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Prenatal ethanol exposure programs an increased susceptibility of non-alcoholic fatty liver disease in female adult offspring rats

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ABSTRACT

Prenatal ethanol exposure (PEE) induces dyslipidemia and hyperglycemia in fetus and adult offspring. However, whether PEE increases the susceptibility to non-alcoholic fatty liver disease (NAFLD) in offspring and its underlying mechanism remain unknown. This study aimed to demonstrate an increased susceptibility to high-fat diet (HFD)-induced NAFLD and its intrauterine programming mechanisms in female rat offspring with PEE. Rat model of intrauterine growth retardation (IUGR) was established by PEE, the female fetus and adult offspring that fed normal diet (ND) or HFD were sacrificed. The results showed that, in PEE + ND group, serum corticosterone (CORT) slightly decreased and insulin-like growth factor-1 (IGF-1) and glucose increased with partial catch-up growth; In PEE + HFD group, serum CORT decreased, while serum IGF-1, glucose and triglyceride (TG) increased, with notable catch-up growth, higher metabolic status and NAFLD formation. Enhanced liver expression of the IGF-1 pathway, gluconeogenesis, and lipid synthesis as well as reduced expression of lipid output were accompanied in PEE + HFD group. In PEE fetus, serum CORT increased while IGF-1 decreased, with low body weight, hyperglycemia, and hepatocyte ultrastructural changes. Hepatic IGF-1 expression as well as lipid output was down-regulated, while lipid synthesis significantly increased. Based on these findings, we propose a "two-programming" hypothesis for an increased susceptibility to HFD-induced NAFLD in female offspring of PEE. That is, the intrauterine programming of liver glucose and lipid metabolic function is "the first programming", and postnatal adaptive catch-up growth triggered by intrauterine programming of GC-IGF1 axis acts as "the second programming".

Introduction

Ethanol is a common component of the human diet. Approximately 7% of pregnant women consume alcoholic beverages, and in some communities, nearly 50% of women of reproductive age are binge drinkers (Muhajarine et al., 1997; Williams and Gloster, 1999). Prenatal ethanol exposure (PEE) may induce developmental toxicity and anomalies in fetus and offspring. The spectrum of possible clinical manifestations due to PEE is broad, sometimes manifested as the fetal alcohol syndrome (FAS). Intrauterine growth retardation (IUGR) is one of the main characteristics of the FAS (O'Leary, 2004), mainly characterized by low birth weight (Silveira and Horta, 2008). Human and animal studies highlighted that subjects born with IUGR are prone to central redistribution of adipose tissue and are at high risk for developing metabolic syndrome (MS), type 2 diabetes, and cardiovascular disease (Hernandez and Mericq, 2011; Levy-Marchal and Czernichow, 2006; Reinehr et al., 2009).

Non-alcoholic fatty liver disease (NAFLD) is considered to be the hepatic manifestation of the MS (Greenfield et al., 2008). Epidemiological studies have shown that the incidence of NAFLD has increased over the

Abbreviations: ACCα, acetyl-CoA carboxylase α; ACTB, β actin; ADIPOR2, adiponectin receptor 2; AMPKα, adenosine monophosphate activated protein kinase α; APOB, apolipoprotein B; CORT, corticosterone; CPT1α, carnitine palmitoyltransferase 1α; FASN, fatty acid synthase; FOXO1, fork-head transcriptional factor O1; GAPDH, glyceraldehyde-phosphate dehydrogenase; GC, glucocorticoid; GC–MS, gas chromatography–mass spectrometry; GLUT2, glucose transporter 2; G6Pase, glucose-6-phosphatase; GSK3β, glycogen synthase kinase 3β; HFD, high-fat diet; HMGCR, HMG-CoA reductase; HNF4, hepatocyte nuclear factor 4; HPRT1, hypoxanthine phosphoribosyltransferase 1; IGF-1, insulin-like growth factor-1; IGFBP3, insulin-like growth factor binding protein 3; IGF-1R, IGF-1 receptor; INSR, insulin receptor; IRS1, insulin receptor substrate 1; IRS2, insulin receptor substrate 2; IUGR, intrauterine growth retardation; JAK2, Janus kinase 2; LEPR, leptin receptor; MS, metabolic syndrome; mTORC2, mammalian target of rapamycin complex 2; MTTP, microsomal triglyceride transfer protein; NAFLD, non-alcoholic fatty liver disease; ND, normal diet; PEE, prenatal ethanol exposure; PPARα, peroxisome proliferator activated receptor α; SREBP1c, sterol regulatory element binding protein-1c; TG, triglyceride.

