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Reprint of "*In utero* exposure to benzo[a]pyrene increases adiposity and causes hepatic steatosis in female mice, and glutathione deficiency is protective"☆

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HIGHLIGHTS

- Metabolic effects of prenatal exposure to the pollutant benzo[a]pyrene were examined.
- Benzo[a]pyrene-exposed female offspring had increased adipose tissue and body weights and hepatic lipid.
- Glutathione-deficient offspring were resistant to these effects.

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs), including benzo[a]pyrene (BaP), are ubiquitous environmental pollutants found in tobacco smoke, air pollution, and grilled foods. Reactive metabolites and reactive oxygen species generated during PAH metabolism are detoxified by reactions involving glutathione (GSH). Early life exposures to tobacco smoke and air pollution have been linked to increased risk of obesity and metabolic syndrome. We investigated the independent and interactive effects of prenatal exposure to BaP and GSH deficiency due to deletion of the modifier subunit of glutamate cysteine ligase (*Gclm*), the rate-limiting enzyme in GSH synthesis, on adiposity and hepatic steatosis in adult female F1 offspring. We mated $Gclm^{+/-}$ dams with $Gclm^{+/-}$ males and treated the pregnant dams with 0, 2, or 10 mg/kg/day BaP in sesame oil by oral gavage daily from gestational day 7 through 16. We analyzed metabolic endpoints in female $Gclm^{-/-}$ and $Gclm^{+/+}$ littermate F1 offspring. Prenatal BaP exposure significantly increased visceral adipose tissue weight, weight gain between 3 weeks and 7.5 months of age, hepatic lipid content measured by oil red O staining, and hepatic fatty acid beta-oxidation gene expression in $Gclm^{+/+}$, but not in $Gclm^{-/-}$, female offspring. Hepatic expression of lipid biosynthesis and antioxidant genes were decreased and increased, respectively, in $Gclm^{-/-}$ mice. Our results suggest that reported effects of pre- and peri-natal air pollution and tobacco smoke exposure on obesity may be mediated in part by PAHs. GSH deficiency is protective against the metabolic effects of prenatal BaP exposure.

1. Introduction

In recent years, it has become increasingly clear that various aspects of the intrauterine environment, such as exposure to environmental pollutants, influence the developmental origins of obesity and other risk factors for cardiovascular disease (Janesick and Blumberg, 2011a,b; La Merrill and Birnbaum, 2011).

Maternal smoking during pregnancy is associated with increased risk of obesity, diabetes, and hypertension in offspring (Ino, 2010; Morley et al., 1995; Oken et al., 2005; Power and Jefferis, 2002). Children of mothers who smoked during pregnancy were more likely to display hallmarks of metabolic syndrome, including higher body mass index (BMI), higher LDL and lower HDL concentrations, higher triglycerides, and higher systolic and diastolic blood

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pressure at eight years of age (Huang et al., 2007). Maternal exposure to second-hand tobacco smoke during pregnancy has been associated with increased BMI in offspring at 2 and 3 years of age (Braun et al., 2011). Gestational treatment with nicotine decreased pancreatic islet size and number and caused weight gain, adipocyte hypertrophy, glucose intolerance, and insulin resistance in male rats (Somm et al., 2008). However, tobacco smoke is a complex mixture, and the effects of other components of tobacco smoke such as PAHs have not been studied for their ability to prenatally program obesity. Tobacco smoke contains numerous PAHs, such as benzo[a]pyrene (BaP). The total carcinogenic PAH content of one cigarette has been estimated at 25–250 ng (Lodovici et al., 2004; Shopland et al., 2001). PAH exposure also occurs with exposure to sidestream tobacco smoke. Sidestream or second-hand tobacco smoke contains 10-fold higher concentrations of PAHs than mainstream smoke, or about 2.3–3.9 μg total PAHs and 0.5–1.2 μg carcinogenic PAHs per cigarette (Lodovici et al., 2004).

Particulate matter (PM) air pollution, especially the fine particulate fraction ($PM_{2.5}$), is rich in PAHs, and PAHs in PM are thought to mediate many adverse effects of PM (Lewtas, 2007). Concentrations of PAHs in ambient urban air are 10-fold higher than in rural air. Total PAH intake from ambient air has been estimated at 0.2 μg/day (range 0.02–3 μg/day) (ATSDR, 1995; Menzie et al., 1992). Long-term exposures to $PM_{2.5}$ were associated with increased cardiovascular mortality in a large study of participants from many US cities (Pope et al., 2004). Another multi-city study found that long term $PM_{2.5}$ exposure was associated with increased incidence of nonfatal cardiovascular events and of deaths from cardiovascular diseases in postmenopausal women (Miller et al., 2007). Several recent studies found increased risk of insulin resistance and type II diabetes with exposure to traffic-related air pollution (Krämer et al., 2010; Pruett et al., 2011) and $PM_{2.5}$ (Pearson et al., 2010; Xu et al., 2011) and increased risk of hypertension with exposure to $PM_{2.5}$ (Fuks et al., 2011). Exposure to traffic-related air pollution during childhood was associated with increased attained BMI (Jerrett et al., 2010). Early life exposure of mice to $PM_{2.5}$ increased adiposity, and caused insulin resistance and vascular dysfunction (Xu et al., 2010).

The other major source of PAH exposure in non-smokers is through the diet. Studies in the US and Europe have estimated that average daily intake of PAH from food is 1–17 μg/day, with the higher intakes associated with frequent consumption of grilled or smoked foods (ATSDR, 1995; Menzie et al., 1992).

