Circulating Levels of Phthalate Metabolites Are Associated With Prevalent Diabetes in the Elderly

P. Monica Lind, PhD1
Björn Zethelius, MD, PhD2,3
Lars Lind, MD, PhD4

OBJECTIVE—Phthalates are ubiquitous industrial high-volume chemicals known as ligands to peroxisome proliferator–activated receptors (PPARs). Because PPAR-γ agonists modulate insulin sensitivity and are used to treat type 2 diabetes, we investigated whether circulating levels of phthalate metabolites are related to prevalent type 2 diabetes.

RESEARCH DESIGN AND METHODS—A total of 1,016 subjects, aged 70 years, were investigated in the Prospective Investigation of the Vasculature in Uppsala Seniors Study. Four phthalate metabolites were detected in almost all participant sera by an API 4000 liquid chromatograph/tandem mass spectrometer. Type 2 diabetes was defined as the use of pharmacological hypoglycemic agents or a fasting plasma glucose >7.0 mmol/L.

RESULTS—A total of 114 subjects were shown to have diabetes. Following adjustment for sex, BMI, serum cholesterol and triglycerides, educational level, and smoking and exercise habits, high levels of the phthalate metabolites monomethyl phthalate (MMP) (P < 0.01), mono(2-ethylhexyl) phthalate (MEP) (P < 0.05), and mono(2-ethylhexyl) phthalate, were associated with an increased prevalence of diabetes. Using the fasting proinsulin-to-insulin ratio as a marker of insulin secretion and the homeostasis model assessment-insulin resistance index as a marker of insulin resistance, MEP was mainly related to poor insulin secretion, whereas MEP and MMP mainly were related to insulin resistance.

CONCLUSIONS—The findings in this cross-sectional study showed that several phthalate metabolites are related to diabetes prevalence, as well as to markers of insulin secretion and resistance. These findings support the view that these commonly used chemicals might influence major factors that are regulating glucose metabolism in humans at the level of exposure of phthalate metabolites seen in the general elderly population.

Phthalates (phthalate diesters) are a large group of ubiquitous, industrial high-volume chemicals that are commonly used as plasticizers in, for example, polyvinyl chloride plastics to make plastic products more flexible. Therefore, phthalates are found in numerous household products, such as food packaging, furniture, and toys, and in medical devices, such as tubing and intravenous bags. Certain plastics may contain up to 40–50% phthalate by weight. In addition, phthalates are used in personal care products, such as cosmetics, and also in pharmaceuticals. Because phthalates are additives and, as such, not covalently bound to the plastic, they can easily leach and transfer to air and food. As a consequence thereof, humans are exposed to phthalates through inhalation, ingestion, and dermal exposure, and exposure is unavoidable because of the abundance of plastic in our society. Phthalates are rapidly degraded into the respective phthalate monoesters in phase I reactions catalyzed by lipases and esterases. The respective monoesters are eliminated in the urine as glucuronide conjugates or are further metabolized, and it is in fact the monoester metabolites that have been claimed to be responsible for adverse health effects (1–8).

Although they have relatively short half-lives in humans, phthalates have been associated with a number of health problems, including increased risk for adverse reproductive development, obesity, asthma, atherosclerosis, and allergies (9–12).

It has been known for several years that phthalates can bind to members of the nuclear peroxisome proliferator–activated receptors (PPARs) (13–19). These receptors are known to be involved in adipose tissue and lipid homeostasis, and the natural ligands mainly are fatty acids. Pharmaceutical compounds have been developed as agonists for PPAR-α and -γ available on the market, such as fibrates and glitazones, are known to influence fat distribution and change lipid status (20,21). Furthermore, PPAR-γ antagonists are known to influence glucose homeostasis via reduction of insulin resistance (22) and are used for the treatment of type 2 diabetes (20).