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years. The prevalence of NAFLD is 12%–24% in the Asia-Pacific region (Park et al., 2006) and 20%–30% in Europe and the United States (Bedogni et al., 2005). Recently, NAFLD has been included among persistent IUGR-dependent metabolic dysfunctions (Alisi et al., 2012). A population-based study shows that in 90 Italian children with biopsy-proven NAFLD, the prevalence of IUGR with NAFLD was approximately four-fold higher than the average IUGR prevalence in children (Nobili et al., 2008). Animal experiments also confirmed that rats with IUGR due to prenatal hypoxia showed enhanced vulnerability for NAFLD in adulthood (Cao et al., 2012). Pregnancy-food-restricted IUGR rats also displayed increased triglyceride (TG) synthesis and lipid accumulation in liver (Choi et al., 2007). All these findings indicate that NAFLD has a fetal origin.

Although recent studies have confirmed an increased susceptibility to NAFLD in IUGR offspring after birth, the underlying mechanism remains unclear. Studies have suggested that hepatic lipid de novo synthesis might induce the fetal-original hepatic steatosis (Postic and Girard, 2008), mainly referring to lipogenic transcription factor-sterol regulatory element binding protein-1c (SREBP1c) and the key enzyme of lipid synthesis–fatty acid synthase (FASN) (Horton et al., 2003). Insulin-like growth factor-1 (IGF-1) is the main factor of IUGR caused by an unfavorable intrauterine environment and postnatal catch-up growth (Qiu et al., 2004). In liver, IGF-1 could activate the mammalian target of rapamycin complex 2 (mTORC2) *via* activation of the protein kinase B pathway, thereby inducing expression of SREBP1c and mediating hepatic lipid synthesis (Hagiwara et al., 2012). Therefore, changes in lipid synthesis mediated by the hepatocyte IGF-1 signaling pathway might lead to the high susceptibility to NAFLD in IUGR offspring.

Our previous research showed that PEE could result in the fetus being over-exposed to maternal glucocorticoid (GC) and IUGR (Liang et al., 2011), which may occur through the inhibition of the liver IGF-1 signaling pathway (Aros et al., 2011). Further studies revealed that rat offspring of PEE could undergo a period of catch-up growth in association with dyslipidemia and hyperglycemia (Nammi et al., 2007), these animals have a decreased whole body insulin sensitivity (Chen and Nyomba, 2004). Interestingly, female offspring with IUGR were found to be more susceptible to diet-induced hepatic steatosis (Carbone et al., 2012). However, it is unclear whether female PEE offspring are at higher risk for developing NAFLD and whether such NAFLD is related to the hepatic IGF-1 pathway and catch-up growth. Here, we established a rat IUGR model by PEE, and evaluated the susceptibility of female adult offspring to HFD-induced NAFLD. Further, we explored its intrauterine programming mechanisms by observing the metabolic phenotypes and gene expression of hepatic glucose and lipid metabolism before and after birth.

Materials and methods

Materials. Ethanol (analytical pure grade and chromatographic pure grade) was obtained from Zhen Xin Co., Ltd. (Shanghai, China) and Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Isoflurane was purchased from Baxter Healthcare Co. (Deerfield, IL, USA). Rat corticosterone (CORT) ELISA kits were obtained from Assaypro LLC (Saint Charles, Missouri, USA). Rat IGF-1 ELISA kits were purchased from RD Systems, Inc. (Minneapolis, MN, USA). Glucose oxidase assay kits were provided by Mind Bioengineering Co., Ltd. (Shanghai, China). Total cholesterol (TCH) and TG assay kits were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Reverse transcription and quantitative PCR (Q-PCR) kits were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). GeXP multiplex gene expression analysis kits were purchased from Beckman-Coulter Inc. (Fullerton, CA, USA). The oligonucleotide primers for rat Q-PCR genes (PAGE purification) and GeXP multiplex gene expression analysis (HPLC purification) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Other chemicals and agents were of analytical grade.

