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Developmental cigarette smoke exposure II: Hepatic proteome profiles in 6 month old adult offspring

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ABSTRACT

Utilizing a mouse model of 'active' developmental cigarette smoke exposure (CSE) [gestational day (GD) 1 through postnatal day (PD) 21] characterized by offspring low birth weight, the impact of developmental CSE on liver proteome profiles of adult offspring at 6 months of age was determined. Liver tissue was collected from Sham- and CSE-offspring for 2D-SDS-PAGE based proteome analysis with Partial Least Squares-Discriminant Analysis (PLS-DA). A similar study conducted at the cessation of exposure to cigarette smoke documented decreased gluconeogenesis coupled to oxidative stress in weanling offspring. In the current study, exposure throughout development to cigarette smoke resulted in impaired hepatic carbohydrate metabolism, decreased serum glucose levels, and increased gluconeogenic regulatory enzyme abundances during the fed-state coupled to decreased expression of SIRT1 as well as increased PEPCK and PGC1α expression. Together these findings indicate inappropriately timed gluconeogenesis that may reflect impaired insulin signaling in mature offspring exposed to 'active' developmental CSE.

1. Introduction

Maternal environmental exposures, nutrition, and lifestyle play critical roles in fetal growth and development [1–4]. According to the Center for Disease Control and Prevention, approximately 20% of women smoke cigarettes at some time during pregnancy [5] though both epidemiological evidence and animal studies suggest that consumption of tobacco products during pregnancy is hazardous to the fetus [5–15]. Maternal cigarette smoke exposure during gestation is associated with a host of adverse reproductive outcomes, congenital anomalies, and a high incidence of intrauterine growth restriction and resulting infant low birth weight [5,11,12,14–16]. Moreover, children manifest adverse outcomes of fetal exposure to tobacco smoke that persist into childhood such as an increased incidence of respiratory infections and asthma as well as a propensity towards infant/childhood behavioral and cognitive deficits [5,11,12,14–16]. Interestingly, more recent studies have established compelling linkages between pre-/perinatal exposures to cigarette smoke and an increased risk of offspring obesity and metabolic disease [17–25]. While the ability of prenatal and early-life environmental exposures such as cigarette smoke to elicit developmental programming and long-lived alterations in adult health/disease risk has been well documented [1,4,26–31], the precise biological mechanisms by which such exposures dysregulate the development, function, and adaptability of the organism's key metabolic regulatory tissues/organs are still largely unknown.

Cigarette smoke contains a multitude of combustion gases, heavy metals, flavorings and other additives including addictive substances such as nicotine [32–35]; The toxins in cigarette smoke are generally transferred across the placenta to the embryo/fetus [36], exposing rapidly proliferating and differentiating cells/tissues to a mixture of over 8000 toxic substances [37]. Although a primary site of exogenous compound detoxification in adult humans [38,39], the liver has limited capacity to detoxify such compounds in the embryo/fetus [40–42] and, as such, the developing organ is functionally challenged, in a premature state, by this myriad of toxins. The diversity of potentially toxic substances in cigarette smoke, along with multiplicity of metabolic organs that are targeted by

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exposure, makes it difficult to elucidate the mechanisms through which developmental exposure to cigarette smoke elicits long-lived metabolic consequences in exposed offspring. Key insights may be found by examining alterations in genomic/epigenomic transcriptomic, proteomic, and metabolomic signatures of the primary metabolic tissues between exposed and non-exposed offspring. Such strategies have been utilized successfully to delineate the mechanistic underpinnings of 'developmental reprogramming' and long-lived metabolic consequences of *in utero* and early-life xenobiotic exposures such as cigarette smoke [43–52].

In order to elucidate the cellular and molecular mechanisms underlying the linkage between developmental exposure to cigarette smoke and an increased risk of offspring metabolic disease, we have utilized a well-characterized mouse inhalation exposure model which simulates 'active' smoke exposure spanning both fetal and early neonatal developmental periods (GD1-PD21) and examined the impact of such exposure on the proteome of liver, kidney and hippocampus at varied times during the offspring's lifespan. The present report details the impact of developmental cigarette smoke exposure on hepatic proteome profiles of offspring at 6 months of age – 5 months past cessation of their exposure – from littermates of pups from the same litters utilized in our prior study of developmental (GD1-PD21) cigarette smoke exposure. Parallel studies from our laboratory concerning the impact of such developmental CSE on liver, kidney and hippocampus proteome profiles at weaning (PD21) [53–55] and at adulthood [56,57] documented an impact of exposure on tissue metabolic activity. We previously reported that weanling (PD21) offspring who were developmentally exposed to cigarette smoke exhibited hepatic oxidative stress, impaired gluconeogenesis, altered lipid metabolism, impaired small molecule and amino acid metabolism, and impaired cellular morphology networks [54]. In the present report, we document sustained deficits in offspring growth, suppressed serum blood glucose levels, as well as disruption of basal gluconeogenesis in the fed state demonstrating a *continued impact of developmental CSE* on metabolic pathway function in adult offspring aged 6 months well past the cessation of exposure.

