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Developmental bisphenol A (BPA) exposure leads to sex-specific modification of hepatic gene expression and epigenome at birth that may exacerbate high-fat diet-induced hepatic steatosis

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ABSTRACT

Developmental bisphenol A (BPA) exposure increases adulthood hepatic steatosis with reduced mitochondrial function. To investigate the potential epigenetic mechanisms behind developmental BPA-induced hepatic steatosis, pregnant Sprague–Dawley rats were dosed with vehicle (oil) or BPA (100 μg/kg/day) from gestational day 6 until postnatal day (PND) 21. After weaning, offspring were either challenged with a high-fat (HF; 45% fat) or remained on a control (C) diet until PND110. From PND60 to 90, both BPA and HF diet increased the fat/lean ratio in males only, and the combination of BPA and HF diet appeared to cause the highest ratio. On PND110, Oil-HF, BPA-C, and BPA-HF males had higher hepatic lipid accumulation than Oil-C, with microvesicular steatosis being marked in the BPA-HF group. Furthermore, on PND1, BPA increased and modified hepatic triglyceride (TG) and free fatty acid (FFA) compositions in males only. In PND1 males, BPA increased hepatic expression of FFA uptake gene *Fat/Cd36*, and decreased the expression of TG synthesis- and β-oxidation-related genes (*Dgat*, *Agpat6*, *Cebpα*, *Cebpβ*, *Pck1*, *Acox1*, *Cpt1a*, *Cybb*). BPA altered DNA methylation and histone marks (H3Ac, H4Ac, H3Me2K4, H3Me3K36), and decreased the binding of several transcription factors (Pol II, C/EBPβ, SREBP1) within the male *Cpt1a* gene, the key β-oxidation enzyme. In PND1 females, BPA only increased the expression of genes involved in FFA uptake and TG synthesis (*Lpl*, *Fasn*, and *Dgat*). These data suggest that developmental BPA exposure alters and reprograms hepatic β-oxidation capacity in males, potentially through the epigenetic regulation of genes, and further alters the response to a HF diet.

Introduction

Various dietary, behavioral, and genetic factors contribute to the increased incidence of metabolic syndrome worldwide, and recent data suggest that environmental endocrine disrupting chemicals (EDCs) may also be important factors in the growing epidemic of obesity and metabolic disorders. Although a number of EDCs have been shown to disrupt normal reproductive outcomes (Unuvar and Buyukgebiz, 2012), more recently several EDCs, including the ubiquitous plasticizer bisphenol A (BPA), were shown to alter metabolic processes and have been associated with obesity and adulthood chronic diseases (Olsen et al., 2012; Trasande et al., 2012).

The Developmental Origins of Health and Disease Hypothesis suggests that exposure to in utero dietary or environmental stressors can program the adulthood phenotype, and this has been confirmed in humans and experimental animal models (Barker, 2004; Hanley et al., 2010; Tamashiro and Moran, 2010). In animals, maternal dietary manipulations during pregnancy have been shown to contribute to

Abbreviations: *Acox1*, acyl-CoA oxidase 1, palmitoyl; *Agpat6*, 1-acylglycerol-3-phosphate O-acyltransferase 6; *Ash1l*, absent, small, or homeotic-like 1 L; BPA, bisphenol A; BW, body weight; *Cebpα*, CCAAT/enhancer binding protein (C/EBP), alpha; *Cebpβ* or C/EBPβ, CCAAT/enhancer binding protein (C/EBP) beta; *Cpt1a*, carnitine palmitoyltransferase 1a; *Cyba*, cytochrome b-245, alpha polypeptide; *Cybb*, cytochrome b-245, beta polypeptide; *Dgat1*, diacylglycerol O-acyltransferase 1; *Dnmt1*, *Dnmt3a*, or *Dnmt3b*, DNA methyltransferase 1, 3a, or 3b; EDC, endocrine disrupting chemical; *Fabp1*, fatty acid binding protein 1; *Fasn*, fatty acid synthase; *Fat/Cd36*, fatty acid translocase/cluster determinant 36; FFA, free fatty acid; H3Ac or H4Ac, acetylated histone H3 or H4; H3Me2K4, di-methylated histone H3 at lysine residue 4; H3Me3K9, H3Me3K27, or H3Me3K36, tri-methylated histone H3 at lysine residue 9, 27, or 36; HF, high-fat; *Hdac1* or *Hdac3*, histone deacetylase 1 or 3; *Kmt2b* or *Kmt2c*, lysine(K)-specific methyltransferase 2b or 2c; *Lpl*, lipoprotein lipase; NAFLD, non-alcoholic fatty liver disease; *Pck1*, phosphoenolpyruvate carboxykinase 1 (soluble); PND, postnatal day; SREBP1, sterol regulatory element binding protein 1; TG, triglyceride; TSS, transcription start site.

