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Associations between urinary biomarkers of polycyclic aromatic hydrocarbon exposure and reproductive function during menstrual cycles in women☆

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ABSTRACT

Essentially all women are exposed to polycyclic aromatic hydrocarbons (PAHs), formed during incomplete combustion of organic materials, including fossil fuels, wood, foods, and tobacco. PAHs are ovarian toxicants in rodents, and cigarette smoking is associated with reproductive abnormalities in women. Biomonitoring of hydroxylated PAH (OH-PAH) metabolites in urine provides an integrated measure of exposure to PAHs via multiple routes and has been used to characterize exposure to PAHs in humans. We hypothesized that concentrations of OH-PAHs in urine are associated with reproductive function in women. We recruited women 18–44 years old, living in Orange County, California to conduct daily measurement of urinary luteinizing hormone (LH) and estrone 3-glucuronide ($E_1$3G) using a microelectronic fertility monitor for multiple menstrual cycles; these data were used to calculate endocrine endpoints. Participants also collected urine samples on cycle day 10 for measurement of nine OH-PAHs. Models were constructed for eight endpoints using a Bayesian mixed modeling approach with subject-specific random effects allowing each participant to act as a baseline for her set of measurements. We observed associations between individual OH-PAH concentrations and follicular phase length, follicular phase LH and $E_1$3G concentrations, preovulatory LH surge concentrations, and periovulatory $E_1$3G slope and concentration. We have demonstrated the feasibility of using urinary reproductive hormone data obtained via fertility monitors to calculate endocrine endpoints for epidemiological studies of ovarian function during multiple menstrual cycles. The results show that environmental exposure to PAHs is associated with changes in endocrine markers of ovarian function in women in a PAH-specific manner.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants formed during incomplete combustion of organic materials such as wood, tobacco, fossil fuels, and food (ATSDR, 1995). Data from the National Health and Nutrition Examination Survey (NHANES) on concentrations of hydroxylated PAHs (OH-PAHs) in the urine of representative samples of Americans show that essentially all Americans are exposed to PAHs (Li et al., 2008; NHANES, 2009). For non-smokers who do not consume grilled or roasted foods, air pollution is the largest source of exposure. Residents of urban areas have higher inhalation exposure to PAHs than do residents of rural areas (Menzie et al., 1992).

Exposure to tobacco smoke can be the principal source of PAH exposure for smokers. Sidestream smoke has 10-fold higher concentrations

☆ The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the National Institute for Occupational Safety and Health (NIOSH). Mention of any company or product does not constitute endorsement by NIOSH.

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of PAHs than mainstream tobacco smoke, and second hand smoke is another major source of exposure (Lodovici et al., 2004; Lu et al., 2008; Shopland et al., 2001). PAHs have been measured in human serum and ovarian follicular fluid (Neal et al., 2008). The latter study showed that follicular fluid of women smokers had significantly elevated levels of the PAH benzo[*a*]pyrene (BaP) compared to follicular fluid of nonsmoking women.

Many PAHs are mutagenic and carcinogenic (ATSDR, 1995; IARC, 1983, 2010) and are potent ovarian toxicants and ovarian tumorigens in rodents. Both single high doses (Mattison, 1979; Mattison and Nightingale, 1982; Mattison and Thorgeirsson, 1979; Mattison et al., 1980; Takizawa et al., 1984) and multiple lower doses (Borman et al., 2000) of BaP, 9,12-dimethyl-1,2-benzanthracene (DMBA), and 3-methylcholanthrene destroy immature primordial and primary follicles in mice and rats. DMBA concentration-dependently increases reactive oxygen species and induces apoptosis in cultured rat antral follicles (Tsai-Turton et al., 2007). BaP inhibits growth, survival, and estradiol and anti-Müllerian hormone secretion of cultured mouse secondary follicles (Neal et al., 2007; Sadeu and Foster, 2011).

Epidemiological studies have linked smoking to decreased fecundability (probability of pregnancy) (Alderete et al., 1995; Baird and Wilcox, 1985; Hassan and Killick, 2004; Jensen et al., 1998) and earlier onset of menopause (Harlow and Signorello, 2000; Mattison et al., 1989; van Noord et al., 1997). In a prospective study in which urinary estrogen and progesterone metabolites were measured daily during multiple menstrual cycles, exposure to environmental tobacco smoke in nonsmoking women was associated with lower concentrations of estrone conjugates during the 20-day window around ovulation (Chen et al., 2005).

