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Associations between circulating levels of bisphenol A and phthalate metabolites and coronary risk in the elderly

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ABSTRACT

Studies have pointed out associations between various chemicals with estrogenic activity and cardiovascular disease. Being ubiquitous, the plastic additive substances bisphenol A (BPA), and phthalates have been detected in almost all types of analyzed human samples. The aim of this study was to investigate whether circulating levels of BPA and/or four selected phthalate metabolites are associated to coronary risk in an elderly population. In the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study, coronary risk was assessed by the Framingham Risk Score (FRS) together with circulating serum levels of BPA and the four phthalate metabolites monoisobutyl phthalate (MiBP), monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-(2-ethylhexyl) phthalate (MEHP) in 1016 subjects aged 70 years. BPA, MEHP, and MMP were associated to LDL-cholesterol and MEHP to HDL-cholesterol, MEP to diastolic blood pressure and MiBP to fasting glucose when the compounds were investigated one by one. After Bonferronni correction, only the relations for MMP to LDL-cholesterol ($p<0.0001$), MEP to diastolic blood pressure ($p<0.0002$), and MiBP to fasting glucose ($p<0.0001$) remained significant. MMP was associated to the FRS ($p=0.02$), but after Bonferronni correction, this association was not significant. In conclusion, associations were found between MMP and LDL-cholesterol, MEP and diastolic blood pressure, and MiBP and fasting glucose. We did not observe any strong associations between BPA nor any of the four phthalate metabolites and Framingham Risk Score in this elderly population.

1. Introduction

Bisphenol A (BPA) and phthalates are high volume chemicals that have been used as components in plastics for a long period of time. BPA is mainly used for making polycarbonate plastic but is also widely used in the production of other plastics, in epoxy resins lining food and beverage containers, and in cash receipts. Phthalates are derivatives of phthalic acid used primarily as plasticizers to make plastic products more flexible. Consumer products containing plastics include food packaging materials, children's toys, and water pipes but phthalates are also used in medical devices such as bags for blood and in personal care products such as cosmetics. Both BPA and phthalates can migrate out of the plastic product and into the environment (Halden, 2010; Heudorf et al., 2007; Koch and Calafat, 2009; Schettler, 2006; Schug et al., 2011).

Ingested BPA is mainly conjugated with glucuronic acid which is eliminated in the urine. A fraction of the absorbed BPA may also be distributed to body storage sites followed by a slow, low-level release of BPA into the bloodstream (Fernandez et al., 2007). Metabolism and elimination of phthalates is more complex. The phthalates are metabolized to the corresponding monoester metabolites, which are eliminated in the urine as glucuronide conjugates or are further metabolized (Frederiksen et al., 2007; Wittassek and Angerer, 2008).

BPA and phthalates act as endocrine-disrupting compounds (EDCs), i.e., compounds capable of causing dysfunction in hormonally regulated body systems. The main action attributed to BPA is estrogen-like activity, while the phthalates function mainly as anti-androgens (Talsness et al., 2009; Wolstenholme et al., 2010; vom Saal et al., 2007). Most plastic products release chemicals having estrogenic activity (Schug et al., 2011). These

Abbreviations: BMI, body mass index; BPA, bisphenol A; CV, cardiovascular; CHD, coronary heart disease; DBP, dibutyl phthalate; DEHP, diethylhexyl phthalate; DiBP, diisobutyl phthalate; EDC, endocrine disrupting compounds; LOD, limit of detection; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEP, monoethyl phthalate; MiBP, mono-isobutyl phthalate; MMP, monomethyl phthalate; NHANES, National Health and Nutrition Examination Survey; PPAR, peroxisome proliferator activated receptor; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors

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chemicals are widespread in all kinds of different environments all over the world and have been detected in human urine, milk, saliva, serum, plasma, ovarian follicular fluid, and amniotic fluid. For reading more about exposure, toxicology, and metabolism of BPA and phthalates, see e.g., the following articles (Heudorf et al., 2007; Olsen et al., 2012; Wittassek and Angerer, 2008).

Elevated circulating levels of environmental contaminants and pollutants have been found to be associated with prevalent coronary heart disease (CHD), a number of cardiovascular (CV) risk factors such as hypertension, obesity, and diabetes, and metabolic syndrome (Dirinck et al., 2010; Kreiss et al., 1981; Sergeev and Carpenter, 2010; Uemura et al., 2009). Urinary levels of BPA were also recently found to be associated with CHD in the NHANES sample (Lang et al., 2008; Melzer et al., 2010). There are also a recent study suggesting that exposure to BPA and phthalates, measured in serum, is associated with atherosclerosis (Lind and Lind, 2011).

The Framingham Risk Score (FRS) is the most commonly used scoring system aiming at predicting the risk of cardiovascular disease in an individual within a certain time period in research and clinical practice. FRS is a scoring system that uses age, gender, smoking history, blood pressure, HDL-cholesterol, LDL-cholesterol, and blood glucose levels to estimate coronary event risk over the course of ten years among individuals without previously diagnosed CHD (Wilson et al., 1998).

The aim of this cross-sectional study was to investigate whether there is a relationship between circulating levels of BPA and/or four common phthalate metabolites and FRS and/or for the CV risk factors included in the score. For these aims, we used the population-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study (Lind et al., 2005) in which we have data on CV risk factors and circulating levels of BPA and four different phthalate metabolites in over 1000 subjects (Olsen et al., 2012).

