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Dietary and inhalation exposure to polycyclic aromatic hydrocarbons and urinary excretion of monohydroxy metabolites – A controlled case study in Beijing, China

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ABSTRACT

Daily dietary and inhalation exposures to 16 parent polycyclic aromatic hydrocarbons (PAHs) and urinary excretion of 13 monohydroxy metabolites (OHPAHs) were monitored for 12 non-smoking university students in Beijing, China, during a controlled feeding experiment. The relationship between the urinary excretion of OHPAHs and the uptake of PAHs was investigated. The results suggest severe exposure of the subjects to PAHs via both dietary and inhalation pathways. Large increase of most urinary OHPAHs occurred after the ingestion of lamb kabob. Higher concentrations of OHPAHs were observed for female subjects, with the intakes of parent PAHs lower than those by males, likely due to the gender differences in metabolism. It appears that besides 1-PYR, metabolites of PHE could also be used as biomarkers to indicate the short-term dietary exposure to PAHs and urinary 3-BaA may serve as the biomarker for inhalation intake of high molecular weight PAHs.

1. Introduction

It is well recognized that exposure to polycyclic aromatic hydrocarbons (PAHs) can cause adverse health effects including cancer (Boström et al., 2002). Relying heavily on coal and biomass fuels for energy, emission of PAHs in China accounts for one fifth of the global total (Shen et al., 2013), leading to severe contamination of environment (Liu et al., 2007a; Tao et al., 2004) and food (Xia et al., 2010). Human expose to PAHs via dietary, inhalation, and dermal contact (Boström et al., 2002), among which, dermal contact can be minimal for non-occupational population (Li, 2007). Due to PAHs' low solubility, exposure through drinking water does not contribute much either. Many evidences suggest that food ingestion dominates overall PAH intake among nonsmokers who are not subject to occupational exposure (Li, 2007; Suzuki and Yoshinaga, 2007; Viau et al., 2002).

PAHs in uncooked food originate from contaminated soils, water, air, or animal feed (Phillips, 1999; Zhao et al., 2012). In general, PAH contents in meat are higher than those in cereals and vegetables (Xia et al., 2010). Relatively high PAH concentrations are often found in processed food, especially grilled and smoked meat (Alomirah et al., 2011). For example, it was reported that the total concentration of 16 parent PAHs ($pPAH_{16}$) in grilled duck breast was as high as 319 ng/g, compared with 8.5 ng/g in the steamed dishes (Chen and Lin, 1997). The enriched PAHs during the process of grilling or smoking are likely to arise from the pyrolysis of fat at high temperature and adsorption of the PAHs emitted from combusted fuels (Lijinsky, 1991). It has been demonstrated that ingestion of these heavily PAH-polluted food can result in increased health risks like colorectal adenomas and postmenopausal breast cancer (Sinha et al., 1999; Steck et al., 2007).

Urinary OHPAHs have long been regarded as the biomarkers to assess internal exposure to PAHs (Jongeneelen et al., 1985). Among a variety of OHPAHs, 1-hydroxypyrene (1-PYR) is the most preferable tracer. Enhanced levels of 1-PYR have been reported in urine samples from workers occupationally exposed to PAHs, smokers, or others who have consumed PAH-polluted food and they were often found to be significantly correlated with PAH exposure (Li et al., 2012; Merlo et al., 1998; Sobus et al., 2009). However, discrepancies among different authors were sometimes reported on the relationship between PAH intake and urinary excretion, especially for population in general environmental settings (Merlo et al., 1998;

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Suzuki and Yoshinaga, 2007; Viau et al., 2002). The correlation between PAH intake and urinary excretion might be confounded by the inaccurate estimation of PAH exposure doses, neglection of other possible exposure routes, or inter individual variations of urinary OHPAHs (Viau et al., 2002; Scherer et al., 2000).

In this controlled study, PAHs in indoor and ambient air and daily dishes, to which a group of 12 university students exposed, were monitored during an 11-day experiment. The activity and food consumption of the subjects were strictly controlled and the relationship between parent PAH exposure and urinary OHPAH was addressed using the mean value of male and female subjects to eliminate inter-individual variability. Meanwhile, urinary excretion of OHPAHs was characterized and relative contributions of ingestion and inhalation pathways, differences in exposures and excretions between genders, excretion kinetics, and potential exposure biomarkers are discussed.

