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Prenatal caffeine ingestion induces transgenerational neuroendocrine metabolic programming alteration in second generation rats

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ABSTRACT

Our previous studies have demonstrated that prenatal caffeine ingestion induces an increased susceptibility to metabolic syndrome with alterations of glucose and lipid metabolic phenotypes in adult first generation (F1) of intrauterine growth retardation (IUGR) rats, and the underlying mechanism is originated from a hypothalamic–pituitary–adrenal (HPA) axis-associated neuroendocrine metabolic programming alteration in utero. This study aims to investigate the transgenerational effects of this programming alteration in adult second generation (F2). Pregnant Wistar rats were administered with caffeine (120 mg/kg·d) from gestational day 11 until delivery. Four groups in F2 were set according to the cross-mating between control and caffeine-induced IUGR rats. F2 were subjected to a fortnight ice water swimming stimulus on postnatal month 4, and blood samples were collected before and after stress. Results showed that the majority of the activities of HPA axis and phenotypes of glucose and lipid metabolism were altered in F2. Particularly, comparing with the control group, caffeine groups had an enhanced corticosterone levels after chronic stress. Compared with before stress, the serum glucose levels were increased in some groups whereas the triglyceride levels were decreased. Furthermore, total cholesterol gain rates were enhanced but the high-density lipoprotein-cholesterol gain rates were decreased in most caffeine groups after stress. These transgenerational effects were characterized partially with gender and parental differences. Taken together, these results indicate that the reproductive and developmental toxicities and the neuroendocrine metabolic programming mechanism by prenatal caffeine ingestion have transgenerational effects in rats, which may help to explain the susceptibility to metabolic syndrome and associated diseases in F2.

Introduction

Caffeine is a xanthine alkaloid widely present in coffee, tea, soft drinks and some drugs. In adult individuals, although caffeine intake can improve the body's metabolic state (Carrieri et al., 2012; Panchal et al., 2012; Sugiura et al., 2012; van Dam, 2006), epidemiological investigations and animal experiments have demonstrated that prenatal caffeine ingestion induces adverse effects on reproductive (pregnancy and implantation) and embryo development, such as increased prematurity, spontaneous abortion, intrauterine growth retardation (IUGR) and other disease risks (Fortier et al., 1993; Kuczkowski, 2009; Sengpiel et al., 2013).

Our previous studies (Huang et al., 2012; Y. Liu et al., 2012; Xu et al., 2012a,b) have confirmed that prenatal ingestion of caffeine could give rise to IUGR in rodents, which may be associated mainly with the fetal over-exposure to maternal glucocorticoid (GC), resulting in a hypothalamic–pituitary–adrenal (HPA) axis-associated neuroendocrine metabolic programming alteration and a susceptibility to adult metabolic syndrome (MS) at the dose of caffeine 120 mg/kg·d. MS is manifested as the following problems: the functional development of HPA axis was inhibited; and the glucose and lipid metabolic function and their corresponding blood phenotypes were changed in utero; and as a result, a low basal activity and enhanced stress sensitivity of HPA axis as well as the GC-dependent alteration of blood glucose and lipid metabolic phenotypes were shown after birth. Altered HPA axis

Abbreviations: ACTH, adrenocorticotropin-releasing hormone; CORT, corticosterone; F1, first generation; F2, second generation; GC, glucocorticoid; GD, gestational day; GR, glucocorticoid receptor; HDL-C, high-density lipoprotein-cholesterol; HPA, hypothalamic–pituitary–adrenal; IUGR, intrauterine growth retardation; LDL-C, low-density lipoprotein-cholesterol; MR, mineralocorticoid receptor; MS, metabolic syndrome; PW, postnatal week; PM, postnatal month; TCH, total cholesterol; TG, triglyceride; 11β-HSD2, 11β-hydroxysteroid dehydrogenase 2.

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Table 1
F1 mating principle and F2 grouping.

F1 (n)		F2 groups
♂	♀	
Normal (6)	Normal (6)	Control ♂ × control ♀ (CC)
Normal (6)	IUGR (6)	Control ♂ × caffeine ♀ (CCa)
IUGR (6)	Normal (6)	Caffeine ♂ × control ♀ (CaC)
IUGR (6)	IUGR (6)	Caffeine ♂ × caffeine ♀ (CaCa)

IUGR: intrauterine growth retardation.

function has been linked to several adult-onset diseases, such as insulin resistance and hypertension in rats and humans (Nyirenda et al., 2001; Phillips, 2002; Reynolds et al., 2001).

Although the transgenerational effects of adverse prenatal environmental factors on offspring have been identified, the underlying mechanism is still a hot issue in recent years (Grossniklaus et al., 2013). Several studies have shown that prenatal environmental factors could adversely affect HPA axis functions and have transgenerational effects (Bertram et al., 2008; Iqbal et al., 2012; Long et al., 2013). However, whether the reproductive and developmental toxicities including the HPA axis-associated neuroendocrine metabolic programming alteration by prenatal caffeine ingestion could be maintained to the next generation is still unknown. Based on our previous studies, the present study aims to verify the transgenerational effects of the neuroendocrine metabolic programmed mechanism as well as the transgenerational effects of reproductive and developmental toxicities in adult second generation (F2) offspring by prenatal ingestion of caffeine (120 mg/kg·d), mainly via observing the changes of HPA axis activity, and changes in glucose and lipid metabolism before and after chronic stress. These observations will provide a valuable experimental basis for explaining the transgenerational effects of susceptibility to metabolic syndrome in F2 as well as in first generation (F1).