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the development of non-alcoholic fatty liver disease (NAFLD) in offspring (Aagaard-Tillery et al., 2008; McCurdy et al., 2009), and recent studies suggest that prenatal low-dose BPA exposure can also modify markers of hepatic energy metabolism at weaning (Somm et al., 2009) and lead to steatosis in adult animals (Jiang et al., 2014). While the precise mechanisms behind these observations are not completely understood, in cell models, BPA's effect on hepatocyte metabolism has been proposed to occur through endoplasmic reticulum stress (Asahi et al., 2010), the production of reactive oxygen species (Huc et al., 2012), and decreased fatty acid β-oxidation (Grasselli et al., 2013).

Carnitine palmitoyltransferase 1a (CPT1A) in the liver is the regulatory enzyme for the transport of long-chain fatty acids into the mitochondria for subsequent β-oxidation (Nakamura et al., 2014). In humans, CPT1A deficiency causes hepatomegaly, hepatic steatosis, and metabolic perturbations (Bennett and Santani, 1993), while in rats, *Cpt1a* expression was shown to be down-regulated during steatosis in male rats consuming a high-fat diet (Xie et al., 2010), while a moderate overexpression of *Cpt1a* in obese male rats increased hepatic fatty acid β-oxidation and decreased hepatic triglyceride accumulation (Stefanovic-Racic et al., 2008). *Cpt1a* expression has been shown to be regulated by the binding of transcription factors (including C/EBPβ and SREBP1) (Attia et al., 2011; Thakran et al., 2013) in cell culture, as well as with altered DNA methylation in humans (Frazier-Wood et al., 2014; Irvin et al., 2014). However, despite the critical regulatory role of this gene in hepatic energy metabolism and lipid accumulation, and the proposed role of BPA exposure in NAFLD development, no studies have specifically assessed the gene's potential epigenetic regulation in response to developmental BPA exposure. Therefore, the present study first determined the effect of prenatal exposure to BPA, with or without a post-weaning HF dietary challenge, on adult liver pathophysiology. We then investigated whether the adulthood phenotypes observed were associated with neonatal changes in hepatic lipid metabolism and fatty acid homeostasis — at the biochemical, genetic, and epigenetic levels, with a special focus on *Cpt1a*. Furthermore, because BPA is known to affect males and females differently, and because NAFLD development has been shown to be sex-specific (Lazo et al., 2013), we also considered offspring's sex in our analyses.

