## Letter to the Editor

# Caffeine Exposure Causes Immune Dysfunction and Intrauterine Growth Restriction Retardation in Rats*

ZHANG Wen Zhong[1], SUN Na Na[2], HU Yang[1], CAO Yu[3,#], and Amber Sheeks[4,#]

This study was conducted to assess the effects of caffeine on the dam and the physical development of rat offspring. Pregnant Sprague-Dawley rats (20 per dose group) were administered caffeine by gavage at 0 (control), 5, 20, and 80 mg/kg body weight (bw) daily from gestational day 6 through lactation using caffeine dissolved in water. The developmental toxicity of caffeine was evaluated. Dams in the 80 mg/kg bw group exhibited splenic atrophy. The litter weights were significantly lower in the 20 and 80 mg/kg bw groups than the untreated control ($P < 0.05$), with a greater reduction in the higher dose group. The number of female offspring was reduced in the 20 and 80 mg/kg bw groups ($P < 0.05$). Some of the offspring were eaten by the dam following birth, which accounted for half of the litters in the 80 mg/kg bw group ($P < 0.05$). Offspring body weight in the 80 mg/kg bw group was lower than that in the control during the lactation period ($P < 0.05$). Exposure to caffeine during pregnancy can cause maternal immune dysfunction and intrauterine growth restriction retardation (IUGR); the developmental toxicity of caffeine varied with gender, as females were more sensitive than males. The no observed adverse effect level was 5 mg/kg bw based on the IUGR.

Caffeine is the main alkaloid found in coffee beans and tea leaves. It has been reported that 90% of North Americans consume caffeine daily, and caffeine consumption in China is increasing annually. A total of 68%–74% of pregnant women in the United States consume caffeine daily, with total consumption ranging from 125 to 193 mg/day (1.6–2.5 mg/kg bw)[1]. Caffeine is absorbed rapidly and completely after oral intake; bioavailability is 90%–100%, and blood concentration peaks within 20–40 (3–120) min. The absorption rate is proportional to the caffeine dose in humans and animals. Caffeine is lipotropic, so it is distributed throughout the whole body, including the brain, by passing through the blood-brain barrier and to the developing fetus by passing through the placental-blood barrier. Although caffeine may enhance certain functional abilities, toxic effects and addiction are possible, particularly in frequent and high-dose consumers[2]. The current recommended intake amount during pregnancy is < 300 mg daily, and > 300 mg daily increases the risk of hallucinations and caffeine overconsumption poisoning, which is characterized by tension, irritability, anxiety, muscle twitching, insomnia, headache, and heart palpitations, among other symptoms.

Prenatal caffeine exposure reportedly improves the exercise performance of adult offspring[3]. Definitive conclusions are not possible due to limitations in models and the different experimental endpoints among studies. Therefore, the dose-dependent effects of early life exposure on parents require additional investigation. The objective of this study was to examine the potential developmental toxicity of caffeine during pregnancy and lactation.

All studies were conducted according to the laboratory management regulations of Beijing regarding animal welfare and experimental ethics and approved by the China National Center for Food Safety Risk Assessment Standing Committee on Ethics in Animal Experimentation (2009043). The experiments were performed following all other relevant guidelines and regulations. Caffeine (99.9% purity) was purchased from Sigma (St. Louis, MO, USA). A total of 120 male and mature unbred virgin female Sprague-Dawley rats were purchased from

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1. Department of Safety Engineering, North China Institute of Science & Technology, Sanhe 065201, Hebei, China; 2. Key Laboratory of Food Risk Assessment, Ministry of Health China National Center for Food Safety Risk Assessment, Beijing 100021, China; 3. Center for Public Health Surveillance and Information Service, Chinese Center for Disease Control and Prevention, Beijing 102206, China; 4. Muskingum University, OH 43762, USA

the Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China) [license number: SCXK (JING) 2006–0009] and used for breeding at approximately 70-days-of-age. The rats were maintained at the experimental animal center of the Chinese Center for Disease Control and Prevention. Each female rat deemed suitable according to health and body weight was mated with a resident mature male after 2 weeks of acclimation to the laboratory conditions. The appearance of a vaginal plug was used as an indicator of successful mating and considered gestational day 0 (GD 0). Pregnant rats were weighed and randomly divided into four groups of 25–30 animals each. The groups were identified by unique markings. From GD 6 to postnatal day (PND) 21, pregnant rats were housed singly and administered caffeine by oral gavage at 0, 5, 20, or 80 mg/kg bw. The day of birth was regarded as PND 0. Exposure to caffeine was continued throughout pregnancy and lactation. The body weights of the offspring and dams were recorded twice weekly. All dams and offspring were housed in the same room under controlled temperature (25 ± 3 °C), relative humidity (50% ± 10%), and illumination (12-h/12-h light/dark cycle) throughout the entire experimental period, and were provided distilled water and regular rat chow *ad libitum*. All dams and pups were carefully observed once daily for their general health condition, including mortality and signs of toxicity.

All parameters are expressed as mean ± standard deviation. Homogeneity of variance was examined by Levene's test. If Levene's test demonstrated no significant deviations, group means were compared by one-way analysis of variance followed by post-hoc LSD tests for the pairwise comparisons. Datasets with a heterogeneous variance were analyzed by the Games–Howell multiple comparisons test.

Median per capita caffeine consumption in the United States is about 143 mg/day (about 1.8 mg/kg bw; 1.6 mg/kg bw for women and 2.2 mg/kg bw for men), and 4% of the Americans consume > 400 mg/day (about 5.2 mg/kg bw; 4.5 mg/kg bw for women and 6.6 mg/kg bw for men)[4]. Therefore, the minimum dose in this study was 5 mg/kg bw to model this high level of consumption. Gestational body weight and food consumption during gestation did not differ among the caffeine dose groups of dams. However, compared with the controls (no caffeine), absolute spleen weight and the spleen/body weight ratio were significantly lower in the 80 mg/kg bw group of dams, with a dose-response relationship ($P$ < 0.05). No significant differences were observed in the liver, kidneys, or adrenal glands (Table 1). A decrease in the spleen index indicates immune dysfunction[5]. No study has reported that caffeine harms the immune system of pregnant women, but epidemiological studies have shown that caffeine consumption during pregnancy leads to a higher risk of allergic reactions in offspring[6].

In a large Swedish cohort ($n$ = 67,569), coffee consumption during pregnancy caused a shorter gestation (SGA), which is a strong predictor of neonatal intensive care admissions and an increased risk of neonatal death[7]. In the present study, the litter weights were significantly lower in the 20 and 80 mg/kg bw groups than in the untreated controls ($P$ < 0.05), with a greater reduction in the higher dose group. The number of female offspring decreased in the 20 and 80 mg/kg bw groups (Table 2). Some of the offspring were eaten by the dam following birth, accounting for half of the litters in the 80 mg/kg bw group. This behavior is an indicator of severe intrauterine growth restriction retardation (IUGR) and teratogenicity, and such a phenomenon may be related to maternal immune dysfunction. Brent et al.[8] reported that the teratogenic threshold of caffeine is 80–100 mg/kg bw, which is consistent with the present study. In addition, fewer female pups were born in the 20 and 80 mg/kg bw dose groups, suggesting that the developmental toxicity of caffeine differs between the genders, with female fetuses being more sensitive than male fetuses. Guanghui et al.[9] reported that fetal metabolites are

**Table 1.** Absolute and relative organ weights

| Group (mg/kg) | Spleen (g) | Spleen/body (%) | Liver (g) | Liver/body (%) | Kidney (g) | Kidney/body (%) | Paranephros (g) | Paranephros/body (%) |
|---|---|---|---|---|---|---|---|---|
| 0 | 0.90 ± 0.11 | 0.29 ± 0.22 | 13.75 ± 1.31 | 4.43 ± 0.37 | 2.11 ± 0.17 | 0.68 ± 0.03 | 0.09 ± 0.02 | 0.030 ± 0.007 |
| 5 | 0.93 ± 0.15 | 0.30 ± 0.05 | 13.35 ± 1.01 | 4.25 ± 0.21 | 2.25 ± 0.29 | 0.72 ± 0.10 | 0.09 ± 0.02 | 0.027 ± 0.008 |
| 20 | 0.87 ± 0.16 | 0.28 ± 0.05 | 13.25 ± 1.06 | 4.32 ± 0.28 | 2.11 ± 0.21 | 0.69 ± 0.63 | 0.08 ± 0.02 | 0.026 ± 0.007 |
| 80 | 0.75 ± 0.2* | 0.23 ± 0.06* | 12.11 ± 4.03 | 3.75 ± 1.20 | 2.14 ± 0.24 | 0.67 ± 0.07 | 0.10 ± 0.02 | 0.029 ± 0.008 |