2. Material and methods

2.1. Subjects

All subjects eligible to the study were living in the community of Uppsala, Sweden and were 70 years old. The subjects were randomly chosen from the register of community residents. A total of 1,016 subjects participated, giving a participation rate of 50.1 percent. The study was approved by the Ethics Committee of the University of Uppsala.

2.2. Basic Investigation

All subjects were investigated in the morning after an overnight fast. No medication or smoking was allowed after midnight. An arterial cannula was inserted in the brachial artery for blood sampling. The participants were asked to answer a questionnaire about their medical history, smoking habits, and regular medication.

Blood pressure was measured by a calibrated mercury sphygmomanometer in the non-cannulated arm to the nearest mmHg after at least 30 min of rest, whereupon the average of three recordings was used. Lipid variables and fasting blood glucose were measured by standard laboratory techniques (Carlsson et al., 2010). From these data, the FRS was calculated (Wilson et al., 1998). Basic characteristics are given in Table 1.

Approximately 10 percent of the cohort reported a history of coronary heart disease, 4 percent reported stroke and 9 percent diabetes mellitus. Almost half the cohort reported any CV medication (45 percent), with antihypertensive medication being the most prevalent (32 percent). Fifteen percent reported use of statins, while insulin and oral antiglycemic drugs were reported in 2 and 6 percent, respectively (Lind et al., 2005).

2.3. Chemical analyses

Human serum were analyzed for the levels of Bisphenol A (BPA) and ten phthalate metabolites at ALS Environmental Canada, Serum samples (0,5 ml) was analyzed for levels of BPA and ten phthalate metabolites using isotope liquid chromatograph/tandem mass spectrometer (API4000LC-MS/MS), following the general procedures presented by the Centers for Disease Control and Prevention as previously described more in detail (Olsen et al., 2012). Briefly quality control of the analysis was maintained by analyzing a method blank (calf serum) and two spiked calf serum samples (20 ng/mL, all analytes) along with every17 samples. The detection limits (LOD), was 0.2 ng/mL. Four out of the ten phthalate metabolites, namely mono-(2-ethylhexyl) phthalate (MEHP); mono-ethyl phthalate (MEP); mono-isobutyl phthalate (MiBP); and mono-methyl phthalate (MMP), were detectable in all, but 5–12 subjects (at least 96 percent of subjects). The fact that some subjects showed undetectable levels rules out a general contamination of these compounds. Only the four metabolites with detectable levels were used in the statistical analysis. For the rest of the metabolites, 31–100 percent of the observations were below the detection limit.

Table 1
Baseline characteristics in the investigated sample. Values are given in means and (standard deviation, SD).

n	1016
Females (percent)	50.2
Height (cm)	169 (9.1)
Weight (kg)	77 (14)
Waist circumference (cm)	91 (12)
BMI (kg/m^2)	27.0 (4.3)
Waist/hip ratio	0.90 (0.075)
SBP (mmHg)	150 (23)
DBP (mmHg)	79 (10)
Serum cholesterol (mmol/L)	5.4 (1.0)
LDL-cholesterol (mmol/L)	3.3 (0.88)
HDL-cholesterol (mmol/L)	1.5 (0.42)
Serum triglycerides (mmol/L)	1.3 (0.60)
Fasting blood glucose (mmol/L)	5.3 (1.6)
Antihypertensive treatment (percent)	31.5
Statin use (percent)	14.7
Current smoking (percent)	11

2.4. Statistics

Since all compounds were skewed, the variables were log-transformed to achieve normal distributions. Relationships between BPA and phthalate metabolites and continuous risk factors were evaluated by linear multiple regression models. Gender, BMI, serum cholesterol and triglycerides, hypertension, smoking and diabetes mellitus were used as confounders in the analysis, except for the confounding variable being analyzed as the outcome it the respective regression model. Thus, if fasting glucose was the dependent variable, all confounding variables except diabetes were included in the model, etc.. Interactions between the BPA and phthalate metabolites and gender regarding risk factors were evaluated by introducing an interaction term (BPA and phthalate metabolites x gender) in the models. Logistic regression was used when the effects of smoking on levels of BPA and phthalate metabolites were evaluated, adjusting for confounders as described above.

The independency of the compounds in their relation to the Framingham Risk Score was evaluated by adding all of the BPA and phthalate metabolites as independent variables together with gender in a multiple linear regression model. Finally, to check for possible non-linear relationships, multivariable fractional polynomial models were used that evaluate whether the powers of -2, -1, 0.5, 2 or 3 fit the regression model better than the linear term (power 1) for each variable in the model.

The statistical program package STATA 11 (College Station, TX, USA) was used.

3. Results

Mean values or proportions of the established risk factors are shown in Table 1.

3.1. BPA and phthalate metabolites and CV risk factors

Of the investigated compounds, BPA, MEHP, and MMP were associated to LDL-cholesterol; for HDL-cholesterol, only BPA and MEHP were associated ($p < 0.05$, Table 2). There were also associations for MEP to blood pressure, for MiBP vs fasting glucose, and for MMP vs BMI and smoking when the compounds were investigated one by one (Table 2). After Bonferroni