Methods

Dosing of BPA and animal experimental design during gestation and weaning. Timed-pregnant Sprague–Dawley rats (n=6/treatment group) were obtained from Charles River Laboratories (Wilmington, MA, USA) on gestational day 2, individually housed in ventilated cages in a temperature-controlled environment, and fed a modified AIN-93G diet and water ad libitum. Beginning on gestational day 6 until postnatal day (PND) 21, dams were randomized into two groups, and daily orally dosed using a 1 mL Eppendorf pipette with either vehicle control (tocopherol-stripped corn oil) or BPA (100 μg/kg/day BW). This dose has been shown to affect metabolism (including increased body weight and altered glucose homeostasis) and reproductive outcomes (including altered estrous cyclicity and hormone levels) in previous rodent models of perinatal exposure (Rubin et al., 2001; Liu et al., 2013). A relatively similar perinatal BPA exposure dose (50 μg/kg/day BW) led to steatosis in adult offspring (Jiang et al., 2014; Wei et al., 2014), and adult exposure to BPA at 50 and 500 μg/kg/day BW increased hepatic triglycerides in mice (Marmugi et al., 2012). Immediately after birth, litter sex distributions and offspring body weight were recorded, male and female pups (n=6, one of each sex from each dam) were removed for sampling, and the remainder of the pups were completely mixed within each treatment group, and 4 male and 4 female pups were randomly selected and placed back with each dam within the respective control or BPA-treated groups for cross-fostering. PND1 livers were snap-frozen in liquid nitrogen and stored at −80 °C for all subsequent biochemical and molecular analyses.

Animal experimental design after weaning using a high-fat dietary challenge. On PND22, male and female pups were once again randomized into new cages (within treatment groups), with 2–3 male or female offspring per cage, and separated into two dietary groups: control (C: 64% carbohydrate, 20% protein, and 16% fat) or high-fat diet (HF: 35% carbohydrate, 20% protein, and 45% fat) (Table 1), which yielded 4 experimental post-weaning groups: Oil-C, Oil-HF, BPA-C, and BPA-HF. All animals were single-housed after the onset of puberty until the end of the study (PND110), when tissues were collected and either snap-frozen in liquid nitrogen and stored at −80 °C or fixed in 10% formalin for histopathology analysis.

Body weight, cumulative energy intake, and body composition throughout the study. Body weight and food intake were measured every 4 days beginning on PND22. Energy intake after weaning was calculated by weighing pellets remaining on the fourth day, subtracting from initial food supplied, and multiplying daily calculated intake by the caloric composition of each diet (4.0 kcal/g in control and 4.8 kcal/g in HF). Body composition was measured on PND1, at weaning on PND21, after the onset of puberty on PND60, and on PND90 using the EchoMRI-700 Body Composition Analyzer (Echo Medical Systems, Houston, TX), which allows for measurement of fat and lean mass in conscious and unrestrained animals. On PND1 and PND21, male and female pups were scanned as a litter, and on PND60 and PND90 offspring were scanned individually.

Liver histopathology and staining in adult offspring. At the end of the study (PND110), liver samples from adult animals were fixed in 10% formalin, embedded in paraffin, and 3 μm histological slides were prepared and stained with hematoxylin and eosin. Formalin-fixed tissues were embedded in OCT, and slides were prepared with a cryostat and stained with Oil-Red-O. Histopathological evaluation was performed by a board-certified pathologist. Samples were first assigned a score for overall hepatic vacuolation severity, and then independently graded for either macro- or micro-vacuolation. Pathological findings were graded from 1 (minimal) to 5 (severe). For all analyses, the pathologist was blind to the treatment, and reported all information using a numerical code.

Hepatic triglyceride (TG) content, free fatty acid (FFA) content, and FFA composition in PND1 offspring. Hepatic lipids were extracted and purified from frozen livers from 6 male and 6 female PND1 pups from each maternal treatment group (oil or BPA) using the Bligh & Dyer

Table 1
Diet composition.[a]

%	Control (C)		High-fat (HF)	
	Gram	kcal	Gram	kcal
Protein	20	20	24	20
Carbohydrate	64	64	41	35
Fat	7	16	24	45
kcal/g	4.0		4.8	
Ingredients				
Casein	200	800	200	800
L-Cystine	3	12	3	12
Corn starch	397.5	1590	105	420
Maltodextrin	132	528	132	528
Sucrose	100	400	100	400
Cellulose	50	0	50	0
Soybean oil	70	630	70	630
Lard	0	0	130	1170
Mineral mix	35	0	35	0
Vitamin mix	10	40	10	40
Choline bitartrate	2.5	0	2.5	0

[a] Research Diets, Inc, New Brunswick, NJ