2. Methodology

2.1. Experimental design

Six male and six female university students, all nonsmokers, participated in this study, and oral informed consents were obtained individually. All the students lived on campus of Peking University and commuted on foot or by bicycle between dormitories and their office/laboratory building once or twice a day (<15 min per trip). None of the individual subjects left the campus during the study period. Demographic profiles including age, gender, height, and weight are listed in Table S1. The study was conducted from Dec. 21 to Dec. 31, 2010. A number of popular dishes were selected and all 12 subjects had exactly the same menu including three meals and a night snack at fixed hours and 2.5 L of bottle water every day (Table S2). Lamb kabob was purchased from a street vendor near the campus, while all other food was bought from the university cafeterias where the majority of students eat their meals. Daily activities of the students were self-recorded and summarized in Table S3.

2.2. Sample collection

An aliquot of each dish selected was mixed and stored at −20 °C prior to analysis. Daily (24 h) air samples were collected at four sites (laboratory, student's offices, student's dormitory, and outdoors) using active samplers (1.5 L/min, XQC-15E, Tianyue, China) for particulate (glass fiber filters, GFFs, 30 mm diameter) and gaseous (polyurethane foam plugs, PUFs, 22 mm diameter × 76 mm, 0.024 g/m^3) phase PAHs, respectively. The GFFs were baked at 450 °C for 6 h and equilibrated in a desiccator (25 °C) for 24 h prior to weighing and sampling. PUFs were purified by Soxhlet extraction using acetone, dichloromethane, and *n*-hexane successively for 8 h each. Three urine samples (100 mL each) were collected from each subject every other day (Dec. 22, 24, 26, 28, and 30) immediately before, 8 h after, and 24 h after the lunch.

2.3. Sample extraction and analysis

The extraction and cleanup procedure of food and air samples can be found elsewhere in detail (Xia et al., 2010, 2013). Briefly, food samples were extracted with 20 mL of acetonitrile using a microwave-accelerated reaction system (CEM Mars Xpress, USA) and further extracted with *n*-hexane. PUFs were Soxhlet extracted with 150 mL of *n*-hexane/acetone mixture (1:1, v/v) for 8 h and GFFs were extracted with 25 mL of the same solvent mixture using the microwave-accelerated reaction system. The extracts were concentrated and transferred to silica/alumina chromatography columns for purification. Internal standards (NAP-d_8, ACE-d_{10}, ANT-d_{10}, CHR-d_{12}, and Perylene-d_{12}, J&K Chemical, USA) were spiked before analysis.

PAHs were analyzed by a gas chromatograph using a DB-5MS capillary column, equipped with a mass spectrometer (Agilent 6890/5973, USA). The oven temperature was programmed at 80 °C for 1 min, increased to 270 °C at a rate of 5 °C/min, held for 2 min, to 290 °C at 3 °C/min, held for 1 min, then to 305 °C at 10 °C/min and held for 12 min. Helium was used as the carrier gas. Target PAHs were identified based on the retention time and qualitative ions of standards in selected ion monitoring mode and were quantified by the internal standards. Monitored PAHs included naphthalene (NAP), acenaphthene (ACE), acenaphthylene (ACY), fluorene (FLO), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo(a)anthracene (BaA), chrysene (CHR), benzo(b)fluoranthene (BbF), benzo(k) fluoranthene (BkF), benzo(a)pyrene (BaP), diben(a,h)anthracene (DahA), indeno(1,2,3-cd)pyrene (IcdP), and benzo(g,h,i)perylene (BghiP).

Urinary OHPAH was extracted based on the method of Fan et al. (2006) with slight changes accordingly. An aliquot of 20 mL of urine sample was thawed and enzymolyzed with 40 μl of β-glucuronidase/sulfatase (G0876, Sigma–Aldrich, USA) in a mixture of 2 mL of 0.1 mol/L hydrochloric acid, and 5 mL of 0.5 mol/L sodium acetate and acetic acid buffer at 37 °C for 16 h and centrifuged at 2000 rpm for 10 min. The supernatant was extracted and concentrated by solid-phase extraction using ENVITM-18 column (Supelclean, USA), which was previously activated by 5 mL of methanol (GR, Beijing Tongguang, China) and 10 mL of ultrapure water successively. The column was washed with 10 mL of ultrapure water and 10 mL of 30% methanol (v/v) in turn and the analyte was eluted with methanol (8 mL) and concentrated to 200 μL by a gentle stream of nitrogen. Another aliquot of 20 mL of urine sample was centrifuged at 2000 rpm for 10 min and the supernatant was diluted 10 times by the mobile phase solution (95% 0.02 mol/L sodium dihydrogen phosphate/5% methanol, v/v) for the measurement of urinary creatinine, according to the method of Tsikas et al. (2004).

