performed by liquid chromatography–tandem mass spectrometry. Internal and external quality assurance and quality control protocols were employed in order to ensure the reliability of the obtained results. Phthalate metabolite concentrations were adjusted for urinary dilution by creatinine levels (Dirtu et al., 2013).

Statistical analysis Statistical calculations were performed using SPSS, version 20.0 (SPSS, Chicago, IL). Normality of distribution was verified using the Kolmogorov–Smirnov test. All phthalate levels displayed a skewed distribution. After transformation ($y=\log(x+1)$), all levels with the exception of 5CxMEPP were transformable to normality. Both Pearson and Spearman rank correlation analyzes were performed if appropriate and a Bonferroni correction was applied (significance set at $p<0.005$). To detect differences in PMs levels between groups, an ANOVA test was performed. We compared subjects with normal glucose tolerance with subjects with abnormal glucose tolerance. Abnormal glucose tolerance was defined as the presence of any of the following: IFG, IGT, IFG+IGT, type 2 diabetes mellitus. Standard linear regression was performed to assess the impact on AUC glucose and insulin levels, HOMA-IR, HOMA-B, Belfiore Index, Insulinogenic Index, Matsuda Index and the ratio AUC insulin/AUC glucose. Participants with type 2 diabetes mellitus were excluded for the analysis of HOMA-IR, HOMA-B, Belfiore Index, Insulinogenic Index, Matsuda Index and the ratio AUC insulin/AUC glucose because glucose toxicity per se may cause insulin resistance and beta cell function impairment. Two steps of adjustments were used: (1) adjustment for gender only and (2) multiple adjustments for gender, age, PAL, current smoking behavior, current medication use and BMI. Results of the regression analysis were considered significant at $p<0.05$.

3. Results

Study population The study population consisted of 38 men (31%) and 85 women (69%). Mean age was 41 ± 12.5 years and mean BMI was 38.7 ± 5.4 kg/m² (Table 1). Ninety-one participants (69%) of our population regularly took medication. Ten participants (8.1%) were diagnosed with previously unknown type 2 diabetes, 42 individuals (34.1%) had IGT, 2 (1.6%) and 6 (4.9%) individuals had IFG or combined IFG+IGT respectively. All participants had a normal kidney function, with a mean serum creatinine of 0.76 ± 0.14 mg/dl and a mean estimated creatinine clearance of 101 ± 18 mL/min/1.73 m² (MDRD) (Table 1).

Anthropometric data BMI was weakly but significantly related to MCPP ($r=-.27$, $p=0.002$) and 5Oxo-MEHP ($r=-.25$, $p=0.004$). Please see SI Table 1 for an overview of all correlation analyzes.

Glucose metabolism HbA1c levels were negatively related to MCPP ($r=-.26$, $p=0.004$) and MEHP ($r=-.30$, $p=0.001$). The correlation analysis between PMs and fasting glucose, fasting insulin and 2 h glucose and 2 h insulin levels was not significant. Please see SI Table 1 for an overview of all correlation analyzes.

No differences in urinary PMs levels could be detected between patients with NGT on one hand and patients with IGT, IFG, IFG+IGT or T2DM on the other hand.

The relationship between PMs and AUC glucose was assessed using linear regression analysis adjusted for gender, identifying MCPP, M*n*BP and MBzP as significantly associated with AUC glucose. (Table 2). Following multiple adjustments however, only MBzP levels were significantly related to AUC glucose (Table 2). 5HO-MEHP, 5Oxo-MEHP and M*n*BP levels were significantly associated with AUC insulin in a gender adjusted linear regression model. Following multiple adjustments, 5Oxo-MEHP and M*n*BP remained significant (Table 2). Both gender and multiple adjusted regression analyzes indicated several PMs as significantly associated with the Belfiore Index. (Table 2). Regression analysis could not identify any PM as significantly related to the fasting based estimates HOMA-IR or HOMA-B, but MEP was significantly associated with HOMA-IR in the multiple adjusted model. (Table 2) Several PMs were significantly associated with the Matsuda index,