2. Materials and methods

2.1. Animal exposures

Adult C57BL/6J mice were purchased from Jackson Labs (Bar Harbor, ME). Animals were housed and maintained in the University of Louisville Research Resources Center, an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility. Animals were maintained in a controlled temperature/humidity environment with a 12 h light/dark cycle and free access to Purina LabDiet 5015 and water throughout the duration of the experiment both prenatal and postnatal exposure periods as well as following cessation of exposure to cigarette smoke. Female mice were age-matched at the outset of the study and timed pregnancies were obtained by overnight mating of a single mature male with two nulliparous females. The presence of a vaginal plug was considered evidence of mating and the time designated as gestational day 1 (GD 1). Pregnant mice were weighed and randomly assigned to either the Sham exposure (Sham, n = 9) or Cigarette Smoke Exposure (CSE; n = 9) groups. Animals were exposed from GD1, throughout the entirety of gestation; following parturition maternal animals were exposed with offspring until postnatal day 21 (PD21).

Animals were exposed to ambient air or cigarette smoke for 6 h per day, 7 days per week, from GD1-PD21. A Teague TE-10C whole body smoke inhalation exposure system (Teague Enterprises; Davis, CA)[58] was utilized to generate and deliver mixed mainstream/sidestream cigarette smoke at the rate of 40 cigarettes smoked per 6 h period. Cigarette smoke was generated from Philip Morris Marlboro Red brand cigarettes^{TM} (Philip Morris; Richmond, VA; 15 mg of tar/cigarette; 1.1 mg nicotine/cigarette; additives), selected since it represents the most popular brand of cigarettes consumed among 18–25 year olds – the age group containing the majority of maternal smokers [59–62]. Cigarettes were smoked using the standard Federal Trade Commission method: a two second, 35 cm^3 puff, once a minute for a total of 9 min [58]. For the duration of the exposure period, the dam/litter combinations were individually housed in the cages utilized for exposures. For quality control purposes, dual exposure chambers (one receiving cigarette smoke and one receiving ambient air) were characterized twice during each daily exposure session for: total suspended particulates (TSP), temperature, carbon monoxide levels, and humidity (Table 1) [54].

Tail blood was collected from representative animals immediately following the 6 h exposure session at various time points throughout the 6 week exposure regimen for determination of plasma cotinine levels. Cotinine, the principal metabolite of nicotine, is a well-documented marker of 'active' tobacco smoking and passive/environmental tobacco smoke exposure [63–66]. Cotinine levels were assayed by electrospray tandem mass spectrometry (ESI–MS/MS) utilizing a direct inject platform (Nanomate) coupled to a 7 T LTQ-FT-ICR-MS.

Following discontinuation of cigarette smoke exposure on PD21, offspring were maintained at normal temperature and humidity without exposure to any agent. At 6 months of age, following behavioral and cognitive assessments [67], offspring were euthanized by asphyxiation with carbon dioxide followed by thoracotomy and cardiac puncture. Tissues were harvested and stored at −80 °C until analysis. Proteome profiling of the liver and other tissues was conducted on identically exposed littermates at the time of weaning (PD21) [53–55]. The current report of liver proteome profiles from 6 month old animals that were previously developmental exposed to cigarette smoke serves as the lead manuscript for the coordinated three part evaluation of tissue specific proteome profiling studies, and includes the companion studies on kidney and hippocampus proteome profiles [56,57].

2.2. 2D-SDS-PAGE

The liver samples (Sham n = 4; CSE n = 6) were homogenized in sample preparation buffer [7 M urea, 2 M thiourea, 40 mM dithiothreitol (DTT)]. Protein concentration for each of the samples was determined using the Bradford Assay [68]. Four hundred micrograms of protein in rehydration buffer (8 M urea, 2% CHAPS, 2 μl IPG buffer pH 3–10, 2.5 mg/mL DTT, 0.002% bromophenol blue) was applied to IPGphor Drystrips (Nonlinear, 3–11, 180 mm x 3 mm x 0.5 mm, GE Healthcare, Piscataway, NJ). First dimension separation by isoelectric focusing at 22,000 V hours (Vhrs) was performed with a hold at 100 V until further processing. The IEF strips were stored at −80 °C for 1 h followed by: 1) equilibration for 60 min in reducing buffer (6 M urea, 75 mM Tris-HCl pH 8.8, 29.3% glycerol, 2% SDS, 0.002% bromophenol blue with 3.5 mg/mL DTT) and 2) equilibration in alkylating buffer (same buffer with 45 mg/mL iodoacetamide instead of DTT) for an additional 30 min. Second dimension SDS-PAGE separation (25 cm x 20.5 cm 15% polyacrylamide gels) was performed overnight (18 h; 100 V). Protein spots were visualized by Colloidal Coomassie Blue G-250.

2.3. Image analysis

Gels were scanned using an Epson Expression 10000 XL scanner with transparency attachment. Densitometric analysis of gel images was performed with Progenesis SameSpots software (Non-