Because it has been reported that phthalate levels in humans are associated with obesity (10,23), a well-known effect of PPAR-γ receptor activation, and because obesity is an important risk factor for diabetes development (24), we hypothesized that high levels of phthalates in humans also might be associated with diabetes. A recent small study performed in Mexico supports this hypothesis (25). To further evaluate this hypothesis, we used data from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study, in which we have measured circulating levels of phthalate metabolites in almost 1,000 elderly individuals. As a secondary objective, we also investigated if the phthalate metabolite levels were associated with markers of insulin secretion and resistance, two major features involved in the regulation of glucose metabolism.

From the1Department of Occupational and Environmental Medicine, Uppsala University, Uppsala, Sweden; the2Department of Public Health/Geriatrics, Uppsala University, Uppsala, Sweden; the3Medical Products Agency, Uppsala, Sweden; and the4Department of Medicine, Uppsala University Hospital, Uppsala, Sweden.

Corresponding author: P. Monica Lind, monica.lind@medsci.uu.se.

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The limit of detection (LOD) was 0.2 ng/mL. All analytes (except for MiBP) were measured using standard laboratory techniques. Continuous variables are given as means (SD) or median (25th and 75th percentiles). Proportions (%) are given for smoking, antihypertensive treatment, and statin use.

### Table 1—Baseline characteristics in the investigated sample

<table>
<thead>
<tr>
<th></th>
<th>All subjects</th>
<th>Women</th>
<th>Men</th>
<th>Diabetes</th>
<th>Nondiabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1,016</td>
<td>509</td>
<td>507</td>
<td>119</td>
<td>897</td>
</tr>
<tr>
<td>Means (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of female subjects (%)</td>
<td>52</td>
<td></td>
<td></td>
<td>42</td>
<td>51</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>1,016</td>
<td>169 (9.1)</td>
<td></td>
<td>175.8 (6.5)</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>1,016</td>
<td>77.3 (14.4)</td>
<td></td>
<td>83.5 (13)</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>1,004</td>
<td>91.2 (11.6)</td>
<td></td>
<td>94.7 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>1,013</td>
<td>5.3 (1.6)</td>
<td></td>
<td>9.6 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>1,012</td>
<td>149.6 (22.7)</td>
<td></td>
<td>154.7 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>1,012</td>
<td>78.7 (10.2)</td>
<td></td>
<td>79.3 (10.3)</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1,013</td>
<td>1.5 (0.4)</td>
<td></td>
<td>1.36 (0.4)</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>1,011</td>
<td>3.4 (0.9)</td>
<td></td>
<td>3.2 (0.9)</td>
<td></td>
</tr>
<tr>
<td>Serum triglycerides (mmol/L)</td>
<td>1,013</td>
<td>1.3 (0.6)</td>
<td></td>
<td>1.5 (0.8)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>1,016</td>
<td>27.0 (4.3)</td>
<td></td>
<td>27.0 (3.7)</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma insulin (mU/L)</td>
<td>1,007</td>
<td>9.2 (7.1)</td>
<td></td>
<td>9.0 (2.4)</td>
<td></td>
</tr>
<tr>
<td>HOMA-IR (mU/L × mmol/L)</td>
<td>1,004</td>
<td>2.4 (3.6)</td>
<td></td>
<td>2.6 (4.2)</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma proinsulin (pmol/L)</td>
<td>1,002</td>
<td>10.8 (10.7)</td>
<td></td>
<td>12.3 (12.1)</td>
<td></td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>1,015</td>
<td>11</td>
<td></td>
<td>15.4 (13.7)</td>
<td></td>
</tr>
<tr>
<td>Antihypertensive treatment (%)</td>
<td>1,007</td>
<td>31</td>
<td></td>
<td>24.9 (20.3)</td>
<td></td>
</tr>
<tr>
<td>Statin use (%)</td>
<td>1,016</td>
<td>15</td>
<td></td>
<td>4.9 (2.6–15.2)</td>
<td></td>
</tr>
<tr>
<td>MEHP (ng/mL) [median]</td>
<td>1,003</td>
<td>4.5 (2.0–15.5)</td>
<td></td>
<td>4.2 (2.1–17.4)</td>
<td>4.5 (2.0–15.8)</td>
</tr>
<tr>
<td>MEP (ng/mL) [median]</td>
<td>1,003</td>
<td>11.6 (7.2–17.5)</td>
<td></td>
<td>11.6 (7.2–18.5)</td>
<td>11.4 (7.0–17.0)</td>
</tr>
<tr>
<td>MiBP (ng/mL) [median]</td>
<td>1,003</td>
<td>13.5 (9.3–29.3)</td>
<td></td>
<td>13.5 (9.1–33.3)</td>
<td>13.3 (9.2–24.5)</td>
</tr>
<tr>
<td>MMP (ng/mL) [median]</td>
<td>1,003</td>
<td>1.5 (0.8–3.1)</td>
<td></td>
<td>1.5 (0.8–3.2)</td>
<td>1.5 (0.8–3.0)</td>
</tr>
</tbody>
</table>