***Note.*** Data presented as mean ± SD of 25 dams per caffeine dose group. *$P$ < 0.05 *vs.* control.

**Table 2.** Effects of maternal caffeine administration on surviving pup number and litter weight

| Group (mg/kg) | Dams | Litter weight (g) | Female pups (No.) | Male pups (no.) |
|---|---|---|---|---|
| Control | 25 | 98.85 ± 31.86 | 7.4 ± 1.9 | 7.6 ± 3.5 |
| 5 | 25 | 92.25 ± 32.30 | 6.4 ± 1.9 | 7.4 ± 4.4 |
| 20 | 25 | 77.62 ± 16.03* | 5.1 ± 2.2* | 7.5 ± 3.5 |
| 80 | 25 | 73.90 ± 20.66* | 5.6 ± 3.3 | 7.4 ± 1.9 |

***Note.*** *$P < 0.05$ *vs.* control.

**Table 3.** Effects of maternal caffeine administration on offspring's body weight during lactation

| Group (mg/kg) | Offspring | PND 4 | PND 7 | PND 11 | PND 14 | PND 17 | PND 21 |
|---|---|---|---|---|---|---|---|
| Control | 20 | 10.44 ± 1.18 | 16.60 ± 1.90 | 26.31 ± 3.14 | 34.10 ± 3.38 | 41.28 ± 3.74 | 57.49 ± 5.86 |
| 5 | 20 | 10.31 ± 1.09 | 16.11 ± 1.82 | 25.57 ± 3.30 | 33.08 ± 4.28 | 39.75 ± 5.41 | 53.92 ± 7.12 |
| 20 | 20 | 10.25 ± 1.21 | 15.83 ± 1.62 | 25.53 ± 2.60 | 33.19 ± 3.93 | 39.78 ± 4.83 | 54.84 ± 5.60 |
| 80 | 20 | 8.21 ± 1.27* | 12.42 ± 2.01* | 20.62 ± 3.15* | 27.39 ± 3.89* | 33.43 ± 4.39* | 45.24 ± 5.75* |

***Note.*** *$P < 0.05$ *vs.* control. PND, postnatal day.

different between the genders after prenatal caffeine exposure. The dose-dependent decrease in litter weight was consistent with the increased risk of IUGR. Brent et al.[8] reported that the threshold dose of caffeine for developmental toxicity in rats is about 30 mg/kg bw. However, according to the present study, the threshold dose of caffeine for developmental toxicity should be < 20 mg/kg bw. A large-scale case-control study in Japan that included 7,252 SGA infants (7.6% of the total) and 4,281 preterm infants found a dose-dependent relationship between maternal caffeine intake and SGA risk in the range of 205.5–5080.0 mg/day (3.4–84.8 mg/kg bw)[10].

In the present study, the mean preweaning body weight (PND 1–PND 21) of offspring (males and females combined) was significantly lower in the 80 mg/kg bw group than the controls ($P < 0.05$, Table 3). The threshold dose for prenatal and postnatal exposure is different, as studies have reported that 100 mg/kg bw caffeine does not lead to neurobehavioral toxicity in adult mice. In the present study, the no observed adverse effect level (NOAEL) of caffeine was 5 mg/kg bw based on the IUGR (as manifested by a lower litter body weight and the number of female pups) during pregnancy. The NOAEL of caffeine is 20 mg/kg bw based on the body weight of postnatal offspring during lactation. The limitations of this study include using experimental groups with higher than typical human intake of caffeine per kg bw to determine health outcomes. This toxicity model indicates that caffeine consumption should be limited during pregnancy and lactation.

No potential conflicts of interest exist.

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#Correspondence should be addressed to CAO Yu, E-mail: caoyu@chinacdc.cn, Tel/Fax: 86-10-58900405; Amber Sheeks, E-mail: asheeks@muskingum.edu

Biographical note of the first author: ZHANG Wen Zhong, male, born in 1976, PhD, Professor, majoring in toxicology related study.

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