PAHs require metabolism by microsomal cytochrome P450 enzymes and epoxide hydrolase to dihydrodiols, such as BaP-7,8-*trans*-dihydrodiol to exert toxicity (Kleiner et al., 2004; Shimada and Fujii-Kuriyama, 2004). This dihydrodiol can undergo further oxidation by cytochrome P450s to 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-benzo(a)pyrene (BPDE) or can be metabolized by aldo-keto reductases to BaP-7,8-dione (Xue and Warshawsky, 2005). BPDE forms bulky DNA adducts in the nucleus and mitochondria and is mutagenic (Allen and Coombs, 1980; Denissenko et al., 1996; Mass et al., 1993). BaP-7,8-dione is an arylhydrocarbon receptor (AHR) ligand, enabling it to be shuttled to the nucleus, where it undergoes redox cycling, generating reactive oxygen species (ROS) and oxidative DNA lesions, such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-OHdG) (Park et al., 2009). Glutathione transferase-mediated conjugation with glutathione (GSH) is a major Phase II biotransformation/detoxification pathway for BPDE and BaP-7,8-dione metabolites of BaP (Jernström et al., 1996; Romert et al., 1989; Xue and Warshawsky, 2005). As a major cellular antioxidant, GSH also detoxifies ROS that are produced as a result of BaP metabolism.

GSH is synthesized in two ATP-dependent reactions. The first, rate-limiting reaction is catalyzed by GCL, a heterodimer composed of a catalytic (GCLC) and a modifier (GCLM) subunit (Franklin et al., 2009; Griffith, 1999). Mice that lack *Gclc* die during embryonic development (Dalton et al., 2000, 2004; Shi et al., 2000). Mice that lack *Gclm* survive and reproduce, but have low GSH concentrations (Giordano et al., 2006; Yang et al., 2002). *Gclm* null mice have increased sensitivity to acetaminophen and domoic acid toxicity (Giordano et al., 2006, 2007; McConnachie et al., 2007). Our previous studies showed that $Gclm^{-/-}$ mice are more sensitive to the gonadal toxicity of gestational exposure to BaP than wild type littermates (Lim et al., 2013; Nakamura et al., 2012) and that female $Gclm^{-/-}$ mice are subfertile (Nakamura et al., 2011). In contrast, *Gclm* null mice are protected against diet-induced steatohepatitis, showing upregulation of hepatic antioxidant genes and downregulation of triglyceride synthesis and fatty acid β-oxidation (Haque et al., 2010; Kendig et al., 2011). Nonalcoholic hepatic steatosis, also called nonalcoholic fatty liver disease, is an independent risk factor for Type 2 diabetes and is prevalent in individuals with metabolic syndrome (Sung and Kim, 2011). *GCLC* polymorphisms are associated with increased risk of progression of nonalcoholic hepatic steatosis to nonalcoholic steatohepatitis in humans (Oliveira et al., 2010).

In our studies designed to test the modifying effects of GSH deficiency on the ovarian and testicular toxicity of prenatal BaP exposure (Lim et al., 2013; Nakamura et al., 2012), we observed increased weight gain in the BaP-exposed female offspring. We therefore investigated the effects of prenatal BaP exposure and *Gclm* genotype on adiposity and hepatic steatosis in these offspring.

2. Materials and methods

2.1. Materials

All chemicals and reagents were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma–Aldrich (St. Louis, MO) unless otherwise noted.

2.2. Animals

Gclm null mice were generated by disrupting the *Gclm* gene by replacing exon 1 with a beta-galactosidase/neomycin phospho-transferase fusion minigene (Giordano et al., 2006; McConnachie et al., 2007). The mice were backcrossed 8 times onto a C57BL/6J genetic background (B6.129-$Gclm^{tm1Tjka}$; hereafter referred to as $Gclm^{-/-}$). Mice for these experiments were generated at the University of California Irvine (UC Irvine) by mating $Gclm^{+/-}$ males with $Gclm^{+/-}$ females. Offspring were genotyped by PCR using primers for both the native *Gclm* sequence and the *β-Geo* sequence on DNA extracted from tail or toe snips as previously described (Giordano et al., 2006). All mice were housed in an American Association for the Accreditation of Laboratory Animal Care-accredited facility, with free access to deionized water and soy-free laboratory chow (Harlan 2019, 23% of calories from fat), on a 14:10 h light–dark cycle. Temperature was maintained at 21–23 °C. The experimental protocols were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 1996) and were approved by the Institutional Animal Care and Use Committee at UC Irvine.

2.3. Monitoring of estrous cycles

Estrous cycle stage in individually housed adult female mice was evaluated every morning by microscopic examination of fresh vaginal lavage fluid obtained in 0.9% sodium chloride (Cooper et al., 1993).

2.4. Experimental protocol

$Gclm^{+/-}$ female mice were mated with $Gclm^{+/-}$ or $Gclm^{-/-}$ male mice on the afternoon of proestrus based on vaginal cytology. Females were checked for vaginal plugs the following morning. The day of vaginal plug detection in the female was designated gestational day (GD) 1. Dams were treated by oral gavage with 10 mg/kg benzo[a]pyrene in sesame oil daily from GD7 to GD16 (Block 1) or 2 mg/kg/day from GD7 to GD16 (Block 2). Control animals were gavaged with the same volume (2 ml/kg) of sesame oil alone in both blocks. Dams were randomly assigned to treatment group using a random number table. The dosing regimen in Block 1 was based on a previous study in CD-1 mice, which showed that offspring treated with this dose had reduced fertility compared to controls but were not completely infertile (MacKenzie and Angevine, 1981). The lower dose was used in the second block because of an apparently, but not statistically significantly, increased intrauterine mortality of $Gclm^{-/-}$ female fetuses in Block 1, which resulted in only one litter of eight BaP-treated litters having any $Gclm^{-/-}$ female offspring (Lim et al., 2013). Dams

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