Continuous variables are given as means (SD) or median (25th and 75th percentiles). Proportions (%) are given for smoking, antihypertensive treatment, and statin use.
phthalate (MiBP), and monomethyl phthalate (MMP), were detectable in all but 5–12 subjects (at least 96% 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% of the observations were below the LOD. The measured serum concentrations of MEHP, MEP, MiBP, and MMP are given in Table 1. The analysis of the phthalates took place 5–8 years following the collection of the samples.

**Insulin and proinsulin measurements**

At the laboratory of the Department of Public Health and Caring Sciences/Geriatrics, University Hospital, Uppsala, plasma proinsulin and insulin concentrations were determined using the Proinsulin ELISA and the Insulin ELISA immunoassays (Mercodia, Uppsala, Sweden) on a Bio-Rad Coda automated EIA analyzer (Bio-Rad Laboratories, Hercules, CA).

**Calculations of insulin secretion and resistance**

The ratio of fasting proinsulin to insulin was used as an index of insulin secretion, since this ratio increases with failing β-cell function (27,28). The homeostasis model assessment-insulin resistance (HOMA-IR), as an index of insulin resistance, was calculated as fasting insulin × glucose/22.5 (29).

**Statistics**

All four phthalate metabolites, as well as the indices of insulin secretion and resistance, were skewed toward high levels but were normally distributed following ln-transformation. Relationships between phthalate metabolites and prevalent diabetes were evaluated by logistic regression models, first using the phthalate metabolites as continuous variables and thereafter following division of the phthalate metabolites into quintiles. For the continuous analysis, two steps of adjustments were used: 1) adjustment for sex only and 2) multiple adjustments for sex, serum cholesterol and triglycerides, BMI, smoking and exercise habits, and educational levels. In the quintile analysis, P values are given for linear trend analysis as well as for quadratic trend. In the quintile analysis, only multiple-adjusted P values are given.

A similar approach was used when relating phthalate metabolites to either the proinsulin-to-insulin ratio or to HOMA-IR index, except that linear regression was used. In this analysis, only nondiabetic subjects were included. General additive models were used (30) to visualize the relationship between some of the phthalate metabolites and prevalent diabetes in a continuous fashion in Supplementary Fig. 1. These models were adjusted for the same covariates as the multiple-adjusted models described above.

**RESULTS**—Mean values or proportions of the established risk factors are shown in Table 1. Relationships between the four evaluated phthalate metabolites and prevalent diabetes in logistic regression models using continuous variables, both MEP and MiBP were significantly related to diabetes following adjustment for sex only. Following multiple adjustments, however, only MiBP levels were significantly related to diabetes (odds ratio [OR] 1.30 [95% CI 1.10–1.55], P = 0.0025; for details see Supplementary Table 2). In Supplementary Table 2, details on different levels of adjustments are presented.

