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Excretion of Di-2-ethylhexyl phthalate (DEHP) metabolites in urine is related to body mass index because of higher energy intake in the overweight and obese

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ABSTRACT

A higher body mass index (BMI) has been positively associated with the rate of excretion of di-2-ethylhexyl phthalate (DEHP) metabolites in urine in data from the National Health and Nutrition Examination Survey (NHANES), suggesting an association between DEHP exposure and BMI. The association, however, may be due to the association between body mass maintenance and higher energy intake, with higher energy intake being accompanied by a higher intake of DEHP. To examine this hypothesis, we ran a Monte Carlo simulation with a DEHP physiologically-based pharmacokinetic (PBPK) model for adult humans. A realistic exposure sub-model was used, which included the relation of body weight to energy intake and of energy intake to DEHP intake. The model simulation output, when compared with urinary metabolite data from NHANES, supported good model validity. The distribution of BMI in the simulated population closely resembled that in the NHANES population. This indicated that the simulated subjects and DEHP exposure model were closely aligned with the NHANES population of interest. In the simulated population, the ordinary least squares regression coefficient for log(BMI) as a function of log(DEHP nmol/min) was 0.048 (SE 0.001), as compared with the reported value of 0.019 (SE 0.005). In other words, given our model structure, the higher energy intake in the overweight and obese, and the concomitant higher DEHP exposure, describes the reported relationship between BMI and DEHP.

1. Introduction

The notion that certain environmental agents ("obesogens") can cause obesity is grounded on experimental data (Grün and Blumberg, 2006; Hao et al., 2012), and has some support from human data (Legler et al., 2015; Heindel et al., 2015). For example, the cross-sectional data among adults with low-level exposure were found to consistently support a positive association of DDT metabolites with obesity (Tang-Péronard et al., 2011). However, a pharmacokinetic analysis of this association in humans indicated that the association may be an artefact, due to the combined effect of trends in exposure, long half-life of metabolites, obesity-dependent half-life, and timing of the studies of the persistent compounds (Wolff et al., 2007).

Some non-persistent compounds, including selected phthalates, have also been suspected human obesogens. In a recent analysis of data from the 2009–2010 National Health and Nutrition Survey (NHANES), Christensen et al. (2014) examined the association of body mass index (BMI) with di-2-ethylhexyl phthalate (DEHP) and five other phthalate metabolites in urine, and found small, but statistically significant positive associations for all of them. Christensen et al.'s approach was novel because it used urine metabolite excretion rate as an improved metric of exposure, and with this metric phthalates were more strongly related to BMI than when the traditional methods based on urine metabolite were used (Hays et al., 2015). Now that the BMI-phthalate association has been more clearly demonstrated in NHANES data, the reason for the association deserves careful scrutiny so that proper weighting can be given to the findings in causal assessments.

The major source of exposure to phthalates is food (Fromme et al., 2007a). Energy intake from food is positively correlated with body mass (Swinburn et al., 2009). The BMI-DEHP association may be due to

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higher body mass causing higher energy intake, with higher energy intake being accompanied by a higher intake of DEHP. The effect of excess energy intake on weight gain is well recognized. However, what has been only more recently appreciated is that once weight has been gained, the baseline energy requirement has increased disproportionally. As weight is gained, more lean tissue is needed to maintain mobility. Lean tissue is more energetically expensive to sustain than adipose tissue. Increasing adiposity results in a baseline energy requirement that is large compared with the energy excess that initially caused the adiposity. In a population with a substantial prevalence of overweight and obesity, the increased energy requirement due to higher body weight dwarfs the energy intake originally leading to the weight gain (Hall et al., 2011). A higher energy intake will correlate with a higher exposure to, e.g., DEHP, for which most exposure is via diet (Wormuth et al., 2006; Schecter et al., 2013; Mervish et al., 2014).

To address the question of whether increased food intake due to higher weight could account for the increase in urine DEHP metabolites with greater body mass index, we considered the quantitative relationships at each step (e.g., how much body weight influences DEHP exposure, how much DEHP exposure influences the rate of excretion of metabolites, etc.) We did this with a Monte Carlo simulation with a DEHP physiologically-based pharmacokinetic (PBPK) model for adult humans to simulate urinary excretion rate of DEHP metabolites in the NHANES population studied by Christensen et al. (2014). The choice of a PBPK model platform for the analysis of the overall quantitative relationship was natural because in such models body size routinely affects exposure, the relationship between exposure and excretion of metabolites is intrinsic to the method, and assessing the influence of population variation in exposure and body size on the association is straightforward. While Christensen et al. reported several phthalates, we focused on DEHP because detailed data on the concentration of DEHP in duplicate-plate food collections was available, allowing for estimation of daily intake based on energy intake.