As for the dose of caffeine used in the present study. Human epidemiological evidence indicate that caffeine is associated with human IUGR (Peck et al., 2010). Caffeine intake in pregnant women of 300 mg/d (5 mg/kg·d) according to the World Health Organization (WHO) was associated with increased risk for small for gestational age (SGA) (Guilbert, 2003). Some studies have shown that caffeine intake of some pregnant women is >300 mg/d (Boylan et al., 2008; Fenster et al., 1991). Using the dose conversion between humans and rats (human:rat 1:6.17) (Reagan-Shaw et al., 2008), the dose of 120 mg/kg·d in the present study is about 4 times higher than the reported data (Boylan et al., 2008; Fenster et al., 1991). Furthermore, the caffeine concentration in maternal blood was 254 ± 11 μM (49 ± 2 μg/ml) after intake of 120 mg/kg·d caffeine in our studies (Wang et al., 2013), which is only 1.6 times higher than the clinical signs of intoxication (~30 μg/ml), but did not reach the dose with fatalities (~80 μg/ml). In our previous studies (Xu et al., 2012b), we had used 20, 60 and 180 mg/kg·d of caffeine and observed some multi-index changes in 20 mg/kg·d and a clear dose–effect relationship. To achieve the typical IUGR model, the dose of 120 mg/kg·d caffeine was used, to verify the transgenerational effects in the present study.

Materials and methods

Chemicals and reagents

Caffeine (CAS #58-08-2, >99% purity) was purchased from Sigma-Aldrich Co., Ltd. (St Louis, MO, USA). Rat adrenocorticotropin-releasing hormone (ACTH) kit was obtained from Beijing North Biotech Institute (Beijing, China). ELISA kit was purchased from Assaypro (St Charles, USA) for serum corticosterone (CORT) concentration. Glucose oxidase assay kit was provided by Shanghai Mind Bioengineering Co., Ltd. (Shanghai, China). Triglyceride (TG) and total cholesterol (TCH) assay kits were from Sangon Biotech Co., Ltd. (Shanghai, China). High-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) assay kits were from Chuang-Ye Biotech Co., Ltd. (Zhejiang, China). Isoflurane was purchased from Baxter Healthcare Co. (Deerfield, IL, USA). All other chemicals and reagents were of analytical grade.

Animals and treatments

F0: Specific pathogen free Wistar rats weighing 180–220 g (female)/260–300 g (male) were obtained from the Experimental Center of Hubei Medical Scientific Academy (License no. SCXK 2010–2011, Hubei, China). All subsequent 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. Animals were allowed to acclimate for one week before subjected to experimental conditions. For mating, two female rats were placed together with one male rat overnight, with gestational day (GD) 0 designated as the day on which evidence of mating was observed (i.e. a vaginal plug or vaginal smear with sperm cells). Caffeine (120 mg/kg·d) administration of rats from GD11 to GD20 was performed as described before (Xu et al., 2012a).

F1: Pregnant rats that were allowed to deliver spontaneously at term, based on birth date in postnatal week (PW) 1 of pups were screened and put into batches with the following screening principles: ① pregnant rats with litter size ≥ 10 were selected and the pups were normalized to 10; ② control group keeps non-IUGR pups, caffeine group keeps IUGR pups; ③ the ratio of

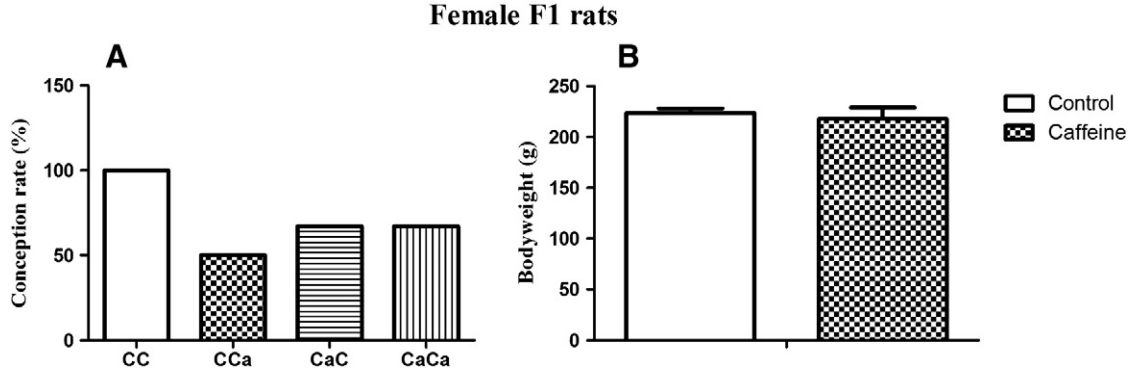

Fig. 1. Effects of prenatal caffeine ingestion (120 mg/kg·d) on gestation day (GD) 0 bodyweights, conception rates in first generation (F1). A: conception rates of the first two estrous cycles in F1; B: body weights on GD0 in F1. CC: control♂–control♀; CCa: control♂–caffeine♀; CaC: caffeine♂–control♀; CaCa: caffeine♂–caffeine♀. Mean ± S.E.M., n = 6. $^{*}P < 0.05$, $^{**}P < 0.01$ vs control.