No significant interactions between the four phthalate metabolites and sex, obesity, smoking, and education levels were detected when interaction terms between the phthalate metabolites and these four confounders were introduced in the models. A sensitivity analysis for these four confounders is presented in Supplementary Table 3. Of note is that the OR for MiBP is higher in men than women (1.48 [95% CI 1.19–1.85] in men and 1.08 [0.80–1.46] in women). However, the interaction term between MiBP and sex regarding prevalent diabetes is not significant (P = 0.11).

In quintile analysis, MEP and MiBP, as well as MMP, showed a significantly elevated prevalence of diabetes in the highest quintile compared with the lowest (OR 2.00–2.50), and the linear trend tests were significant. Furthermore, for MEP the quadratic trend test was significant. The highest OR was seen in the third quintile (OR 2.87 [95% CI 1.37–6.03]; for additional details see Table 3).

**Phthalates versus the proinsulin-to-insulin ratio**

MEHP and MiBP were significantly related to a high proinsulin-to-insulin ratio, both following sex adjustment only and following multiple adjustments, respectively. MEP was weakly related to the proinsulin-to-insulin ratio in an inverse way in linear regression models using continuous variables (Table 4). In quintile analysis, the quadratic trend test for MiBP was significant, being consistent with the finding that the highest proinsulin-to-insulin ratio was seen in the third quintile (for details see Supplementary Table 4).

**Phthalates versus the HOMA-IR index**

MEP and MMP were significantly related to a high HOMA-IR index, both following sex adjustment only and following multiple adjustments, respectively (Table 4). In quintile analysis, the quadratic trend test for MEP was significant, being consistent with the finding that most of the effect on HOMA-IR was seen in the third quintile (for details see Supplementary Table 5).

In the analysis of HOMA-IR and the proinsulin-to-insulin ratio, no diabetic subjects were included because the diabetic state or pharmacological treatment for diabetes may affect the proinsulin and insulin measurements.

**Table 2—Relationships between four phthalate metabolites and prevalent diabetes**

<table>
<thead>
<tr>
<th></th>
<th>Sex adjusted</th>
<th>Multiple adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>MEHP</td>
<td>1.05 (0.92–1.21)</td>
<td>0.461</td>
</tr>
<tr>
<td>MEP</td>
<td>1.30 (1.00–1.69)</td>
<td>0.049</td>
</tr>
<tr>
<td>MiBP</td>
<td>1.25 (1.07–1.46)</td>
<td>0.006</td>
</tr>
<tr>
<td>MMP</td>
<td>1.12 (0.95–1.33)</td>
<td>0.174</td>
</tr>
</tbody>
</table>

The phthalate metabolites are used as ln-transformed continuous variables in the analysis. The first models are just sex adjusted, whereas the multiple-adjusted models used sex, serum cholesterol and triglycerides, BMI, smoking and exercise habits, and educational levels as confounders.
**Phthalates and diabetes in the elderly**

**Table 3—Relationships between four phthalate metabolites and prevalent diabetes.**

<table>
<thead>
<tr>
<th>Quintile</th>
<th>MEHP</th>
<th>MEP</th>
<th>MiBP</th>
<th>MMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>1.17</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>0.77</td>
<td>0.65</td>
<td>0.65</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>0.24</td>
<td>0.19</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>5</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
</tbody>
</table>

The phthalate metabolites are divided into quintiles in the analysis, and the first quintile is used as the referent. The values for linear trend and quadratic trend are adjusted for sex, serum cholesterol and triglycerides, BMI, smoking and exercise habits, and educational levels.

**DISCUSSION**—The current study showed that some of the measured circulating phthalate metabolites, MEP, MMP, and MiBP, were associated with prevalent diabetes in the present cross-sectional study. Furthermore, MiBP levels were inversely related to a marker of poor insulin secretion, whereas MMP and MEP levels were related to insulin resistance, two major factors involved in regulation of an impaired glucose metabolism and independent risk factors for type 2 diabetes development.