The concentrations of OHPAHs were determined by a high performance liquid chromatography equipped with XDB-C18 capillary column (4.6 × 250 mm, 5 μm) and a fluorescence detector (Agilent 1100/1200, USA). The injection volume was 20 μL. OHPAHs were separated by gradient elution (methanol/water, v/v) at a flow rate of one mL/min with 50, 90, and 50% methanol at 0–30, 30–35, and 35–45 min, respectively. The excitation wavelengths were 227, 272, 256, 240, and 275 nm and the emission wavelengths were 355, 336, 370, 387, and 430 nm for 0–13, 13–18, 18–22, 22–25, and 25–32 min, respectively. 1-hydroxynaphthalene (1-NAP), 2-hydroxynaphthalene (2-NAP), 2-hydroxyfluorene (2-FLO), 2-hydroxyphenanthrene (2-PHE), 3-hydroxyphenanthrene (3-PHE), 4-hydroxyphenanthrene (4-PHE), 9-hydroxyphenanthrene (9-PHE), 1-PYR, 3-hydroxybenzo(a)anthracene (3-BaA), 3-hydroxychrysene (3-CHR), 6-hydroxychrysene (6-CHR), 3-hydroxybenzo(a)pyrene (3-BaP), 9-hydroxybenzo(a)pyrene (9-BaP) were quantified. Urinary creatinine was detected by a variable wavelength detector (Agilent 1100, USA) at 235 nm with a retention time of 10 min.

2.4. Quality control

Reagent and procedure blanks were measured together with each batch of samples, and subtracted from the results. At least two replicates were measured for each sample. The detection limits of PAHs ranged from 0.23 to 1.42 ng/mL and 0.53 to 1.32 ng/mL for the gaseous and particulate phase air samples, respectively, and from 0.053 to 0.25 ng/g for the food samples. Method recoveries determined by spiking the sampling matrix with PAH standards (PPH-10JM, Chem Service, USA) ranged from 66 to 143% and 87 to 154% for gaseous (five duplicates) and particulate (four duplicates) phase PAHs, respectively. 2-Fluoro-1,1′-biphenyl and *p*-terphenyl-d_{14} (2.0 μg/mL, J&K Chemical, USA) were spiked before extraction as surrogates, the recoveries of which were 64–92% for food samples, 54–86% for gaseous phase PAHs, and 65–110% for particulate phase PAHs, respectively. The detection limits of OHPAHs in the urine samples ranged from 0.00165 to 0.55 μg/L. Recoveries of OHPAHs in urine samples spiked with standards were 88–123%.

2.5. Data analysis

The dietary intakes were calculated based on the measured PAH concentrations in food and the corresponding food consumptions. The daily inhalation exposures to PAHs were estimated according to the measured air concentrations, the daily activity pattern, and the respiration rate of 243.2 L/d per unit body weight (Zhang et al., 2009). The total daily intakes of individual subjects were the sums of dietary and inhalation exposures and reported as ng/kg d body weight. Creatinine-corrected concentrations were used for urinary OHPAHs and expressed as μmol/mol creatinine. SPSS 13.0 (SPSS Inc., USA) was used for statistical analysis at a significance level of 0.05.

3. Results and discussion

3.1. PAHs in food and air

The measured $pPAH_{16}$ in the food varied from 11 ± 5.0 ng/g in banana to 350 ± 84 ng/g in lamb kabob, which was generally within the same ranges of similar food reported in other places (see details in Table S4). For example, the concentrations of PYR in all dishes except lamb kabob (0.051–8.0 ng/g) were comparable to those in the daily diets of Canadians (Viau et al., 2002). The measured BaP concentrations in lamb kabob and fried steak were similar to those measured in barbecued meats (Alomirah et al., 2011; Chen and Lin, 1997). The $pPAH_{16}$ in lamb kabob was also in the same range of those in meat kebab in Kuwait (Alomirah et al., 2011). Food contamination in China is regulated by National Food Safety Standard and the maximum level of BaP allowed is 5 ng/g for grain and smoked and grilled meat (PRC, 2012), which is identical to European Commission standard (EC, 2006). Among all the food tested, BaP was only detected in lamb kabob (1.1 ± 1.6 ng/g) and fried steak (0.9 ± 1.2 ng/g), both of which were lower than the national standard.