Animals and treatment. Specific pathogen free (SPF) Wistar rats (200–240 g) were obtained from the Experimental Center of Hubei Medical Scientific Academy (No. 2009-0004, Hubei, China). The animal experiments were performed in the Center for Animal Experiment of Wuhan University (Wuhan, China), which has been accredited by Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). All animal experiment procedures were approved by and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Chinese Animal Welfare Committee.

The designed experiments were divided into 3 separate batches, including the experiment of the offspring fed with HFD, fed with normal diet (ND) and the fetal offspring. Each experiment was independent of each other, including breeding, processing and detecting.

Animals were housed (room temperature: 18–22 °C; humidity: 40%–60%), acclimated, and mated. Upon confirmation of mating by the appearance of sperm in a vaginal smear, the day was taken as gestational day (GD) 0. Pregnant rats were transferred to individual cages and randomly divided into control and ethanol groups ($n = 8$ for each group). Ethanol group was given ethanol 4 g/kg·d by gavage administration (Chen and Nyomba, 2003) from GD11 to GD20, while control group was given the same volume of distilled water. The pregnant rats were kept until normal delivery (GD21), and on postnatal day 1 (PD1) the numbers of pups were normalized to 8 pups per litter to assure adequate and standardized nutrition until weaning (postnatal week 4, PW4). After weaning, one female pup per litter was randomly selected from each group and fed with HFD (providing 18.9% kcal from protein, 61.7% kcal from carbohydrate and 19.4% kcal from fat) (Zhang et al., 2013) *ad libitum*. The body weight was measured weekly and the corresponding growth rate was calculated as follows (Xu et al., 2012). At PW24, after recording the food intake for three days and the rectal temperature at 23:00, the offspring rat was anesthetized with isoflurane and decapitated. Serum was prepared and stored at −80 °C for metabolic phenotype analyses. The liver was dissected, randomly selected, partly fixed in 4% paraformaldehyde solution for histological examination, and the rest immediately frozen to stored at −80 °C for gene expression analyses.

$$\text{Body weight growth rate } (\%) = \frac{\text{Body weight of PW x} - \text{Body weight of PW 1}}{\text{Body weight of PW 1}} \times 100$$

For the experiment of fetal rats, 8 randomly selected pregnant rats with 10–14 live fetuses from each group were anesthetized with isoflurane and euthanized on GD20. The female fetuses were quickly removed to weigh, and IUGR was diagnosed when the body weight of an animal was two standard deviations lower than the mean body weight of the control group (Engelbregt et al., 2001). Serum samples from each littermate were pooled together and immediately frozen at −80 °C for metabolic phenotype analyses. One fetal liver from each group was randomly selected and routine fixed for histological and ultra-structural examination, and the rest of the fetal livers from each group were immediately frozen stored at −80 °C for gene expression analyses.

For the experiment of adult rats fed with ND (providing 22% of its energy content as protein, 63% as carbohydrate, and only 5% as fat), the animal treatment was the same as the above adult rats fed with HFD except for the diet type.

Blood sample analysis. Serum corticosterone (ELISA), IGF-1 (ELISA), glucose (biochemical assay) and TG (biochemical assay) were detected by assay kits following the manufacturer's protocol. The blood ethanol concentration was detected by gas chromatography–mass spectrometry (GC–MS). The GC–MS system used for analysis was an Agilent 5975C with a quadrupole mass spectrometer (Agilent). Data acquisition and analysis were performed using standard software supplied by the manufacturer. Samples of 0.1 ml of serum were mixed with 0.9 ml of