**Comparison with the literature**

In a recent cross-sectional study of Mexican women, self-reported diagnosis of diabetes was related to several phthalate metabolites (25). However, neither MEP nor MiBP was found to be significantly related to diabetes in the Mexican study, and MMP was not evaluated. The current study has the advantage that three times as many diabetic patients were included. Furthermore, we used the combination of self-reported diabetes and measurements of fasting glucose and/or use of pharmacological treatment of diabetes to define prevalent diabetes. The addition of fasting glucose measurements resulted in the addition of another 31 diabetic case subjects to the 85 subjects that reported a history of diabetes. Thus, if self-reported diagnosis only and no fasting glucose measurements had been used to define cases, 26% of the diabetes cases would have been misclassified and observations made would have been diluted.

In the sensitivity analysis in the current study, a higher OR for MiBP regarding diabetes was found in men compared with women (1.48 vs. 1.08). However, the interaction term between MiBP and sex regarding diabetes was not significant, so future studies have to determine whether this observed relationship between MiBP and diabetes really differs between men and women.

In an evaluation of male participants in the National Health and Nutrition Examination Survey 1999–2002, high levels of MBP and monobenzyl phthalate (MBzP), and MEP were associated with insulin resistance (23). Thus, the finding of a relationship between MEP and a high HOMA-IR index is consistent between the studies. MBP and MBzP were measured in the PIIVUS study, but because only a small fraction of the participants showed detectable levels in the circulation, we were not able to perform any meaningful evaluation of these phthalate metabolites. To the best of our knowledge, no other study has investigated whether phthalate metabolites are related to markers of insulin secretion.

**Measurement of phthalates and sources of exposure**

Because the parent phthalates are so abundant, it is a hopeless task to measure those compounds without major contamination. Therefore, usually their metabolites, not abundant in the environment, are measured in the circulation or in the urine (31). The majority of previous studies on human phthalate exposure is compromised on...
urine testing. Measurements in the urine have the advantage that higher levels are found and thereby more metabolites could be properly detected. Thus, a limitation of the current study is that only four phthalate metabolites could be investigated in detail with regard to diabetes.

In the current study, MiBP, MMP, MEP, and MEHP were detectable in almost all analyzed serum samples. The common feature of the metabolites MEP, MiBP, and MMP is that all these three metabolites are derived from degradation of associated phthalate parent compounds (diethyl phthalate, di-isobutyl phthalate, and dimethyl phthalate, respectively) used as solvents/carriers of fragrances used in personal care products. Di-2-ethyl phthalate, with the metabolite MEHP, is on the other hand mainly used as plasticizer to make plastic compounds more flexible.

In a Danish study, the correlations between levels of 13 metabolites in different matrices (urine, semen, and serum) were examined in 60 young men. Both MEP and MiBP levels were correlated in serum and urine, indicating that serum levels could be used as biomarkers of human exposure (1). In the Danish study, MiBP, MEP, MBzP, mono-(2-ethyl-5-oxohexyl) phthalate, and mono-(2-ethyl-5-hydroxyhexyl) phthalate were found in lower levels and in fewer samples, but the LOD was higher than in the current study. Frederiksen et al. (1) found higher levels and a detection rate of monoisononyl phthalate, whereas mono-n-octyl phthalate was not detectable in any of their samples. For these metabolites, their LOD was comparable with the current study.

Sources of exposure of these compounds and comparisons with the levels in other studies are described in more detail in our previous recent publication by Olsén et al. (26) and at http://www.atstdr.cdc.gov/.

Possible mechanisms

Because phthalate metabolites are known ligands to PPARs (13–19), receptors known to influence glucose homeostasis, impairments in PPAR-signaling pathways are most likely to contribute to the actions of phthalates on glucose metabolism and diabetes development. PPAR activation has been shown to be involved in different steps in glucose homeostasis, such as influence on insulin resistance (22) and on insulin secretion (32), influence on circulating levels of lipids (21), and altering the amount of visceral and subcutaneous fat (21,33). Addi-


References


Phthalates and diabetes in the elderly