2. Methods

2.1. PBPK model

A PBPK model of DEHP for adult humans has been published (Gentry et al., 2011). That Gentry model was based on allometric scaling of a rat model to human physiology (ICRP, 2002), with adjustment of the rates of MEHP metabolism and urinary clearance to fit the human data that were available at the time which was the Anderson et al. (2011) cumulative excretion in urine. The addition of the plasma data that were not included in Anderson (Covance, 2010) along with the DEHP and MEHP whole blood time-course data (Kessler et al., 2012) provided a more robust dataset to estimate uptake and clearance of DEHP, MEHP and the oxidative metabolites after oral exposure to DEHP.

The Gentry et al. (2011) model was for DEHP and its primary metabolite, mono-(2-ethylhexyl)–phthalate (MEHP). We extended this model to include three additional secondary metabolites: 2-Ethyl-5-hydroxy-hexylphthalate (MEHHP), 2-Ethyl-5-carboxy-pentylphthalate (MECPP), and 2-Ethyl-5-oxy-hexylphthalate (MEOHP)(Fig. 1). The four DEHP metabolites included in the DEHP model corresponded to all measured DEHP metabolites reported in the 2009–2010 NHANES data (CDC, 2009), and were the same ones analyzed by Christensen et al. (2014). For DEHP, the model includes liver, gastrointestinal (GI) lumen, GI wall, fat, rest of the body (lumped remaining tissues), and blood. Excretion of DEHP to urine was described as clearance from plasma (MEHP) or the volume of distribution (oxidative metabolites) compartments. The MEHP submodel includes GI, liver and rapidly and slowly perfused lumped compartments. The glucuronide conjugate of MEHP submodel was simplified from MEHP to include GI lumen, liver and a lumped remaining perfused tissue. Each oxidative metabolite was given its own single volume of distribution compartment and either was further metabolized to the corresponding glucuronide conjugate or excreted directly into urine. To reduce the number of parameters needing estimation, the oxidative metabolism of MEHP was described as a single saturable metabolic pathway and the fraction of each oxidative metabolite was estimated from the available human data. All model constants are reported in Table 1.

2.2. Human data

Two controlled oral bolus studies were used to estimate the rate constants for metabolism and urinary clearance rates of DEHP and its metabolites. Kessler et al. (2012) reported the time-course blood concentration of DEHP and MEHP (free representing non-conjugated monoester and total representing the sum of the conjugated and non-conjugated monoester) after a single oral bolus (645 ± 20 μg DEHP-D4/kg BW) of labelled DEHP. DEHP was ingested as an emulsion consisting of an aqueous saccharose solution (70% w/v). Data were reported for blood DEHP and MEHP (free and total) at 0.25 h before ingestion, at 0.00 h, and at 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10 and 24 h after ingestion. Additional blood samples were collected in three of the four subjects at 5.0, 7.0 and 9.0 h after ingestion. Total urine voided within a time-interval was collected using screw-capped polypropylene bottles. The end times of each collection interval were 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10, 12, 14, 18, 22, 26, 30, 34, 38, 42 and 46 h after ingestion. The total amount of MEHP, MEHHP and MEOHP in urine normalized to BW (nmol/kg BW) was reported.

Anderson et al. (2011) reported the cumulative excretion of MEHP, MEHHP, MEOHP and MECPP after a single oral bolus of labelled DEHP in 20 volunteers. The study included analysis of plasma samples for DEHP metabolite concentrations; however, these data were not reported in the publication. The high dose group (0.78 mg per individual) was administered in olive oil on a piece of bread as a part of a standard breakfast. Urine samples were collected over time intervals of pre-dose, 0–4, 4–8, 8–12, 12–24, 24–36, and 36–48 h post dose. Plasma samples were collected pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h. A low dose (0.31 mg per individual) was also administered on a separate occasion; however, MEHP and its metabolite concentrations in plasma were nearly all below the limit of quantification (LOQ) so only the data from the high dose group was used in our modeling. While urine samples were analyzed to report total (free plus conjugate) MEHP and its metabolites, plasma samples were analyzed with and without enzymatic deconjugation with glucuronidase in order to report the free and total concentrations of monoester and its metabolites separately.

2.3. Model parameterization

As noted above, the model presented in Gentry et al. (2011) was primarily based upon allometric scaling of the rat DEHP model because little information was available on the glucuronide conjugation or oxidative metabolism in humans. The Kessler data were used to estimate the oral absorption and hydrolysis of DEHP to MEHP in the GI lumen to provide the best fit to the reported plasma concentrations of monoester and its metabolites as well as diester itself. The Kessler whole blood time-course dataset had two notable features. The first was the detection of the appreciable mass of DEHP that was absorbed systemically. The second notable feature was the biphasic absorption pattern seen in the individual DEHP blood concentration time profile data (Kessler et al.'s Fig. 2). It is unclear from Kessler's data alone as to the specific mechanism; however, the biphasic absorption may be due to the emulsion used to dose subjects or the pulsatile nature of stomach emptying. In order to capture the two peaks in the whole blood data, a 2nd GI compartment was added along with a delay in both hydrolysis and absorption in this compartment in order to allow time to pass prior to the mass being available for metabolism or absorption. The delay ranged from 2 to 4 h depending on the subject-specific blood profile of