PAHs are readily absorbed through the lungs, gut, and skin (ATSDR, 1995). PAHs generally require metabolic activation to exert toxicity. They are first oxidized by cytochrome P450 (CYP) enzymes to epoxides, which undergo hydrolysis by epoxide hydrolase to diols, which can be oxidized by CYPs to diol epoxides or converted to catechols by aldo-keto deductases (Penning, 2004; Verma et al., 2012; Xue and Warshawsky, 2005). The diol epoxide metabolites are DNA damaging mutagens, and the catechols undergo redox cycling, generating reactive oxygen species that can damage cellular macromolecules (Denissenko et al., 1996; Penning, 2004; Verma et al., 2012; Xue and Warshawsky, 2005). The epoxides can also spontaneously rearrange to monohydroxylated intermediates, which are excreted as conjugates in the urine or bile (Verma et al., 2012). Biomonitoring of these hydroxylated PAH metabolites in urine provides an integrated measure of PAH exposure via multiple exposure routes (Li et al., 2008).

Although the altered reproductive function observed in women who smoke or who are exposed to environmental tobacco smoke has been postulated to be due to exposure to PAHs in tobacco smoke, no studies have directly examined the associations between PAH exposure biomarkers and measures of hypothalamic-pituitary-ovarian axis function in women. We hypothesized that PAH exposure causes ovarian dysfunction manifested as altered urinary luteinizing hormone (LH) and estrogen metabolite profiles and even anovulatory menstrual cycles.

2. Methods

2.1. Study participants

Study participants were recruited for a pilot study to lay the groundwork for a subsequent larger, adjunct study to the planned National Children's Study (NCS). The purpose of the pilot study was to test the feasibility of recruiting women who were not intending to become pregnant and not using hormonal contraception for a study of the association between urinary PAH metabolites measured once per menstrual cycle and urinary reproductive hormone concentrations measured daily using a microelectronic dipstick monitor for six menstrual cycles.

Initially, women were recruited based on NCS Vanguard Study protocols (Baker et al., 2014; Montaquila et al., 2010). For the Vanguard Study, Orange County, California was divided into 15 geographical strata, which were each then further divided into 11 geographical units (GUs). Each GU was then divided into 10 segments or 'neighborhoods'. In a two-stage sampling process, 15 of those segments, totaling 15,000 households, were sampled by the NCS Coordinating Center for screening. The remaining segments were not further studied under the NCS Vanguard Study. Women for the current study were recruited from four segments that were not selected for the NCS Vanguard Study. Two segments from predominantly non-Hispanic white GUs (in Irvine, CA) and two segments from predominantly Hispanic GUs (in Santa Ana and Costa Mesa, CA) were selected to assure representation of the two largest ethnic groups in Orange County. Initially eligible women were identified and recruited by door-to-door contact in the home, with follow-up telephone and email contact by study staff. Subsequently, when the NCS shifted to other recruitment strategies, eligible women for the present study were recruited at public events such as health fairs at universities and colleges, work places and events sponsored by community groups. The current study population is thus a convenience sample. Baseline study visits and sample collection occurred between October 2010 and July 2012.

2.2. Inclusion and exclusion criteria

Eligible women were between the ages of 18–44, residing in Orange County California, who were not pregnant, currently not planning to conceive, not using hormonal contraception, did not have a history of surgical sterilization, treatment with antineoplastic drugs or radiation therapy to the pelvis and did not have conditions known to cause infertility by mechanisms other than ovarian failure (pelvic inflammatory disease, endometriosis). Women who had recently been pregnant or breastfeeding were asked to delay starting the study until they had one full menstrual cycle after the birth if not breastfeeding or after they stopped breastfeeding.

2.3. Participation and compensation

Recruits were told that they would be responsible for testing their first morning void urine daily for reproductive hormones using a dipstick fertility monitor, collecting one urine sample during each of the study menstrual cycles, completing a daily diary and a monthly questionnaire, and potentially taking one or more home pregnancy tests. Participants were given the option of keeping the fertility monitors upon completion of the study or of receiving a payment of $100 upon returning the monitor. Written, informed consent was obtained from all participants in person by a member of the study staff. All participants were fluent in English or Spanish. The study was approved by the University of California Irvine Institutional Review Board (Study Number 2009-7034).

2.4. Baseline study visit

After completing informed consent, participants were instructed to go to one of the two Orange County locations of the UC Irvine Institute for Clinical and Translational Science (ICTS) for their baseline visit 5–9 days prior to their next menses onset. Study staff administered the standardized NCS preconception questionnaire (NCS, 2007) to obtain information about the participant's demographics, medical history, reproductive history, tobacco smoke exposure, exercise history, occupational history, residential history, housing characteristics, use of chemicals (e.g. cleaning agents, pesticides) in and around the home and yard, and pets. Questions relating to tobacco, alcohol, and illicit drug use were taken from the NCS First Trimester Maternal In-Person Questionnaire (NCS, 2007). We refer to these variables hereafter as baseline covariates.